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VISUAL EFFECTS OF RESPIRATORY DISTURBANCE

DESMOND MICHAEL CONNOLLY

Doctor of Philosophy

CITY UNIVERSITY

APPLIED VISION RESEARCH CENTRE

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Declaration

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Abstract

BACKGROUND. Mild hypoxia is well known to impair scotopic (night) vision while moderate to severe hypoxia is required to compromise photopic (day) vision. However, the severity of hypoxia required to affect mesopic (twilight) vision, when both rod and cone photoreceptors contribute to visual perception, is unknown. This question is relevant to night flying at low altitude when mild hypobaric hypoxia might influence the visual performance of healthy aircrew operating in dimly illuminated cockpits and flight decks. Comparative studies indicate that increased rod oxygen consumption in dim light promotes outer retinal hypoxia under normal respiratory conditions. This might increase the susceptibility of the outer retina to the deleterious effects of exogenous hypoxia. This thesis aims to identify and quantify effects of respiratory disturbance on visual performance across the low photopic to mid-mesopic range. As well as mild to moderate hypoxia, the effects of hyperoxia and hypocapnia (hyperventilation) are considered.

METHODS. Cardio-respiratory status was monitored closely in all experiments, including breath-by-breath mass spectrometry for respired partial pressures of oxygen and carbon dioxide. An initial dark adaptation study in a hypobaric chamber assessed the effects of hypoxia, hyperoxia and hypocapnia on threshold sensitivity to dim flash stimuli, enabling definition of a mesopic adaptation procedure that was independent of respiratory condition. Hyperventilation increases flicker sensitivity, so the potential for hypocapnia to lower mesopic flicker thresholds and thereby confound subsequent studies was assessed under progressive hypocapnia. Subsequent experiments examined the visual perception of 12 healthy volunteers (6 male and 6 female) under low photopic, upper mesopic and mid-mesopic viewing conditions while breathing gas mixtures to establish normoxic, mildly hypoxic or hyperoxic respiratory states. Visual parameters comprised spatial contrast sensitivity, threshold chromatic sensitivity, visual processing speed, temporal contrast sensitivity and low contrast acuity, the last incorporating assessment of pupil size at each light level and respiratory condition. Visual stimuli were display-based and comprised foveal Gaussian Gabor patch gratings; the City University Colour Assessment and Diagnosis Test; the Useful Field of View® Test; threshold Frequency Doubling Technology perimetry; and the City University Contrast Acuity Assessment Test. Breathing gases were masked from the subjects and exposure orders were balanced and randomised between males and females. In some experiments hypocapnia was induced by voluntary hyperventilation. Vision testing was conducted binocularly and monocularly where practicable. Repeated measures designs favoured initial statistical analyses using balanced Analysis of Variance followed by various parametric and non-parametric *post hoc* analyses.

RESULTS. The achievement of scotopic sensitivity during dark adaptation is oxygen dependent, being delayed progressively by worsening hypoxia but hastened by hyperoxia and hypocapnia, such that rod photoreceptors are functionally hypoxic, in the dark, under normal respiratory conditions. Mesopic flicker sensitivity is highly correlated with the severity of hypocapnia but the magnitude of the effect is slight and unlikely to be meaningful. At the fovea, spatial contrast sensitivity is resistant to respiratory disturbance but threshold chromatic sensitivity is increasingly vulnerable to hypoxia as light level decreases in the mesopic range. Both spatial contrast sensitivity and chromatic sensitivity exhibit clear binocular summation. Mild hypoxia tends to delay visual processing speed and may be relevant to the extraction of visual information from complex scenes. Effects of hypoxia to impair and hyperoxia to enhance temporal contrast sensitivity vary with light level and retinal eccentricity but also support a progressive effect of hypoxia with decreasing mesopic luminance. Low contrast acuity is compromised by mild hypoxia at photopic and mesopic luminance but is enhanced by hyperoxia, relative to normoxia, in the mesopic range, implying further oxygen-dependent functional impairment. Mild hypoxia consistently induces pupillary miosis in the low photopic to mid-mesopic range.

CONCLUSIONS. Mild hypoxia compromises numerous visual attributes and its effects may be promoted by endogenous, rod-driven, outer retinal hypoxia with decreasing mesopic luminance. In dim light hyperoxia enhances some aspects of visual sensitivity relative to performance breathing air, implying oxygen-limited functional impairment under normal respiratory conditions, presumably due to increasing rod oxygen consumption in dim light. These effects have implications for the use by aircrew of supplementary oxygen at modest altitudes. Hypocapnia enhances visual sensitivity but is unlikely to be meaningful except, perhaps, in the scotopic range. The outer retina is functionally hypoxic in the mesopic range under normal respiratory conditions and this may have implications for the aetiology of retinal pathology. Effects of respiratory disturbance on pupil size warrant further consideration.

Abbreviations and Symbols

SI Units (from *Le Système International d'Unités*) are represented conventionally. Other units, symbols and abbreviations that have been used are listed below.

2-AFC	Two-alternative forced-choice
4-AFC	Four-alternative forced-choice
8-AFC	Eight-alternative forced-choice
α	Alpha (significance level)
Δ	Delta (change)
η	Eta (viscosity)
π	Pi
°	Degrees of visual angle
°C	Degrees Celsius (temperature)
°N	Degrees North (latitude)
[ ]	Concentration
$[Ca^{2+}]_i$	Intracellular concentration (of calcium ion)
[Hb]	Haemoglobin concentration
A	'Air' condition (normoxia)
AD	Anderson-Darling (test of normality)
ADP	Adenosine diphosphate
amsl	Above mean sea level
ANOVA	Analysis of variance
Ar	Argon
AR5	Aircrew respirator Mark 5
atm	Atmosphere
ATP	Adenosine triphosphate
ATPD	Ambient temperature and pressure, dry
BBB	Blood-brain barrier
bpm	Beats per minute
BRB	Blood-retinal barrier
C	Contrast
Ca^{2+}	Calcium ion
CAA	Contrast Acuity Assessment test
CAD	Colour Assessment and Diagnosis test
CAT	Contrast acuity threshold
CFF	Critical flicker/fusion frequency
cGMP	Cyclic guanosine monophosphate
CI	Confidence interval
CIE	Commission Internationale d'Éclairage
CO_2	Carbon dioxide
CoV	Coefficient of variation
cpd	Cycles per degree
cps	Cycles per second
CRP	Corneo-retinal potential
CRT	Cathode ray tube
CSF	Contrast sensitivity function
D	Dioptres
D	Optical density
dB	Decibel
df	Degrees of freedom
EEG	Electroencephalogram
ERG	Electroretinogram
FDT	Frequency Doubling Technology
F	Flow
F_IO_2	Fractional inspired oxygen concentration
FM100	Farnsworth-Munsell 100-Hue Test

ft	Feet (altitude amsl)
GC	Ganglion cell
GCL	Ganglion cell layer
GL	Ground level
GTP	Guanosine triphosphate
H	Hypoxia condition (14.1% oxygen)
H^+	Hydrogen ion
H_2O	Water
Hb	Haemoglobin
HCO_3^-	Bicarbonate ion
I	Intensity
ICAO	International Civil Aviation Organisation
INL	Inner nuclear layer
INQ	Inferior nasal quadrant
IO	Inside observer
IOP	Intraocular pressure
IPL	Inner plexiform layer
IS	Inner segment
ITQ	Inferior temporal quadrant
K	Ocular rigidity coefficient
K^+	Potassium ion
K-cell	Koniocellular cell
L	Luminance
L	Length
L-cone	Long wavelength-sensitive cone
LGN	Lateral geniculate nucleus
Log_{10}	Logarithm (to base 10)
MANOVA	Multivariate ANOVA
MAP	Mean arterial (blood) pressure
Mb	Megabyte
M-cell	Magnocellular cell
M-cone	Medium wavelength-sensitive cone
MFR	Mean firing rate
mm Hg	Millimetres of mercury
MT	Movement time
N	Number (of subjects)
N_2	Nitrogen
Na^+	Sodium ion
ND	Neutral density
NFL	Nerve fibre layer
Ngb	Neuroglobin
O	Oxygen condition (100% oxygen)
O_2	Molecular oxygen
OD	Optical density
ONL	Outer nuclear layer
OPL	Outer plexiform layer
OS	Outer segment
P	Pressure
p	p value (statistical significance)
P_a	Arterial pressure
P_aCO_2	Arterial partial pressure of carbon dioxide
P_ACO_2	Alveolar partial pressure of carbon dioxide
P_AN_2	Alveolar partial pressure of nitrogen
P_aO_2	Arterial partial pressure of oxygen
P_AO_2	Alveolar partial pressure of oxygen
P_B	Barometric pressure
PC	Personal computer
PCO	Pressure chamber operator
PCO_2	Partial pressure of carbon dioxide
P-cell	Parvocellular cell
PDE	Phosphodiesterase
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
$P_{ET}O_2$	End-tidal partial pressure of oxygen
pH	$-\log_{10}$ hydrogen ion concentration
PH_2O	Saturated water vapour pressure
P_IO_2	Inspired partial pressure of oxygen
PIPs	Pseudoisochromatic plates
PO_2	Partial pressure of oxygen

psig	Pounds per square inch (gauge)
P_v	Venous pressure
QO_2	Oxygen consumption
r	Radius
R	Resistance
R	Respiratory exchange ratio
R*	Activated rhodopsin
R-G	Red-green
rms	Root mean square
ROS	Rod outer segment
RPE	Retinal pigment epithelium
RQ	Respiratory quotient
RT	Reaction time
S	Sensitivity
S_aO_2	Arterial haemoglobin oxygen saturation
S-cone	Short wavelength-sensitive cone
SD	Standard deviation
SE	Standard error of the mean
sf	Spatial frequency
SNQ	Superior nasal quadrant
SRS	Subretinal space
STP	Standard temperature & pressure
STPD	Standard temperature & pressure, dry
STQ	Superior temporal quadrant
T	Transmittance
UFOV	Useful (functional) field of view
UFOV®	The Useful Field of View test
V	Volume
VA	Visual acuity
VER	Visual evoked responses
VSG	Video signal generator
Y-B	Yellow-blue

1 Introduction

1.1 Overview

1.1.1 The nature of human vision

Sensing fluctuations in ambient electromagnetic radiation, human vision provides a dynamic and near-instantaneous perception of the environment that is limited more by physics and geography than biology. Visual perception can offer detailed information on form, depth, texture, colour and motion, and operates between extremes of light intensity that may range by over one billion billion-fold, from near total darkness to bright noonday sunlight reflecting from a layer of snow.

The eyes are the rapidly and automatically adaptive sensors that enable visual sensation between these extremes. Their optics harness light incident upon their external surfaces to produce, with no conscious effort, a representative image of the environment that is focused upon a matrix of 125 million photoreceptors in each retina. Phototransduction converts the image into patterns of electrical signals that undergo complex interactive pre-processing within the neural tissue of the retina. The output from each retina is transmitted to the brain by only a million or so ganglion cells, each coding multiple strands of information.

In the brain, these signals undergo considerable further bilateral processing in multiple interconnected centres. The image is deconstructed, for example in relation to colour, contrast, boundaries and orientation, and reconstructed for most efficient use. Many cells in these pathways respond variably to different patterns of stimulation, providing information on multiple visual parameters to other areas while forming a complex network of feed-forward, feedback and cross-coupled loops that effect spatial and temporal filtering, modulation, adaptation and amplification mechanisms.

The end result is the perception by the conscious mind of a real-time, three-dimensional image of the external scene that integrates awareness of self and enables rapid and complex interaction with the environment. Reliable evidence of the past may be assessed, current activity witnessed, and future events anticipated and encouraged or avoided, even from afar. From moment to moment and day to day, this miracle is relied

upon without reservation yet is largely taken for granted, despite facilitating virtually every conscious activity and being essential for many.

However, the incredible performance of the healthy human visual system masks an inherent vulnerability. For given viewing conditions, sensitivity to a faint or challenging visual stimulus will be limited by a probabilistic threshold, below which it will tend not to be seen or discriminated. If that threshold is elevated slightly, reducing sensitivity and compromising visual performance, while still allowing sufficient of the external scene to be appreciated, subjects are likely to remain unaware of the deficiency to which they are exposed. The extent of what cannot be seen cannot possibly be appreciated, while that which remains visible may satisfy subjective criteria for normality. An individual may believe unreservedly that his eyesight is as good as it should be for the prevailing viewing conditions, yet his visual performance may be degraded significantly, depriving him of potentially useful, even vital, information. In few occupations may this be more important than in military aviation, when safety-critical responses and mission success may depend fundamentally on optimal visual performance. In a contribution to an open forum web-based aviation medicine group in 2003, a commentator noted:

> "... one night at 8,000 feet (and well below any [regulatory] requirements for supplemental oxygen) for a bit over an hour, it was amazing to me how drastically my color vision improved with just a few puffs of oxygen ... I'd read that this would occur, but had no idea how large the difference would be. I felt no other symptoms of hypoxia ... but being able to much more clearly make out the color of lights in the approach environment was a big help". (McManus, M.)

This thesis will examine the extent to which subtle visual impairment may result from aviation-related disturbances of normal respiratory physiology.

1.1.2 Relation to past work

Early balloonists noticed impaired vision during flight at high altitude, with difficulty reading a mercury column although distant vision was unaffected (Van Liere and Stickney, 1963, p 335). Recognition of the fundamental importance of good eyesight to military aviators during World War I stimulated early research into the effects of altitude-related hypoxia (oxygen deficiency) on the eye and vision (Wilmer and Berens, 1918). The need to fly and fight at night during World War II prompted considerable further research into the visual effects of systemic hypoxia, particularly on dark adaptation and contrast discrimination at low light levels. During the 1970s Kobrick

conducted a series of studies into the effects of hypoxia on the extent of the visual field and central versus peripheral attention. Otherwise the psychophysical effects of respiratory disturbance have received sporadic attention since the end of World War II.

Moderate to severe hypoxia, equivalent to breathing air at altitudes of 4572 m (15,000 ft) and over, compromises many aspects of cone photoreceptor-mediated visual performance under daylight (photopic) conditions, while mild hypoxia is well known to impair rod photoreceptor-mediated night (scotopic) vision. However, the extent to which mild hypoxia might compromise visual performance under twilight (mesopic) conditions, when both cones and rods contribute, is unknown and is the core concern of this thesis. This has direct relevance to contemporary aircrew visual performance when flying at night, as cockpits and flight decks are typically illuminated in the mesopic range.

It is worthwhile to explore this question now for three main reasons. Firstly, over the last 25 years much work has explored the oxygen (O_2) requirements and metabolism of the eye, driven by a desire to understand the pathophysiology of disease processes such as glaucoma, diabetic retinopathy and age-related macular degeneration. Secondly, respired gas composition is now monitored routinely, breath by breath, using respiratory mass spectrometry, allowing close control and documentation of alveolar gas pressures during respiratory challenges. Finally, the rapid development of computer-driven display technology has made available a plethora of new and sensitive vision testing techniques, effectively of limitless variety.

Studies investigating the effects of respiratory disturbance on visual performance are often vulnerable to the same criticisms. Many have tended to control well either visual or respiratory adaptation, but rarely both. Many have used relatively insensitive vision tests or have failed to document adequately the imposed respiratory conditions. Others have ignored possible effects of hyperventilation to induce hypocapnia, that is, to lower carbon dioxide (CO_2) levels, and thereby confound both vision testing and respiratory state. None of these observations should apply to the work reported here.

1.1.3 Scope

This thesis explores the extent to which losses of visual sensitivity may result from relatively mild disturbances of respiratory physiology typical of acute but modest altitude exposure. In this context 'acute' is taken to mean exposures to steady equivalent

altitudes that are of rapid onset (minutes) but short duration (no more than an hour), while 'modest' relates to altitudes which young, healthy individuals would be expected to tolerate for many hours, following abrupt exposure, without noticing any deleterious effects. This has direct relevance to aviators who fly routinely without the benefit of supplementary O_2, for example in helicopters, light aircraft, gliders and partially pressurised or unpressurised transport aircraft, and especially under conditions which would normally be expected to challenge visual performance, such as at night and in poor weather. Bagshaw (2006) provides an introduction to the visual tasks faced by contemporary aviators. It is emphasised that this work specifically does not encompass 'chronic' altitude exposures typical of terrestrial ascent, as when mountaineering, these being characterised by slow onset, progressive severity and extended duration (days), while affording variable opportunity for adaptation and acclimatisation.

1.1.4 Aims

The primary aim of this thesis is to identify possible effects of mild acute hypoxia on fundamental aspects of human visual performance at low light levels, thereby defining more clearly the limits and vulnerabilities of the human visual system at altitude. In so doing, the findings are considered for any insights they provide into the underlying physiology of the human visual system. Finally, the findings are considered from an applied perspective for their implications in relation to aircrew visual performance. These aims are revisited in the Discussion in Chapter 11.

1.1.5 New findings

The following are believed to be new findings resulting from this work:

Rod photoreceptors are functionally hypoxic, in the dark, under normal respiratory conditions, that is, breathing air at sea level (Chapter 4).

The timing of cone rod inflection on a mixed dark adaptation curve is delayed by hypoxia and hastened by hyperoxia and hypocapnia (Chapter 4).

For a simple mesopic stimulus, there is a linear correlation between critical flicker/fusion frequency and severity of hypocapnia (Chapter 5).

Mild hypoxia impairs foveal chromatic sensitivity progressively as background mesopic light levels fall from 1.67 to 0.21 cd m^{-2} (Chapter 7).

Mild hypoxia compromises visual processing speed and thereby impairs the extraction of information from cluttered visual scenes (Chapter 8)

Mild hypoxia impairs temporal contrast sensitivity, beyond the fovea, at a photopic background luminance of 100 cd m^{-2} (Chapter 9).

Mild hypoxia impairs and supplementary O_2 enhances temporal contrast sensitivity across the central visual field at a mesopic background luminance of about 1 cd m^{-2} (Chapter 9).

Mild hypoxia consistently compromises acuity to low contrast stimuli at photopic and mesopic background levels of 12, 1 and 0.1 cd m^{-2} (Chapter 10).

Supplementary O_2 enhances acuity to low contrast stimuli at a mesopic background luminance of 0.1 cd m^{-2} (Chapter 10).

1.2 Visual Perception

Authoritative accounts of ocular anatomy, biochemistry and physiology, optometry, the visual system, human visual performance and visual psychology are available elsewhere (for example, Schwartz, 2004; Kaufman and Alm, 2003; Bruce, Green and Georgeson, 2003; Forrester *et al*, 2002). The following is a very limited account of the human visual system intended to be relevant in the context of respiratory disturbance and to support subsequent discussion of the experiments that follow. In developing this account various other reference works have also been consulted repeatedly (particularly Rodieck, 1998; Hubel, 1995; Zeki, 1993; Boff and Lincoln, 1988). The full list of reference texts consulted is provided in the Bibliography.

1.2.1 Light enters the eye

Light entering the eye must pass through the tear film, conjunctiva, cornea, aqueous humour, pupil, crystalline lens, vitreous gel, inner retinal layers, and various membranes that separate these structures, before reaching the photosensitive layer of the outer retina (Figure 1.1). While its course will be affected whenever it passes between tissues of differing optical density (OD), light is refracted primarily by the cornea and secondarily by the crystalline lens, the shape of the latter being modified involuntarily by the ciliary musculature to adjust the focus of the retinal image. The normal refractive power of the cornea is approximately 43D (range 38 to 48D) while that of the lens is 19D (range 17

to 26D), together generating a typical refractive power of around 59D at rest (Boff and Lincoln, 1988, p54). Thus, the cornea contributes over two thirds of the refractive power of the eye. The developed lens and vitreous gel are essentially avascular and may be regarded as exhibiting negligible metabolic activity. It is necessary to recognize, however, that the elasticity of the lens capsule decreases with age, causing loss of accommodative power (presbyopia), such that the near point of focus starts to recede rapidly during the fifth decade of life.

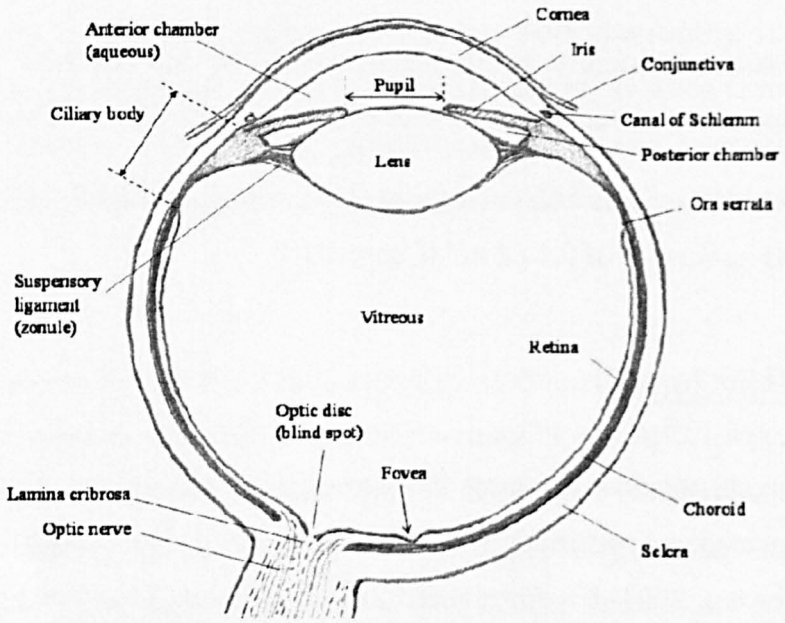


Figure 1.1 Sketch of a horizontal section through right eye (after Pirenne, 1948, p 3)

The amount of light entering the eye is determined by the area, and hence diameter, of the pupil, which in adults may range from about 2 to 9 mm. This near circular aperture is controlled by the balanced and mutually antagonistic actions of the sphincter and dilator muscles of the iris, which are under autonomic control. Pupillary constriction (miosis) in response to steady bright light and dilatation (mydriasis) in response to steady dim light are not monotonic, being subject to initial overcompensation and oscillation before settling to a 'steady' state over one and 20 minutes respectively. Pupillary responses to brief changes in light level are complex and involve consideration of latency, amplitude and duration. The pupil also constricts with accommodation to a near visual target and is smaller when viewing monocularly rather than binocularly. Apart from these reflexes, many other factors influence pupil size including age, target colour, contrast patterns, the structure of the visual field, drugs, level of conscious arousal, cognitive activity, fatigue, emotional state and psychological factors, but probably not refractive error or gender (Winn, Whitaker, Elliott *et al*, 1994).

Even under steady state conditions, the pupil is in constant motion, showing rhythmic fluctuations, and there is considerable variation in pupil size between individuals.

In bright light a fully constricted pupil with a diameter of only 2 mm will have an area of about 3.1 mm^2, reducing the aperture of the lens, limiting the effects of spherical and other optical aberration, mitigating against blurring of the retinal image and so aiding acuity and depth of focus. In the dark, mydriasis allows more light to pass through to the retina, sacrificing acuity for sensitivity. A maximally dilated pupil with a diameter of 9 mm will have an area of about 63.6 mm^2, allowing about 20 times more light to enter the eye than when fully constricted. This amounts to little more than one order of magnitude. However, human eyes may be exposed to a range of light intensities spanning over 12 $\log_{10}$ units. Within this range, human vision is very effective over around 7 $\log_{10}$ units yet, at any moment, is generally performing within an 'active range' of only 1-2 $\log_{10}$ units. The pupil is unlikely to change in diameter by more than 1-2 mm under such conditions, so the pupil provides only a limited contribution to the process of visual adaptation. Nonetheless, pupil size is an important determinant of retinal illumination, the amount of light that may contribute to the visual process, and hence visual thresholds and sensitivity. It also controls the depth of field and aberrations of the eye, while responding to a wide range of stimuli (Barbur, 2004a).

Not all of the light reaching the eye and passing through the pupil will be sensed in the outer retina. The ocular media are not perfectly lucent and some light will be absorbed while some will be scattered. For example, short wavelength (blue) light will be absorbed increasingly by an ageing and yellowing lens as well as by the yellow macular pigment of the central retina. Furthermore, not all the light reaching the retina is involved in phototransduction. Beyond the photoreceptors is a pigmented layer just one cell thick, the retinal pigment epithelium (RPE), which has many important functions (Strauss, 2005). One of these is to absorb stray light, minimizing light scatter and internal reflections within the eye, and thereby reducing glare.

1.2.2 The outer retina – phototransduction

An average human retina contains around 90 million rod photoreceptors and about five million cones, the latter comprising three varieties (Curcio, Sloan, Kalina *et al*, 1990). A detailed account of the nature of vertebrate rods and cones is given by Ebrey and Koutalos (2001). Rods and cones are named and distinguished by the morphology of their outer segments, although this varies considerably, even within the same retina. The

photoreceptors are closely arrayed in a layer one cell thick, and are aligned with their long axes parallel to that of incident light. This maximizes the likelihood of photon capture in the stack of many hundreds of highly fluid, outer segment disc membranes containing the light sensitive pigment that is characteristic of each receptor type. Each receptor contains about 10^9 molecules of photopigment. The outer segment tips abut the RPE, with which each photoreceptor forms a functional unit, and together they comprise the 'outer retina', nourished primarily by the blood of the choroid capillary plexus (choriocapillaris) which lies immediately beyond the RPE (Figure 1.2).

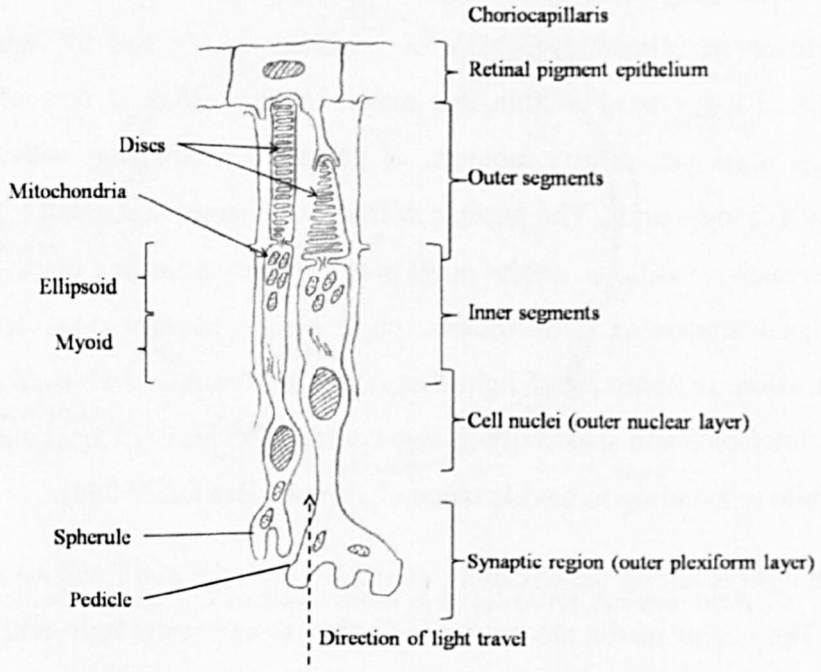


Figure 1.2 Simple schematic of main outer retinal components with a rod (left) and cone (right)

The long, slim rod outer segments (ROS) each contain up to ~1000 discs holding the membrane-bound, photosensitive pigment, rhodopsin. The discs are produced at the base of the outer segment and, over about 10 days, move to the tips where they are phagocytosed by the apical microvilli of the RPE. The rod inner segments comprise an outer half, or ellipsoid, containing many densely-packed mitochondria, and an inner half, the myoid, containing other organelles, including Golgi apparatus and smooth endoplasmic reticulum, as well as glycogen, all indicating a highly active metabolic and synthetic nature. Compared to rods, cones are generally shorter, wider at the base of the outer segment and tend to be more conical, although they are long, slender and tightly packed at the fovea. Unlike rod discs, the outer segments of cones comprise discs formed from intricately folded layers of the cell wall membrane, and hence they are in

free communication with the inter-photoreceptor space. They have a longer life span than rod discs. Cone ellipsoids contain about 600 mitochondria per cell, more than rods, suggesting an even greater metabolic and energy requirement.

The details of vertebrate phototransduction and retinoid metabolism are complex and are reviewed elsewhere (McBee, Palczewski, Baehr *et al*, 2001; Pepe, 2001). The following account is simplified and intended to illustrate the exceptional photoreceptor requirement for biological energy.

In a steady 'dark' state, the cation channels of the ROS are held open by bound cyclic guanosine monophosphate (cGMP), sustaining an influx of cations that tends to depolarize the cell membrane and maintain the release of neurotransmitter (glutamate). The cations comprise mainly sodium (Na^+) and calcium (Ca^{2+}) ions, (80% and 15% respectively), with some magnesium. Cation exchange pumps in the outer segments work constantly to extrude the Ca^{2+}, requiring intracellular potassium ions (K^+) to be sacrificed and expelled together with the Ca^{2+}, in exchange for yet more Na^+ which enters the cell. Accordingly, Na^+ pumps in the inner segments strive to expel the excess Na^+ and recover the lost K^+. The darker it is, the greater the influx of cations in the outer segments, the more depolarized the cell membrane becomes, the more transmitter is released and the harder the exchangers and pumps have to work to expel the Ca^{2+} and Na^+. Water accompanies the movement of electrolyte and it has been stated that, in complete darkness, the equivalent of the entire rod cytosolic fluid volume is pumped every 15 to 30 s (Arden, 2001; Arden, Sidman, Arap *et al*, 2005; both referencing Hagins, Ross, Tate *et al*, 1989). This continuous influx and extrusion of cations constitutes the 'dark current'. The dark level of cGMP is maintained by balancing its synthesis by guanylate cyclase and its hydrolysis by cGMP phosphodiesterase (PDE).

The light-sensitive photoreceptor pigments comprise an opsin (a protein retained within the photoreceptor disc membrane) and a chromophore, 11-*cis* retinal, an aldehyde of vitamin A. In rods, an absorbed photon isomerizes the 11-*cis* retinal of rhodopsin which initiates a cascade of extremely fast reactions that are amplified as they proceed. The activated form of rhodopsin, metarhodopsin II (or R*), moving within the fluid disc membrane, encounters and activates molecules of a G protein, transducin, which in turn stimulates PDE. As a result, the cytosolic concentration of cGMP falls and the cGMP-gated ion channels in the rod outer segments close, reducing the influx of Na^+ and Ca^{2+} ions. The intracellular Ca^{2+} concentration, or $[Ca^{2+}]_i$, then falls as Ca^{2+} continues to be

extruded by Na^+/Ca^{2+}, K^+ exchangers, while the inner segment pumps expel the Na^+, and so the cell membrane tends to hyperpolarize (Pugh and Cobbs, 1986).

To recover from light excitation, R* is deactivated through multiple phosphorylation by rhodopsin kinase and capping of the molecule with the protein arrestin (in competition for the binding site of transducin). Transducin is deactivated and the stimulated hydrolysis of cGMP by PDE is decreased. This enables cGMP levels to recover, re-opening the ion channels in the outer segments, allowing the $[Ca^{2+}]_i$ to rise and once again tending to depolarize the cell membrane. The roles of cGMP and Ca^{2+} in phototransduction and receptor adaptation are discussed in Pugh and Lamb (1990). The release of neurotransmitter from the photoreceptor terminal is also influenced by Ca^{2+} entering the cell through voltage-gated ion channels, indicating a further role in the control of photoreceptor signalling, as well as in contributing to the dark current.

The isomerised chromophore is reduced by retinal dehydrogenase whereupon it is able to leave its membrane-bound opsin. This all-*trans* retinal is then reduced to retinol and transported to the RPE. There, all-*trans* retinol is esterified and converted to 11-*cis* retinol before being oxidized to 11-*cis* retinal ready for transportation back to the photoreceptor. The details of this process remain uncertain, as is the biological imperative to locate it in the RPE, rather than keeping it locally in the photoreceptor. The removal of photoisomerised chromophore from inactivated rhodopsin allows dephosphorylation to occur, paving the way for regeneration of photosensitive rhodopsin with the addition of a new molecule of 11-*cis* retinal. The light sensitive molecule is then ready to capture its next photon.

The rod stacks of membrane discs are considerably taller than those of cones and have a greater likelihood of capturing a photon, making rods more sensitive to light. Measurement of the minimum energy necessary for vision was reported by Hecht, Shlaer and Pirenne (1942), from which it was deduced that a single quantum of light is sufficient to activate a single rod, although 6-10 quanta are required to stimulate nearby rods in a brief time interval to enable perception of light. In contrast, at maximal cone sensitivity (absolute cone threshold) it appears that at least 5-7 quanta must be absorbed by each of two cones for detection of a light stimulus (Pandey Vimal, Pokorny, Smith *et al*, 1989). The reproducibility of rod single-photon responses has been confirmed experimentally (Baylor, Lamb and Yau, 1979; Rieke and Baylor, 1998). Thus, the rod membrane potential is influenced by low light intensities that cones cannot detect. On the other hand, cones function well at light intensities that saturate the rod transduction

cascade, cause them to hyperpolarize maximally, and 'bleach' substantial quantities of the rhodopsin photopigment.

1.2.3 The outer retina – adaptation to light and dark

The 'active range' of visual sensitivity, covering only 1-2 $\log_{10}$ units, has to shift up (light adaptation) and down (dark adaptation), depending on the prevailing ambient light conditions, in order to enable visual sensation across the range of intensities that may confront the eye. This is effected primarily through a complex process of photoreceptor adaptation that is intimately related to phototransduction. Reviews of the subject have been published by Fain, Matthews, Cornwall *et al* (2001) and by Lamb and Pugh (2004). The following is greatly simplified.

Photoreceptors quickly adjust to moderate changes in light intensity but complete recovery of sensitivity, following exposure to illumination of an intensity sufficient to bleach a significant proportion of the visual pigment, takes much longer. When intense illumination ceases, photoreceptors experience a 'dark light', a temporary reduction in sensitivity that may be quantified as an 'equivalent background light'. In effect, the presence of bleached pigment simulates a steady background light intensity, or partial light adaptation, even in complete darkness. The magnitude of the reduction in rod sensitivity correlates with the extent of bleaching but far exceeds the reduction in quantum catch efficiency that might be expected. This is attributed to the persistence of photoproducts of rhodopsin activation and their decay intermediates which continue to activate the phototransduction cascade and require inactivation. Quenching of R* and deactivation of transducin occur relatively quickly, but the decay of phosphorylated and arrestin-bound intermediates and the regeneration of photopigment take longer (Firsov, Kolesnikov, Golobokova *et al*, 2005). There may also be an effect of bleached opsin mediated through changes in $[Ca^{2+}]_i$ (Fain, Matthews and Cornwall, 1996). In short, a high $[Ca^{2+}]_i$ in darkness may prolong the life of R*, while the reverse is true in light, when $[Ca^{2+}]_i$ decreases. Finally, the process of regenerating 11-*cis* retinal and reintegrating it to form rhodopsin is time consuming. For all these reasons, rod adaptation to dark occurs slowly.

Rods also respond to increases in background light intensity that do not produce substantial proportions of bleached pigment and appear to have two temporal phases of light adaptation (Calvert, Govardovskii, Arshavsky *et al*, 2002). The first is fast, occurring within seconds of the onset of light, is probably mediated by Ca^{2+} feedback

upon phototransduction cascade components and may provide an 80-fold reduction in sensitivity. A slower phase lasting tens of seconds was only observed at continuous light intensities that suppressed over half the dark current, and provided an additional sensitivity loss of up to another 40-fold before rod saturation occurred. The mechanism of the slow phase is uncertain. Together they provided a total range of light adaptation of over 3000-fold. The fast phase explains the ready loss of dark-adapted rod sensitivity with only brief light exposure.

Cones, on the other hand, can function in the presence of a significant amount of bleached pigment. They are able, therefore, to operate over a much broader range of light intensities. Although they cannot match the extreme sensitivity to low light achievable by rods, cone pigment regeneration is relatively rapid, and so they adapt to low light levels much quicker than rods, achieving close to full sensitivity in 10-12 minutes, even after a substantial bleach.

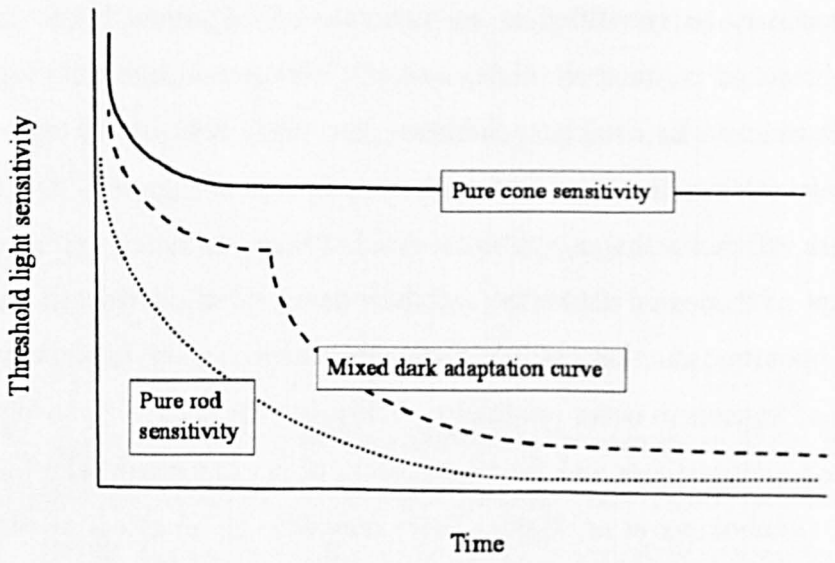


Figure 1.3 General appearances of cone, rod and mixed dark adaptation curves (after Forrester, Dick, McMenamin *et al*, 2002)

Figure 1.3 illustrates the general appearance of threshold sensitivity curves of cones and rods during adaptation to dark. Measurement of pure cone adaptation may be achieved by using a long wavelength stimulus to which only cones are sensitive, and illustrates that cone adaptation is relatively rapid, approaching an absolute sensitivity asymptote after only a few minutes in the dark. Pure rod adapation can be measured in rod

monochromats, subjects without cone photoreceptors, demonstrating slower adaptation but achieving far greater sensitivity to light.

Following a significant retinal bleach, psychophysical estimation of threshold sensitivity using a stimulus to which both cones and rods are sensitive will initially measure threshold cone sensitivity, settling towards a cone plateau, until rod sensitivity exceeds that of cones. At that stage, rod thresholds will be measured and the subsequent time course of rod sensitivity will be followed until an asymptote at absolute sensitivity is approached, up to an hour or more after a substantial bleach. The inflection point between the cone and rod threshold curves, in mixed cone and rod dark adaptation, is referred to here as cone rod inflection or the 'cone rod break', illustrated by the mixed curve in Figure 1.3. The generation of unambiguous cone rod breaks during dark adaptation demands a prior retinal bleach to deprive rods of sufficient photopigment to ensure a clear delay in regeneration of sensitivity by comparison to cones.

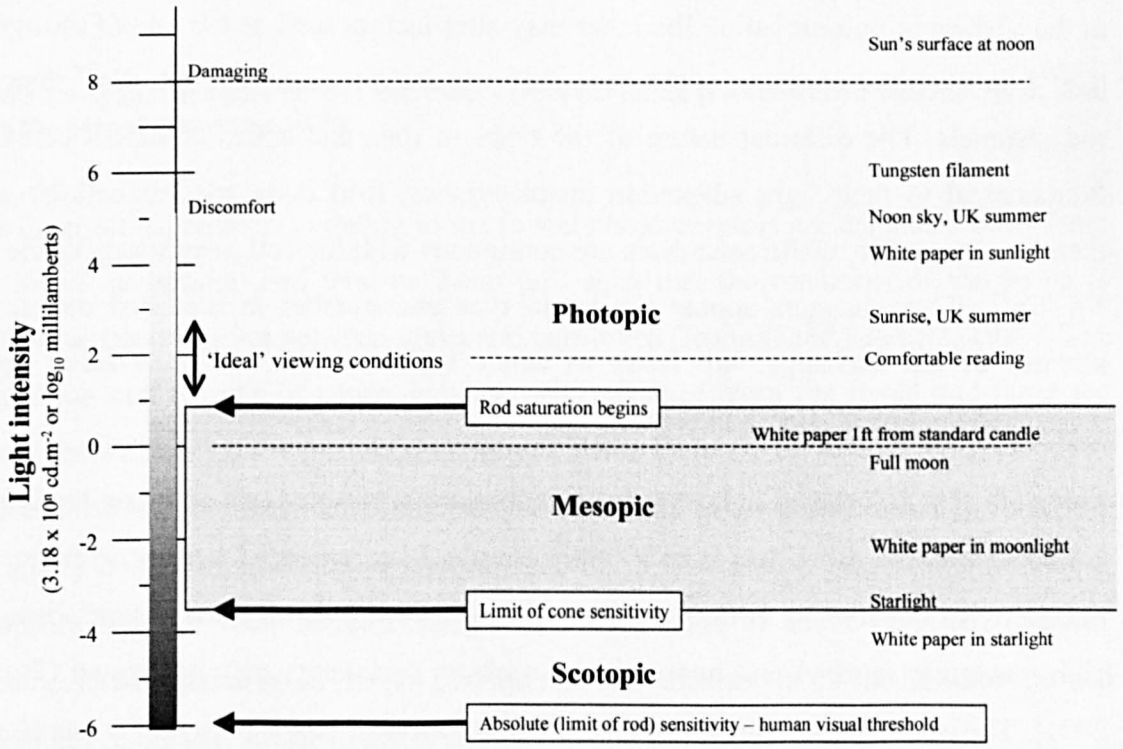


Figure 1.4 The light intensities confronting the eye (after Boff and Lincoln, 1988)

Thus, the adaptation ranges of rods and cones are discrete but overlap. The rod dark current ceases completely at photopic (daylight) levels of light intensity and rod responses become 'saturated', that is, rods are maximally hyperpolarised, transmitter

release is minimal and fluctuations in light intensity above this level do not influence their output (Figure 1.4). Accordingly, vision in the photopic range is mediated purely by cones. Conversely, scotopic (night) vision, at intensities below that of the cone rod break, is the exclusive domain of the more sensitive rods. In between these regions lies the mesopic range of 'twilight' intensities, typical of dawn and dusk, at which both rods and cones contribute to visual perception (Figure 1.4).

1.2.4 The duplex nature of the retina

Rods and cones operate over different ranges of light intensity and also adapt to them differently. Rods have a high absolute sensitivity, a slow response speed and a slow rate of adaptation to dark. Although they are less sensitive in the dark, cones adapt sooner to reductions in light level, have a fast response, and do not saturate, continuing to signal at higher light levels. These differences between rods and cones are thought to result primarily from differences in the biochemical components of the phototransduction process, the nature of the outer segment disc membranes and perhaps from differences in the surface to volume ratio. The latter may alter factors such as the rate of change of $[Ca^{2+}]_i$ in the outer segment, as the cone channels are more permeable to Ca^{2+} than the rod channels. The different nature of the discs in rods and cones is almost certainly fundamental to their light adaptation characteristics. Rod discs are intracellular and, therefore, vesicular, while cone discs are continuous with the cell membrane. While the Na^+/Ca^{2+}, K^+ exchangers appear similar in type and number in rods and cones, the kinetics of the exchanger are faster in cones which would lead to a faster light-stimulated decline in $[Ca^{2+}]_i$ in cones.

There are also differences in how rods and cones are distributed across the retina; Figure 1.5 illustrates this using data from a single sample. In common with other primates, the human retina contains an avascular region of high receptor density that is specialised for high resolution (acuity) and upon which an object of interest may be fixated (Provis, 2001). The central visual field is observed by the macula lutea, a region of retina almost 6 mm in diameter with its centre located 4 mm temporal and slightly inferior to the optic disc. The fovea is a shallow disc with a diameter of 1.5 mm at the centre of the macula. Foveal receptors are predominantly cones with a peak density in the central foveola, a rod-free zone that averages 0.35 mm in diameter and contains from 100,000 to over 300,000 cones per mm^2. The 0.5 mm diameter ring of retinal tissue surrounding the

fovea is the parafovea, while the rest of the macula comprises a further concentric ring, 1.5 mm in diameter, termed the perifovea (Polyak, 1941).

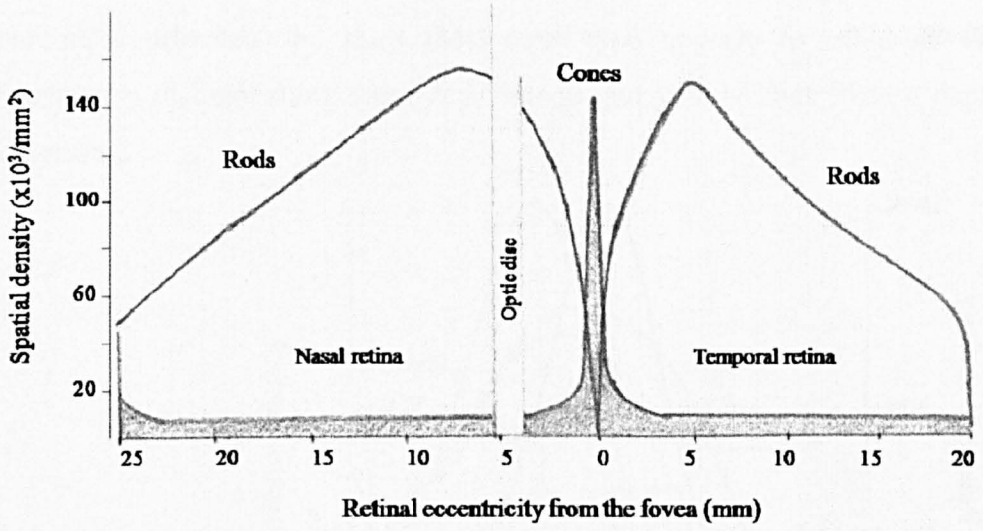


Figure 1.5 Spatial densities of rods and cones across the retina (redrawn from Rodieck, 1998, p43; from the data of Østerberg, 1935).

The inner retinal elements relating to the foveal photoreceptors are displaced away from the foveal depression and tend to 'heap up', such that the thickness of the retina is greatest at the transition between fovea and parafovea (Iwasaki and Inomata, 1986). The proportion and density of cones decrease with distance from the fovea and there are relatively few outside the macula. In contrast, while there are no rods at the very centre of the fovea and relatively few in the macula compared to the periphery, rods do appear in rapidly increasing numbers with distance from the fovea, reaching a peak density at approximately 5-6 mm, corresponding to a visual angle of about 18° from fixation. Curcio, Sloan, Kalina *et al* (1990) provide a recent assessment of the distribution and variability of the spatial density of rods and cones in the human retina.

Another difference between rods and cones is their differing sensitivities to light of different wavelengths, that is to say, their spectral sensitivity. The wavelengths of electromagnetic radiation that constitute visible light extend from approximately 360 to 830 nm, the blue and red ends, respectively, of the visible spectrum. Rods are maximally sensitive to wavelengths of ~510 nm and are responsible for the scotopic luminous efficiency curve, V'(λ) (Figure 1.6). Thus, rods are relatively insensitive to

light at the far red end of the spectrum. In contrast, the three cone types are responsible for photopic sensitivity, producing a net luminous efficiency curve, V(λ), that is shifted towards the red end of the spectrum, with a peak at around 555 nm. This explains the phenomenon described by Purkinje, by which, as light levels fall at dusk and rods begin to contribute more to visual perception, red objects seem to darken while blue ones appear to glow.

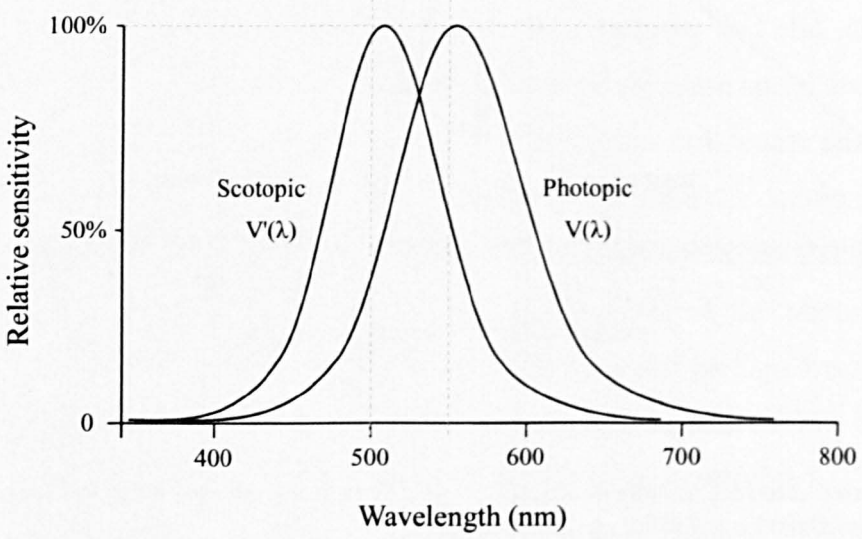


Figure 1.6 Normalised photopic and scotopic spectral sensitivity curves (approximate)

A single receptor type with a single photopigment cannot enable perception of colour, as any variation in such a receptor's electrical response resulting from a change in the spectral composition of incident light could also be caused by a difference in light intensity alone. It would be impossible to distinguish between contrast and colour with a single receptor type. However, each of the three types of cone photoreceptors in the human retina contains a discrete photopigment, cyanolabe, chlorolabe and erythrolabe. These absorb light maximally at approximately 420, 531 and 558 nm respectively. Their corresponding receptors are known as short-wavelength sensitive (S-), medium-wavelength sensitive (M-) and long-wavelength sensitive (L-) cones respectively (Figure 1.7). The photopic luminous efficiency curve or V(λ) results mostly from summation of the M- and L-cone signals (Figure 1.6). This difference in sensitivity to the spectral composition of light is the basis for the perception of colour. As a result the S-, M- and L-cones are commonly termed blue, green and red, although their spectral sensitivities overlap considerably and peak L-cone sensitivity does not lie in the red part

of the visible spectrum. Furthermore, the nature of colour vision signal processing does not relate back to the specific spectral sensitivities of the photoreceptors. Nonetheless, the three cone pigments underpin the trichromatic nature of normal human colour vision such that any particular colour can be matched by a combination of intensities of just three monochromatic lights drawn from the visible spectrum. In comparison to dichromatic primates, the third (M-) cone type appears to offer an evolutionary advantage in distinguishing some red, orange and yellow hues from a dappled green background.

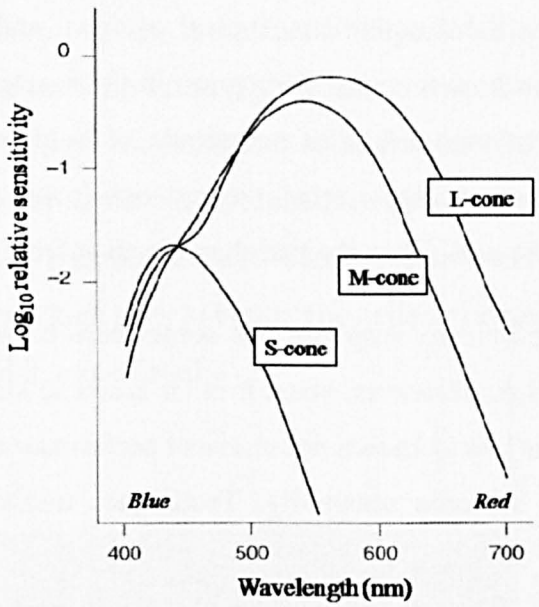


Figure 1.7 Relative sensitivities of S-, M- and L-cone photoreceptors (redrawn from Schwartz, 2004; after Boynton, 1979; based on data of Smith and Pokorny, 1975).

Returning to their distribution in the retina, S-cones constitute between 10-18% of human cones and are arrayed in a regular lattice, but are relatively sparse in the fovea. L- and M-cones are difficult to distinguish and estimations of their relative ratio and distribution vary considerably between studies and individuals. However, it seems likely that L-cones predominate at the fovea (Gowdy and Cicerone, 1998). At the fovea, L- and M-cones form a regular hexagonal mosaic with a minimum centre-to-centre spacing of 2-3 μm. Cone density decreases rapidly with distance from the fovea and visual acuity (VA) falls accordingly.

Thus, human eyes may be regarded as having a duplex visual system based on two distinct classes of photoreceptor. The cones are responsible for photopic (daylight) vision with high central acuity, owing to their concentration in the macula with little or no pooling of the outputs from adjacent receptors. They also enable perception of colour

through their differing spectral sensitivities and they adapt quickly to changes in light level. However, they are relatively insensitive in dim light. The more numerous rods provide scotopic (night) vision with very high sensitivity but with poor central acuity as there are relatively few rods in the central retina and none in the centre of the fovea. Scotopic vision is also monochromatic as all rods have the same visual pigment (rhodopsin) and hence the same spectral sensitivity. Rods adapt slowly to reductions in light level but are readily desensitised when exposed to bright light.

1.2.5 The mesopic range

Under relatively dim, 'twilight' conditions, typical of dawn and dusk, both rods and cones are active and contribute to visual perception in the mesopic range (Figure 1.4). This range spans three to four orders of magnitude of light intensity, varying with retinal location. At the fovea, photopic sensitivity extends down to as little as 2-3 cd m^{-2} while rods contribute more usefully in the periphery, even at ten-fold greater luminance.

The mesopic luminous efficiency response lies somewhere between the photopic and scotopic curves (Figure 1.6). However, since it is far easier to study either rod or cone responses in isolation, far less is known about visual performance in the mesopic range than either photopic or scotopic sensitivity. Techniques traditionally used to study mesopic vision have included heterochromatic brightness matching or flicker photometry, but while these provide information about distinct features of the visual system, differences between them necessarily produce inconsistent photometric data in relation to mesopic sensitivity. Unsurprisingly, while photopic and scotopic visual performance have been examined under respiratory disturbance, very little has been done to evaluate mesopic vision in this way.

Generally speaking, at the upper end of the mesopic range cone vision predominates, while the reverse is true as light levels fall and cones approach the limit of their sensitivity. Not only do the relative contributions of rods and cones vary across the mesopic range in a manner that cannot be modelled or predicted easily, but the relative contributions of the different cone types also varies with light level. Furthermore, there are increasing numbers of reports of interactions between rods and cones that no doubt also vary with luminance. Differences in how rod and cone signals are processed will be discussed in the sections that follow.

1.2.6 The inner retina – cellular organisation

A schematic of the general structure of the retina is illustrated in Figure 1.8. Further detail is available in a review of mammalian retinal architecture and a more recent study of macaque retina (Wässle and Boycott, 1991; Dacey, 1999).

In essence, vertical processing of the visual signal is effected through transmission of the photoreceptors' signals to their affiliated bipolar cells, which in turn convey the information to their associated ganglion cells. However, the process is subject to considerable lateral influence from the horizontal and amacrine cells, in the outer (OPL) and inner (IPL) plexiform layers respectively (Figure 1.8). Thus, considerable pre-processing of visual information takes place within the neural tissue of the inner retina. The ganglion cells (GC) form the innermost cellular layer (GCL) of the retina and their nerve fibres cross its inner surface to merge at the optic disc, where they are responsible for the 'blind spot', before exiting the eye in the optic nerve (Figure 1.1). Thus, the GC provide the visual output from eyes to brain. The cellular organisation of the inner retina will now be considered in more detail.

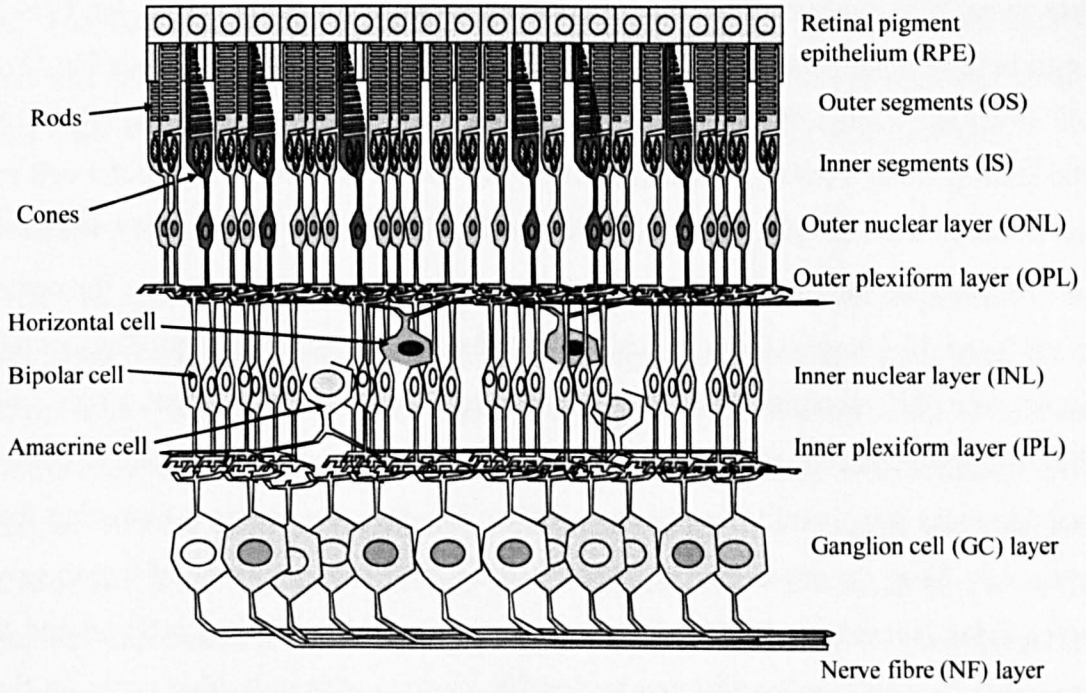


Figure 1.8 Schematic of the general organisation of the neural elements of the retina

Photoreceptors

The cell bodies of rods and cones are connected to specialised and expanded synaptic terminals known as spherules and pedicles respectively (Figure 1.2). Rod spherules synapse with telodendria from up to five bipolar and horizontal cells but do not contact other rod spherules. Cone pedicles are broader and contain up to 12 indentations, each with a triad of neuronal connections. The central process of each triad is a midget bipolar cell dendrite but all indentations may connect with the same midget bipolar cell. Each cone is usually connected to all (four to six) local horizontal cells, which have dendrites connecting to the lateral processes of the indentations. Each pedicle also has numerous shallow synapses to flat diffuse bipolar cells, while processes from cone pedicles also contact other cone pedicles, as well as rod spherules, through gap junctions. This may allow rod signals to be passed by cone bipolar cells under some circumstances and may also enable other rod-cone interactions.

Bipolar cells

The retina contains around 35,000,000 bipolar cells of several varieties. Their (single or multiple) dendrites pass outwards from the cell body in the inner nuclear layer (INL) to synapse primarily with photoreceptors but also with horizontal cells, while a single axon passes inwards to synapse with ganglion and amacrine cells. In the fovea, the ratio of cone to bipolar to GCs can be 1:1:1. It is said that a 2 mm lesion centred on the fovea could affect an average of 225,000 cones corresponding to 25% of the total GC output to the brain (Provis, Penfold, Cornish *et al*, 2005). In the periphery one bipolar cell can receive stimuli from 50-100 rods. About 20% of bipolar cells are rod bipolars, which are most dense around the macula. They have a receptive field that is small near the central retina (15 μm, 10-20 rods) and larger in the periphery (30 μm, 30-50 rods). They usually synapse with AII amacrine cells. Midget bipolar cells usually connect single cone pedicles to individual amacrine and midget GC. The near 1:1 ratio of midget cells to cones decreases peripherally. In contrast, diffuse bipolar cells accept information from many cones. Their dendrites fan out up to 70-100 μm to end in clusters of five to seven cone pedicles (sometimes 15-20). There is extensive overlap of these cells around the fovea. Blue S-cone bipolars may make specific contact with only blue cones in their territory.

Horizontal cells

Horizontal cells have extensive lateral connections and are of two main types. Type A (HII in primates) has larger, spidery dendritic trees and much larger receptive fields than Type B (HI), which are smaller and bushier. These dendritic trees contact only cones. Near the fovea each HI cell contacts about seven cones, but up to 18 peripherally, while HII dendrites contact about twice as many cones. HI cells have a long axon that ends in extensive arborisation that is post-synaptic only to rods, contacting up to 100 spherules. Each rod connects with at least two horizontal cells while each cone connects with three or four of each type. A third type of horizontal cell is also described in the human retina (HIII). The overlap between horizontal cells is considerable and any one area of retina may be served by up to 20 horizontal cells. They have an integrative role in retinal processing and the three human types may have colour-specific roles.

Amacrine cells

Amacrine cells are diverse with at least 25 different types in the human retina. Their numerous dendrites ramify and terminate mainly in the synaptic complexes formed by bipolar and GC processes in the IPL. Their dendritic fields are highly variable. These cells are thought to play a role in modulating (probably inhibiting) the signals reaching GC. They have a crucial role in rod (scotopic) vision as all rod bipolars synapse with amacrine cells, with none connecting directly to GC. The lateral extensions of amacrine cells have widespread connections to other amacrine, ganglion and bipolar cells.

Ganglion cells

There are approximately 1,000,000 GC in each retina and their axons form the nerve fibre layer (NFL) on its inner surface, leaving the eye at the optic disc to form the optic nerve. Hence the overall ratio of photoreceptors to GC is around 100:1. While cones contributing to high acuity vision at the centre of the fovea may have dedicated GC at a ratio of 1:1, the ratio increases away from the fovea. Rod outputs are also pooled increasingly with movement towards the periphery. There are up to seven layers of ganglion cell bodies in the central retina, thinning to as few as one cell layer in the periphery. They receive information primarily from bipolar and amacrine cells and are classified according to their cell body size and dendritic tree spread, branching pattern and branching level in the IPL. These vary considerably in form and size and correlate with retinal location and function (receptive field size).

Midget GC synapse with amacrine cells and only one midget bipolar cell (and hence usually only one cone). Dendritic spread is small in the central retina but increases 10-fold at an eccentricity of only a few mm. Neighbouring midget GC dendritic fields do not overlap but form mosaics. They are also known as P-cells as they project to the parvocellular layers of the lateral geniculate nucleus (LGN) of the brain. Midget GC provide the anatomical basis for small receptive fields and high VA in the fovea. Diffuse (parasol) GC receive information from a large field and from all types of bipolars except midget bipolars. They occur across the retina but with smaller dendritic fields nearer the fovea than in the periphery. They are also known as M-cells as they project to the magnocellular layers of the LGN.

Other retinal cells

In addition to these main neural elements, interplexiform cells are thought to provide a feedback link between the plexiform layers. The human retina also contains three types of glial cells. Microglial cells are resident immune cells that are important in defending against invading microorganisms, initiating inflammatory responses and tissue repair. Retinal astrocytes are present in vascularised regions of the inner retina (NFL, GCL and IPL), linking the superficial vascular elements with the vitreo-retinal border or inner limiting membrane. However, the most important retinal glia are the Müller cells. These span the thickness of the retina and contact or ensheath all the neural elements. They form a dense regular pattern and provide an anatomical link between the neural elements and the compartments with which they need to exchange molecules, namely the retinal vessels, the vitreous and the subretinal space (SRS), the latter forming the pathway to the choroidal vessels via the RPE. They have numerous vital roles in the retina that encompass glucose metabolism, regulation of blood flow, maintenance of the blood-retinal barrier (BRB), recycling of neurotransmitters, neuronal excitability, acid-base balance, ion homeostasis, and recycling of photopigments (Bringmann, Pannicke, Grosche *et al*, 2006).

1.2.7 The inner retina – signal processing

Each GC has a roughly circular receptive field within which stimulation will affect the cell's firing rate. The concentric receptive fields of GC were first described by Kuffler (1953). The response of the cell depends on whether the visual stimulus falls in the central circular area of the field or in the surrounding ring of the field. Some cells respond with a burst of signals to onset of stimulus in the centre or offset in the

surround (centre-ON) while some respond to the opposite, offset in the centre or onset in the surround (centre-OFF). Thus, the centre and surround are said to be antagonistic. However, the visual signal is modified long before it reaches the GC.

All vertebrate photoreceptors hyperpolarise (that is, they tend to turn OFF) in light, reducing the release of transmitter from their synapses. The situation becomes rapidly more complex as this signal is processed by the neural layers of the retina. Bipolar cells include hyperpolarizing (OFF) types that preserve the sign from the photoreceptor signal, and depolarizing (ON) types, in which the sign is reversed. This provides the signal for the receptive field centre response of the bipolar cells. ON and OFF bipolar cells synapse in different sublaminae of the IPL, the ON layer being closer to the GC (more vitreal). Like photoreceptors, horizontal cells hyperpolarize to light. As well as providing the spatially antagonistic receptive field of bipolar cells, they appear to provide feedback to cones that may modulate receptor sensitivity. Amacrine cells always depolarize in response to light but this may be transient or sustained. They are particularly responsive to moving stimuli, may produce ON and OFF responses to changes in illumination in their receptive fields, and may provide the basis of the antagonistic surrounds of the receptive fields of GC.

The GC response will follow the nature of change of a stimulus (increasing output with a stronger stimulus, decreasing with a weaker one) if it is falling in the ON area of the field. It will change in the opposite direction if it falls in the OFF region (increasing output with a weakening stimulus and decreasing with a stronger one). Whether a GC is centre-ON or centre-OFF is determined by the nature of the connections between the photoreceptor and bipolar cells that feed it. Complicating matters further, the GC response characteristics vary. Some respond linearly to light/dark gratings with no net change in output when summed responses to the centre and surround stimulation are zero. Others, however, have a non-linear response and will always signal the presence of a grating. These responses will then differ when faced with gratings moving across the cells' receptive fields, the first rising and falling against a background signal, and the second rising and falling against an elevated baseline. These features provide the basis for recognizing and signalling differences and changes in light intensity, or spatial and temporal contrast sensitivity.

Most retinal GC have concentric ON or OFF receptive fields that fall into one of two additional varieties. Some are 'colour opponent' in that they have antagonistic centre and surround regions that have peak sensitivities at different wavelengths of light. With

white light these cells will continue to exhibit spatial antagonism but the response to coloured light will be excited by some wavelengths and inhibited by others. These are the P-cells referred to earlier, which provide information about both luminance and wavelength in the centres and surrounds of their receptive fields. On the other hand, the 'broad band' M-cells do not exhibit colour opponency. They will respond little to a monochromatic light filling the receptive field but strongly to a difference in luminance between centre and surround, across a broad band of wavelengths. The difference arises particularly because P-cells are driven by a single type of cone. However, there are other differences. P-cell responses are tonic, that is, sustained stimulation results in a sustained response, while M-cell responses are phasic or transient, fading quickly if a stimulus does not change. The receptive fields of both types increase with eccentricity from the fovea but the centres of M-cell fields are consistently larger than those of P-cells. The smaller receptive fields of P-cells oblige more of them to cover the visual field and they outnumber M-cells eight-fold. Finally, the contrast threshold of M-cells is about 10 times lower (that is, they have a 10-fold greater contrast sensitivity) than P-cells.

Small bistratified GC have dendrites in both sublaminae of the IPL and appear to have a specific centre-only depolarising (ON) response to S-cone stimulation and a hyperpolarising (OFF) response to M- and L-cones. This suggests that the S-cone signal path through the retina is discrete from that of M- and L-cones.

The responses of the inner retina are dynamic in nature and adapt to the stimulation being received. The process of lateral inhibition enhances the signalling of boundaries between different types of visual information. Bipolar signals are rectified, probably by amacrine cells, so that only the positive or negative component is passed, which may account for the non-linear responses of some GC to changes in light intensity. Rod and cone signals are generally processed separately but do interact and the 'periphery effect' means that GC are influenced by light falling far from their receptive fields. Spatial summation of centre and surround information occurs over a large area in the OPL (via horizontal cells) and also over a smaller area in the IPL (amacrine cells). A further non-linear M-cell response to changes in contrast is seen, becoming more transient with increasing contrast and providing a 'contrast gain control' that is believed to operate in the IPL. This maintains sensitivity to small changes in contrast over a wide operating range independent of changes in average illumination and illustrates the dynamic nature of the retinal circuitry, modulating its responses to changes in the pattern of input. There

are also mechanisms that adjust to changes in mean contrast over time. Rod outputs are pooled over wider areas of retina at lower light intensities and different retinal pathways may process rod information at different dim light intensities. Considerable adaptation to the visual scene therefore takes place in the inner retina as well as at the level of the photoreceptors.

1.2.8 From eye to brain

Behind the eyes, the two optic nerves join in the midline at the optic chiasma to allow the decussation of nerve fibres, such that those carrying information from the nasal half of each retina (temporal visual field) cross to the contralateral side. The fibres then continue in the optic tracts to synapse in the LGN of the thalamus. After decussation, each side of the central visual system processes information relating to the contralateral half of the visual field. However, the fovea enjoys bilateral cortical representation.

The bulk of the GC output passes to the LGN and comprises two main sets of signals travelling in broadly parallel pathways. These are a colour-opponent signal which passes from P-type GC to the parvocellular layers of the LGN, and a broad-band, luminance contrast signal from M-cells which synapse in the magnocellular layers. These synapses in the LGN form two magnocellular and four parvocellular laminae which are easily distinguished and comprise three layers from each eye, arranged precisely in overlying retinotopic maps of the contralateral half of the visual field. These maps are therefore supplied by GC mapping the ipsilateral temporal retina and the contralateral nasal retina. Thus, images of the same object formed on each retina are processed together in the same part of the brain. The alternating excitatory and inhibitory arrangement of parallel rows of LGN cells aligned in a particular direction provides the basis of orientation selectivity in the visual cortex.

The characteristics of LGN cells are similar to those of the GC that signal them. There are four varieties of colour opponent P-cells. The more common are driven by antagonistic inputs from M- and L-cones and are either excited by red and inhibited by green (+R −G) or vice versa (+G −R). The less common are driven by both M- and L-cones in one part of the receptive field and by S-cones in the other and show blue-yellow opponency (+B −Y or +Y −B).

P-cells respond optimally to contrast gratings drifting at 5 to 10 Hz with a near linear response as a function of contrast. At light levels above about 40 cd m^{-2} contrast

sensitivity appears to be mediated primarily by P-type GC. The magnocellular pathway responds optimally to relatively high temporal frequencies (20 Hz) and its response to lower frequencies declines more rapidly than that of the parvocellular pathway. When stimulated optimally, M-cells are 5 to 10 times more sensitive than P-cells. M-cells have greater contrast gain but the response is linear only over a limited range of low contrasts and their responses tend to saturate at high contrasts. Magnocellular lesions have no effect on the spatial contrast sensitivity function.

In sum, the parvocellular pathway appears important for discrimination of colour, texture, fine shape, pattern and stereopsis while magnocellular function is important for detecting complex and fast motion and for discriminating between steady and rapidly flickering stimuli. The P-cell pathway conveys information about light patterns at all spatial frequencies (*sf*), but only at low to medium temporal frequencies, while the M-cell pathway transmits information about high temporal and low *sf*. The P-cell pathway also conveys information about luminance contrast at high *sf* and chromatic contrast at low *sf*. The function of the M-cell pathway appears primarily to be to draw attention to objects moving in the peripheral retina and is therefore also important for relative motion sensation.

There are also koniocellular (K-) layers in the LGN, named after the small size of the cell bodies, lying ventral to each of the primary M- and P-layers. Those GC feeding the K-layers do not usually have concentric receptive fields but may respond to light onset, offset or both, in any part of the field, or only respond to moving spots of light. Others have colour-opponent responses without concentric fields. The small bistratified GC are amongst this group of K-cells and project to the middle two K-layers in the LGN. They appear to have a special role in S-cone signal processing. The two most dorsal K-layers have particularly large receptive fields and are driven by GC with widely branching dendrites. The two most ventral K-layers are driven by GC similar to those of the retino-tectal pathway.

1.2.9 Visual cortex and beyond

A simple outline follows of the nature of the visual processing that takes place within the main cortical regions. Zeki (1993) provides further detail and a starting point for more detailed study of visual processing in the brain.

The axons of the LGN cells form the optic radiation, or geniculo-cortical pathway, projecting to the visual cortex in the occipital region of the brain. The visual cortex is highly organised. The 'functional architecture' of Hubel and Wiesel (1962) describes columns of cells that are retinotopically mapped with each half of the visual field represented on the contralateral cortex. The striate cortex, or V1, comprises six main layers of cells, with sublaminae, and the axons of LGN cells pass to most of these layers. The cortical cells form a complex network of vertical and horizontal connections between and within layers and the number of different cell types and the complexity of their connections is much greater than in the retina. A greater allocation of cortex is given over to mapping the central visual field than to mapping the periphery, and the fovea has a disproportionately large representation. Some layers of cells respond to stimuli presented to only one eye, while others have binocular fields that respond to stimuli presented to either eye (bilateral cortical representation), although they may respond more strongly to one or the other and hence show ocular dominance. Cells sharing the same ocular dominance are grouped together in bands.

Some of the cells in the visual cortex are sensitive to the contrast and phase of a grating but have elongated receptive fields that make them respond to particular orientations of a bar, edge or grating, enabling orientation selectivity or tuning in response to the patterns of LGN input. Thus, the striate cortex acts as a spatial filter with cells having narrow bandwidths of *sf* to which they respond when their directional selectivity is satisfied. Other cells respond to the presence of a grating irrespective of phase (non-linear response) or to the ends of bars or edges that fall in their receptive fields. Many respond weakly to stationary stimuli and have direction-selective responses to moving patterns. Some exhibit colour opponency and others are double-opponent with differing colour-opponent properties in the various parts of their receptive fields, responding to orientated edges defined by colour difference alone.

Staining with cytochrome oxidase reveals dark 'blobs' and lighter 'inter-blobs'. P-cells supply both but M- and K-cell input is concentrated in the blobs, which are most visible in selected layers. The sequencing of orientation preference is also patterned across the cortex. The blobs may contain cells tuned to low *sf* and are capable of undertaking Fourier-type transforms to analyse small patches of the retinal image. Inter-blob regions are tuned to a wider range of frequencies and moving from blob to inter-blob may be associated with a systematic increase in preferred *sf*.

Natural images can be decomposed into spatial patterns of light intensity that may be detected by 'tuned' filters comprising oriented edges and bars similar to the receptive fields of cortical cells. This type of analysis has been extended to properties of detection of motion and colour that also correspond to some cortical cell characteristics. It appears that the striate cortex exploits the redundancy in natural images to allow highly efficient coding of the visual scene from the GC output. Lesions of the striate cortex cause specific visual field deficits that allow prediction of the location of the cortical damage with great accuracy.

V1 is surrounded by other (extra-striate or pre-striate) visual areas that are less easily defined and may have full, partial or disorderly retinotopic representation. However, these visual association areas must be intact if the visual scene is to be interpreted normally. Each of the visual areas in the occipital lobe communicates with several others, usually with reciprocal pathways. There are over 300 neural projections linking extra-striate areas and most can be classified as either ascending or descending, according to whether they lead away from or towards V1. Inputs to these areas arise mainly from V1 but also from K-cells of the LGN and from the pulvinar nucleus (which is supplied directly from the retina and also via the superior colliculus, discussed below). Areas V2 and V3 surround and have point-to-point connections with V1 and so are retinotopically mapped, but the retinal quadrants are represented in a different spatial arrangement in each of the three areas, while V3 is a mirror image of V2 but without its characteristic metabolic architecture. Extending laterally from V3, V4 receives parvocellular signalling as central (foveal) input from V1 but mostly relayed via V2, and appears to have a strong role in colour vision. V5, anterior to V4, receives input from V1 and provides adjacent areas with input. This 'V5 complex' receives predominantly magnocellular signalling from V1 and via V2 and appears selectively responsive to different kinds of motion; for the purpose of this review V5 is regarded as synonymous with the middle temporal area. Medial to V3, V6 lies anteriorly, receives input from V2, has a very disorderly retinal map and may be involved with spatial representation. Many of these regions have 'association' areas.

Each human eye receives light from the visual field across a horizontal visual angle of about 150°. However, there is considerable overlap of the fields from two eyes such that both retinas receive light from the central visual field across a horizontal visual angle of about 120° and the full horizontal extent of the field of view from two eyes is just over 180° (Figure 1.9). Since the visual field is divided along the vertical meridian

with the information from each half being presented to the contralateral cortex, the question arises as to how the brain reconstructs the visual image with a seamless join. The area of V1 that represents the vertical meridian is connected and matched to its contralateral counterpart via the corpus callosum, and the corresponding areas of the pre-striate cortex are similarly paired. Thus, although the visual fields of the two eyes overlap considerably, and although their information is channelled into separate hemifields, perception of the two visual fields is fused. The binocularity of the central field enhances the discrimination of spatial and temporal patterns of visual information and enables stereopsis (3-dimensional perception) as well as providing non-stereoscopic binocular cues for depth perception. Additionally, summation of the information from two eyes enhances sensitivity, for example to contrast. Binocular concordance also appears to offer advantages in relation to shorter visual reaction times and more rapid accomplishment of visual tasks (Justo, Bermudez, Perez *et al*, 2004).

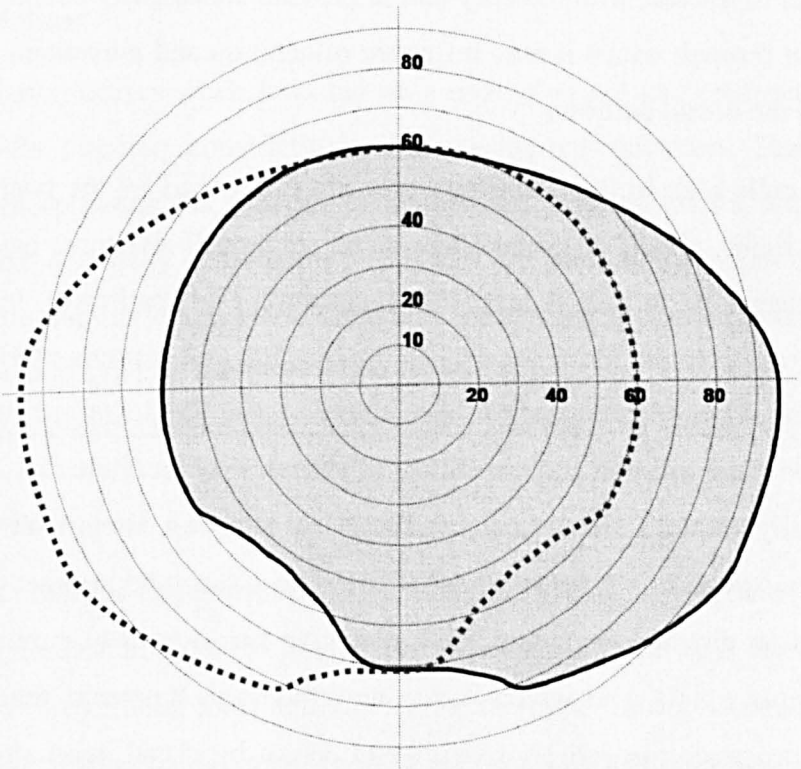


Figure 1.9 Overlap of the visual fields in degrees of visual angle (after Boff and Lincoln, 1988, p 61)

After V1 and V2, and stretching beyond the occipital lobe to many other areas of the brain that have visual functions, the connections between visual areas appear to form two main, and to some extent hierarchical, pathways, albeit with overlaps and interconnections between them. The first is a 'dorsal' pathway running via V3 to the middle temporal area, on to the medial superior temporal area and then to the parietal

lobe. This pathway is magnocellular in nature with an additional direct connection to the middle temporal area from V1 cells that are driven by M-cell output from the LGN. The dorsal pathway is specialised for processing spatial layout and image motion information, and appears important for rapid and precise orientation and movement of the body with respect to the environment, but may not contribute to conscious perception of the visual scene. It also appears to be involved in a network with many other areas of the brain concerned with controlling eye, head and limb movements.

The 'ventral' pathway runs to V4 and then to the posterior and anterior infero-temporal areas of the temporal lobe. This pathway is supplied with predominantly parvocellular information and appears to provide hierarchical processing of colour, colour patterns, shape, form and identity, and hence detailed spatial arrangement. In addition, V4 appears important for strategies of visual attention, while parts of the infero-temporal areas appear important specifically for facial features and recognition. The ventral pathway appears to interact with memory and to provide the conscious representation of the environment through which it may influence orientation and movement over longer timescales than the dorsal pathway.

While parietal cells high in the dorsal pathway are influenced by the positions of the eyes, head and limbs, effects of visual attention on single cell responses have also been demonstrated elsewhere in both dorsal (V3) and ventral (V4) pathways. In the ventral pathway, cells may respond more strongly to the location and features of visual stimuli to which an animal has been trained to focus attention. For the dorsal pathway, learned expectations about the location and movement of stimuli may facilitate fast and accurate control of visually guided actions. Lower in the visual pathway, attentional effects have also been demonstrated in V1 when potential distractors surround a target of attention. With V1, attention directed towards a cell's receptive field seems to diminish the area outside the receptive field over which it may be influenced. It appears that no level of cortical visual processing is entirely passive and driven by visual input alone. Instead, areas of cortex may be activated by attending to corresponding elements of the visual field away from fixation, implying feedback from other cortical areas. Thus, even throughout the cortex, various strategies of visual adaptation are at work.

1.2.10 Special visual pathways

Pupillary light reflexes

Both pupils constrict when bright light enters one eye, the receiving eye demonstrating the direct light reflex, while its partner exhibits the consensual light reflex. The afferent pathway for these reflexes is via GC fibres that do not go to the LGN but pass to the superior colliculus and pretectal nuclei of the midbrain. These communicate with their contralateral partners and, bilaterally, with the accessory oculomotor nuclei. Thus uncrossed fibres generate the direct reflex while crossed fibres are responsible for the consensual reflex. Parasympathetic autonomic fibres from the accessory oculomotor nuclei run with the oculomotor nerves and synapse in the ciliary ganglion in the orbit. Postganglionic fibres pass as the short ciliary nerves into the sclera and to the sphincter pupillae of the iris.

Accommodation

Accommodation occurs when focusing on a nearby object and comprises convergence of the eyeballs, pupillary constriction and increasing lens thickness. This again involves pathways incorporating the superior colliculus and pretectal nuclei, and cortical input, feeding to the accessory oculomotor nuclei and the oculomotor nuclei themselves. The former intiate pupillary constriction and also innervate the smooth muscle fibres of the ciliary muscle to thicken the lens, while the latter innervate the skeletal muscle of the medial recti, the extrinsic ocular muscles which turn the eyes inward.

Eye movements

The motor cortex for rapid, voluntary eye movements (saccadic system) is in the frontal eye field in the frontal lobe. Horizontal eye movements to the right are mediated by the contralateral (left) frontal lobe, and vice versa, the upper motor neurons crossing in the midbrain and passing to the pons. From there, neurons of the horizontal pontine gaze centre send axons to the ipsilateral abducent nucleus while others cross again to climb back to the contralateral oculomotor nucleus. The cranial nerves from these nuclei then innervate the lateral and medical recti, respectively. Vertical eye movements are mediated by simultaneous activity of both frontal eye fields. Crossed and uncrossed fibres pass to the pretectal region of the midbrain where axons are projected bilaterally to the oculomotor and trochlear nuclei of the midbrain. The trochlear nerve then

supplies the superior oblique muscle of the eye, while the superior and inferior recti and the inferior oblique are supplied by the oculomotor nerve.

Similarly, there are two pathways, one horizontal and one vertical, for smooth pursuit (following or tracking) eye movements. This time, the upper motor neuron cell bodies are located in the anterior occipital lobe. Again, horizontal pursuit movements are mediated by the contralateral cortex, with simultaneous bilateral activity controlling vertical pursuit movements. The fibres may pass via the pulvinar nucleus of the thalamus before following a similar course to those controlling saccadic movements. Less well understood pathways also govern reflex eye movements in response to vestibular and neck receptor stimuli, and others help to maintain gaze fixation in relation to movement of subject and target.

1.3 Respiratory Disturbance

Authoritative accounts of respiratory physiology, altitude medicine and physiology, and aviation and aerospace medicine are available elsewhere (Lumb, 2000; Ward, Milledge and West, 2000; Rainford and Gradwell, 2006). The brief review of respiratory and altitude physiology provided here is intended only to support discussion of the respiratory disturbances imposed during subsequent experimental work. Many commonly used units of pressure remain outside the Système International d'unité. For convenience, physiological gas and blood pressures are measured and reported here in mm Hg (1 kPa = 7.501 mm Hg).

1.3.1 Metabolic gases

Inspired O_2 is required for the process of oxidation and the consequent release of energy is fundamental to life. Energy is released whenever electrons are removed from molecules and O_2 is the ultimate 'electron sink'. In most oxidase reactions this energy is released as heat. However, mitochondrial oxidative phosphorylation couples the reaction to the formation of adenosine triphosphate (ATP), from adenosine diphosphate (ADP), creating a high energy phosphate bond that is then used to provide biological energy. ATP cannot be stored and must be produced as required. The 'engine room' of the process is the aerobic phase of the Krebs Cycle, where carbohydrate is broken down to CO_2 and water, accounting for 80% of O_2 consumption (QO_2) in resting man (Denison, 1981). Aerobic metabolism of one molecule of glucose yields 38 of ATP,

providing the primary mechanism of controlled energy release in humans. The CO_2 product gas requires carefully managed elimination from the body to avoid upsetting the normal homeostasis of blood and tissue acid-base balance.

In the lungs, gas exchange takes place with the blood perfusing pulmonary capillaries in the walls of the alveolae. CO_2 diffuses passively but rapidly from the blood into the alveolar gas. Under normal conditions, respiration is carefully controlled to maintain a steady arterial partial pressure of CO_2 (P_aCO_2) at close to 40 mm Hg (SD ± 2 mm Hg), which is reflected in the alveolar partial pressure (P_ACO_2). Inspired gas is saturated with water vapour as it passes to the alveolae of the lungs. At a normal body temperature of 37°C, saturated water vapour pressure (P_{H_2O}) is 47 mm Hg. Thus, both water vapour and CO_2 account for a significant proportion of total alveolar gas pressure at any given ambient pressure or altitude. The net alveolar partial pressure of O_2 (P_AO_2) can be estimated using the alveolar air equation (Equation 1) if the respiratory exchange ratio, R, and the fractional inspired O_2 concentration (F_IO_2) are known.

$$P_AO_2 = P_IO_2 - P_ACO_2\left(F_IO_2 + [\{1 - F_IO_2\}/R]\right)$$

Equation 1 Alveolar air equation

The term in brackets in Equation 1 is a correction factor intended to account for the effect of dietary differences. R is the ratio of CO_2 molecules exhaled to O_2 molecules inhaled and is approximated by the Respiratory Quotient (RQ) at rest. The major products of cellular respiration are CO_2, water and ammonia (NH_3). The RQ relates the rate of CO_2 production by the tissues to the rate of QO_2 and varies with different foods. For carbohydrate the RQ = 1, such that one molecule of CO_2 is produced for each molecule of O_2 consumed. For fat and protein the RQ ≈ 0.7, so unless the diet contains only carbohydrate, some O_2 will be used in processes that do not generate CO_2, and the net RQ will be less than 1.0. For the normal omnivorous diet in the UK, typical of the subjects participating in the experiments reported here, RQ = 0.845 ± 0.013 (Black, Prentice and Coward, 1986). For this work, since all experiments were conducted at rest, R has been assumed to have a value of 0.845.

The inspired partial pressure of O_2 (P_IO_2) of saturated gas entering the alveolae can be calculated easily from Equation 2, where P_B is ambient (barometric) pressure.

$$P_IO_2 = F_IO_2 (P_B - P_{H_2O})$$

Equation 2 Formula for calculating P_IO_2 (saturated)

The normal pulmonary ventilation-perfusion mismatch in upright lungs means that the PO_2 of mixed pulmonary venous blood is about 4 mm Hg lower than that of mixed (end-expiratory) alveolar gas, as some blood will pass through relatively poorly ventilated areas of lung (base) while some well ventilated areas of lung will be relatively poorly perfused (apex). This 'shunt' has much less effect on CO_2, such that the mixed pulmonary venous gas tension is < 1 mm Hg greater than the mixed P_ACO_2. The PO_2 of pulmonary venous blood is further reduced by mixing of blood from venous shunts (bronchial and thebesian veins) so that the net alveolar-arterial PO_2 difference is about 8-10 mm Hg. At sea level, the resulting normal arterial PO_2 (P_aO_2) is approximately 95-100 mm Hg and the normal mixed venous PO_2 in blood returning to the lungs is 40 mm Hg.

1.3.2 Carriage of gases in the blood

The vast majority of the O_2 transported by the blood is carried in chemical combination with haemoglobin (Hb), with a much smaller component in physical solution in accordance with Henry's Law (Appendix 1). The concentration of dissolved O_2 at a gas tension of 100 mm Hg is only 0.3 ml STPD [1] per 100 ml of blood (or 0.3 vol %). The degree of arterial haemoglobin O_2 saturation (S_aO_2) bears a sigmoid relationship to PO_2 that is illustrated by the Hb O_2 dissociation curve in Figure 1.10. Typically, S_aO_2 is estimated using a pulse oximeter to measure peripheral O_2 saturation or S_pO_2. Here the term S_aO_2 is used throughout to signify the parameter being estimated.

In contrast with dissolved O_2, at a gas tension of 100 mm Hg arterial Hb will be ~98.5% saturated, so the concentration of O_2 in chemical combination is slightly over 20 vol %, assuming [Hb] = 15 g dl^{-1}, pH = 7.4, P_aCO_2 = 40 mm Hg, and body temperature = 37°C. The sigmoid nature of this relationship facilitates O_2 transfer in the lungs and tissues. The mixed venous Hb O_2 saturation is about 74% (15.5 vol %) at rest, and 50% (10.5 vol %) during exercise requiring an O_2 uptake of 1.0 litre (STPD) per minute.

[1] STPD - standard temperature and pressure, dry.

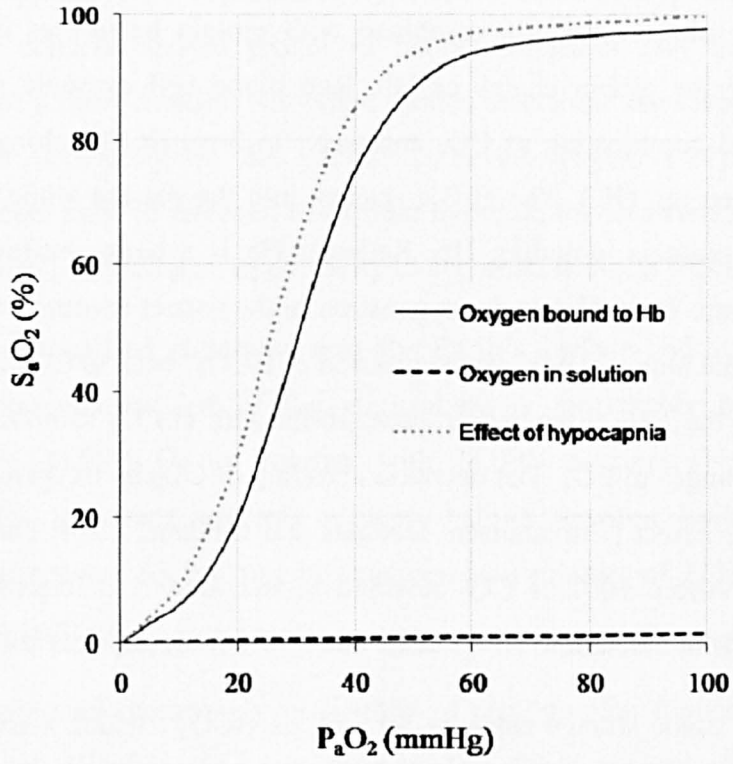


Figure 1.10 The haemoglobin O_2 dissociation curve (after Ernsting, Ward and Rutherford, 2006)

Decreasing pH (acidosis), hypercapnia or increasing partial pressure of CO_2 (PCO_2), and increasing temperature all act to shift the dissociation curve to the right, favouring delivery of O_2 to working tissues. Equally, increasing pH (alkalosis), decreasing PCO_2 (hypocapnia) and decreasing temperature tend to shift the curve to the left (Figure 1.10). The effect on the dissociation curve due to pH and PCO_2 is known as the Bohr Effect and facilitates appropriate gas transfer to and from the blood both at the lungs and in the tissues (Bohr, Hasselbalch and Krogh, 1904). The curve will be shifted to the right in mixed venous blood returning to the lungs, as the increased PCO_2 will reduce the pH in the red blood cells. As CO_2 is released into the alveolae the curve shifts to the left, increasing the affinity of Hb for O_2. Equally, as O_2 is released to the tissues so the curve will move to the right once more as CO_2 passes into the blood.

CO_2 is far more soluble than O_2 and diffuses readily through the tissues in accordance with Fick's Law (Appendix 1). Thus, there is far more CO_2 than O_2 in simple solution and CO_2 requires much shallower gas tension gradients to promote the movement of similar amounts of gas to and from capillary blood. The P_aCO_2 is determined by P_ACO_2, which is itself determined by the ratio of CO_2 production to alveolar ventilation, and hence is independent of ambient pressure.

Some 90% of the CO_2 carried by the blood is in the form of bicarbonate ion (HCO_3^-), with 5% in physical solution and 5% combined with protein buffers as carbamino compounds, including the globin chains of Hb. Red blood cell carbonic anhydrase facilitates the chemical combination of CO_2 and water to form HCO_3^- along with the corresponding hydrogen ion (H^+). The HCO_3^- passes into the plasma while the H^+ is buffered by red cell proteins including Hb. Reduced Hb is a better buffer for CO_2 carriage in the carbamino form. Hence deoxygenation in the tissues favours CO_2 uptake while oxygenation in the lungs favours the conversion of HCO_3^- back to CO_2 for release to the alveolae. Thus the CO_2 dissociation curve shifts with Hb O_2 saturation and is thereby affected by changes in PO_2. The decreased binding of CO_2 by oxygenated Hb is known as the Haldane Effect (Christiansen, Douglas and Haldane, 1914; Dautrebande and Haldane, 1921). About 60% of CO_2 released in the lung is delivered there as [HCO_3^-], with ~30% from carbamino compounds and ~10% from physical solution.

There is a substantial tissue storage capacity for CO_2 as HCO_3^-, which acts as a vital buffer through which acid-base balance is regulated, as shown in Equation 3.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Equation 3 Homeostatic mechanism of the bicarbonate buffer system

The tissues tolerate acidosis poorly as enzyme systems are compromised by increasing [H^+]. Accordingly, [H^+] is closely regulated through tight respiratory control of alveolar ventilation, so maintaining a normal acid-base equilibrium in the blood and tissues. The fundamental importance of CO_2 in controlling [H^+] and pH, is evident from Equation 4.

$$pH = 6.1 + \log_{10}\left([HCO_3^-] / \{0.03 \times PCO_2\}\right)$$

Equation 4 The Henderson-Hasselbalch Equation

1.3.3 Hypoxia

Hypoxia may be defined, simply, as a state of oxygenation that is inadequate to meet tissue requirements. Broadly, hypoxia may result from a reduced O_2-carrying capacity of the blood (anaemic hypoxia), impaired tissue perfusion (ischaemic hypoxia), disturbed tissue utilisation of O_2 (histotoxic hypoxia), or reduced oxygenation of arterial blood (hypoxic hypoxia). Thus, hypoxia may be local or systemic, and often results from disease processes. Exposure to low ambient pressure, during altitude exposure,

imposes hypobaric (low pressure) hypoxia, a form of hypoxic hypoxia. This is of particular concern to two groups of people. Aviators may be exposed to hypoxia acutely, for a short duration but with a sudden onset, and the severity of the hypoxia can be severe, depending on the altitude achieved (Figure 1.11). On the other hand, mountaineers may be exposed to chronic hypoxia, for an extended duration but with a gradual onset, affording an opportunity to acclimatise that is denied to aviators.

Apart from localised emissions near the Earth's surface and a variable water vapour content, the composition of the atmosphere is remarkably constant, with dry air comprising 20.95% O_2 by volume, with 78.09% nitrogen (N_2), 0.93% argon (Ar), 0.04% CO_2, and trace amounts of neon, helium, krypton, hydrogen and xenon. For practical purposes, dry air may be regarded as a mixture of 21% O_2 with a balance of N_2, that is, inert gas.

As with pressure, commonly used units of altitude also remain outside the Système International d'Unités. For convenience, this work reports altitude interchangeably using both metres (m) and feet (ft) above mean sea level (amsl). Use of the former derives typically from terrestrial altitude exposure (mountaineering) while the latter is more usual in respect of aviation.

Barometric pressure falls almost exponentially with increasing altitude amsl, halving at ~18,000 ft, halving again by ~33,700 ft, and yet again at ~48,000 ft, falling at 100,000 ft to one-hundredth that at sea level. The precise nature of the relationship between atmospheric pressure and altitude fluctuates with temperature during ascent, and therefore with latitude and time (diurnal and seasonal), as well as local meteorological conditions. The work presented here is based on the International Civil Aviation Organisation (ICAO) Standard Atmosphere (1964), which represents the pressure and temperature characteristics of the real atmosphere at latitude 45°N. Throughout this thesis reference will be made to specific pressure altitudes or 'equivalent' altitudes. These are atmospheric pressures expressed in terms of the altitude corresponding to that pressure in the ICAO Standard Atmosphere (1964). With respect to the Standard Atmosphere (1964), the resulting decrease in barometric pressure with increasing altitude is shown in Figure 1.11.

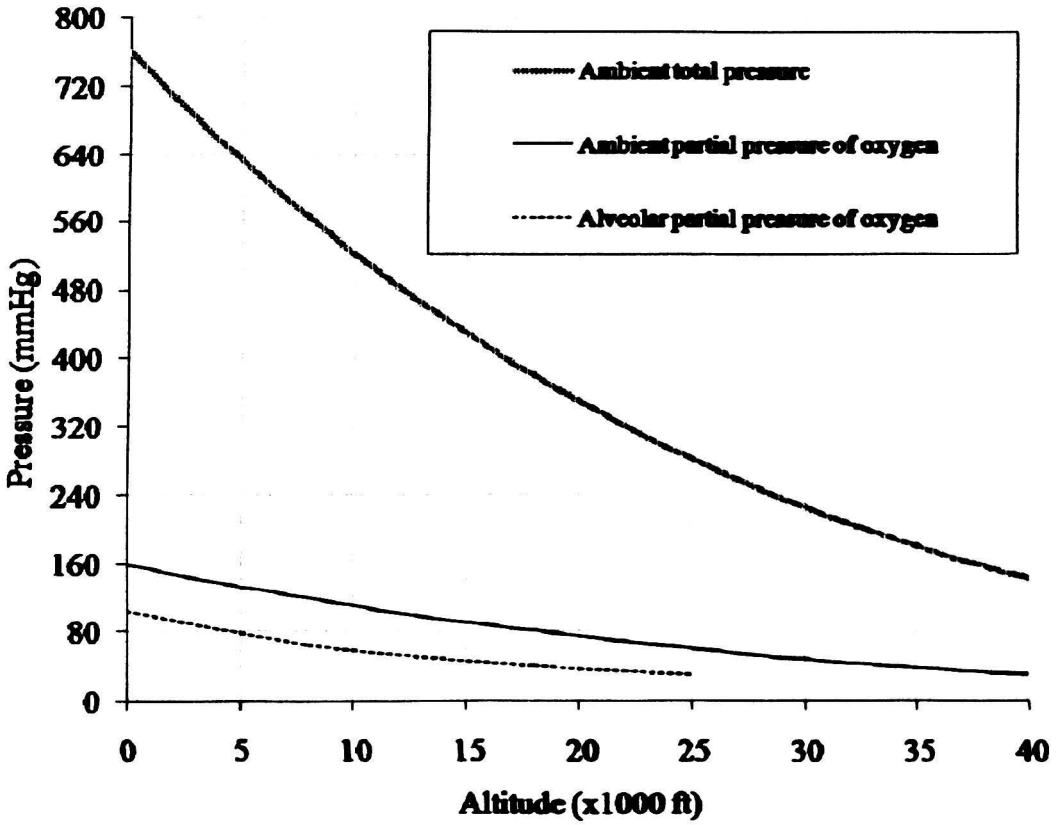


Figure 1.11 Ambient and alveolar pressure-altitude relationships

In accordance with Dalton's Law of partial pressures (Appendix 1), the partial pressure of atmospheric O_2 will also fall almost exponentially with ascent, in proportion to its fractional concentration (Figure 1.11). Thus, while the F_IO_2 will remain the same (0.2095) at altitude, the P_IO_2 will decrease and so must the P_AO_2. Figure 1.11 also includes a plot of mean P_AO_2 measured from 30 seated, resting subjects during acute hypobaric (low pressure) exposures to equivalent altitudes up to 25,000 ft (Gradwell, 2006, p45). Hypobaric hypoxia is an inevitable consequence of breathing air during ascent.

Figure 1.12 shows the P_AO_2 and P_ACO_2 measured from 30 subjects breathing air at rest for 10 to 20 minutes at ambient pressure at various equivalent altitudes. P_AO_2 falls approximately linearly with ascent until an altitude of 8000 to 10,000 ft is achieved, whereupon the hypoxic drive to ventilation is sufficient to lower the P_ACO_2 progressively with further ascent. This hypocapnia helps, partially, to offset the further fall in P_AO_2 that would otherwise occur. Hypoxic ventilatory drive is itself countered by the tendency of hypocapnia to depress respiration in an attempt to maintain normal acid-base balance. Furthermore, the respiratory exchange ratio adjusts during the initial

exposure to acute hypobaric hypoxia. Thus individual gas tension responses to acute hypobaric hypoxia are highly variable, particularly at lower altitudes. Pulmonary diffusion characteristics are such that P_aO_2 at altitude remains about 8-10 mm Hg less than P_AO_2, if the individual is at rest, while P_ACO_2 and P_aCO_2 tensions are effectively identical. However, at modest altitude, even within subjects at rest, P_aO_2 and therefore S_aO_2 are extremely labile, responding with great sensitivity to changes in alveolar ventilation.

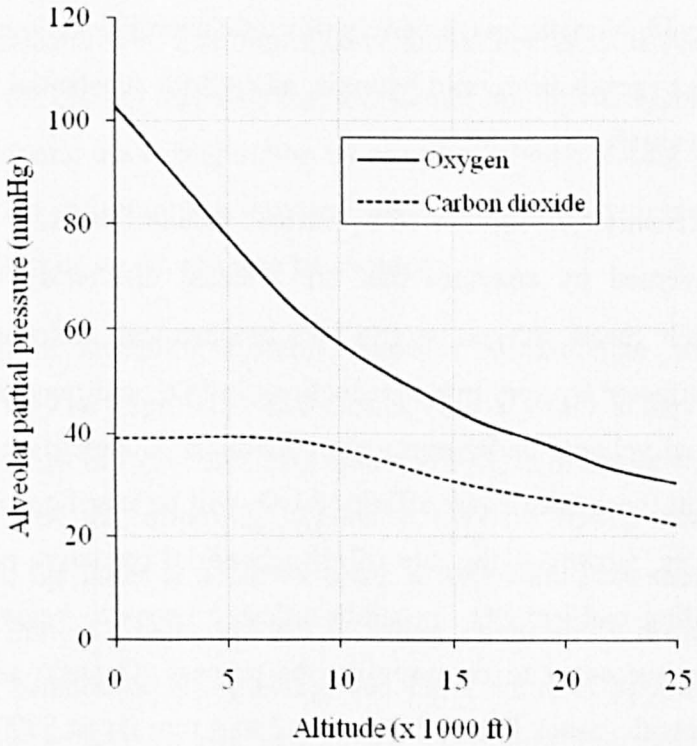


Figure 1.12 Alveolar gas tensions during acute steady altitude exposure (data of Gradwell, 2006)

The cardiovascular responses to acute hypobaric hypoxia are well known. Heart rate starts to increase at equivalent altitudes above about 6000 to 8000 ft, reaching 10-15% above sea level value at 15,000 ft, 20-25% at 20,000 ft, and doubling by 25,000 ft. Cardiac output increases proportionately, as stroke volume is unchanged. However, mean arterial blood pressure (MAP) is largely unaffected by even moderate hypobaric hypoxia, as pulse pressure increases and blood flow is redistributed. Acute hypobaric hypoxia sufficient to lower P_aO_2 to less than 45 mm Hg will increase cerebral blood flow but until then it will be determined exclusively by P_aCO_2. The conflict between hypoxic cerebral vasodilatation and hypocapnic cerebral vasoconstriction usually results

in a net reduction in cerebral flow above about 8000 to 10,000 ft but a net increase over about 16,000 to 18,000 ft.

Acute hypobaric hypoxia can be sufficiently severe to compromise ATP production and cellular function. In an otherwise healthy individual the most immediate consequences of systemic hypoxia will be neurological, and may range in severity from minor and transient cognitive effects through to rapid loss of consciousness, coma and, eventually, death. Acute exposures to altitudes up to 10,000 ft are typically asymptomatic, and resting exposures to 15,000 ft are usually well tolerated unless prolonged or accompanied by cold. Nonetheless, a variety of subtle neuropsychological and cognitive effects are known to result from mild hypoxia, albeit with substantial variability within and between individuals.

This differential sensitivity is influenced by various factors. The rates of O_2-consuming reactions are governed by enzymes that are affected differently by reduced PO_2. Oxygenases, which insert oxygen atoms into biochemical molecules to form new compounds, are affected by very minor reductions in PO_2, and may already be running at less than optimal velocity under normoxic conditions. Competition between enzyme systems means that those with a low affinity for O_2 will be sacrificed in favour of those with a high affinity. However, the rate of mitochondrial oxidative phosphorylation is unaffected by falling ambient PO_2, possibly unless it drops to below 0.5 mm Hg, so severe hypoxia is necessary to compromise the process (Gayeski and Honig, 1988). However, the cerebral venous PO_2 falls by only 2 to 4 mm Hg at 8000 ft, where effects have been documented. Thus, while the mechanism by which mild hypoxia impairs cerebral function is unclear, it is not explained by failure of oxidative phosphorylation. At higher altitudes tests of mental performance may be impaired both by hypoxia and by any secondary hypocapnia, the latter causing cerebral vasoconstriction, decreased perfusion, and respiratory alkalosis, increasing the pH of cerebral tissue.

Hypoxia impairs simple response times, complex choice responses, serial choice reaction times and increases error rates (McFarland, 1932; Tune, 1964; Ledwith, 1970). Study of the recognition of visual stimulus orientation demonstrated response time (motor) decrements at 7,000 ft while accuracy (sensory) deficits became apparent at 12,000 ft (McCarthy, Corban, Legg *et al*, 1995). Short and long-term memory tasks may begin to be impaired when P_AO_2 falls below ~60 mm Hg (above ~8000 ft) but psychomotor and cognitive performance appear well preserved until 10,000 ft is exceeded and P_AO_2 falls below 55 mm Hg (Bartholomew, Jensen, Petros *et al*, 1999).

Complex pursuit, choice-reaction time and eye-hand coordination tasks begin to be affected above ~12,000 ft. Visual reaction time is impaired above this altitude once S_aO_2 falls below about 81-82%, corresponding to a P_aO_2 of ~45 mm Hg (Fowler and Prlic, 1995). Conceptual reasoning (Maag, 1954) and vigilance (Figarola and Billings, 1966) are compromised by moderately severe hypoxia at 17,000 ft.

Tests that are sufficiently sensitive may demonstrate cognitive effects of very mild hypoxia. If visual performance is to be assessed under hypoxia then any tendency for such cognitive effects to confound the results must be avoided. Accordingly, when assessing visual sensitivity to a stimulus, tests should not measure reaction or response times. Similarly, the chosen response method should not be vulnerable to psychomotor error, such as 'pressing the wrong button by mistake'. Vision testing should not rely on short-term memory and complex response tasks, which are vulnerable at equivalent altitudes of 12,000 ft or so, should also be avoided.

For the purposes of achieving systemic hypoxia to challenge normal physiology, hypoxic hypoxia may be imposed by two means, both of which achieve quantifiable and comparable reductions in P_AO_2, albeit by reducing P_IO_2 in different ways. The first is to reduce ambient pressure, imposing hypobaric hypoxia during actual or simulated altitude exposure; the latter is achieved using a hypobaric (decompression) chamber. The second is to impose a reduction in F_IO_2 using a reduced O_2 breathing gas mixture, typically with a balance of N_2. Clearly, this latter form of hypoxic hypoxia is not hypobaric but appropriate P_AO_2 can be achieved that mimic those produced at altitude.

1.3.4 Hyperventilation

Hyperventilation may be defined as pulmonary ventilation in excess of metabolic need, that is, in excess of the requirement for controlled elimination of CO_2 from the body. Accordingly, its cardinal feature is hypocapnia, a reduction in P_ACO_2, and thereby P_aCO_2, that promotes the elimination of CO_2 from the tissues. Thus, hyperventilation lowers the arterial and tissue $[H^+]$ through a mass action effect on the bicarbonate buffer system (Equation 3), increasing pH and generating a respiratory alkalosis. Increasingly severe hyperventilation and hypocapnia occur as a secondary response to progressive hypoxia once the P_AO_2 falls below about 55 to 60 mm Hg (Figure 1.12).

Hypocapnia induces intense cerebral vasoconstriction and reduces cerebral blood flow in a linear fashion over the physiologically tolerable range between about 20 and 50 mm

Hg, such that a reduction in P_ACO_2 from 40 to 20 mm Hg reduces cerebral blood flow by 50% (Kety and Schmidt, 1946; Kety and Schmidt, 1948). It is likely that the effects of gross hyperventilation result from a combination of ischaemic cerebral hypoxia and cerebral alkalosis, including performance decrement, electrophysiological changes and eventual loss of consciousness. While a performance decrement may be seen when P_ACO_2 falls below 30 mm Hg, symptoms usually only begin below about 25 mm Hg and are generally mild above 20 mm Hg. They include light-headedness, dizziness and paresthesiae (tingling) around the lips and extremities.

The significance of hypocapnia here is four-fold. Firstly, even mild hypocapnia is known to exert effects on visual performance and consideration should be given to establishing the level of hazard that such effects might pose. Secondly, hyperventilation may occur as a secondary phenomenon in response to hypoxia and may therefore confound interpretation of the visual effects of the primary respiratory insult. Thirdly, some inexperienced research subjects who are asked to wear a mask may hyperventilate involuntarily. Finally, discomfort, from any cause, may provoke hyperventilation.

Hypoventilation and subsequent hypercapnia can occur, but usually result from disease, rebreathing of expired gas or inhalation of an increased fractional concentration of CO_2, such as for research purposes. The effects of hypercapnia are considered below in relation to past studies of ocular physiology and visual performance, as this has helped illuminate responses to other metabolic gas disturbances, but hypercapnia has not been imposed experimentally during the course of this work.

1.3.5 Hyperoxia

Supplementary O_2 is used to prevent acute hypoxia in aviators during altitude exposure. Breathing gas regulators can automatically increase the F_IO_2 of inspired gas, up to 100% O_2, to maintain an adequate P_AO_2. Breathing 100% O_2 promotes denitrogenation of the body as N_2 is washed out of the tissues as a consequence of the reduced alveolar partial pressure (P_AN_2). In theory, when breathing 100% O_2, the alveolar air equation simplifies to that shown in Equation 5. However, in practice, even after breathing 100% O_2 for two hours, the P_AN_2 is still 3-5 mm Hg, owing to its delayed release from some tissues, so the P_AO_2 will usually be slightly less than that predicted by Equation 5.

$$P_AO_2 = P_B - P_{H_2O} - P_ACO_2$$

Equation 5 Simplified alveolar air equation breathing 100% O_2

At most, 1.39 ml STPD of O_2 can combine with each g of Hb, so, if the [Hb] of whole blood is 15 g dl^{-1}, its maximum O_2 carrying capacity is 20.8 ml STPD of O_2 per 100 ml of blood (20.8 vol %). Following admixture of shunted blood, P_aO_2 is increased to around 650 mm Hg when breathing 100% O_2 at one atm. Hb may be regarded as completely saturated at a PO_2 of 200 mm Hg, but the quantity of O_2 in physical solution increases linearly with PO_2 to reach approximately 1.95 ml (STPD) per 100 ml of blood at 650 mm Hg (Figure 1.13).

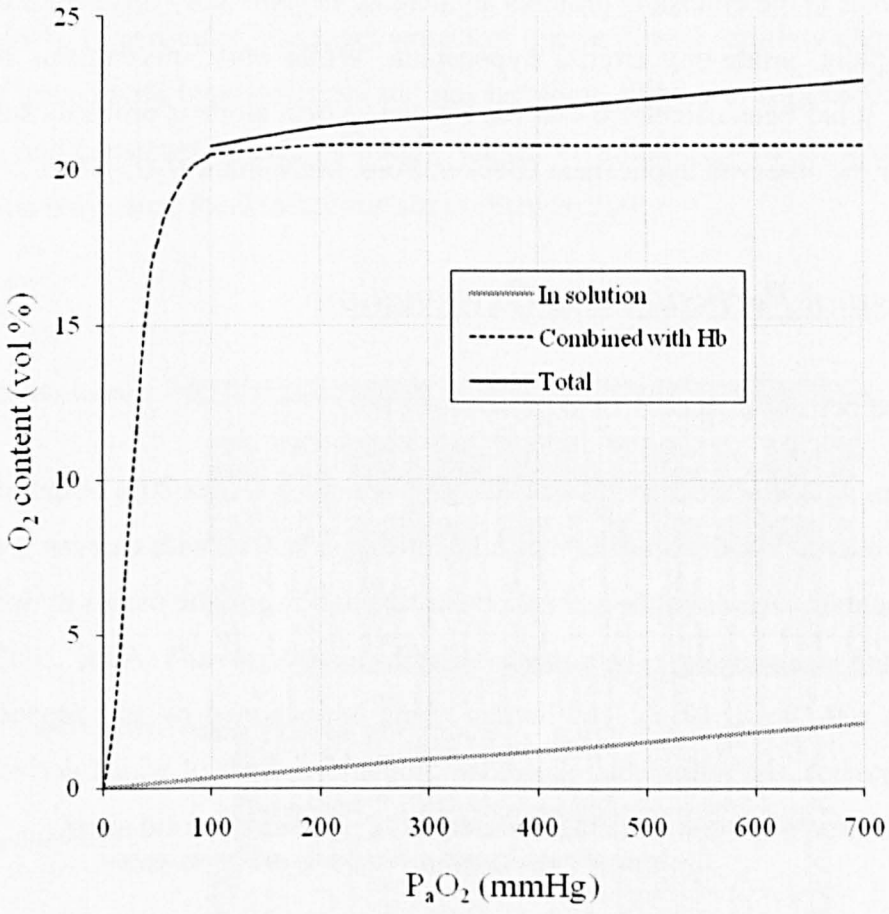


Figure 1.13 Blood O_2 content under conditions of hyperoxia

Thus some 8.5% of the total O_2 content of arterial blood is gas in physical solution when breathing 100% O_2 near sea level. Furthermore, some 1.35 vol % of the O_2 in physical solution can be delivered from this blood before any significant desaturation of Hb occurs. In falling from 650 mm Hg to a normal P_aO_2 of ~100 mm Hg, S_aO_2 will fall by only ~1.5%, liberating ~0.3 vol % of O_2, while some 1.65 vol % will be given up from solution.

The paradoxical hypocapnia that accompanies hyperoxia was identified long ago but is often forgotten (Christiansen, Douglas and Haldane, 1914; Dautrebande and Haldane, 1921). It has been quantified in recent work using gas mixtures containing from 30% to 75% O_2 (Becker, Polo, McNamara *et al*, 1996) and is attributable to the Haldane Effect, whereby highly oxygenated Hb binds less CO_2 in the carbamino form. The effect is exaggerated when hyperoxic gas is administered and S_aO_2 approaches 100%. Mixed venous O_2 saturation increases by ~10% to ~80%, venous Hb binds less CO_2 and transport of the gas from the tissues will be impaired. The resulting tendency towards tissue acidosis in the brainstem prompts an increase in respiratory drive from the central chemoreceptors, promoting arterial hypocapnia. While other mechanisms have been postulated, it has been calculated that the Haldane Effect alone is probably sufficient to account for the observed hypocapnia (Becker, Polo, McNamara *et al*, 1996).

1.4 Retinal Perfusion and Oxygenation

1.4.1 The retinal and choroidal circulations

The nervous system makes up 2% of resting body mass but uses 20% of the inhaled O_2. The retina may be regarded as specialised neurological tissue with an even greater QO_2 than grey matter. Details of the normal ocular circulation and the means by which it has been studied are available elsewhere (Cioffi, Granstam, and Alm, 2003; Harris, Kagemann and Cioffi, 1998). The human retina is nourished by and depends on two vascular systems, the retinal and choroidal circulations, both of which derive from the ophthalmic artery, a major intracranial branch of the internal carotid artery.

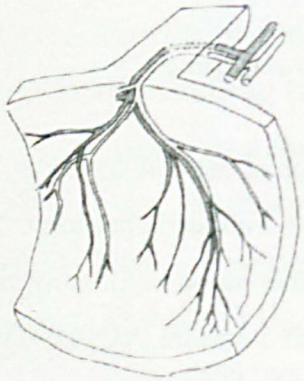


Figure 1.14 Central retinal vessels (redrawn from Forrester, Dick, McMenamin *et al*, 2002)

The retinal circulation supplies the inner (vitreal) retina by means of the central retinal artery (Figure 1.14). This vessel branches from the ophthalmic artery and penetrates the optic nerve about 10 mm behind the globe, travelling within the nerve to emerge at the optic disc, where it branches into four vessels to supply the four quadrants of the inner retina.

These vessels traverse the inner surface of the retina mainly in the NFL and feed two layers of inner retinal capillaries which lie approximately at the levels of the GCL and the INL, (Figure 1.15) albeit with variation across the breadth and depth of the retina (Zhang, 1994). In particular, the development of the perifoveal capillary plexus appears intimately linked with hypoxic stress and has led some authors to suggest that macular O_2 supply and consumption are 'on a knife edge', such that even minor impairment of oxygenation may cause local metabolic stress (Provis, 2001).

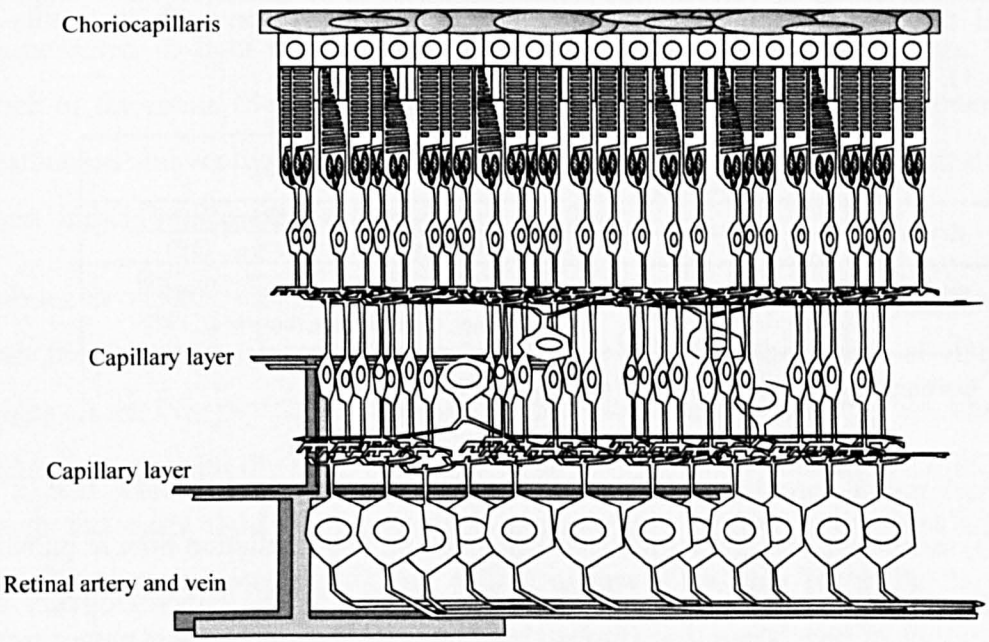


Figure 1.15 Schematic diagram of the blood supply to the retina

The lack of inner retinal capillaries at the fovea suggests a compromise between oxygenation and interference with vision. The retinal capillaries are not densely packed, with relatively large distances between neighbouring vessels (Funk, 1997). Tight junctions between retinal capillary endothelial cells help to maintain the functional

BRB, as the RPE does for the outer retina, and thus the principle of the blood-brain barrier (BBB) is extended to the neural retina.

The uveal circulation accounts for 95% of the intraocular blood volume, the bulk of it in the choroid, which receives 65-85% of the total ocular blood. Posterior ciliary arteries branch from the ophthalmic artery and penetrate the rear of the globe near the optic nerve to radiate within the outer layer of the choroid. These vessels feed arterioles in the middle layer which themselves supply an inner network of capillaries known as the choriocapillaris. This is organised in a retinotopic lobular arrangement, corresponding to local pressure gradients, to form a trabecular meshwork of highly fenestrated, densely-packed, wide-bore vessels with a spongy nature and upon which the RPE may be conceived to float (Zhang, 1994; Hirata and Nishiwaki, 2006). Blood leaves the choroid and drains via the vortex veins with an admixture of blood from the anterior uvea.

The organisation of the choroid suggests a requirement for a layer of arterial blood as close as possible to the RPE and, beyond that, the photoreceptors. Estimates of choroidal flow exceed those of the inner retinal system by some ten to twenty-fold (Table 1-1).

	Flow ($\mu l\ min^{-1}$)	Reference
Retina	34	Riva, Grunwald, Sinclair *et al*, 1985
	80	Feke, Tagawa, Deupree *et al*, 1989
Choroid	900	Langham, Farrell, O'Brien *et al*, 1989
	670 (males) 842 (females)	Yang, Hulbert, Batterbury *et al*, 1997

Table 1-1 Estimates of human retinal and choroidal blood flow

The (inner) retinal circulation is characterised by a relatively low flow rate, a low venous O_2 saturation, and autoregulation. The mean retinal circulation time in humans is about 3-5 s, being less near the macula and greater toward the periphery. Delivery of O_2 to the inner retina is achieved by desaturation of Hb, such that the saturation of retinal venous blood is only about 60% (Hickam, Frayser and Ross, 1963). Overall blood flow to the central retina is greater than to the periphery, except that the avascular fovea must be nourished via the choroid.

The choroidal capillaries are unusually large and tend to be arranged in parallel, promoting low resistance and a high flow rate. In contrast to the more conventional retinal circulation, the choroidal system may be likened to an arteriovenous fistula providing a sheet of fast-moving, well-oxygenated blood. It is characterised as having

little autoregulation, a very high flow rate and very high venous O_2 saturation (Elgin, 1964). The difference in arteriovenous O_2 content is slight, amounting to only ~1 vol % (Wangsa-Wirawan and Linsenmeier, 2003). Although the flow through the retinal circulation may be only 5% of that through the choroid, it is estimated that ~21% of the O_2 consumed by the cat retina is delivered from the retinal circulation (Alm and Bill, 1972b). This still means that 80% is delivered from the choroid, although the figure in primates may be closer to 65% (Cioffi, Granstam, and Alm, 2003).

1.4.2 The retinal oxygen tension profile

Microelectrode studies of the PO_2 profile across the cat retina demonstrate a steep PO_2 gradient falling from a maximum at the choriocapillaris to a minimum near the ONL. PO_2 then rises with further movement toward the vitreous and reaches a relatively uniform tension of 20-30 mm Hg across the innermost retinal layers (Alder, Cringle and Constable, 1983). Early studies suggested that frog retinal QO_2 was greater in darkness than light. This prompted an *in vivo* study in rhesus monkey in which pre-retinal PO_2 was measured in light and dark using O_2 microelectrodes placed 0.1 mm from the surface of the retina (Stefánsson, Wolbarsht and Landers, 1983). Four monkeys were anaesthetised and ventilated with 100% O_2. The pre-retinal PO_2 fell in darkness and rose in light, indicating increased retinal QO_2 in the dark.

Linsenmeier (1986) subsequently used microelectrodes to measure the PO_2 profile across the thickness of the cat retina in light and dark. The effect of illumination changes on the PO_2 profile was localised to the outer retina where PO_2 was observed to oscillate in time with the respiratory cycle. The PO_2 gradient moving from the choroid through the outer third of the retina is considerably steeper under dark-adapted as opposed to light-adapted conditions, falling to just a few mm Hg at the level of the photoreceptor inner segments. Linsenmeier's results are reproduced in Figure 1.16. At rod saturating levels of light an increase in PO_2 to a new minimum of 20-30 mm Hg can be seen near to the inner segments. It seems clear that these effects on PO_2 result primarily from altered O_2 requirements at the level of the photoreceptor inner segments. The results suggest that the outer retina consumes around twice as much O_2 under dark-adapted than light-adapted conditions. Most remarkably, in darkness the tissue PO_2 approached 0 mm Hg at the level of the photoreceptor inner segments, that is, the tissue was virtually anoxic, suggesting that all the available O_2 was being utilised by the inner

segment mitochondria. This suggests that PO_2 may fall sufficiently low in darkness to compromise oxidative phosphorylation (Wangsa-Wirawan and Linsenmeier, 2003).

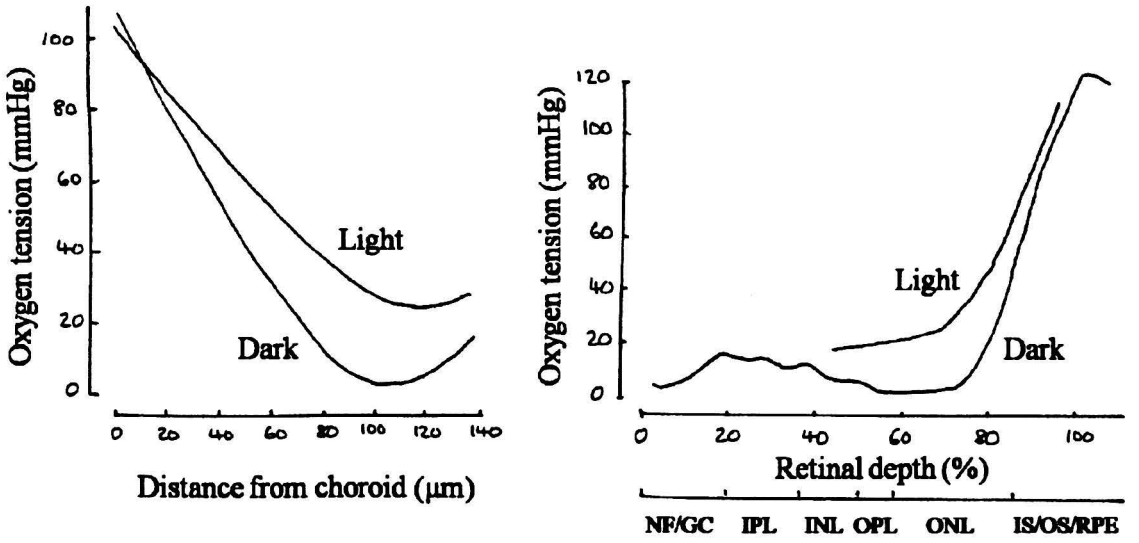


Figure 1.16 PO_2 profile of cat retina in light and dark (redrawn from Linsenmeier, 1986)

Similar studies have been conducted in the macaque and the rat, which, in common with the cat, rhesus monkey and man, also have dual retinal blood supplies (Ahmed, Braun, Dunn *et al*, 1993; Yu and Cringle, 2002). Although tissue PO_2 did not fall quite as low as in the cat, a sufficient trough was demonstrated to suggest that oxidative metabolism in photoreceptor inner segment mitochondria might be compromised (Wangsa-Wirawan and Linsenmeier, 2003). The PO_2 trough near the photoreceptor inner segments implies a boundary region between the retinal and choroidal sources of O_2, around the level of the ONL/OPL, where its availability depends on the gas flux from both (Figure 1.16).

Detailed studies of inner retinal PO_2 profiles suggest that the dominant O_2 consuming layers of the inner retina are the OPL and the deeper (OFF-centre GC signalling) region of the IPL (Yu and Cringle, 2001; Yu and Cringle, 2002). In contrast to the dramatically increased outer retinal O_2 requirement in the dark, consumption across the inner retina appears much steadier in both light and dark (Braun, Linsenmeier and Goldstick, 1995). The QO_2 of the inner and outer retina has been quantified in light and dark (Cringle, Yu, Yu *et al*, 2002). Inner retinal consumption (primarily in the IPL) was slightly greater (mean 184 nL O_2 min^{-1} cm^{-2}) than that of the outer retina (mean 148 nL O_2 min^{-1} cm^{-2}) under light-adapted conditions ($p < 0.01$). In the dark, outer retinal consumption

increased by nearly 50% ($p < 0.001$) with a time constant for the decrease in outer retinal PO_2 of just under 15 s. Inner retinal QO_2 was unaffected. This suggests that the region of retina most vulnerable to O_2 deficiency will vary with light level and that the degree of hypoxia required to compromise function will vary at different retinal locations and under different viewing conditions.

Inner retinal blood flow can increase between 40-70% after transition from light to dark, providing an extra $\sim 10^{14}$ O_2 molecules to the inner retina every second, which may match the increased outer retinal requirement (Feke, Zuckerman, Green *et al*, 1983). The greater O_2 demand from the photoreceptors in the dark, deepening the O_2 sink at the level of the inner segments, will demand a greater flux from the retinal as well as the choroidal circulation. Inner retinal autoregulation maintains a steady inner retinal tissue PO_2 that compensates for the increased outer retinal demand. The arrangement in the macaque supports this view (Ahmed, Braun, Dunn *et al*, 1993). The macaque retina is more like the human retina than others studied but the PO_2 profile is also similar to that observed in the cat. In the parafoveal macaque retina in light, the choroid meets almost all the retinal O_2 requirement with a monotonic PO_2 gradient from the choroid to the inner retina. However, when dark-adapted, 10% of the photoreceptor O_2 requirement is supplied by the retinal circulation.

Although outer retinal O_2 demand in light is much less than in the dark, choroidal flow remains high and may assist with heat removal to maintain a stable temperature in the outer retina, especially the macula. In monkeys flow increases in response to light or light-generated heat and the increased flow may be detected as an elevation in ocular surface temperature, which has also been demonstrated in humans (Parver, Auker and Carpenter, 1983). The effect of light-dark-light transitions on choroidal perfusion has been studied in humans and shows a reduction in choroidal flow in darkness (Longo, Geiser and Riva, 2000; Fuchsjager-Mayrl, Polska, Malec *et al*, 2001). This may allow choroidal flow to dissipate the heat produced in the outer retina at higher light intensities. However, a reduced choroidal flow in darkness also allows a greater O_2 extraction per unit volume of choroidal blood at a time when the photoreceptor O_2 requirement is at its greatest. The mechanism by which this change in choroidal flow is mediated is unclear (Fuchsjager-Mayrl, Malec, Amoako-Mensah *et* al, 2003).

1.4.3 Outer retinal demand for energy and oxygen

The primary visual stimulus is the absorption of light by the photoreceptors and the processes of phototransduction require considerable amounts of biological energy. Photoreceptors are amongst the most metabolically active cells in the body. In rods, substantial amounts of ATP and guanosine triphosphate (GTP) are required to power the cell membrane ion pumps and to synthesise cGMP, as well as to supply the GTP hydrolysed to inactivate transducin, and the ATP for rhodopsin phosphorylation. Rod inner segments have an extremely high QO_2 of 15-20 ml O_2 (STP) per 100 g of tissue per minute (ml $100g^{-1}$ min^{-1}) in the dark and their metabolism is O_2-limited (Wangsa-Wirawan and Linsenmeier, 2003). As a result, any reduction in PO_2 due to even mild hypoxia might be expected to reduce the flux of O_2 to the inner segments and compromise rod QO_2 in darkness.

The high choroidal blood flow is essential to maintain the O_2 'pressure head' and so deliver sufficient O_2 by diffusion to the inner segments. Some 55-65% of all retinal mitochondria are densely packed into the photoreceptor inner segments and they depend on the choroid to satisfy their high O_2 demand. Since the maintenance of high choroidal PO_2 requires a high choroidal blood flow (1400 ml $100g^{-1}$ min^{-1}) together with a low O_2 extraction per unit volume of choroidal blood (otherwise the PO_2 would fall), the result is a high choroidal venous O_2 saturation. Mathematical modelling of microelectrode studies suggests that this vascular profile is essential to maintain normal photoreceptor function in the dark when the O_2 demand is greatest (Linsenmeier and Padnick-Silver, 2000). A choroidal blood flow of at least 500 ml $100g^{-1}$ min^{-1} is required to sustain an outer retinal O_2 demand of 4.5 ml $100g^{-1}$ min^{-1}. A less conservative assumption for outer retinal O_2 demand of 5 ml $100g^{-1}$ min^{-1} would necessitate a choroidal flow of 1250 ml $100g^{-1}$ min^{-1} (within the measured range of 1020 to 2600 ml $100g^{-1}$ min^{-1}). It was also deduced that increased choroidal QO_2 per unit volume of blood could never fully compensate for a decreased choroidal flow rate.

Cones contain more mitochondria than rods and their ellipsoids are larger with a higher mitochondrial density. Mitochondria occupy 74-85% of cone ellipsoids and 54-66% of rod ellipsoids in macaque (Hoang, Linsenmeier, Chung *et al*, 2002). When adjusted for outer segment volume, cones contain up to 10 times more mitochondria than rods. The authors suggest that this is unlikely to be due to a substantially greater metabolic requirement in cones, not least since the production of ATP is O_2-limited rather than

mitochondria-limited in rods in the dark. They propose that the extra mitochondria may play an optical (waveguide) role in cones.

A subsequent study has analysed mitochondrial characteristics and QO_2 in mice rods and cones (Perkins, Ellisman and Fox, 2003). Essentially the differences between rod and cone mitochondria were attributed to cones producing more ATP than rods because they have higher energy requirements. The authors noted that the number of mitochondria per cell, relative mitochondrial volume per cell, levels of cytochrome oxidase and mitochondrial cristae surface area all reflect aerobic energy production capacity. As well as a five-fold increase in cGMP turnover during continuous illumination, the many faster responses of cones are likely to drive an increased requirement for ATP in light. These include differences in relation to increased Ca^{2+} permeability in outer segments; an 8-10 times faster Na^+/Ca^{2+}, K^+ exchanger; higher levels of GTPase activating protein; five-fold faster regeneration of visual pigment; and much greater Na^+/K^+-ATPase activity in inner segments. Thus, cones require more ATP and O_2 in light than rods, yet QO_2 in cones, as in rods, increases in the dark when ATP production is O_2-limited rather than mitochondrion-limited (Ahmed, Braun, Dunn *et al*, 1993). This and other inconsistencies remain to be explained (Hoang, Linsenmeier, Chung *et al*, 2002). An additional consideration is that metabolic requirements for synaptic transmission appear greater for rods than cones, with larger numbers of mitochondria in cone pedicles than rod spherules (Perkins, Ellisman and Fox, 2003).

1.4.4 Ocular pressures, volumes and flows

Pressure occlusion of the ocular circulation causes local hypoxia and results in loss of vision in as little as 4 s (Craik, 1940; Anderson, 1968). The time to visual loss is delayed by increasing P_aO_2 (Carlisle, Lanphier, and Rahn, 1964). This allows estimation of the normal retinal O_2 uptake which ranges from 7.0 to 9.7 ml min^{-1} per 100 ml of 'wet' retina (Saltzman, Anderson, Hart *et al*, 1964). The recovery of vision following release of pressure is also rapid (4-6 s) and is faster breathing air than 100% O_2, so visual recovery is likely to depend on restoration of the inner retinal circulation, which is subject to vasoconstriction under hyperoxia. This is supported by the observation that adding CO_2 to the hyperoxic breathing gas delays visual loss under ocular pressure, as it will counter hyperoxic vasoconstriction and so maintain retinal oxygenation and visual function. Thus, preservation of vision relies on continuing inner retinal perfusion and oxygenation, with both influenced by changes in pressure and respiratory disturbance.

Poiseuille's Law gives the flow, F, of a liquid of viscosity η along a tube of length L and radius r, where ΔP is the pressure difference at each end of the tube (Equation 6).

$$F = (\pi r^4 / 8\eta L)\, \Delta P$$

Equation 6 Poiseuille's formula for the flow of liquid along a tube

Perfusion pressure through a blood vessel is the difference between arterial and venous pressure ($P_a - P_v$), leading to Equation 7 as a formula for flow through a blood vessel.

$$F \propto (P_a - P_v)\, r^4 / \eta L$$

Equation 7 Generic formula for blood flow

MAP is usually calculated as equal to diastolic blood pressure plus one third of the pulse pressure (that is, one third of the difference between systolic and diastolic pressures). Assuming MAP of ~100 mm Hg at heart level, then the hydrostatic effect of a column of blood 25 cm high in an upright individual suggests a pressure in the internal carotid artery, at the level of the ophthalmic branch, of ~80 mm Hg. A further pressure drop along the ophthalmic artery (demonstrated in animals) is likely to reduce the arterial pressure in the vessels entering the human eye (P_a) to around 60-70 mm Hg. The venous pressure at which blood drains from the eye approximates to the intraocular pressure (IOP). Assuming a normal IOP of 15-20 mm Hg, the ocular perfusion pressure ($P_a - P_v$) will be about 45-50 mm Hg (Cioffi, Granstam and Alm, 2003).

Assuming that vessel length and viscosity remain constant, then a general formula for blood flow through the eye is given by Equation 8.

$$F \propto (P_a - IOP)\, r^4$$

Equation 8 General formula for blood flow through the eye

Equation 8 indicates that the principal determinant of resistance to flow is vessel calibre and that the other main factors influencing ocular blood flow are arterial pressure and IOP. These are closely related. The correlation between IOP and venous pressure, the dependence of IOP on arterial pressure and the relationship between IOP and intraocular blood volume have all been demonstrated in the cat (Macri, 1964). The relationship between retinal blood flow and vessel diameter is consistent with Poiseuille flow, changing in proportion to the fourth power of vessel diameter (Feke, Tagawa, Deupree

et al, 1989). For the inner retina, volumetric flow rate, determined from mean blood velocity and vessel diameter, correlates with diameter of both arteries and veins ($p < 0.001$) and total arterial and venous volumetric flow rate correlates with vessel calibre (Riva, Grunwald, Sinclair *et al*, 1985).

Water is virtually incompressible, so changing the fluid volume within a tense elastic container will change the internal pressure of the container. The eye is such a structure and the basic exponential form of the ocular pressure-volume (P-V) relationship is well known. Friedenwald (1937) plotted $\log_{10}$ IOP against ΔV to produce a linear relation. He designated the slope of this line as the rigidity coefficient and proposed its use as a measure of ocular rigidity, that is, 'the resistance which the eyeball offers to a change in intraocular volume'. The relationship is summarised in Equation 9, where ΔV is the change in intraocular volume associated with a change in IOP from P_1 to P_2, and K is the rigidity coefficient, a constant that reflects the elasticity of the wall of the eye.

$$\Delta V = (\log_{10}P_2 - \log_{10}P_1) / K$$

Equation 9 Friedenwald's ocular pressure-volume relationship

The P-V relationship is very variable but has been refined by various authors to account for complications arising, for example, from changes in elasticity of the ocular shell with volume and pressure, and because distension of the wall of the eye is affected by the flow of the viscous blood within it. The basic relationship between IOP and intraocular volume within the living human eye is shown in Figure 1.17 (Eisenlohr, Langham and Maumenee, 1962). Kiel (1995) examined rabbit ocular P-V curves obtained at different MAP, and also measured choroidal blood flow and blood volume, demonstrating that the P-V relationship varies widely depending on arterial pressure but supporting the general appearance of the relationship shown in Figure 1.17.

Baseline IOP and the initial rate of rise in IOP are both dependent upon and vary with MAP. That is, the gradient of the initial slope of the increase in pressure with increasing volume correlates with the increase in MAP. IOP tends to 'plateau' as it approaches MAP, but once MAP is exceeded IOP increases at a rate that is independent of MAP. As IOP approaches MAP so the perfusion pressure falls and choroidal blood flow ceases. In contrast to the traditional view of the ocular P-V relationship as a single exponential function, Kiel therefore demonstrated a range of curves varying with MAP.

He concluded that the ocular P-V relationship is heavily dependent on systemic arterial pressure due to its effects on choroidal blood flow and blood volume.

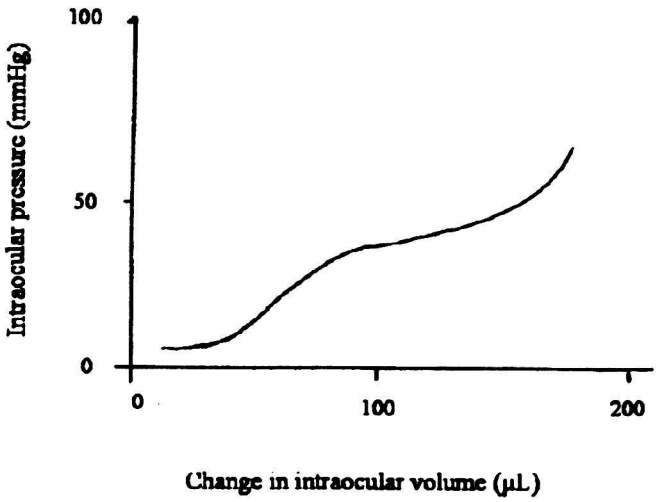


Figure 1.17 The living human ocular pressure-volume relationship (redrawn from Morgan, 2003; after Eisenlohr, Langham and Maumenee, 1962).

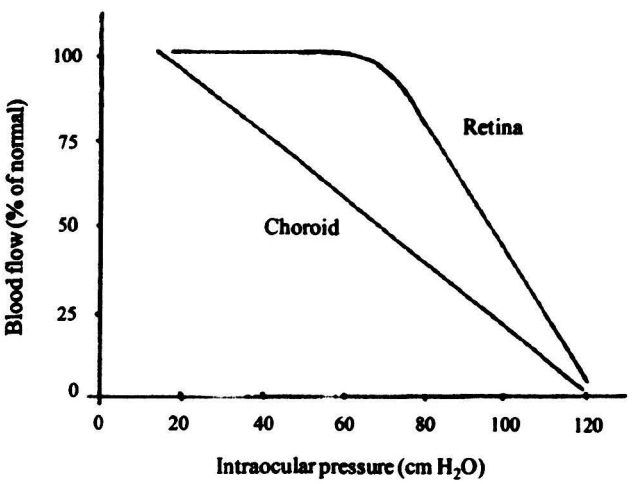


Figure 1.18 Retinal and choroidal blood flow responses to increased intraocular pressure (redrawn from Cioffi, Granstam and Alm, 2003)

The low resistance choroidal flow is usually regarded as passive and without significant autoregulation, responding approximately linearly to fluctuations in perfusion pressure. Other parameters being stable, it will respond predictably to changes in arterial or

venous pressure. Flow will decrease as IOP increases if arterial pressure is held steady (Figure 1.18). This passive response of the choroidal circulation means that both choroidal venous O_2 saturation and choroidal P_{O_2} should fall with increasing IOP, as volume flow of blood through the choroid is reduced (Alm and Bill, 1970; Yancey and Linsenmeier, 1979).

Despite responding to changes in blood pressure and IOP, the high choroidal flow rate maintains the supply of O_2 to the outer retina by virtue of the very many unit volumes of blood to pass through the choriocapillaris. The normal choroidal flow is so great that the arteriovenous PO_2 and PCO_2 differences are small and decreased flow will not alter local tissue PO_2 and PCO_2 sufficiently to affect choroidal vascular resistance, at least until very low flows are achieved. Furthermore, increasing the arteriovenous PO_2 difference may tend to compensate for some reduction in choroidal flow.

However, the increased O_2 requirements of the dark-adapted eye are vulnerable to a reduction in choroidal PO_2 secondary to an increase in IOP that reduced perfusion pressure to around 40 mm Hg (Yancey and Linsenmeier, 1989). The inner retinal PO_2 was little affected but choroidal PO_2 fell substantially, by about 0.5 mm Hg for each mm Hg reduction in perfusion pressure. It was concluded that increasing IOP leads to decrease in choroidal PO_2 and sufficient distal retinal hypoxia to impair photoreceptor QO_2. It was suggested that this would slow the photoreceptor ion pumps.

More recently it has been suggested that the relationship between choroidal blood flow and perfusion pressure is bilinear, with some evidence of passive autoregulation for moderate reductions in perfusion pressure (Riva, Titze, Hero *et al*, 1997). In fact, Friedman suggested as long ago as 1970 that the choroidal circulation does demonstrate a mild degree of autoregulation (Friedman, 1970). Other work indicates a more complex and dynamic interaction between arterial pressure, IOP, choroidal blood volume and choroidal flow, suggesting limited choroidal autoregulation that may be most effective when arterial pressure varies and IOP is uncontrolled (Kiel and van Heuven, 1995).

In contrast, autoregulation of the inner retinal circulation in response to changes in perfusion pressure was first demonstrated by measuring vitreal PO_2 close to the retina of the cat (Alm and Bill, 1972a). Autoregulation of inner retinal flow is effective in countering elevations of IOP that reduce perfusion pressure to only 25 to 35 mm Hg. Small, stepwise elevations in IOP show that it is net perfusion pressure, rather than absolute IOP, that determines GC responsiveness. Retinal neuronal transmission is only

completely suppressed when net perfusion pressure falls below ~20 mm Hg (Grehn, 1981; Grehn, Grüsser and Stange, 1984). The effect of altering perfusion pressure on human retinal blood flow, using artificial changes in IOP, demonstrates rapid changes in vascular resistance that are due to an active process (Riva, Grunwald and Petrig, 1986). Although autoregulation was evident at perfusion pressures as low as 10 mm Hg (IOP of ~42 mm Hg), it was only fully effective as long as perfusion pressure was not lowered by more than 50% (IOP < 27-30 mm Hg). Earlier work supports a range of IOP at which full retinal autoregulation can be achieved as between ~6-30 mm Hg (Riva, Sinclair and Grunwald, 1981; Grunwald, Sinclair and Riva, 1982).

This review has demonstrated a requirement to consider possible effects on IOP, the ocular P-V relationship, retinal and choroidal blood flow, and net retinal oxygenation that may result either directly from reduction in ambient pressure, or as physiological responses to changes in PO_2 or PCO_2, or as a result of changes in arterial or perfusion pressure secondary to changes in respiratory condition. This applies whether the latter are induced using breathing gas mixtures, through altered patterns of ventilation, or through changes in ambient pressure (hypobaric decompression).

1.5 Respiratory Effects on the Eye and Visual Pathway

1.5.1 Cornea and anterior chamber

The high corneal content of hydrophilic glycosaminoglycans ensures that the normal cornea is well hydrated but also that it swells readily when its external integrity is lost due to injury. An endothelial pump actively transports water out of the stromal layers into the aqueous and there is also a net flux of ions and water towards the epithelium and tear film. Visual halos, glare and veils occur due to light scattering when the cornea is hypoxic, while corneal oedema and clouding may produce significant loss of VA and even frank blurring of vision (Meek, Dennis and Khan, 2003).

Much research into corneal oxygenation relates to contact lens wear or tear film function. Neither aspect is considered likely to have influenced the current study. However, for the experiments that follow it was clearly relevant to consider the possibilities of corneal hypoxia and swelling as confounding factors, as well as the use by subjects of contact lenses. The possibility was also considered that changes in ambient pressure might alter the corneal curvature through a direct physical effect.

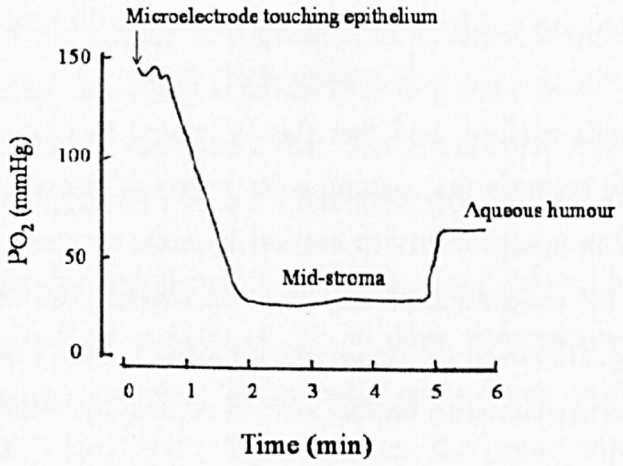


Figure 1.19 Rabbit cornea PO_2 profile (redrawn from Kwan, Niinikoski and Hunt, 1972)

The outer epithelial surface of the cornea and outer stromal layers obtain O_2 from the tear film and hence from ambient air when the eyelids are open, or from the palpebral circulation when they are not. The corneal epithelium appears resistant to short-term hypoxia (O'Leary, Wilson and Henson, 1981). The deeper endothelial surface has long been held to receive O_2 from the anterior uveal circulation (in the iris) via the aqueous humour. Corneal function may therefore depend on both ambient and systemic oxygenation, supported by the *in vivo* PO_2 profile across the open rabbit eye when breathing air, shown in Figure 1.19 (Kwan, Niinikoski and Hunt, 1972).

The use of O_2 microelectrodes demonstrated that PO_2 falls abruptly with movement into the cornea across both the epithelial (and anterior stromal) and endothelial surfaces, while remaining at a steady minimum level through the mid-stroma. This minimum PO_2 was estimated to be 29-30 mm Hg in three rabbits. Thus the mid-stroma does not require significant amounts of O_2 and it is oxygenation of the inner and outer corneal surfaces, from the aqueous humour and ambient air respectively, which is important. Also, PO_2 remained stable with movement through the aqueous humour at a mean of 72 (SE ± 5) mm Hg, somewhat higher than previous estimations, before falling to a steady plateau of only ~20 mm Hg soon after penetrating the lens surface (lenticular epithelium).

Klyce (1981) induced stromal oedema in the isolated rabbit cornea when the tear side was made severely hypoxic, but oedema was prevented as long as the PO_2 in the

aqueous humour was held above 40 mm Hg. He attributed the swelling to hypoxic interference with corneal metabolism and accumulation of lactate within the stroma as a result of anaerobic glycolysis. More recently Bonanno (2001) proposed that hypoxic impairment of endothelial cell function is particularly important, specifically the control of stromal ion and fluid balance, and that this is related to changes in intracellular endothelial pH and the acidosis that accompanies hypoxia. These factors may help to explain the variability in susceptibility to corneal hypoxia between individuals but do not fully account for the magnitude of the effect on stromal swelling (Nguyen, Soni, Brizendine *et al*, 2003). However, the requirement for the current work is to identify the minimum PO_2 required to maintain a normal state of corneal hydration and lucency.

Measuring the corneal thickness of three subjects' eyes bathed in hypoxic gas mixtures under air-tight goggles, for 3.5 h, the critical ambient O_2 concentration required to maintain normal corneal hydration (and so prevent swelling) was derived to be between 1.5-2.5%, equivalent to an ambient PO_2 of only ~11-19 mm Hg at sea level (Polse and Mandell, 1970). The humidity of the gas did not appear to affect the outcome. It is reported that this result was supported by Carney (1974) studying four eyes exposed to 2% O_2 for 2 h (Holden, Sweeney and Sanderson, 1984). A subsequent study of pre-corneal PO_2 ranging between 6.9-20.2 mm Hg, for 4 h, extrapolated that the minimum required to avoid oedema lay between 23-37 mm Hg, noting that there was considerable variation between individuals (Mandell and Farrell, 1980).

Holden, Sweeney and Sanderson (1984) concluded that the PO_2 required to prevent corneal swelling in their group of eight subjects was 74 mm Hg, although this conclusion is based on data obtained after 8 h of exposure. The data obtained during the first 2-3 h of the experiment are more ambiguous, with early data under 21.4% O_2 (that is, with a PO_2 close to that of normal air) showing more corneal swelling than 10.1% (74 mm Hg) O_2 and appearing little different to the 7.5% (55 mm Hg) O_2 condition at 2 h. Thus, it is inappropriate to apply the headline result from this report to the current study, in which exposures to hypoxia were planned to last no longer than an hour. Even so, an equivalent altitude in excess of 18,000 ft would be necessary to drop ambient PO_2 below the suggested pre-corneal minimum of 74 mm Hg to prevent hypoxic swelling.

At sea level, with the eyes open, the corneal epithelium will be exposed to an ambient PO_2 of ~160 mm Hg. Under the closed eyelid, the cornea derives up to two thirds of its O_2 from the palpebral circulation. The palpebral conjunctival PO_2 in rabbit is 70 ± 13 mm Hg suggesting a relatively high tissue PO_2 is required to provide for the cornea

beneath the closed eye (Kwan and Fatt, 1971), which is not to say that a lower tension might not prevent corneal oedema. In the human, the palpebral conjunctiva provides an O_2 source that has been gauged as equivalent to an ambient gas concentration of 7.7% O_2, or a PO_2 of around 55 mm Hg at sea level (Efron and Carney, 1979; Fatt and Bieber, 1968). Weissman (1986) concluded that the pre-corneal PO_2 required to prevent swelling was 30 mm Hg, with a 95% confidence interval (CI) from 23 to 36 mm Hg.

Turning now to consider endothelial hypoxia, rats exposed to a hypobaric environment equivalent to breathing air at 5500 m, for 30 days, demonstrated changes suggesting chronic endothelial hypoxia while the epithelial surface was unaffected (Mastropasqua, Ciancaglini and Di Tano, 1998). This indicates that inner corneal hypoxia may be significant even if the ambient PO_2 is apparently adequate, although this does not mean that brief hypoxia will impair visual performance. Various invasive studies have tried to estimate the PO_2 in the anterior chamber of humans and animals (for example, Hoper, Funk, Zagorski *et al*, 1989; Helbig, Hinz, Kellner *et al*, 1993; Fitch, Sweberg and Livesey, 2000). They suggest that the iris vasculature delivers O_2 to the aqueous but also that the anterior chamber PO_2 is influenced by ambient PO_2.

The anterior chamber PO_2 has also been estimated non-invasively, challenging the idea that the endothelial surface is oxygenated by the aqueous. In contrast to the data illustrated in Figure 1.19, a non-invasive estimate of mid-anterior chamber PO_2 has been reported at only 23 (SD ± 3) mm Hg, reducing to 4 ± 2 mm Hg after 20 minutes of contact lens wear and returning to normal when the lens was removed (McLaren, Dinslage, Dillon *et al*, 1998). This suggests that the bulk of the O_2 reaching the mid-anterior chamber comes from ambient air and supports earlier work demonstrating that the O_2 content of the cornea and anterior chamber decreases if access to atmospheric O_2 by the corneal epithelium is restricted (Stefansson, Foulks and Hamilton, 1987; Polse and Decker, 1979; Stefansson, Wolbarsht and Landers, 1983; Barr and Silver, 1973). With a contact lens in place, the PO_2 gradient in the anterior chamber was reversed, suggesting that some anterior chamber O_2 does come from the uveal circulation.

Recent optical sensor measurements of normoxic PO_2 in the anterior, posterior and vitreous chambers (Shui, Fu, Garcia *et al*, 2006) are shown in Figure 1.20. These also support a falling PO_2 tension with movement posteriorly from the endothelial surface of the cornea, suggesting that the full thickness of the cornea is supplied with O_2 from ambient air. The distribution of O_2 in the posterior chamber indicates that some enters the aqueous from the uveal circulation.

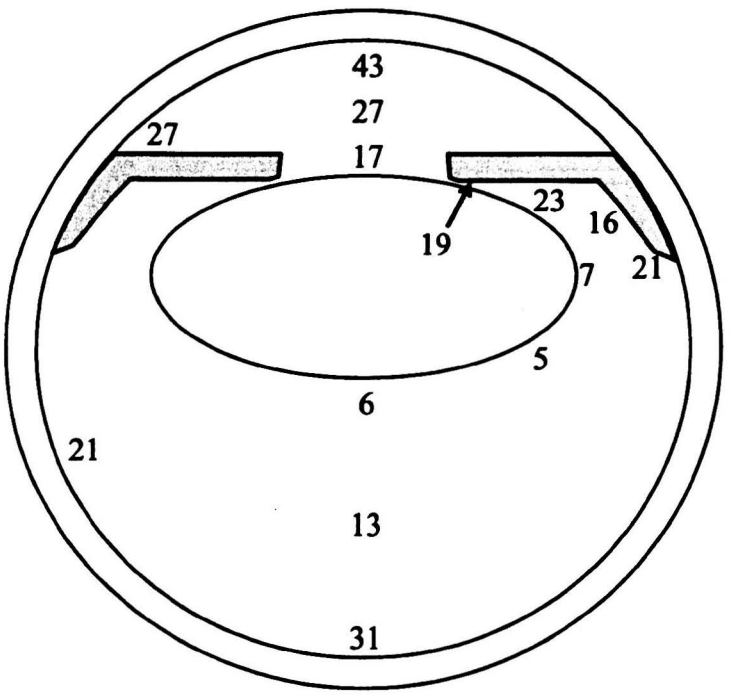


Figure 1.20 Mean normoxic PO_2 (mm Hg) in rabbit eye (after Shui, Fu, Garcia *et al*, 2006)

The results obtained when the animals were hypoxic, breathing 13-15% O_2 (S_aO_2 of 75-80%) are shown in Figure 1.21. Despite the marked reduction in PO_2 in most measurement locations with moderate systemic hypoxia, the PO_2 in the aqueous adjacent to the endothelial surface of the cornea was unchanged, suggesting that the cornea would remain adequately oxygenated under this degree of systemic hypoxia.

Studies have considered whether increased ambient pressure might alter corneal surface contour. One reported altered keratometry readings in Navy divers exposed to pressures of 4 and 5 atm, but noted similar changes in readings taken from a steel ball. Hence, the study did not infer altered corneal shape (Welsh, Bennett and Kislin, 1975). A subsequent study reported no effect on keratometry at pressures up to 3 atm (Gallin-Cohen, Podos and Yablonski, 1980). It was concluded that there was insignificant change in the contour of the globe under hyperbaric exposure and, since the eyeball contains no free gas that might expand, it is reasonable to assume that this holds equally true for modest hypobaric exposures that are insufficient to promote the evolution of gas from solution. This is unheard of other than during rapid (explosive) decompression to altitudes substantially in excess of 18,000 ft, that is, to ambient pressures of far less than 0.5 atm. Furthermore, visual manifestations of neurological decompression illness are only reported above this altitude (Fitzpatrick, 1994).

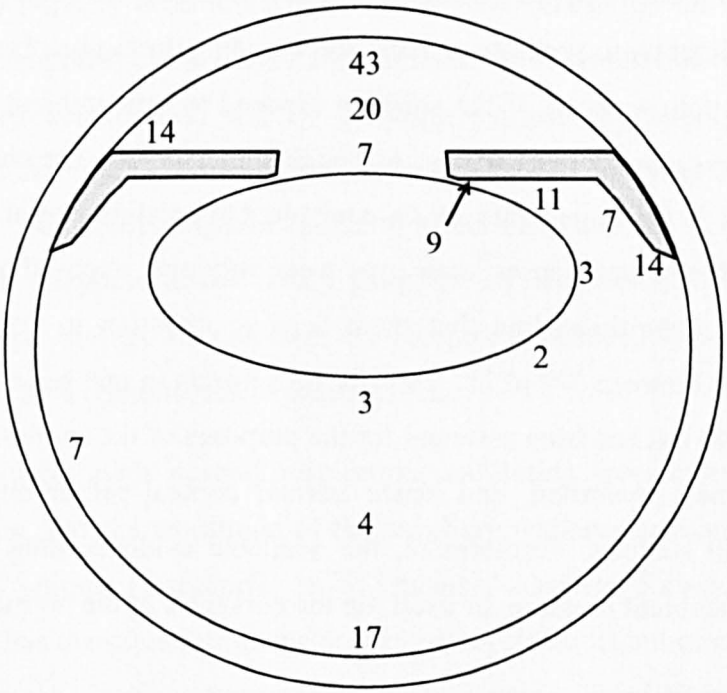


Figure 1.21 Mean hypoxic PO_2 (mm Hg) in rabbit eye (after Shui, Fu, Garcia *et al*, 2006)

A number of hypobaric chamber studies have been conducted on subjects wearing soft contact lenses to assess their suitability for wear at altitude. Although some studies have demonstrated bubble formation or corneal irritation underneath contact lenses at altitude, it is generally accepted that altitude exposure does not, of itself, alter corneal shape or contact lens fit (Eng, Rasco and Marano, 1978; Brennan and Girvin, 1985; Flynn, Miller, Tredici *et al*, 1987). Furthermore, numerous studies have examined the effects of altitude exposure and hypoxia to induce corneal flattening and a hyperopic shift in eyes that have undergone radial keratotomy (White and Mader, 1993; Mader and White, 1995; Ng, White, Parmley *et al*, 1996; Creel, Crandall and Swartz, 1997; Winkle, Mader, Parmley *et al*, 1998; McMann, Parmley, Brady *et al*, 2002). In these studies control eyes have been unaffected while the changes in those with keratotomy are attributable to the resulting hypoxia rather than the decompression *per se* (Ng, White, Parmley *et al*, 1996; Winkle, Mader, Parmley *et al*, 1998).

The equivalent altitudes of relevance to the experiments that follow are 10,000 ft and 15,000 ft, where the respective ambient barometric pressures are 523 mm Hg and 429 mm Hg, and the ambient PO_2 will be 110 mm Hg and 90 mm Hg respectively, well above the likely pre-corneal threshold necessary to prevent corneal hypoxia and swelling. The respective P_aO_2 at these altitudes will be ~50 mm Hg and ~37 mm Hg respectively, sufficiently low to promote a fall in aqueous PO_2. However, from the

evidence presented above, it seems highly unlikely that this will compromise corneal oxygenation sufficiently to promote oedema and clouding during brief exposures. In the experiments that follow, none of the subjects exposed to low ambient pressure in the hypobaric chamber wore contact lenses. Although some subjects breathing the hypoxic gas mixture (14.1 % O_2, to generate a P_aO_2 equivalent to breathing air at 10,000 ft) wore soft, gas permeable contact lenses, their eyes were only ever exposed to ambient air at one atm. Hence, notwithstanding that there is wide variation in corneal O_2 uptake between subjects, between 3-9 $\mu l\ hr^{-1}\ cm^{-2}$ for 68 subjects in one series (Larke, Parrish and Wigham, 1981), it has been assumed for the purposes of the studies conducted here that normal corneal hydration, and hence normal corneal refraction, is maintained throughout by all subjects. Furthermore, the available evidence does not support an effect of altered ambient pressure, of itself, on the curvature of the normal cornea.

1.5.2 Iris and pupil

The structures of the anterior uvea have a rich vasculature. The anterior and long posterior ciliary arteries travel forwards beneath the extraocular muscles, entering the globe to supply the ciliary apparatus and iris. A corresponding ciliary venous sytem drains the anterior uvea. The autonomic nervous control of the dilator and constrictor muscles of the iris is illustrated in Figure 1.22.

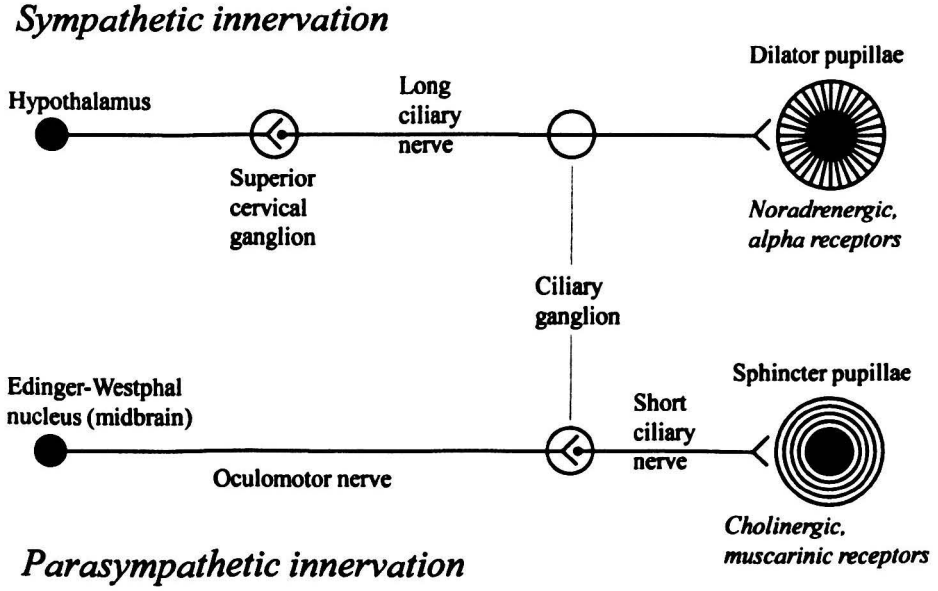


Figure 1.22 Schematic diagram of the autonomic innervation of the iris muscles

The size of the pupil is determined by the balanced actions of the sympathetic and parasympathetic supplies to the dilator and sphincter muscles respectively. Thus, miotic agents (pupillary constrictors) act by blocking sympathetic supply or facilitating parasympathetic activity. Conversely, mydriatic agents may be sympathetic agonists or parasympathetic antagonists. Agents known to affect the pupil may act centrally, at the superior cervical ganglion, at the ciliary ganglion, by interfering with neuromuscular transmitter release, storage or reuptake, by receptor blockade, by a direct action on muscle fibres, or by a combination of these effects.

Pupil size fluctuates with normal respiration, exhibiting inspiratory mydriasis and expiratory miosis, and the amplitude of the pupillary response appears proportional to respiratory tidal volume (Borgdorff, 1975; Ohtsuka, Asakura, Kawasaki *et al*, 1988). The pupil cycle has the same rate as the respiratory cycle but is out of phase with it. The autonomic control of this phenomenon is uncertain. There is clear respiratory modulation of sympathetic outflow during inspiration and expiration (Huang, Yu and Cohen, 1999) and pupillary fluctuations have been suggested to reflect changes in sympathetic tone (Calcagnini, Censi, Lino *et al*, 2000). On the other hand, respiratory modulation of pupil size persists following cervical sympathectomy, which suggests mediation by the parasympathetic nervous system (Borgdorff, 1975).

Proposed mechanisms have included chest wall stretch, lung movement, flucuations in blood pressure sensed by arterial baroreceptors, changes in afferent vagal tone and others. It might also be influenced by local fluctuations in PO_2 and PCO_2, in time with the respiratory cycle, at one or more locations along the pathways of autonomic innervation of the pupil. The rest of this section reviews the empirical evidence available relating to effects of altered PO_2 and PCO_2 on pupil size.

Bunge is recorded as examining pupil size under severe hypoxia in 1936, finding minimal changes that did not interfere with visual sensitivity data (McFarland and Forbes, 1940). On the other hand, Züst (1940) investigated pupil size using flash photography in 1940, under different levels of illumination and at ambient pressures of 720, 520 and 320 mm Hg (equivalent to near sea level, and approximately 10,000 ft and 22,000 ft respectively), finding 'Under low pressure, a definite constriction of the pupil and an increase in the light-reflex excitability' (Rose, 1950a). Dyer (1988) noted a study by McFarland, Holway and Hurvich (1942) who used infrared photography to study the pupils of four subjects breathing 17, 14, 12 and 10% O_2, noting a substantial initial constriction followed by a gradual return to the original size, albeit with considerable

variability. As a result of this and the Züst (1940) study, Mercier and Duguet (1950) apparently concluded that pupillary constriction was a reliable correlate of moderate hypoxia and suspected that 'the constriction was due to hypoxic disinhibition of a central "iridoconstrictor centre"' (Dyer, 1988).

However, measurements of human pupil size during dark adaptation while breathing 10% O_2 have been taken directly and indirectly using infrared photography, demonstrating no effect of hypoxia on pupil size (Ernest and Krill, 1971). This rather severe hypoxia would normally promote hyperventilation. Although experienced subjects were used who were trained not to hyperventilate, the success of training was judged only by monitoring Hb saturation using ear oximetry, so hypocapnia cannot be excluded with certainty.

Other studies have indicated that hypoxia promotes mydriasis. Anaesthetised cats decompressed over 140 s to 20,000 ft exhibited no effect on either normal or sympathectomised pupils, while both dilated slightly at 25,000 ft and more obviously at 30,000 ft, constricting again with descent (Gellhorn and Levin, 1945). The normal pupil dilated more than the sympathectomised pupil. By comparing the responses of different eyes in animals treated with combinations of sympathectomy, division of the oculomotor nerve and adrenalectomy, the authors concluded that a major factor in determining pupillary dilatation under hypoxia was inhibition of parasympathetic tone.

Other workers have reported varying degrees of pupillary dilatation at modest altitude. Furuya (1936), possibly conducting the first study designed to investigate the effect of hypobaric hypoxia on pupil size, reported that hypoxia increased pupil size for decompressions over 5000 m (McFarland, Evans and Halperin, 1941). Pupil size tended to normalise with dwell time at altitude and also recovered quickly upon recompression. Breathing O_2 prevented or moderated the dilatation. In another study, three of four subjects exhibited pupillary dilatation of up to 1 mm at altitudes over 17,000 ft (Evans and McFarland, 1938). Hypoxic dilatation has also been associated with altered light reflexes and anisocoria above 20,000 ft (Bietti, 1953).

Remarkably, referring to work by Duguet and Mercier (1952), it is recorded that, under conditions of hypoxia, the pupil may constrict in darkness and dilate in light (Whiteside, 1957)! Over 55% of subjects exhibited pupillary constriction in darkness with only 28% showing dilatation (17% no change), while in light 50% showed dilatation and only 19% constriction (31% no change). Unfortunately the magnitude of the hypoxia and the

nature of the visual conditions are unclear, although the context in which the material is discussed suggests that the hypoxia was severe.

A recent study has examined the effect of hypobaric hypoxia on pupillary reflexes (Cymerman, Muza, Ditzler *et al*, 2003). Pupil diameter, constriction latency, constriction amplitude and saccadic velocity were measured in the dominant eyes of 35 subjects decompressed to an ambient pressure of 459 mm Hg (simulating 13,400 ft). Ambient illumination was maintained at 237 lux. There were no changes in saccadic velocity or constriction amplitude, but pupil diameter and constriction latency were statistically significantly reduced by 5% and 2% within 1 h of reaching 459 mm Hg. The relevant results are reproduced in Table 1-2.

	Mean	SD	SE	Max	Min
Pupil diameter (mm)					
Sea level	6.0	1.0	0.2	7.8	3.7
Altitude	5.7	1.0	0.2	7.5	3.5
Constriction latency (ms)					
Sea level	302	26	4	359	265
Altitude	296	27	5	358	248

Table 1-2 Pupil effects at an equivalent altitude of 13,400 ft (Cymerman, Muza, Ditzler *et al*, 2003)

Reviewing these results, the first impression is not how different the data are between sea level and altitude, but how similar, and the strength of the statistically significant differences between them is surprising. Accordingly, the data were reviewed in more detail. Measurements were repeated 10 times on each subject at ground level (GL) but only 5 times at altitude, giving a total of 350 measurements at sea level and 175 at altitude. It is not stated whether or not these data follow normal distributions, but since they have been subjected to parametric analysis it is assumed that they do. The mean values given are further assumed to be the global means of the entire data sets rather than the means of individuals' averaged values. The SD values given in Table 1-2 are assumed also to reflect the variance across the full data sets. However, the SE values given appear to have been calculated using the number of subjects (that is $\sqrt{35}$) rather than the number of measurements ($\sqrt{350}$ or $\sqrt{175}$), and are therefore ignored. Two-sample *t* tests were conducted on the mean and SD data given in Table 1-2 for pupil diameter and constriction latency, taking into account the number of measurements in each group and assuming equal variance ($\alpha = 0.05$). For the pupil diameter data $t = 3.24$, $p = 0.001$, with 95% CI for the difference in pupil diameter, between sea level and 13,400 ft, ranging from 0.12–0.48 mm. For constriction latency $t = 2.46$, $p = 0.014$, with

95% CI for the difference in latency, between sea level and 13,400 ft, ranging from 1.2–10.8 ms. A reduction in pupil diameter was seen in 29 (83%) of the 35 subjects, with the reduction ranging from 0.1–23.6% (mean 8.2%) of the sea level pupil diameter.

In a parallel study to that in the hypobaric chamber, Cymerman, Muza, Friedlander *et al* (2005) also examined the effect of terrestrial altitude exposure at the summit of Pikes Peak in Colorado, with an ambient pressure of 463 mm Hg. Subjects breathed supplementary O_2 during ascent. Mean pupil diameters were essentially unchanged at sea level, at altitude when breathing supplemental O_2, and after 30 minutes at altitude without supplemental O_2. However, a similar degree of pupillary constriction was seen at 3 h and 24 h of exposure to that witnessed in the hypobaric chamber. Thus, the slight constrictor response to moderate hypoxia may not be an immediate event, or it may have been delayed by the use of supplementary O_2. The authors suggest that the effect of hypoxia is likely to result from central disinhibition of the Edinger-Westphal nucleus, allowing increased parasympathetic constrictor output.

An effect of hypoxia to promote pupillary constriction is consonant with its effect to promote drowsiness and loss of concentration, as fatigue is clearly associated with smaller pupils (Lowenstein, Feinberg and Loewenfeld, 1963; Morad, Lemberg, Yofe *et al*, 2000). Increased psychological arousal is believed to inhibit the Edinger-Westphal nucleus and decrease parasympathetic outflow to the constrictor pupillae while sympathetic tone to the dilator is increased. Conversely, drowsiness is associated with central disinhibition of the midbrain, producing smaller pupils (Kardon, 2003).

The studies finding an effect of hypoxia to dilate the pupil were generally associated with severe hypoxia (> 20,000 ft) and it seems likely that the effect of hypoxia on pupil size is non-monotonic, or perhaps those studies were confounded by some degree of hyperventilation. Overall, it seems reasonable to conclude that mild to moderate hypoxia induces miosis and that some variable degree of dilatation may occur with more severe hypoxia, possibly related to secondary hypocapnia.

Hyperbaric oxygenation promotes pupillary dilatation, but it is also rapidly toxic to the central nervous system, so the effects on the pupil cannot readily be extrapolated to normobaric and hypobaric conditions. The effects of hyperoxia at one atm or less have hardly been reported. Kent (1966) measured pupil size in four subjects during dark adaptation experiments while breathing either air or O_2 at one atm, finding that the

range of variation in mean pupil diameter (five readings each time) was no more than 0.2 mm for any subject.

Wald, Harper, Goodman *et al* (1942) noticed unusual shifts in absolute visual sensitivity but only when an artificial pupil was not used, and suggested that the effect was mediated through changes in pupil size that they believed to be secondary to hyperventilation. Surprisingly, physiological effects of hyperventilation on the pupil have largely been neglected, with only one study found that investigated the effects of brief spells of increased ventilation (3 minutes at a respiratory rate of 20 cycles min^{-1}) on the pupillogram (Gavriysky, 1995). This was sufficient to lower P_aCO_2 to 22-24 mm Hg. Although the effect on baseline pupil size was not reported, hyperventilation was associated with delayed constriction, reduced constriction amplitude and an extended time to maximal constriction, suggesting a tendency towards pupillary dilatation under hypocapnia. This suggests that hypoxia-induced secondary hyperventilation at altitude could offset an effect of hypoxia to constrict the pupil, while cerebral vasoconstriction accompanying the hypocapnia will tend to worsen the cerebral hypoxia.

Most of the experiments that follow examine effects of hypoxia on visual performance net of any effect on pupil size for five main reasons. First, the practical relevance of the results is more directly applied to visual performance at altitude if pupil size is uncontrolled and net performance data represents likely effects outside the laboratory, reflecting the interests of the Sponsor of the work. Secondly, it is, in any event, impossible to eliminate cognitive effects of systemic hypoxia (such as reduced psychological arousal and impaired concentration) from direct effects on visual performance. Thirdly, it was impractical to measure or monitor pupil diameter while either conducting some of the vision tests or wearing some of the respiratory equipment or both. Fourthly, it should be possible to consider the likelihood and possible contribution of an effect of pupil size on the results. Finally, it was intended to measure the effect of imposed respiratory disturbance on pupil size when practicable, and this was achieved in the final experiment.

1.5.3 Lens, ciliary apparatus, and accommodation

The lens has no blood supply and the local O_2 content is lower than in most other regions of the body (Helbig, Hinz, Kellner *et al*, 1993; McLaren, Dinslage, Dillon *et al*, 1998). However, the lens epithelium and outermost regions have many mitochondria and are metabolically active, producing a steep PO_2 gradient with movement towards

the lens core. The core itself is virtually anoxic, with a PO_2 of < 2 mm Hg (McNulty, Wang, Mathias *et al*, 2004). In fact, it appears that this is beneficial in maintaining transparency, and that excessive oxygenation of the core may promote opacification and cataract formation through oxidation of cellular components (McNulty, Wang, Mathias *et al*, 2004; Shui, Fu, Garcia *et al*, 2006). Thus, the developed lens is unaffected by even severe acute hypoxia, and there is no rationale by which to suspect that mild hypoxia might compromise the optical characteristics of the lens. Indeed, it has been stated that 'keeping a lens anaerobically has no effect on transparency' (Davson, 1972, p101).

On the other hand, this raises the possibility that breathing increased O_2 concentrations might compromise lens lucency. However, oxygenation of the lens appears dependent primarily on vitreal PO_2, which itself is influenced by pre-retinal PO_2. Although this is substantially increased in rabbit when breathing 60% O_2 (Shui, Fu, Garcia *et al*, 2006), this is far from true of all mammals with inner retinal circulations (Alder, Niemeyer, Cringle *et al*, 1986; Yu, Cringle and Alder, 1990; Alder, Yu and Cringle, 1991; Yu, Cringle and Su, 2005). In practice, breathing 100% O_2 for periods of a few hours at a time is commonplace and has not been associated with lens opacification in adult humans. In the experiments that follow, 100% O_2 was administered only to normal healthy adults and for no longer than an hour at a time.

Wilmer and Berens (1918) were the first to report a systematic study of the effects of altitude exposure on vision and the eye. They demonstrated that hypoxia caused recession of the near point of accommodation at an equivalent altitude of 15,000 ft in their hypobaric chamber. The effects were quickly corrected to normal with supplementary O_2, and less dramatically by return to sea level, when the rate of recovery was highly variable. Subjects with refractive error showed more marked effects. They also demonstrated that hypoxia hastened accommodative fatigue progressively with altitude. This was apparent from 15,000 ft and became marked at 20,000 ft, but quickly corrected with O_2.

Furuya is recorded as having reported, in 1937, impairment of accommodation above 5,000 m (16,400 ft) which was progressive with continued exposure and which took 40 minutes to recover completely following a 60 minute dwell at altitude (McFarland, Evans and Halperin, 1937). McFarland's own work in the Andes suggested increasingly rapid fatigue of accommodation with progressive ascent, even in acclimatised subjects, beginning from 9,200 ft and becoming pronounced from 15,000 ft (McFarland, 1937). Ohlbaum (1969) noted subsequent studies that support the progressive effect of hypoxia

on accommodative power when breathing air at altitudes between 10,000 ft and 20,000 ft, quantifying a statistically significant loss at 18,000 ft.

Hypoxic impairment of accommodation is presumed to result from ciliary muscle fatigue. Greater vulnerability may be expected with prolonged hypoxia, increasing age and hyperopia. The experiments that follow used emmetropic subjects but microfluctuations in accommodation do occur in young emmetropic eyes at low luminance (Gray, Winn and Gilmartin, 1993) and also relative to respiration and pulsatile blood flow (Winn, Pugh, Gilmartin *et al*, 1990; Winn and Gilmartin, 1992; Collins, Davis and Wood, 1995). However, these are unlikely to have influenced performance on the display-based vision tests at the viewing distances employed in the current study (Gray, Gilmartin and Winn, 2002) while the hypoxic exposures were relatively brief and mild, generally well below an equivalent altitude of ~15,000 ft where a significant effect might be anticipated.

1.5.4 Extraocular muscle balance and convergence

Hypoxia is well known to cause diplopia and frank heterotropia (strabismus or 'squint') in those with 'significant' heterophoria (latent squint). This appears to be particularly true of those with hyperphoria (vertical misalignment of the two eyes) even if there is good binocular fixation at sea level. Perfect horizontal muscle balance between the two eyes, for both near and distant vision, is uncommon. There is usually a tendency towards esophoria (convergence) or exophoria (divergence) that is measurable when fusion of the two images is prevented. Much early work was devoted to establishing the degree to which heterophoria was acceptable in aviation and the nature and extent of the effects of hypobaric hypoxia upon it.

Wilmer and Berens (1918) demonstrated slight but progressive abduction and adduction losses in 25 subjects with normal eyes at equivalent altitudes of 15,000 ft and 20,000 ft in a low pressure chamber. They also demonstrated hypobaric impairment of convergence that qualitatively mirrored the impairment of accommodation. In subjects with reduced VA or known ocular muscle imbalance, hypoxia caused more obvious recession of the near point. Marked convergence fatigue was demonstrated at 15,000 ft and 20,000 ft but was corrected rapidly with O_2. In subjects with poor convergence power, exophoria or hyperphoria, and especially in those with combined exophoria and hyperphoria, marked convergence failure and diplopia often occurred between 10,000 ft and 15,000 ft.

Nicholls' review (1950) notes a number of studies that demonstrated effects of hypoxia on subjects with convergence insufficiency, exophoria or hyperphoria, including a reduced field of binocular fixation, recession of the near point and diplopia. While hypoxia has generally been shown to aggravate convergence insufficiency, particularly when a primary exophoria exists on near testing, this is not necessarily the case for those with a primary esophoria. Hence, it may be anticipated that some subjects' convergence power will appear unaffected by hypoxia, as was noted by Wilmer and Berens (1918).

A 1941 review noted Velhagen's (1937) study of 16 subjects with mild heterophoria using a low-pressure chamber, reporting, at equivalent altitudes above 10,000 ft, a trend towards esophoria for distance vision and a less consistent tendency in favour of exophoria for near vision, and hence convergence insufficiency (McFarland, Evans and Halperin, 1937). McFarland tested acclimatised subjects in the Andes and found no effect on heterophoria for distance, even at 20,000 ft. In contrast, near vision testing revealed a tendency towards exophoria that was measurable from 9,200 ft (McFarland, 1937). Significant fatigue of convergence was found from 15,000 ft. Livingston (1941) supports McFarland's results, with progressive recession of the convergence near point with ascent from 15,000 ft to 19,000 ft, amounting to some 6 cm.

Effects of hypoxia on external ocular muscle function have been claimed at equivalent altitudes from around 10,000 ft. Examining a group of subjects before, during and after a two-week stay at that altitude, Rose (1949) found an initial tendency towards esophoria after 24 hours at altitude, shifting over the course of the subsequent fortnight to a tendency to exophoria. In contrast, Kobrick (1968) found no such effect after 24 hours at 12,800 ft. Ohlbaum (1969) reviewed a number of studies that demonstrated a variable but persistent tendency under hypoxic conditions towards increasing esophoria (or decreasing exophoria) for distant vision (for example, Adler, 1945; Neely, 1951; Ten Doesschate, 1955).

Effects of acute (as opposed to prolonged) hypoxia on ocular muscle balance have generally only been demonstrated at altitudes in excess of 10,000 ft, even in subjects with heterophoria. Subjects with normal muscle balance appear more resistant with effects apparent above about 14,000 ft, although Ohlbaum (1969) was unable to demonstrate a significant effect on lateral phorias, either for distance or near vision, in normal individuals.

Turning to effects of hypoxia on the coordination of ocular movements, by photographing light reflected from the cornea, an early study revealed a significant increase in the time taken for 10 subjects with normal VA and muscle balance to read six lines of print when breathing 12.5% (13,500 ft) and 10.5% O_2 (18,000 ft). There was an increase in variability for the subject group as a whole, but the faster readers under normoxia tended to remain relatively faster under hypoxic conditions (McFarland, Knehr and Berens, 1937a). Generally there was "a decrease in the precision of movements and the maintenance of fixation in reduced O_2. In most cases nystagmoid movements crept into the records in varying amounts and tended to occur in a rhythmic manner". Comprehension was impaired under the hypoxic conditions. While the subjects' ocular performance tended to adapt during an hour's exposure to 12.5% O_2, the average subject's performance showed a steady decrement over an hour while breathing 10.5% O_2. One subject's hyperphoria was revealed under hypoxia.

The same authors reported a similar study of 10 subjects known to have ocular anomalies, including reduced VA, heterophoria or heterotropia (McFarland, Knehr and Berens, 1937b). The defects became manifested or were accentuated under hypoxia. There was a significant increase in the number of regressions made by the experimental group but not by the control group. The reading time per line and the adjustments made during fixations appeared to be sensitive measures of the early effects of hypoxia.

There is considerable variability in ocular and visual performance within and between normal subjects from day to day and even from hour to hour, in the numerous confounding factors that may have an influence, and in the taking of accurate, objective and repeatable measurements. Worse, the effects of hypoxia are also notoriously variable within and between individuals, and are just as notoriously inconsistent between studies. The reports in this section have served to emphasise these lessons, although the effects of hypoxia on ocular muscle balance and convergence discussed above are not believed to have interfered with the studies that follow.

1.5.5 Intraocular pressure

Effects of changes in IOP on visual performance

If abrupt changes in IOP can influence retinal and visual function then the effects of respiratory disturbance on IOP must be considered as a possible mechanism for interfering with visual performance. Certainly abrupt and substantial changes in IOP can

be made to interfere with retinal nerve fibre activity (Grehn, 1981; Grehn, Grüsser and Stange, 1984). Furthermore, effects of elevated perfusion pressure to increase IOP and reduce choroidal flow also compromise electrophysiological indices of retinal activity in a manner that correlates with decreased choroidal PO_2 (Yancey and Linsenmeier, 1988). In another O_2 microelectrode study in the cat, inner retinal PO_2 remained steady despite acutely increased IOP, implying retinal autoregulation, but outer retinal PO_2 fell severely with some areas becoming anoxic (Alder and Cringle, 1989). Outer retinal QO_2 was compromised if perfusion pressure fell below 50 mm Hg indicating an absence of any meaningful choroidal autoregulatory response to this challenge.

In humans, the effect of changes in retinal perfusion pressure on inner retinal blood flow has been studied using artificial changes in IOP, demonstrating rapid changes in vascular resistance that may be attributed to an active process (Riva, Grunwald and Petrig, 1986). Autoregulation was evident at perfusion pressures as low as 10 mm Hg (IOP of approximately 42 mm Hg) and remained fully effective if perfusion pressure fell by no more than 50% (IOP not above 27-30 mm Hg). The range of IOP compatible with full inner retinal autoregulation is ~6-30 mm Hg (Riva, Sinclair and Grunwald, 1981; Grunwald, Sinclair and Riva, 1982). Thus, autoregulation of retinal flow maintains a constant, inner retinal PO_2 despite normal fluctuations in IOP and blood pressure.

While inner retinal autoregulation is likely to preserve inner retinal function unless IOP increases dramatically, the potential exists for modest elevations in IOP to influence outer retinal activity. Accordingly, the likelihood for hypobaric decompression and hypoxic breathing gas mixtures to elevate IOP is now considered further.

Effect of sudden reduction in ambient pressure on IOP

Pinson (1940) used a mercury manometer for direct measurement of the IOP of anaesthetised rabbits during exposure to low ambient pressures in a decompression chamber. The manometer was sensitive enough to indicate the fluctuations in IOP (between 1-4 mm Hg) that accompanied the cardiac and respiratory cycles. Exposures were made to a maximum equivalent altitude of 40,000 ft (> 80% reduction in ambient pressure) at rates of ascent and descent up to 30,000 ft min^{-1}. No significant change in IOP was observed during any of the ascents with only a slight tendency for the IOP to fall, of doubtful significance, when descending from the higher altitudes.

The IOP of dogs has been measured following decompression from 10,000 ft to either 45,000 ft or 80,000 ft over either 1 minute or 1 s (Cooke, 1970). When breathing air and decompressed over 1 minute, IOP almost doubled at 45,000 ft and almost tripled at 80,000 ft. However, when breathing 100% O_2 there was no effect on IOP at 45,000 ft, but IOP doubled at 80,000 ft. Elevations in arterial blood pressure and pulse pressure were seen during the exposures and it is important to note that 100% O_2 is inadequate to prevent severe hypoxia at 80,000 ft. The effects of rapid, high altitude decompression on IOP probably resulted not from the physical reduction in ambient pressure but from the physiological consequences of acute severe hypoxia.

Thus, a substantial reduction in ambient pressure, of itself, appears insufficient to provoke a change in IOP. This is supported by tonometric measurements on 60 human subjects taken at GL and 30,000 ft (presumably breathing supplementary O_2) in a hypobaric chamber. The results suggested that no significant changes in IOP or the facility of aqueous outflow result from acute reduction of ambient pressure (Newton, Clark, Culver *et al*, 1963). None of the hypobaric exposures undertaken during the experiments that follow were in excess of 15,000 ft and it is assumed that reduction in ambient pressure *per se* would not have influenced IOP or ocular perfusion.

Effect of prolonged altitude exposure on IOP

A recent study assessed the effect of acute exposure to 10,000 ft on the IOP of 20 subjects during a flight at that altitude in an unpressurised aircraft (Bayer, Yumuşak, Şahin *et al*, 2004). They found a statistically non-significant reduction in mean IOP of only 1.2 mm Hg, supporting another recent study that found no effect on normal, healthy control eyes (Mills, 2001). In considering other previous studies it is important to distinguish between acute and prolonged exposures to various altitudes. Prolonged exposures (many days) and altitudes over 14,000 ft were associated with substantial falls in IOP in two studies (Brinchmann-Hansen and Myhre, 1990; Cymerman, Rock, Muza *et al*, 2000). Then again, another recent study concluded that terrestrial altitude exposure to 3700 m and above over a period of a few days provokes a statistically significant (but clinically insignificant) increase in IOP that tends to return to baseline with time (Somner, Morris, Scott *et al*, 2007). With respect to more acute but milder hypoxia at lower altitudes, it has been concluded that around 2 h of exposure is required to induce a significant fall in IOP at altitudes of 10,000 to 14,000 ft (Bayer, Yumuşak, Şahin *et al*, 2004).

Effect of altered PO_2 and PCO_2 on IOP

It is necessary also to consider the effects of specific respiratory challenges to produce qualitative responses in IOP. With regard to the effect of hypobaric hypoxia, McFarland, Evans and Halperin (1941) noted in their review a number of early studies that demonstrated an apparently consistent increase in IOP at altitudes from above about 13,000 to 16,000 ft in a low pressure chamber. During continued exposure IOP tended to return to normal and then fell further immediately after descent, before rising again to the original level after about 30 minutes. These responses may have mirrored those of arterial pressure. It appears that hypobaric hypoxia sufficient to prompt a circulatory response may then be associated with a corresponding elevation in IOP. Thus, the absence of a significant increase in arterial pressure in response to a mild hypoxic challenge suggests that IOP is unlikely to be affected.

In contrast, hyperoxia, induced by breathing normobaric 100% O_2, is associated with a clear reduction in IOP (Gallin-Cohen, Podos and Yablonski, 1980). The same study demonstrated the same outcome under hyperbaric conditions and also in rabbits administered O_2 for over 3 h while pH and PCO_2 were controlled. This IOP response is likely to have resulted from reduction of intraocular blood flow, and hence intraocular volume, in response to the hyperoxia.

Significant decreases in IOP have been observed with hyperventilation, correlating with the reduction in P_aCO_2 (to 23.6 mm Hg) and the associated elevation in arterial pH and P_aO_2 (Kaufmann, Schotte and Holtmann, 1971). There was no significant change in blood pressure or heart rate. It was considered that the decrease in IOP resulted from changes in ocular perfusion. The effect of elevating the inspired CO_2 by means of a rebreathing system has also been studied and demonstrated a mild elevation of IOP in response to the respiratory acidosis (Kielar, Teraslinna, Kearney and Barker, 1977). Changes in IOP were related to rates of change of PCO_2 rather than absolute levels. A highly significant ($p < 0.001$) correlation between end-tidal CO_2 tension ($P_{ET}CO_2$) above and below the normal range, and IOP, increasing and decreasing in parallel, has also been demonstrated during anaesthesia (Samuel and Beaugié, 1974). It was suggested that the fall in IOP with reduction in $P_{ET}CO_2$ might be due to choroidal vessel constriction and hence reduced choroidal blood volume, and this has received support (Wilson, Le May, Holloway *et al*, 1974).

In the experiments that follow, blood pressures and $P_{ET}CO_2$ were monitored throughout to exclude possible confounding effects on IOP during hypoxic challenges. While hyperoxia may have induced slight reductions in IOP, the likely absence of deleterious effects on visual performance would exclude changes in IOP as a confounding factor.

1.5.6 Ocular blood flow

Retrobulbar haemodynamics

There is evidence that ophthalmic artery blood flow may not follow the same response pattern to changes in blood gases as the major cerebral arteries and that it may have a specific and distinct autoregulatory response (Bornstein, Gur, Geyer *et al*, 2000). A study of the regulation of cerebral and ocular blood flow found that hypercapnia increased middle cerebral artery flow velocities and fundus pulsation amplitude (reflecting choroidal flow) but did not affect ophthalmic artery mean flow velocity (Schmetterer, Findl, Strenn *et al*, 1997). Another study confirmed an increase in short posterior ciliary artery blood flow under isoxic hypercapnia as well as regional differences in retinal flow, but no effect on pulsatile ocular blood flow (Roff, Harris, Chung *et al*, 1999). On the other hand, hyperoxia does reduce ophthalmic artery blood flow velocity (Roff, Harris, Morrison *et al*, 1998), and hyperbaric O_2 produces a mean 15% decrease in ophthalmic artery blood velocity in normal subjects (Okamoto, Nishimura, Goami *et al*, 1998).

In brief, cerebral arteries, the ophthalmic artery and the ciliary and central retinal branches may have different and varied responses to changes in PO_2 and PCO_2. This suggests differential arterial sensitivity, local effects, and an increased sensitivity of retinal neural tissue to blood gas disturbance than is seen with other structures within the orbit. Rather than consider in detail the very many papers that have studied the varied responses of these vessels to respiratory disturbance, for the purpose of the current work it is sufficient to review briefly the net effects of changes in PO_2 and PCO_2 on the inner retinal and choroidal circulations.

Inner retinal blood flow

PO_2 gradients exist in the vitreous, close to the internal limiting membrane, that result from the local geometry of the retinal circulation (particularly near retinal arteries) and the PO_2 in retinal vessels (Alder and Cringle, 1985). However, the vitreal PO_2 adjacent to the retina but away from retinal vessels appears to reflect the PO_2 of the superficial

inner retinal capillary network, from which it is inferred that there is no significant inner retinal barrier to the diffusion of O_2. The PO_2 adjacent to retinal arteries during air breathing approximates the femoral P_aO_2 so retinal arteries also offer no significant diffusion barrier to O_2. The retinal venous PO_2 when breathing 100% O_2 is only slightly lower than the retinal P_aO_2 on air, suggesting that when breathing 100% O_2 the retinal circulation delivers primarily dissolved O_2 with retinal venous Hb O_2 saturation remaining over 90%.

As with cerebral vessels, inner retinal vessels dilate in response to hypoxia induced either by decompression or by reduction of F_IO_2, and constrict in response to hyperoxia, (Cusick, Benson and Boothby, 1940; Dollery, Hill, Mailer *et al*, 1964; Nichols and Lambertsen, 1969; Kobrick and Appleton, 1971; Fallon, Maxwell and Kohner, 1985). Hypoxic retinal vasodilatation and hyperoxic vasoconstriction have been shown to be autoregulated *in vitro* (Papst, Demant and Niemeyer, 1982). Retinal vasculature responses to altitude exposure have been studied using digital analysis of fundus photographs taken at equivalent altitudes from 8,000 to 15,000 ft and compared to sea level (Brinchmann-Hansen and Myhre, 1990). Retinal arterial vasodilatation may be well established at 8,000 ft with a general, non-linear trend towards progressive dilatation with further ascent. However, hypocapnia may offset the effect of hypoxia at altitudes between about 10,000 and 15,000 ft, before the effect of more severe hypoxia predominates once more at higher altitudes. Unfortunately, the precise respiratory status of these subjects was not documented in this study.

During prolonged stays at 17,500 ft the effect of hypoxia overwhelms that of any concomitant hypocapnia (Frayser, Houston, Gray *et al*, 1971). If so, then at altitudes between 10,000 ft and 15,000 ft the mutually antagonistic relative contributions to retinal vessel diameter of hypoxia and hypocapnia are likely to be highly variable within and between individuals. This is reinforced by the finding in the same study that the variability in vessel response within the same retinas on the same occasions was at least as great as the marked variability between subjects. Nonetheless, under isocapnic conditions retinal arterial flow velocity and arteriovenous passage time are accelerated by hypoxia and slowed by hyperoxia, in proportion to arterial O_2 content (Harris, Arend, Kopecky *et al*, 1994).

Hyperoxic vasoconstriction is accompanied by a clear reduction in blood flow that is dose-dependent (Riva, Grunwald and Sinclair, 1983; Strenn, Menapace, Rainer *et al*, 1997). However, retinal venous Hb O_2 saturation increases and the veins take on the

colour of arterial vessels, so target tissues will remain adequately perfused and oxygenated. Local changes in retinal periarteriolar PO_2 precede the changes in vessel diameter and blood velocity, so changes in tissue PO_2 are opposed by autoregulated effects on blood flow that appear to be mediated by a local effect of O_2, direct or indirect, on retinal vessel walls (Riva, Pournaras and Tsacopoulos, 1986).

Changes in PCO_2 have a profound effect on cerebral perfusion, with moderate hypocapnia promoting intense cerebral vasoconstriction (Kety and Schmidt, 1946; 1948). A similar pattern of response might reasonably be expected in the eye and the effect of raised P_aCO_2 to increase blood flow in all ocular tissues has been demonstrated clearly in the cat (Alm and Bill, 1972b). Neither hyperventilation nor breathing 5% CO_2 in O_2 appear to have a major effect on the diameter of larger retinal vessels (Anderson and Saltzman, 1965; 1967). However, retinal perfusion does decrease in response to hyperventilation-induced hypocapnia, while retinal venous O_2 saturation increases when breathing air with an enhanced fractional concentration of CO_2, indicating increased retinal flow (Frayser and Hickam, 1964). The addition of CO_2 to hyperoxic breathing gas diminishes the retinal vasoconstrictor response to hyperoxia and increases venous O_2 saturation, suggesting an increase in retinal flow and O_2 delivery over that breathing 100% O_2 alone (Hickam and Frayser, 1966; Pakola and Grunwald, 1993; Arend, Harris, Martin *et al*, 1994). Thus, CO_2 influences retinal blood flow without necessarily altering the diameter of larger retinal vessels.

Comparative studies support unequivocal effects of altered P_aCO_2 on retinal flow and availability of O_2 (Tsacopoulos and David, 1973; Tsacopoulos, Baker, Johnson *et al*, 1973). The effect of small changes in $P_{ET}CO_2$ (10%) on human retinal arterial and macular capillary blood velocities have been studied while controlling heart rate, arterial blood pressure, IOP and ocular perfusion pressure (Harris, Arend, Wolf *et al*, 1995). Blood velocity was decreased by hypocapnia and increased by hypercapnia, confirming that the human retinal circulation responds sensitively to even slight changes in PCO_2, and this has since received further support (Sponsel, Harrison, Elliott *et al*, 1997).

Of interest, isocapnic hyperoxia and isoxic hypercapnia have been used to study variations in regional retinal vascular response to blood gases (Chung, Harris, Halter *et al*, 1999). Baseline blood flow in the inferior temporal quadrant was significantly greater than the superior quadrant. The inferior quadrant was less responsive to vasodilatation, failing to increase perfusion under hypercapnia, but responded with significant flow reduction under hyperoxia. In contrast, hyperoxia did not affect the

superior temporal quadrant whereas hypercapnia significantly increased flow there, although a recent study found more consistent vasoconstrictor responses to hyperoxia in all quadrants (Seendy, Lovasik and Kergoat, 2005).

In summary, the response pattern of the human retinal circulation to changes in blood gas tension qualitatively mirrors that of the cerebral circulation, but with more dramatic responses to changing PO_2 and more subtle but nonetheless sensitive responses to fluctuations in PCO_2, possibly with regional variations in response.

Choroidal blood flow

Some 70% of uveal flow passes through the choroid, with 5% to the iris and the rest to the ciliary body. Trokel (1965) examined the effects of respiratory gases on rabbit choroidal blood flow and found that breathing 100% O_2 reduced choroidal blood volume (by 14%) and blood flow (32%) and increased vasomotor tone (69%). In contrast, the addition of 10% CO_2 to air (displacing N_2) significantly increased choroidal blood volume (55%) and flow (61%) with a reduction in vasomotor resistance (32%). The use of a 10% CO_2 and 90% O_2 mixture produced results suggesting that in this instance the hyperoxia will tend slightly to offset the predominant effect of CO_2, producing a 22% increase in blood volume, 15% increase in flow and 13% decrease in peripheral resistance. Other studies have also demonstrated a small decrease in choroidal blood flow with 100% O_2 and increase in blood flow with hypercapnia (Friedman and Chandra, 1972; Alm and Bill, 1972b). Thus rabbit choroidal flow appears to respond to increased breathing gas concentrations of O_2 and CO_2 in a way that mirrors their effect on the human cerebral circulation.

Studies into the effects of hyperoxia and hypercapnia on human choroidal flow produce similar qualitative findings (Schmetterer, Wolzt, Lexer *et al*, 1995). Pulsation amplitude decreased during hyperoxia and increased with hypercapnia, suggesting reduced and increased flow respectively. The effects may be due to metabolic autoregulatory mechanisms that appear more effective in the optic disc region by comparison with the macular region, demonstrating that optic nerve head flow is influenced by both choroidal and retinal circulations. In an attempt to define a treatment regime for central retinal artery occlusion, the prevention of hyperoxic retinal vasoconstriction with hypercapnia was studied using different concentrations of CO_2 in O_2 to optimise oxygenation across the thickness of the retina from the choroidal circulation (Schmetterer, Lexer, Findl *et al*, 1996). Fundus pulsations were reduced in both the

macula (indicating choroidal flow) and optic disc (retinal flow) regions in response to 100% O_2 with a dose-dependent increase in pulsation with addition of CO_2. Addition of 5% CO_2 increased or maintained pulsatile ocular blood flow in the macula and optic disc at a level comparable to that breathing air. However, while other studies in humans have also shown a possible dose-dependent increase in choroidal blood flow in response to hypercapnia, there was little effect of breathing 100% O_2 (Kergoat and Faucher, 1999; Geiser, Riva, Dorner *et al*, 2000). The effect of hypercapnia is supported by a clear demonstration of the effect of isoxic hypercapnia to increase blood flow through the posterior ciliary arteries that supply the choroid (Roff, Harris, Chung *et al*, 1999).

Other animal studies also suggest that choroidal flow responds to changes in respiratory gases similarly to cerebral blood flow, and that choriocapillaris blood flow distribution is affected by changes in IOP and blood PO_2 and PCO_2 (Flower, Fryczkowski and McLeod, 1995). Changes in gas tension may affect the compliance of the walls of feeding choroidal arterioles and thereby the pulses of ciliary artery blood into the choroidal plexus. In turn, these changes would modify the perfusion pressure gradients throughout the plexus and the distribution of blood from the choroidal arteries.

In sum, hypercapnia increases choroidal flow while hyperoxia may tend less obviously to reduce flow, suggesting a limited degree of metabolic autoregulation. The effects on human choroidal flow of hypocapnia do not appear to have been documented. The ability of the choroid to keep blood flow constant despite changes in perfusion pressure is substantial and is not compromised by altered CO_2 levels (Kiss, Dallinger, Polak *et al*, 2001). This choroidal pressure-flow regulation appears to vastly outweigh any slight adjustment that may result from fluctuations in blood gas tensions under normal circumstances. In support of this, the only study found that has examined the effect of hypoxic hypoxia on choroidal (pulsatile ocular) blood flow in humans demonstrated no effect when breathing 12% O_2, balance N_2 (Kergoat, Marinier and Lovasik, 2005).

1.5.7 Retinal oxygen tension profile

There is no physical anastomosis between the retinal and choroidal systems. In rhesus monkeys, transient acute retinal ischaemia of less than 100 minutes produces no obvious retinal damage, but 4 h of such severe hypoxia causes near-total irreversible injury (Gouras and Carr, 1965). Central retinal artery interruption destroyed most of the inner retinal layers including the major proportion of the bipolar cells. The photoreceptors and pigment epithelium remained largely intact. In contrast, occlusion of

choroidal arterioles damages the outer retina due to the loss of perfusion pressure and consequent reduction in blood flow through the choriocapillaris, leading to immediate loss of the normal PO_2 gradient across the outer retina.

The choroid of the cat provides up to 91% of photoreceptor O_2 in the dark, under both normoxic and hypoxic conditions (Linsenmeier and Braun, 1992). In light, the choroid supplied all photoreceptor O_2 until P_aO_2 fell below about 60 mm Hg when the retinal circulation supplied 10%. Photoreceptor QO_2 increased three-fold in the dark by comparison with rod-saturating light intensities. Inner retinal PO_2 was well regulated under hypoxic conditions until the P_aO_2 fell below about 45 mm Hg in the dark, and was less affected in light. In contrast, photoreceptor QO_2 fell by around 50% when P_aO_2 was reduced to 50 mm Hg.

Quantitative appraisal of the relative O_2 contributions from the human choroidal and retinal circulations suggests that when breathing air the choroid supplies O_2 to around 60% of the thickness of the retina (Dollery, Bulpitt and Kohner, 1969). This may increase to 97% of the retina when breathing 70% O_2. However, even 100% O_2 at one atm, delivered to the eye through an intact choroidal circulation, may be insufficient to counteract the effects of severe retinal ischaemia and sustain normal retinal function (Flower and Patz, 1971).

On the other hand, 100% O_2 at one atm, producing a choroidal P_aO_2 of ~400 mm Hg, elevates inner retinal PO_2 in the absence of an effective retinal supply, while 20% O_2 under the same conditions was associated with abnormal electroretinogram (ERG) and cortical visual evoked responses (VER) that were restored to normal by ventilating with 100% O_2 and perfusing the eye through the choroid alone (Landers, 1978). Studies of the cat eye in light conditions with an inoperative retinal circulation suggest that breathing 60% O_2 at one atm can produce normal or higher PO_2 across the full thickness of the retina as a result of diffusion of O_2 from choroidal blood (Wolbarsht, Stefansson and Landers, 1987). Retinal thicknesses and diffusion distances are comparable in cats, rhesus monkeys and humans, so O_2 might diffuse from the choroidal circulation through the full thickness of the retina, given a high enough choroidal PO_2. If the choroid is the only source of retinal O_2, then the effect of increased choroidal O_2 levels or reduced outer retinal consumption is passed through to the inner retinal layers, as would be expected (Cringle, Yu, Alder *et al*, 1999), while addition of hypercapnia augments oxygenation in all layers (Yu, Cringle, Alder *et al*, 1999). The effect of hypocapnia does not appear to have been reported but might be expected to act in the opposite direction.

1.5.8 Ocular electrophysiology

Corneo-retinal potential

The corneo-retinal potential (CRP) is the steady voltage recorded from the cornea to a reference electrode. Early studies conflict over the effects of respiratory disturbance on the CRP (Fenn, Galambos, Otis *et al*, 1949; Drummond and Rebuck, 1981). Marmor, Donovan and Gaba (1985) reported that the CRP rose slowly as S_aO_2 fell to 80% before falling when normoxia was restored. Steinberg (1987) demonstrated clearly that mild hypoxia elevates the CRP at P_aO_2 of 70-80 mm Hg, identifying that the effect originates at the level of the RPE rather than the neural retina and may relate to changes in $[K^+]$ in the SRS surrounding the photoreceptors as a result of altered ion flux. At least in the cat, the SRS appears to shrink in response to systemic hypoxia and to swell with hyperoxia, suggesting considerable fluid movement that is likely to accompany substantial ion shifts (Cao, Govardovskii, Li *et al*, 1996).

Electroretinogram

The ERG is a more sensitive indicator of the effects of hypoxia on retinal function, the 'a' wave relating to photoreceptor activity and the 'b' wave to bipolar cell activity. The 'c wave' is less specific, more variable and affected by RPE, photoreceptor and Müller cell activity. Severe acute hypoxia, breathing 9% O_2, progressively reduced b-wave amplitudes over a 7-minute period and recovered similarly when normoxia was restored (Brown, Hill and Burke, 1957). There was only a marginal effect on the a-wave. The effect on the b-wave of 15% O_2 was also slight.

Subsequent studies have confirmed that the a-wave is more resistant than the b-wave to hypoxia at a P_aO_2 of 20-30 mm Hg or breathing 12% O_2 (Niemeyer, Nagahara and Demant, 1982; Kang Derwent and Linsenmeier, 2000; Tinjust, Kergoat and Lovasik, 2002). These findings suggest that the outer retina is more resistant to hypoxia than the inner retina (in light) once autoregulation fails. A recent study of the pattern ERG under hypoxia induced by breathing 12% O_2 was more specific in identifying that retinal GC are particularly sensitive to transient, mild systemic hypoxia (Kergoat, Hérard, and Lemay, 2006). Another recent study of multifocal ERG responses while breathing 14% O_2 is particularly relevant to the experiments that follow, many of which employed a very similar gas mixture. The results suggest a post-receptoral bipolar cell deficit in the central retina of healthy subjects during acute mild hypoxia (Feigl, Stewart and Brown, 2007). A further multifocal ERG study has demonstrated retinotopic variation in the

magnitude of the effect of moderate systemic hypoxia (10% O_2), with significant inner retinal effects apparent within the central 2° of visual field, becoming less marked with eccentricity (Klemp, Lund-Andersen and Larsen, 2007).

Cat b-wave and GC sensitivity appear largely unaffected by systemic hypoxia until P_aO_2 falls below ~40 mm Hg, due to effective inner retinal autoregulation (Linsenmeier, 1990). Retinal artery occlusion obliterates the cat b-wave and reduces photoreceptor QO_2 by 25% (Braun and Linsenmeier, 1995). Subsequent 100% O_2 restores the b-wave amplitude to only 50% of normal. Traditionally the b-wave has been regarded as a sensitive indicator of hypoxia, but Linsenmeier (1990) noted that this sensitivity is to local tissue hypoxia rather than systemic hypoxia. Outer retinal responses are altered only when P_aO_2 falls below ~60-80 mm Hg, due to effects of hypoxia on photoreceptor metabolism, while retinal ischaemia of any origin is likely rapidly to compromise inner retinal function and thereby affect vision. This is supported by the finding that b-wave amplitude remains stable during increases in MAP up to 225 mm Hg but decreases rapidly if MAP falls below 55 mm Hg, suggesting that pressure autoregulation of the inner retinal circulation fails below that value (Demant, Nagahara and Niemeyer, 1982).

Hypocapnia from voluntary hyperventilation has long been known to affect the ERG a- and b-waves, although little more could be deduced from the earliest studies (Alpern, Faris, Eskildsen *et al*, 1954). The acidosis from hypercapnia has also been shown to affect the outer retinal ERG responses as pH falls below about 7.3 while inner retinal responses began to be affected at pH 7.2 (Niemeyer, Nagahara and Demant, 1982; Linsenmeier, Mines and Steinberg, 1983).

Notwithstanding the foregoing, it must be remembered that ERG responses persist even under profound ischaemia with loss of vision and absent electroencephalography (EEG) activity (Noell, 1951; Lewis and Duane, 1956; Horsten and Winkelman, 1957), so electrophysiological responses are not easily matched to perceptual correlates.

Ganglion cell activity

Recording single GC action potentials, tolerance of hypoxia appeared independent of adaptation state (McRipley, Ahmed, Chen *et al*, 1997). Under normoglycaemic conditions, hypoxia did not compromise photoreceptor sensitivity or signalling until P_aO_2 fell to 30 mm Hg when inner retinal function was also affected. Hypoglycaemia increased sensitivity to hypoxia with b-wave and scotopic thresholds now affected at a P_aO_2 of 50 mm Hg. Restoration of normoglycaemia re-established resistance to hypoxia.

Cat retinal GC mean firing rate (MFR) in response to contrast stimuli has been assessed under graduated hypoxia (Enroth-Cugell, Goldstick and Linsenmeier, 1980). GC function and inner retinal PO_2 remained at normal levels as long as P_aO_2 remained above ~35 mm Hg. More severe arterial hypoxia resulted in a sharp reduction of inner retinal PO_2 and GC sensitivity. Similar results were found when examining discharges from individual GC using a variety of hypoxic breathing gas mixtures, with forced ventilation to maintain arterial normocapnia and normal acid-base balance (Alder and Constable, 1981). GC MFR was unaffected while P_aO_2 remained over 45 mm Hg. Below this, 67% of cells demonstrated a reversible increase in MFR followed by a fall when P_aO_2 dropped below 35 mm Hg, while the remainder of the cells demonstrated only a fall. Below 24 mm Hg there was an initial increase in firing in some cells followed by a complete cessation of activity. Retinal function was unaffected when breathing greater than 10% O_2.

Central visual pathways

Electrical activity can be measured elsewhere in the visual pathway, including the lateral geniculate, optic radiation, visual cortex and associated areas of the brain. Noell and Chinn (1950) examined tolerance to complete anoxia (breathing 100% N_2) on the visual pathway of rabbits and identified that the visual cortex and geniculate were most susceptible, followed by retinal GC, then bipolar cells and finally, photoreceptors. The effect of severe hypoxia on the visual pathway of cats followed the same pattern, with the cortex more susceptible than the lateral geniculate (Kayama, 1974). However, the results are more equivocal in studies investigating effects of milder hypoxia. Consideration of the effects of respiratory disturbance on cortical activity and visual evoked responses, as measured by electrophysiological or more contemporary means, such as functional magnetic resonance imaging or magnetoencephalography, are beyond the scope of this work. However, it must be considered that effects on visual performance may result from central rather than ocular mechanisms.

It is stressed that altered electrophysiological responses may not correlate with effects on visual performance. Visual deficits clearly occur under conditions of respiratory disturbance that do not affect electrophysiological parameters. Similarly, electrical responses to light stimulation can still be observed when an animal is unconscious, or even, apparently, dead. Furthermore, electrophysiological effects in one part of the visual pathway do not necessarily correlate with responses elsewhere. For example, altered ERGs do not imply absent optic tract responses and effects on cortical potentials

do not mean that vision is necessarily disturbed. Also, variations in electrophysiological responses between species and individuals are considerable.

1.6 Respiratory Effects on Visual Performance

1.6.1 Light sensitivity and dark adaptation

Traditionally, the effect of hypoxia on threshold sensitivity to light during adaptation to dark has been assessed by measuring sensitivity to a stimulus of variable intensity at given periodic intervals following offset of a bleaching light, rather than by measuring the time taken to achieve a sensitivity to given stimulus intensities. In this way, using hypoxic breathing gas mixtures, McFarland and Evans (1939) demonstrated an unequivocal effect of hypoxia to elevate threshold sensitivity to light during dark adaptation, as well as the absolute sensitivity that could be achieved (Figure 1.23).

The elevation of threshold sensitivity was exaggerated with more severe hypoxia and was apparent even when breathing 15.7% O_2, equivalent to breathing air at an altitude of only 7,400 ft. Figure 1.23 shows the mean adaptation curves of 20 subjects plotted against a $\log_{10}$ ordinate scale measured in 'dial units', adjusted by the subject to indicate the barely detectable stimulus intensity. Measurements were made every 30 s for the first 5 minutes of dark adaptation and every 60 s thereafter. However, measurements were not instantaneous and the timing accuracy for each data point will have varied within and between subjects, introducing a horizontal (temporal) measurement error that will tend to disguise the inflection point. Furthermore, in taking mean curves from so many subjects, the natural variation in sensitivity between subjects at each measurement time introduces a vertical (sensitivity) measurement error. Unsurprisingly, therefore, the inflection points between the cone and rod portions of the mixed dark adaptation curves are camouflaged and barely apparent (Figure 1.23). Indeed, the cone rod breaks would surely pass unnoticed to an uninformed reviewer asked to produce regression curves for any of the data series. Instead, the timing of the inflection points has been inferred by a 'best guess', and the delay time to cone rod inflection then interpreted as unaffected by hypoxia.

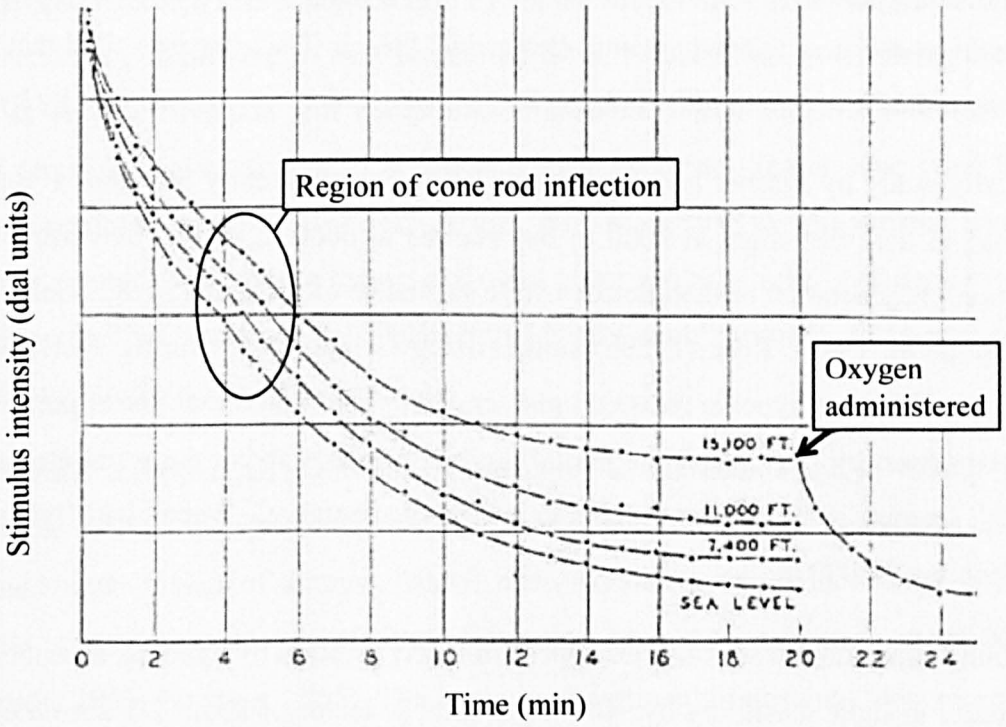


Figure 1.23 Dark adaptation curves of McFarland and Evans (1939)

Figure 1.23 also demonstrates that hypoxic impairment of late rod sensitivity was rapidly corrected with 100% O_2, faster than could be explained through regeneration of visual pigment. However sensitivity worsened again if hypoxia was reinstated. This cannot be due to delayed photochemical regeneration in a retina that is already dark adapted. Together with the apparent lack of any effect on inflection time, this led to the interpretation that 'dark adaptation curves were elevated progressively with increased altitude or diminished partial pressure of O_2' (McFarland and Evans, 1939). It was concluded that hypoxia does not affect the rate of regeneration of photopigment, that its effect was therefore not photochemical in nature, and that a neural mechanism was probably responsible. Work has since shown that hypoxia delays regeneration of rhodopsin *in vitro* (Ostroy, Gaitatzes and Friedmann, 1993).

Numerous other studies have confirmed the effect of worsening hypoxic hypoxia to impair threshold light sensitivity progressively, that is, to elevate the threshold for stimulus detection for a given time in the dark, supporting the contention that the determining variable is the P_AO_2 (McFarland and Edwards, 1937; McDonald and Adler, 1939; McFarland and Forbes; 1940; Wald, Harper, Goodman and Krieger, 1942; Gellhorn and Hailman, 1943; McFarland, Halperin and Niven, 1944; Hecht, Hendley,

Frank and Haig, 1946; Sheard, 1946; Ernest and Krill, 1971; Kobrick and Appleton, 1971). These studies continued the tradition of measuring threshold sensitivity at timed intervals in darkness following controlled retinal bleach. The view prevailed that timing of cone rod inflection and rate of photochemical adaptation were unaffected by hypoxia.

An early study by Fischer and Jongbloed (1935) had apparently recorded a 'delay' in adaptation that was slight at 3000 m but marked at 6000 m, which they attributed to impaired 'regeneration of the photosensitive substance of the retina' (McFarland, Evans and Halperin, 1941). Rose (1950b) summarising German work during World War II, also identified that hypoxia reduces light sensitivity and noted that visual performance was improved by 'increasing the partial oxygen pressure above the usual atmospheric levels'. German aircrew were ordered to use supplementary O_2 when night flying above an altitude of 2,000 m.

Absolute cone and rod sensitivities appear similarly affected by hypoxia, amounting to a reduction in light sensitivity by ~0.4 $\log_{10}$ units (a factor of about 2.5 absolute) when breathing 10.4% O_2, equivalent to breathing air at ~0.5 atm or ~18,000 ft. Extended hypoxia (hours) had no greater effect than shorter exposures (30 min). Sheard (1946) demonstrated that hypobaric hypoxia reduces sensitivity more with eccentricity from the fovea, compromising peripheral rod thresholds more than central cone thresholds. The maximum normalising rod threshold improvement (10° from fixation) was fourfold when breathing O_2 rather than air at 15,000 ft, while the maximum normalising cone threshold improvement was twofold. Ernest and Krill (1971) used careful hypoxic challenges (10% O_2 without hyperventilation) to demonstrate a significantly greater effect on peripheral rod thresholds at 45° eccentricity than those at 5°. Hypoxia elevated both cone and rod thresholds at 5°, with sensitivity to red stimuli compromised more than to blue, suggesting a persistent rod contribution to short wavelength sensitivity in the manner of the Purkinje effect.

Thus rod and cone thresholds are similarly vulnerable to hypoxia in the dark. Both are compromised progressively with eccentricity, as supported when examining the effect of ischaemic hypoxia on absolute visual sensitivity in fully dark-adapted subjects exposed to +Gz (cranio-caudal) sustained acceleration on a human centrifuge (White, 1960). Foveal thresholds were elevated twofold and threefold respectively under +3Gz and +4Gz, while thresholds at 7° rose threefold and fourfold. Hence ischaemic hypoxia also compromises light sensitivity and a cumulative effect with hypoxic hypoxia should be anticipated.

Repeated dark adaptations over 48 h at 15,000 ft in an altitude chamber have demonstrated maximal loss of light sensitivity in the first hour of exposure (Kobrick and Appleton, 1971), improving to near normal by 24 h. Nonetheless, even during 3-week dwells at altitude, hypoxia still compromises absolute visual sensitivity despite any general physiological acclimatization that may have occurred, further supporting P_AO_2 as the determining variable (Kobrick, Zwick, Witt *et al*, (1984). The current work is concerned primarily with acute hypoxia of rapid onset and relatively brief duration ($\leq$ 1 h). While adaptive responses will begin immediately upon exposure, there will be no time for meaningful acclimatization.

It is generally accepted that night vision is impaired at altitudes as low as 4000 to 5000 ft (Tredici, 1985), while various reviews quote unpublished work by Goldie, on the unassisted night vision of aircrew, which demonstrated a 5% reduction in 'pick-up range' at altitudes as low as 4000 ft, increasing to a 40% reduction at 16,000 ft (Whiteside, 1957; Ernsting, 1965). The hypoxic impairment of absolute light sensitivity is shown for various studies as $\log_{10}$ threshold elevation against equivalent altitude breathing air in Figure 1.24, indicating that the hypoxia probably begins to compromise sensitivity at ~5000 ft, equating to a P_AO_2 of ~80 mm Hg.

Figure 1.23 shows that hyperoxia restores absolute visual sensitivity under hypoxic conditions (McFarland and Evans, 1939). Mean absolute light sensitivity of 100 subjects at 5,000 ft improves by over 25% upon breathing 100% O_2 (Pretorius, 1970). Anecdotally, some subjects have achieved greater visual sensitivity with 100% O_2 than before they became hypoxic. Generally, however, studies intended to demonstrate a benefit of hyperoxia over normoxia have failed to do so (Kent, 1966; Connors, 1968).

In contrast to the effect of acute hypoxia, hyperventilation by dark-adapted subjects decreased the visual threshold by a mean of 0.27 log units, doubling light sensitivity (Wald, Harper, Goodman *et al*, 1942). This effect was lost when adding 2% CO_2 to the inspired gas, demonstrating that increased light sensitivity during hyperventilation results from the fall in P_ACO_2 rather than increased oxygenation. Hypocapnia counters the tendency for the visual threshold to rise with hypoxia. Hypercapnia, on the other hand, adding 5% CO_2 to the breathing gas, doubled the visual threshold regardless of ventilation rate. The failure of artificially-induced metabolic acidosis and metabolic alkalosis to affect visual sensitivity contrasts with the effects of hypocapnia and hypercapnia, showing that it is not the change in blood pH *per se* but the change in

P_ACO_2 which is the significant factor, with its concomitant effect on tissue acid-base balance (Alpern and Hendley, 1952).

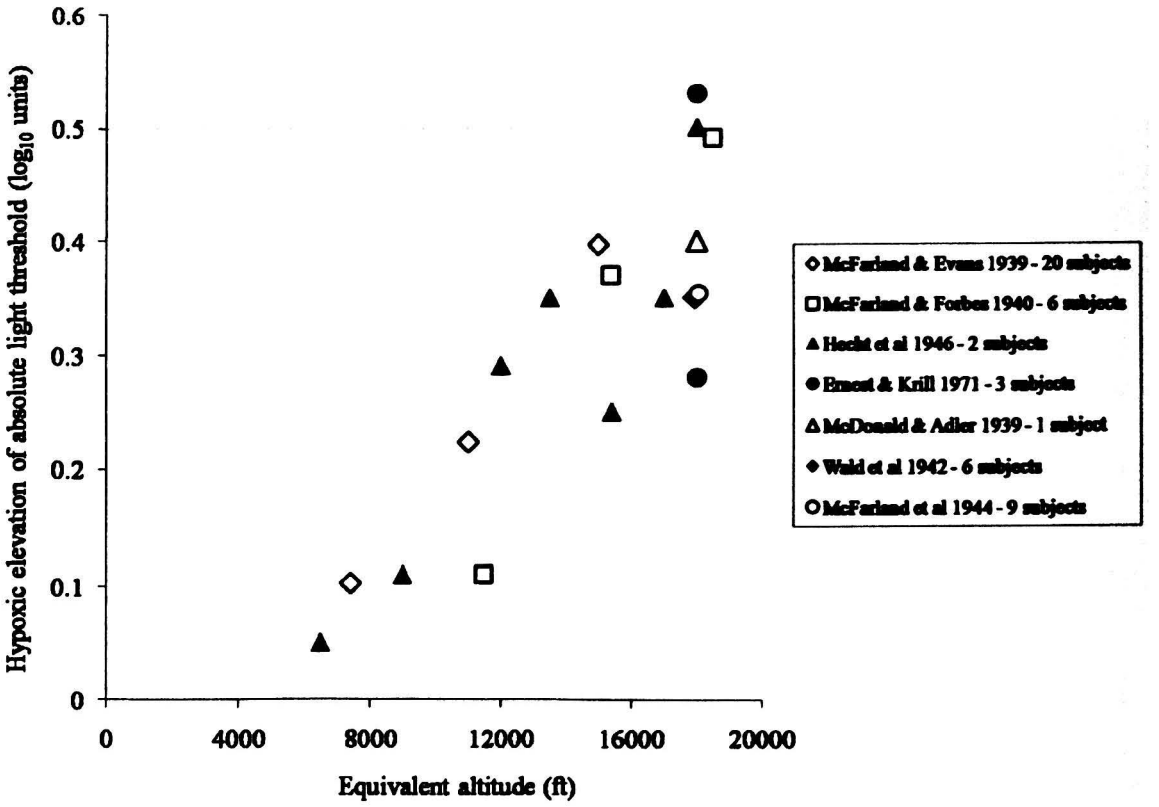


Figure 1.24 Effect of hypobaric hypoxia to impair absolute visual sensitivity

Threshold light sensitivity is compromised by mild hypoxia once P_AO_2 falls below ~80 mm Hg, when the mixed P_aO_2 is likely to be ~70-75 mm Hg and S_aO_2 should still be ~95% (Figure 1.10). Since this effect cannot be photochemical it has usually been attributed to inner retinal hypoxia. Given the capacity of the inner retinal circulation for metabolic autoregulation, it is unlikely that such mild hypoxia would significantly affect inner retinal oxygenation. Given the rod demand for O_2 to maintain the dark current and the reliance of the outer retina upon choroidal P_aO_2 to 'drive' O_2 across to the inner segments, a reduction in the O_2 'pressure head' by 25-30% may well be enough to produce hypoxic impairment of receptoral function that need not be photochemical. Thus, the outer retina appears most vulnerable to hypoxia under dim light conditions.

1.6.2 Contrast sensitivity

Background

Contrast is the difference in brightness between an object and its background. The human visual response detects contrast over a wide range of background light intensity (I), amounting to some 10 $\log_{10}$ units, although only ~2 $\log_{10}$ units contribute usefully to at any given moment. The brightness of a visual target may need to be increased relative to its background to enable detection, giving a threshold differential brightness (ΔI) that may expressed relative to background luminance as the Weber fraction ($\Delta I/I$). As background illumination increases through mesopic and photopic levels, this threshold increases throughout the retina, but to a greater extent with distance from the fovea. Thus, from having a physiological foveal scotoma at scotopic light levels (due to the absence of rods at the fovea), foveal sensitivity increasingly predominates at light levels rising from around 10^{-2} cd m^{-2}. Beyond the fovea, rods and cones contribute almost equally to visual sensitivity at a light intensity of ~10 cd m^{-2}.

Tests of contrast sensitivity commonly use sharply demarcated ('square wave') or sinusoidally graduated contrast gratings of various *sf* of alternate light and dark bars. Sinusoidal gratings are considered to correspond well to mechanisms of visual information processing in the brain. The *sf* of a grating is expressed in cycles per degree (cpd) of visual angle subtended at the retina. The contrast, C, of the grating is then given by considering the highest (L_{max}) and lowest (L_{min}) luminance of the alternating pattern as shown in Equation 10.

$$C = (L_{max} - L_{min}) / (L_{max} + L_{min})$$

Equation 10 Formula for the contrast of a grating

For a grating that is just perceived, the reciprocal of this value may be plotted as the subject's threshold contrast sensitivity for a given *sf*. Young, healthy subjects should achieve contrast thresholds of < 1% (or 1/100) under good viewing conditions, equating to a contrast sensitivity of over 100. Under ideal conditions, subjects may occasionally achieve contrast sensitivities of over 1000. The resulting threshold curve across all *sf* is known as the contrast sensitivity function (CSF). The normal human CSF can be assessed at *sf* ranging from ~0.5 to 20 cpd and are usually represented graphically along the abscissa using a $\log_{10}$ scale ranging from 0.1 to 100. The contrast sensitivity at each *sf* is shown against a $\log_{10}$ ordinate axis, typically ranging from 1 to 1000. The normal

photopic CSF has an inverted 'U' profile. Visual processing enhances contrast sensitivity at *sf* ranging from 1 to 6 cpd, where there is also least within- and between-subject variability, and peak sensitivity is typically between 3 to 4 cpd.

Respiratory disturbance

Early studies on 'visual intensity discrimination' demonstrated reversible impairment of contrast sensitivity under marked hypoxia (8-10% O_2) that was offset by adding 3% CO_2 to the breathing gas (Gellhorn, 1936a; 1936b). Various studies identified loss of contrast discrimination that was more obvious with dimmer background fields and more severe hypoxia. Relative to normoxia, moderate hypoxia (10.8% O_2) compromised differential brightness sensitivity (increased ΔI) at the fovea (McFarland, Halperin and Niven, 1944). The impairment was greater with dimmer background fields and became progressively less pronounced as field intensity increased. Plotting the Weber fraction ($\Delta I/I$) against field intensity (I), hypoxia was shown to displace the resulting 'intensity discrimination curve' by a constant amount, with a mean of 0.356 $\log_{10}$ units. The mean shift in the foveal dark adaptation curves of the same 9 subjects under the same hypoxic challenge was very similar at 0.354 $\log_{10}$ units. The second part of the study considered different hypoxic gas mixtures at a steady low light level. ΔI increased with worsening hypoxia from 7500 ft but was normalised with 100% O_2 at 22,000 ft.

Hecht, Hendley, Frank *et al* (1946) also demonstrated clearly an incremental effect on the Weber fraction of progressively more severe hypoxia, with the effects again most evident at low background field intensities. The effect of hypoxia was 'definite' at 8,000 ft. Using 11.1% O_2 produced a threshold shift of 0.36 $\log_{10}$ units, corroborating the results of McFarland, Halperin and Niven (1944). Neither of these studies considered hypocapnia as a possible confounding factor alongside moderate to severe hypoxia. The relative effects on contrast sensitivity of hypoxia and hypocapnia, and the interaction between them, were studied by Otis, Rahn, Epstein *et al* (1946). Performance on a contrast discrimination test was assessed in relation to alveolar gas composition demonstrating hypoxic impairment at 12,000 ft with rapid deterioration above 18,000 ft (see Figure 1.25 arrow [1]).

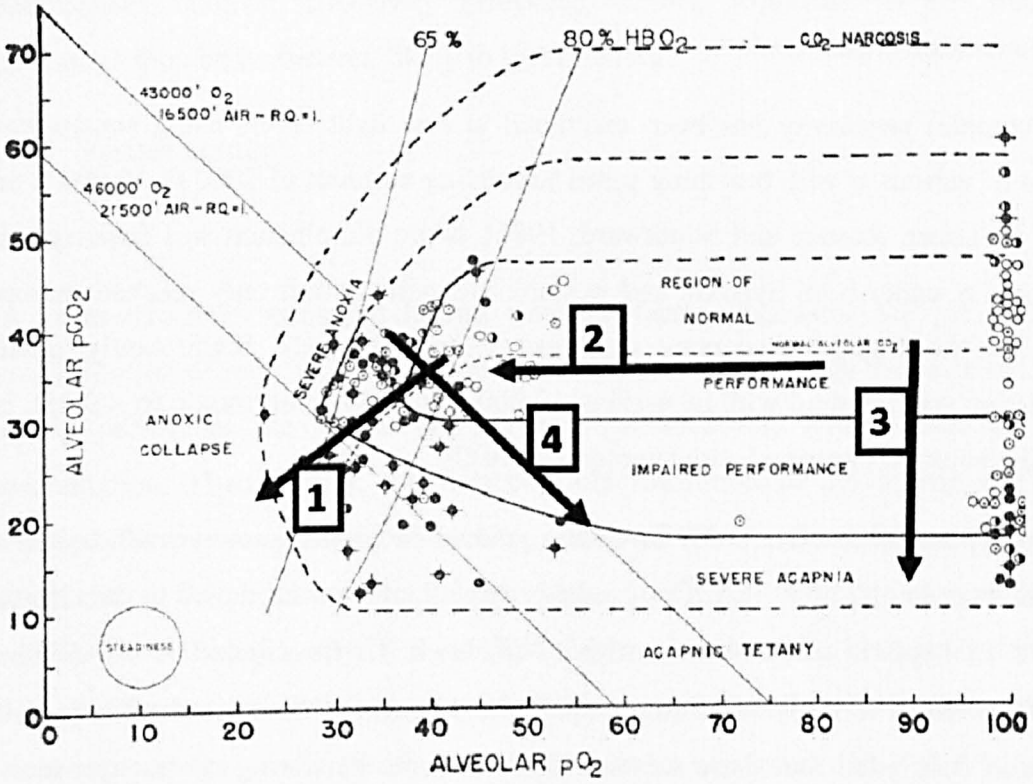


Figure 1.25 Contrast discrimination related to P_AO_2 and P_ACO_2 (Otis, Rahn, Epstein *et al*, 1946)

For a given P_ACO_2, contrast discrimination should worsen with progressive hypoxia, especially when P_AO_2 fell below 40 mm Hg (arrow [2]). Equally, for a given P_AO_2, progressive hypocapnia should compromise contrast discrimination (progression parallel to arrow [3]). However, representation of altitude on this diagram requires a P_AO_2 to benefit from any reduction in P_ACO_2 through secondary hyperventilation (Figure 1.12). Thus, for a given P_IO_2, the resulting P_AO_2 will be greater in subjects with a more pronounced hypoxic ventilatory response. From a point representing the normocapnic P_AO_2 at a given altitude, progression along the relevant diagonal parallel to arrow [4] will be associated with progressively more severe hypocapnia, thereby enhancing P_AO_2 and S_aO_2. As arrow [4] demonstrates, this might initially improve contrast discrimination before compromising performance once P_ACO_2 falls below about 25-30 mm Hg, a level associated with relatively mild hyperventilation that is likely to be asymptomatic. This has the potential to confound interpretation of responses between individuals. Thus, when studying the effect of hypoxia on contrast sensitivity it is essential not to ignore the possible confounding effect of altered P_ACO_2.

Halperin, McFarland, Niven *et al* (1959) demonstrated an impairment of differential brightness sensitivity to cone-dominant foveal stimuli (1° diameter, 0.1 s duration), presented against a dim (moonlight) background field, when breathing 15.85% O_2,

equivalent to breathing air an altitude of 7000 ft. Bolus doses of carbon monoxide compounded the impairment.

Rod (scotopic) sensitivity has been examined at low light levels using square-wave gratings of various *sf* with breathing gases simulating altitudes of 7000 ft, 10,000 ft and 13,000 ft (Leber, Roscoe and Southward, 1986). More illumination was required with increasing *sf* under both hypoxic and normoxic conditions but only reached mesopic levels for the highest frequency gratings (7 and 14 cpd). Significantly greater illumination was required with hypoxia at 13,000 ft than when normoxic ($p < 0.05$), but unaided resolution improved with supplementary O_2.

An epic hypobaric chamber study involving gradual decompression over 40 days to an eventual altitude of 25,000 ft, with subjects breathing ambient air, failed to demonstrate an effect of hypoxia on contrast sensitivity (Kobrick, Crohn, Shukitt *et al*, 1988). A subsequent chamber study of photopic spatial and temporal contrast sensitivity at the fovea also concluded that there was no loss in sensitivity during acute exposures at either 7,000 ft or 12,000 ft, compared with GL (Yap, Garner, Legg *et al*, 1995). Thus, susceptibility of contrast sensitivity to hypoxia appears closely related to light level.

One study has suggested that acute hypobaric hypoxia improves contrast sensitivity (Benedek, Kéri, Grósz *et al*, 2002). Hypobaric hypoxia was imposed at an equivalent altitude of ~18,000 ft in a decompression chamber. Computer-generated, horizontal, sinusoidal contrast gratings, of various spatial and temporal frequencies, were used to establish contrast thresholds for stimulus detection. Measurements were made under normoxia and after 5, 10 and 15 minutes of hypoxia but respiratory gas tensions were not recorded. Significant negative correlations were demonstrated between S_aO_2 and contrast sensitivity to low and medium *sf*. However, contrast sensitivity fluctuated markedly during the 15 minute test period following arrival at altitude and testing was conducted before a respiratory steady state could be established, so it is unlikely that S_aO_2 was stable throughout the test period. The conclusions of this study are counterintuitive and at odds with the others reviewed, indicating the difficulty with assessing hypoxic challenges using S_aO_2 rather than gas tensions. Finally, the relative contributions of hypoxia and hypocapnia are unknown. Hypocapnia is likely to have been present during these exposures and may have confounded the effect of hypoxia at 18,000 ft (Figure 1.25). While the effects of hypocapnia have not been studied using contemporary tests of contrast sensitivity, there is little doubt that acute hypercapnia

compromises contrast sensitivity (Hosking, Evans, Embleton *et al*, 2001), so hypocapnia may be considered likely to be of benefit.

1.6.3 Visual acuity

Background

VA varies across the retina and depends on many factors. Fundamentally, it is related to the distribution of rods and cones. Cones provide greater acuity by virtue of their being densely packed at the fovea, and having dedicated GC and substantial cortical representation. Theoretically, the best possible resolution of the human eye is ~30 seconds of arc, corresponding to Snellen acuity of 6/3. Rods are larger, less densely packed and, in the periphery, may be represented by GC at a ratio of 100:1, producing a best possible rod-only acuity of about 6/60. Light is scattered by the tissue of the inner retina, and the best possible acuity is only achieved at the shallow fovea where the inner retinal components are displaced. At 1° from fixation maximum acuity is 6/9, at 5° it is ~6/20-30 and at 10° it is ~6/30-48 (Tipton, 1984).

Excluding disease and injury, many factors impact upon VA, including the optics of the eye (refractive error, transmission properties, and pupil diameter), environmental factors (such as vibration and hypoxia) and the physical characteristics of the visual stimulus. The latter include stimulus size, context (haze, glare and so forth), luminance, wavelength, contrast, duration, distance, relative motion and so on. With regard to acuity for chromatic stimuli, particularly those presented on a display, luminance contrast appears more important than hue or saturation (Santucci, Menu and Valot, 1982). However, the background colour and resulting colour contrast remain important determinants of acuity. All else being equal, more saturated chromatic stimuli are discerned more easily.

Respiratory disturbance

Early studies suggested that marked hypoxia (8-10% O_2) compromises VA but suffered from marked variability between subjects, failure to consider levels of illumination and insufficiently sensitive test methods. Wilmer and Berens (1918) found the use of Snellen type unsatisfactory and subsequent use of a Snellen chart demonstrated little effect of hypoxia on central acuity even though 'the subjects were severely affected and at times almost stuperous' (Evans and McFarland, 1938).

McFarland and Halperin (1940) studied the effect of hypoxia on foveal VA using a black Landolt C against various red background fields. Under this challenge, examining the ability to resolve a luminous gap between dark contours, VA is poor under dim illumination, improving rapidly at first and then more gradually with increasing light, until maximum acuity is achieved. VA was compromised progressively with reducing light level and with increasingly severe hypoxia from 14.31% O_2 (~10,000 ft) to 10.34% O_2 (18,000 ft). Curves of $\log_{10}$ VA against light level indicate that progressive hypoxia necessitates increasing illumination to maintain VA. Given adequate illumination, hypoxia did not compromise the maximum achievable acuity, while VA rapidly normalised with 100% O_2. The results with 14.31% O_2 clearly suggest an effect of mild hypoxia on VA under dim viewing conditions.

The ability to resolve a dark 'gap' between brighter borders initially improves with increasing illumination, reaches peak acuity, and then declines, such that greater separation of the bright borders is then required for discrimination. Hypoxia (10% O_2) generally impaired discrimination of two points presented at light intensities beyond peak acuity, in effect aggravating the deterioration of VA that accompanies increasing brightness and requiring increased separation for discrimination (Berger, McFarland, Halperin *et al*, 1943). In a sense, this effect of hypoxia is equivalent to making the luminous test borders brighter and cannot be explained by a dimming of field intensity.

Rose (1949) described a reduction in 'twilight' VA at ~10,000 ft in an altitude chamber, measuring the delay in recovery of acuity over 2 minutes following a period of bright light adaptation, but could not reproduce the effect during terrestrial altitude exposure. Other studies have failed to demonstrate effects on photopic VA after 24 h at 12,800 ft (Kobrick, 1968) or during spells lasting 48 h at 15,000 ft (Kobrick and Appleton, 1971). Additionally, a study of VA at GL demonstrated no benefit of supplementary O_2 (Miller, 1958).

The compound effects of low background luminance and poor contrast to compromise VA have been clearly demonstrated using Landolt C targets (Johnson and Casson, 1995). The Snellen VA of an individual achieving 6/6 under optimal conditions will fall to 6/18 under low luminance conditions, and to 6/30 under low luminance with low contrast. The relevance to mesopic vision was clearly stated. It is precisely under such mesopic (rather than photopic) conditions that effects of hypoxia might be expected to be more readily apparent and to have greater functional significance. The effects of hypoxia on contrast acuity at reducing luminance are unknown.

1.6.4 Colour perception

Early studies by Velhagen in 1935 and Schmidt in 1937 are cited as demonstrating altered colour sensitivity and degradation of pre-existing colour deficiency with hypoxia at altitudes as low as 3000 m (Gellhorn and Hailman, 1943; Schmidt, 1950). Many early studies produced conflicting results, often focusing on which colour might be most affected (McDonald and Adler, 1939; McFarland, Evans and Halperin, 1941). They had widely varying methodologies, lacked a standard reference base, and assessed different capabilities under a variety of conditions. Even normoxic assessments within subjects were noted to vary considerably from day to day. Physiological responses to hypoxia were not reported and the possible impact of hyperventilation at higher altitudes was ignored. Accordingly, interpretation of the relevance of these and other subsequent studies is difficult. The more obviously relevant studies are outlined here.

Threshold sensitivity to chromatic stimuli

Hypoxic breathing gas mixtures, equivalent to altitudes from 13,000 to 17,000 ft, reduce the sensitivity to colour of the peripheral visual field in the mesopic range, examined using well-saturated, 0.5° diameter red, green and blue targets (Kobrick, 1968). The effect was exaggerated by more severe hypoxia, dimmer viewing conditions and more prolonged exposure to hypoxia. The effect also appeared more pronounced for red stimuli than for green or blue, and may be attributable, at least in part, to the Purkinje phenomenon.

Ernest and Krill (1971) demonstrated a greater elevation of dark-adapted red thresholds than blue when breathing 10% O_2 using 5° diameter targets at an eccentricity of 5°. A steady S_aO_2 of ~75% was maintained. It was assumed that the blue stimulus would test rod sensitivity while the red would assess cones. All subjects were noted to perceive the red colour at threshold sensitivity but no comment is made about how the blue stimulus was perceived. It was interpreted that cones are more sensitive to hypoxia than rods at this eccentricity.

A more recent study has examined photopic sensitivity thresholds of the central visual field of 48 subjects to white, red, blue and green stimuli presented using a Humphrey field analyser at GL (S_aO_2 97% SD ± 1%) and at ~10,000 ft (S_aO_2 83% ± 3%) in a decompression chamber (Brandl and Lachenmayr, 1994). Hypoxia resulted in "significant ($p < 0.01$) differences in light sensitivity" (English translation from the German original). Threshold sensitivity changes on a logarithmic scale (ΔdB) fell

linearly with S_aO_2 with remarkable correlations for all four stimuli, although the maximum loss was only 1.5dB. There are many difficulties with both the respiratory and visual aspects of this study. The change in threshold sensitivity is presented against a spectrum of S_aO_2 scattered across the range from 97% down to 80%. However, this is surprising given that a single altitude challenge was imposed that should have resulted in reasonably reproducible mean S_aO_2 of ~90% (range ~88-93%), suggesting that the respiratory exposure was poorly controlled and insufficient time was allowed to establish a steady state before vision testing. It is unclear exactly how vision testing was conducted, what calibrations were made, the background field luminance, and how stimulus intensity was controlled. Thus, it is unclear exactly what has been measured and compromised by hypoxia (luminance contrast thresholds perhaps), but it is unlikely to reflect chromatic sensitivity. Saturated and desaturated Panel D15 tests and tests using the Heidelberg anomaloscope were unaffected by hypoxia.

Connors (1968) specifically examined the effect of breathing normobaric 100% O_2 rather than air on foveal thresholds to red (642 nm), green (521 nm), yellow (584 nm) and blue (468 nm) stimuli. Sensitivity to red was consistently greater than for other hues when breathing both air and 100% O_2. Hyperoxia did not affect sensitivity, either for luminance or chromatic thresholds.

Farnsworth-Munsell 100-Hue Test

Various investigators have investigated the effects of hypoxia on colour vision using the Farnsworth-Munsell 100-Hue Test (FM100). Smith, Ernest and Pokorny (1976) imposed moderately severe hypoxia (10% O_2) under two luminance conditions, 37 lux and 1670 lux. Performance was impaired along the tritan (yellow-blue, Y-B) axis, as distinct from the red-green (R-G) axis, at the lower light level. This level of illumination was greater than that required to induce Y-B errors in normoxic subjects (0.2 lux) using the FM100 and suggests sensitivity of the tritan axis to hypoxia, in effect exposing the normal mesopic tritanopia at a higher than usual light level. A minor increase in error rate along the R-G axis was attributed to a non-specific performance decrement. This moderate degree of hypoxia (> 18,000 ft) is such that all subjects reported symptoms when hypoxic. Those undertaking the test condition before the control condition reported that colours seemed brighter in the latter. The authors concluded that the effect was due to inner retinal hypoxia rather than a receptoral mechanism.

During prolonged experimental saturation dives in a hyperbaric chamber, hyperoxia (breathing air at greater than atmospheric pressure) did not affect FM100 performance (Paulson and Ryan, 1981). The authors endorsed the effect of hypoxia on the Y-B axis at low luminance but did not specify the respiratory condition under which vision tests were performed and did not describe their results in any detail.

Vingrys and Garner (1987) examined FM100 performance (standard daylight) at an equivalent altitude of 12,000 ft in a decompression chamber, finding a statistically significant (75%) increase in the number of errors made at altitude in comparison to sea level. However, there was no bias in favour of either the Y-B or R-G axis, suggesting a general loss of colour sensitivity.

A recent study investigated photopic FM100 performance during terrestrial altitude exposure (3000 m) and reported impairment of Y-B colour discrimination. However, the study appears to have been relatively poorly controlled with respect to both visual and respiratory conditions. For example, base altitude tests were conducted in a clinic while tests at 3000 m were conducted at noon, in the open, following a 3-h climb. The method of data analysis also appears somewhat contrived, in that a 125% increase in the number of errors in Sector 4 (from 16 to 36) was not significant and was discounted, while a 75% increase in Sector 1 (31 to 54) and a 50% increase in Sector 3 (40 to 61) were accepted as meaningful (Karakucuk, Oner, Goktas *et al*, 2004).

Anomaloscope and other tests

Vingrys and Garner (1987) also examined Pickford-Nicolson anomaloscope matches at 12,000 ft and while mid-match points were unaffected at altitude, matching ranges were increased for R-G, Y-B and blue-green matches, suggesting a general loss of colour discrimination calculated at a mean of 0.26 $\log_{10}$ units. Although not statistically significant, more R-G errors were made during FM100 and more Y-B errors during anomaloscope testing, with the latter presenting the test stimuli at a relatively low luminance level (6-9 cd m^{-2}).

In two frequently-quoted studies, Richalet *et al* examined effects of prolonged stays at altitude (> 4000 m) on colour vision, particularly the Rayleigh match (Richalet, Duval-Arnould, Darnaud *et al* 1988; Richalet, Rutgers, Bouchet *et al*, 1989). In the first, a consistent increase in the ratio of green to red was required at altitude to match the constant yellow. The second demonstrated a clear diurnal variation in colour vision at altitude which appeared to parallel changes in plasma cortisol levels. Again, decreased

sensitivity to green was noted that was most apparent in the morning, reduced in magnitude during the day and became more pronounced in the evening. Unfortunately, no data are available from the first day at altitude.

A qualitatively similar increase in sensitivity to red (reduced sensitivity to green), assessed using heterochromatic flicker photometry, has been documented when breathing normobaric 10% O_2 (Schellart, Pollen and Van der Kley, 1997). On the other hand, 100% O_2 reduced sensitivity to red (or increased sensitivity to green) by about the same amount (4%). An effect of normobaric hypercapnia (breathing 4% CO_2) to increase red sensitivity relative to green by 1.4% was also statistically significant ($p < 0.05$). However, breathing 2% CO_2 had no effect and hypocapnia was not assessed. The substantial variability within and between subjects was emphasized. In the same study an effect of hypoxia on colour discrimination using Lanthony's Desaturated D15 test was also noted, with errors found mainly along the R-G axis.

Summary

Overall, moderate hypoxia may be said to produce a general impairment of colour sensitivity and colour discrimination while a specific effect of hypoxia on the Y-B axis seems likely, if only at lower levels of illumination. There is considerable variability within and between subjects, even in normal trichromats. The normal perception of colour depends fundamentally on healthy cone function in the outer retina, normal colour-opponent signal processing in the inner retina, and normal chromatic processing mechanisms throughout the rest of the visual pathway. Hypoxia is likely to interfere with colour vision to a varying extent according to its severity, the nature of the colour task, the site of primary susceptibility to hypoxia for that colour task, background light level, the state of visual adaptation, the nature of the chromatic stimulus, retinal eccentricity, time of day and much more. The effects on colour vision of changes in PCO_2, particularly hypocapnia, are largely unknown. Of particular interest, cone function might be compromised by outer retinal hypoxia under circumstances in which rods become increasingly metabolically active and so reduce outer retinal tissue PO_2, that is, under progressively dimmer viewing conditions through the mesopic range.

1.6.5 Visual field

Peripheral visual field

The difficulties associated with gathering reproducible data on the extent of the visual field using different stimuli under widely varying conditions are well known (Ferree and Rand, 1922). Significant variables include stimulus wavelength, saturation, intensity, size, duration and motion; the nature of visual fixation during testing; levels of ambient and background illumination; the state of retinal adaptation; and pupil size. Variations in stimulus intensity alone can radically alter the shape and size of the visual fields and produce contradictory relative field sizes for different stimulus colours on different occasions. There is considerable variation between individuals and within individuals at different times of day. Unsurprisingly, early studies recorded contradictory findings of visual field deficits with hypoxia although there seems little doubt that it does narrow the extent of the peripheral visual fields.

Wilmer and Berens (1918) were first to demonstrate a marked contraction in the visual fields, particularly inferiorly, of 20 subjects at a chamber altitude of 20,000 ft. The magnitude of the effect varied with stimulus type, was less significant at lower altitudes, and normalised over a few minutes with supplementary O_2 or upon returning to GL.

An early review detailed substantially different findings of various investigators (McFarland, Evans and Halperin, 1941). One study found significant contraction of the nasal and superior fields above 14,000 ft, becoming marked above 20,000 ft. Another found temporal and superior field contraction from 16,400 ft. A third found no significant contraction below 26,000 ft with perhaps even a widening of the fields. Halstead (1945) reported insidious functional impairment of the peripheral fields of 13 of 20 subjects repeatedly exposed to altitudes of only 10,000 ft, some of which were not immediately reversible.

For brevity, the results of various other studies of hypoxia are summarised in Table 1-3, including the extended series of related studies by Kobrick and colleagues. In a number of the studies by Kobrick and colleagues the heavy task load appeared to maintain alertness to peripheral stimuli by comparison to the use of infrequent or intermittent stimuli. In none of Kobrick's series of studies is there any mention of possible hyperventilation or hypocapnia which will almost certainly have been present during some exposures.

Hypoxic challenge	Main finding	Comments	Reference
5% O_2 for 10-14 min	Marked contraction of visual field with severe hypoxia and marked secondary hyperventilation. Loss of sensitivity of 10-15% at 30° from fovea	400 subjects aged 16 to 91. Reduced field size above 60 y. Up to 15° loss in STQ, 5-10° loss in INQ, less in SNQ and ITQ. Effect of hypoxia equivalent to increase in age from 25 to 70 y. Impossible to separate effects of hypoxia and hyperventilation	Wolf and Nadroski, 1971
18,000 ft	Progressive field loss over time to 17% after 20 minutes. Rapid recovery on descent	Perimetry assessment using 1° white test target. ? hyperventilation	Smith, 1965
18,000 ft	Progressive reduction in mean field radius over an hour at altitude	Effect first seen at 10,000 ft but statistically significant at 18,000 ft. Very variable between subjects	Birren, Fisher, Vollmer *et al*, 1945
15,500 ft	Reduction in mean field radius of just over 1°	Same methodology as above	Vollmer, King, Birren *et al*, 1945
15,000 ft	Response times abruptly and maximally compromised within 1 h of exposure, recovering over 48 h to near normal	Similar study with and without central monitoring task with repeated testing during 48 h exposures. Performance again worst in upper and lower nasal periphery	Kobrick and Appleton, 1971
> 13,000 ft	Progressive narrowing with altitude of peripheral fields to red, green and blue stimuli	Exaggerated effect with dim light, longer exposure, longer wavelength targets	Kobrick, 1970
> 13,000 ft	Progressive increase in response time / variability and interaction with more peripheral stimulus location	Spatially and temporally randomised flash stimuli. Greatest decrement in nasal field. Least effect in horizontal plane. Decrement maximal after 1 h at altitude	Kobrick and Dusek, 1970
> 13,000 ft	Hypoxia and peripheralisation confirmed to independently affect response times and to have significant interaction	As above with additional central random flash monitoring task that reduced magnitude of response time decrements so increased alertness to peripheral stimuli	Kobrick, 1972
> 13,000 ft	Progressive hypoxic decrement in responses to peripheral stimuli during sustained 3.25 h rapid stimulus task	Performance worse in nasal periphery. Hypoxic effect evident by 8-16 minutes of exposure, maximal by 24-32 minutes, plateau for 1.75 h, normalising by 3 h.	Kobrick, 1974
> 13,000 ft	Hypoxia increased response times to near-threshold peripheral stimuli in mesopic range	Prolonged exposures. Moderately increased response times (8-16 minutes), maximal by 82-90 minutes, normal by 3 h. Magnitude of hypoxic effect less than photopic studies	Kobrick, 1975
> 12,500 ft	Significant reduction in field size reported	Perimeter assessments at altitudes from 8000 to 18,000 ft	Nelson, 1967

Table 1-3 Effects of respiratory disturbance on the peripheral visual field

Hyperbaric O_2 also causes contraction of the visual fields (Nichols and Lambertsen, 1969). However, a study that specifically examined the effect of breathing normobaric 100% O_2 found no effect compared to breathing air (Miller, 1958).

Central visual field

A progressive central visual field disturbance recognised with ascent from 13,000 ft was interpreted as widening of the physiological angioscotoma (Evans and McFarland, 1938). The effect corrected fully with O_2 and was attributed to hypoxia. No effect was observed on a 10° area of field corresponding to the macula. The effect was not attributed simply to retinal vessel dilatation but also to perivascular hypoxia that compromised inner retinal neural function. The effect of altitude upon angioscotoma has been demonstrated at 10,000 ft and appears progressive with prolonged stay at that

altitude over a period of hours (Smith, Seitz and Clark, 1946). On the other hand, administration of 100% O_2 narrows the scotomata in relation to the pattern of retinal vasculature, implying that the effect of O_2 on visual fields is mediated at the retina rather than centrally (Rosenthal, 1939).

Besides his work on stimulus detection in the peripheral visual field, Kobrick (1976) demonstrated effects of hypoxia on target detection along the horizontal meridian across the central 40° or so of visual field. Photographs of a camouflaged soldier against a green field background, taken at different distances and lateral displacements of the soldier from a midline reference, were projected in front of subjects at sea level and at an altitude of 4,300 m (~14,000 ft). Some subjects were deployed to the test altitude via intermediate staging altitudes for 2-4 days. The group deployed directly to altitude demonstrated impaired target detection response time across the range of target sizes (distances) and locations across the central visual field compared with the other groups. Detection of more peripheral targets appears to have been compromised by hypoxia to a greater extent than detection of smaller targets (that is, targets at greater distance).

The well-recognised effect of severe hypoxia at high altitude (7620 m) to decrease central visual field sensitivity has been documented recently (Horng, Liu, Wu *et* al, 2008). On the other hand, central visual thresholds have been examined along four meridians, to 60° either side of fixation, at lower equivalent altitudes of 7,000 and 12,000 ft (Yap, Garner, Legg *et al*, 1995). Group mean S_aO_2 was reduced to 96.8% and 91.2% respectively. The perimeter's white test target was 0.43° in diameter and testing was photopic. No obvious sensitivity deficit was observed.

A study designed to look at the effect of dorzolamide on contrast sensitivity changes due to hypercapnia and hypocapnia found a decrease in sensitivity under hypocapnia of around 0.5 $\log_{10}$ units on Humphrey perimeter testing using the 10-2 programme (Sponsel, Harrison, Elliott *et al*, 1997). The level of hypocapnia was only ~15% below baseline values. By contrast, short-term exposure to mild hypoxia, breathing ambient air at 10,000 ft in a decompression chamber, failed to reveal any effect of this altitude upon visual fields tested using a Humphrey analyser 24-2 programme (Fulk and West, 1990). More specifically, using the same test method in a study to investigate the possibility that the inferior retina might be more susceptible to glaucomatous change, the upper visual hemifield exhibited greater loss of sensitivity than the lower hemifield under conditions of hypercapnia (Roff Hilton, Hosking, Cubbidge *et al*, 2003).

1.6.6 Flicker perception

As its rate of oscillation is steadily increased, at a 'critical' frequency a flickering light source will be perceived as a steady source of light. This is the critical fusion frequency. If the rate is then reduced slowly the light source will once again begin to flicker, providing the critical flicker frequency. The effects of hypoxia and other respiratory conditions on critical flicker/fusion frequency (CFF) have been studied over many years. The CFF is usually measured in cycles per second (cps), each duty cycle comprising one period of light and one of dark.

Early accounts of flicker perception are provided by Simonsen and Brožek (1952) and Landis (1954). The CFF of a stimulus is influenced by numerous factors including its size, brightness, light:dark ratio (flash duration), wave form, surrounding illumination, test method, rate of adjustment, initial flicker rate, pupil size (retinal illumination) and retinal location. CFF increases with $\log_{10}$ intensity over a wide range of illumination. Using a 50:50 light:dark ratio and small stimulus (2°), the maximum range of CFF obtained by varying stimulus intensity is from 3 to 65 cps, while the maximum CFF achievable can be made to exceed 80 cps.

CFF varies widely within and between individuals and may be influenced by age, body temperature, alcohol, other drugs, fatigue, practice and motivation. CFF declines with eccentricity from the fovea. Finally, measurements of CFF differ according to whether the measurement is made through an ascending or descending technique (Ginsburg, 1967).

Hypoxia reduces the CFF (Simonsen and Brožek, 1952; Birren, Fisher, Vollmer *et al*, 1945; Vollmer, King, Birren *et al*, 1946; Lilienthal and Fugitt, 1946; Adler, Burkhardt, Ivy *et al*, 1950; Scow, Krasno and Ivy, 1950; Wolf and Nadroski, 1971). The effect is apparent as low as 10,000 ft and is readily reversed with O_2. CFF values are typically reduced by around 1-2 cps at 10,000 ft and by 2-7 cps at 18,000 ft (reduction by ~10-15% of normoxic CFF). Exposure to an equivalent altitude of only 1850 m has been associated with a reduction in CFF (Sen Gupta, Mathew and Gopinath, 1979). An early study only recorded a significant reduction in CFF following an hour's exposure to 10,000 ft, although a decrease was subsequently observed which was maximal between 2-4 h of exposure (Rokseth and Lorentzen, 1954). On the other hand, repeated testing over 48 h at 15,000 ft demonstrated substantial reductions in CFF within the first hour for all three light:dark ratios tested: 5:95; 50:50; and 95:5 (Kobrick and Appleton,

1971). The effect tended to normalise over the next 24-48 h. EEG changes correlate well with CFF under hypoxia, with or without additional CO_2 in the breathing gas (Gellhorn and Hailman, 1944). The effect on CFF of moderate hypoxia may be offset by breathing 3% CO_2 but severe hypoxia causes a progressive decrement in CFF regardless.

A significant increase in CFF of between 3.5 and 5.3 cps has been reported after only 2 minutes of voluntary hyperventilation, depending on the size and site of the test stimulus (Simonsen and Brožek, 1952). The effect of hyperventilation appears more pronounced for larger retinal images and increasing stimulus size has resulted in a maximum 25% increase in CFF when testing against a dark background and 36% against a light background (Jorgensen, 1955). Granger and Ikeda (1961) detailed the effects of hyperventilation on CFF in relation to varying stimulus intensities and sizes. Plotting frequency against $\log_{10}$ field intensity, hyperventilation displaced CFF curves upwards to a greater extent for larger test fields. At higher intensities and field sizes, CFF often exceeded 40 cps and approached 50 cps with hyperventilation. One study has demonstrated reductions in CFF but attributed this to measurements being taken during the post-hyperventilation 'apnoeic pause', when P_ACO_2 would have been rising (normalising), suggesting that the anticipated increases in CFF had been missed (Baer, 1967). In none of the hyperventilation studies were the magnitude or control of the change in P_ACO_2 measured or monitored.

In contrast, inhalation of CO_2 tends to reduce CFF, particularly at concentrations above 5%, even if the balance of the breathing gas is O_2 (Simonsen and Winchell, 1951; Schaefer and Carey, 1953). Alpern and Hendley (1952) demonstrated that artificially-induced metabolic acidosis and alkalosis did not significantly affect CFF, in contrast to the effect of respiratory acidosis and alkalosis produced by breathing 7% CO_2 (balance O_2) and hyperventilation respectively. Hence, it is not the change in acid-base balance *per se* that is important, but the fact that it is induced by a change in PCO_2. Accordingly, respiratory acidosis decreases CFF and respiratory alkalosis increases it. The effect of hyperventilation to elevate CFF was evident at all light levels.

1.6.7 Visual processing, psychomotor and cognitive performance

One obvious difficulty with imposing systemic hypoxia in an attempt to measure effects on visual performance is that hypoxia will also affect cognitive and psychomotor function that could confound interpretation of vision data if inappropriate test methods

are chosen. To inform test selection and experimental design, this section reviews briefly the effects of hypoxia on cognitive and psychomotor aspects of visual task performance that might have potential to confound testing of the sensory visual system.

Performance on a novel visual orientation task may be impaired by hypoxia at altitudes of only 5000 to 8000 ft (Denison, Ledwith and Poulton, 1966). This was attributed to an effect of hypoxia on the ability to learn a new task rather than to an impairment of cognitive performance *per se*. Many performance-related tests exhibit a learning effect, whereby subjects' results improve with rehearsal until optimal performance is achieved. Accordingly, hypoxia may exaggerate apparent performance decrements in naive subjects if inadequate training is undertaken on a new test before data gathering begins. A variety of other studies both support and dispute this effect.

Vigilance for a signal detection monitoring task may be impaired by hypoxia at PO_2 below ~97 mm Hg, equivalent to breathing air at ~13,000 ft (Cahoon, 1970). However, as with visual task learning, results of other studies disagree as to the extent and significance of such an effect, as well as the minimum altitude at which it might first occur. In some visual monitoring studies conducted using low contrast stimuli or at low background light levels, the main difficulty with interpretation is in distinguishing the component of hypoxic performance deficit that results from attentional impairment as opposed to that due to a loss of visual sensitivity. Similar considerations apply to studies of visual scanning for infrequent target objects of interest.

However, there seems little doubt that if hypoxia is sufficiently severe then response times are slowed and more errors are made (Tune, 1964). Both components of response time, that is, reaction time (RT) and movement time (MT), may be affected by hypoxia, but not as might be anticipated (Ledwith, 1970). A significant increase in RT was seen at only 7000 ft while MT decreased. The addition of a mental workload element to the task made a hypoxic performance decrement manifest at an altitude of only 5000 ft. On the other hand, it is possible also to achieve paradoxical effects whereby enhanced performance results ostensibly from hypoxia. For example, the time required to read briefly presented letters, in good lighting, decreased from 12.1 ± (SD) 3.8 ms to 8.3 ± 1.5 ms following rapid transit to an actual altitude of 3450 m (Schlaepfer, Bartsch and Fisch, 1992). The finding was reproduced using hypoxic breathing gas containing 14.5% O_2, when times decreased from 11.9 ± 1.9 ms to 8.1 ± 1.1 ms. No obvious explanation is made for these findings.

Equivalent altitude	Main finding	Comments	Reference
18,000 ft	Impaired pursuit tracking (psychomotor) performance	Visual tracking task. CFF also compromised.	Scow, Krasno and Ivy, 1950
18,000 ft	Impaired choice reaction time and visual letter search & cancellation deteriorates	CFF decreased at 15,000 ft. Impaired visual tracking & motor pursuit from 15,000 ft. Choice reaction time to extinguish flashing lights with different buttons.	Adler, Burkhardt, Ivy *et al*, 1950
15,500 ft	Increased visual reaction time	Hypoxia disrupts reaction time and movement time components of serial choice reaction time	Fowler, Taylor and Porlier, 1987
15,000 ft	Impaired monitoring performance to a prolonged visual vigilance task	3 h of visual monitoring – distinguish 10 bright from 290 dim 1s light pulses presented every 3s in each 15-minute period	O'Hanlon and Horvath, 1973
15,000 ft	Impairment of an attention-based visual search task after 7 h of exposure (not after 1 h)	8-h hypobaric chamber exposures. Task was detecting a visual target amongst distractors – measured response times, no difference in error rates	Stivalet, Leifflen, Poquin *et al*, 2000
14,200 ft	Impaired visual distinction and memory tasks	Increased error rate with more difficult tasks. No effect at 8250 ft.	Cheng, Ma, Ni *et al*, 1999
14,000 ft	Cognitive responses to visual signals slowed more than to auditory signals	Combined visual-auditory signals can increase response speed	Hongwei, Ruishan and Li, 1997
13,800 ft	Response times worse with hypoxia, decreasing stimulus intensity, and their interaction	Variable name oddball paradigm. Two stimulus intensities were 1.57 and 0.38 cd m^{-2}; background was 0.18 cd m^{-2}. More eye movements under hypoxia	Fowler and Kelso, 1992
13,800 ft	Compromised serial response time task	Greater increase in response latency with dimmer stimuli. Error rate was increased slightly	Fowler, White, Wright *et al*, 1982
12,500 ft	Significant deficits in recall for high memory load readbacks	No deficit for low memory loads or to vigilance task	Bartholomew, Jensen, Petros *et al*, 1999
12,000 ft	Increased error rate for a logical reasoning task	No effect on task learning and no effect at 10,000 ft and below	Green and Morgan, 1985
12,000 ft	*No novel task learning effect*	*Spatial orientation (Manikin) task, serial choice reaction time test, and logical reasoning task in naive subjects, during rest and exercise*	*Paul and Fraser, 1994*
11,900 ft	Continuous calculation test and addition-subtraction reaction time test impaired after 1 h.	More errors and slower performance. Worse at higher altitude of 14,500 ft. Worse at 16,500 ft after 30 minutes.	Wu, Li, Han *et al*, 1998
11,900 ft	4-choice reaction time prolonged and performance worse at 11,900 ft	No effect at 9250 ft for 1 h. Decrement on 4-choice reaction time worse at 14,500 ft. No effect on simple reaction time at any altitude	Li, Wu, Fu *et al*, 2000
11,500 ft	*No effect on a simple vigilance task*	*Light flashes lasting 75ms presented every 1s – detect occasional missed flashes (averaging ~1 every 5 min)*	*Fiorica, Burr and Moses, 1971*
10,000 ft	Impaired numerical processing – more data required but maintained accuracy	Sampled numbered cards one at a time until arrived at decision about whether mean of whole pack greater or less than 74.	Frisby, Barrett and Thornton, 1973
10,000 ft	Visuomotor speed impaired early. Visual reaction time slow by 2 h, worse at 4 h	Varying impairment on different cognitive and neuropsychological tests. Visual digit span memory worse with longer exposure	Vaernes, Owe and Myking, 1984
10,000 ft	'Dose-related' effect on response time from 10,000 ft	Serial choice response time task at two levels of stimulus intensity	Fowler, Elcombe, Kelso *et al*, 1987
9250 ft	Continuous recognition short-term memory impaired at 9250 ft for 1h	Effects worse with increased altitude – progressive performance decreased at 11,900 and 14,500 ft. Increased reaction time consistently earliest feature	Du, Li, Zhuang *et al*, 1999
8000 ft	Much impaired performance on difficult search task, but not easy search task	Easy task, find letter pairs in text (AA, BB etc). Hard task, find alphabetic pairs (AB, FG etc). Improved with practice - ? novel task learning effect	Kelman and Crow, 1969
8000 ft	*No significant effect on a selective attention task*	*Time taken to sort 12 packs of cards with varying alphanumeric characters*	*Kelman, Crow and Bursill, 1969*
8000 ft	Impaired performance on psychomotor test of recent memory	Gedye task – response time to series of left-right light sequences. Possible effect of hypoxia on learning at altitude	Billings, 1974
8000 ft	*No effect on a spatial transformation task*	*No task learning effect but reaction time did decrease with exercise when O_2 saturation not controlled*	*Fowler, Paul, Porlier et al, 1985*
7000 ft	Significantly slowed response times at 7000 ft	Test was hastened judgements of visual stimulus orientation. Judgement accuracy poorer at 12,000 ft	McCarthy, Corban, Legg *et al*, 1995
6000 ft	Impaired vigilance performance for a prolonged visual monitoring task	Same differential signal brightness task as O'Hanlon and Horvath (1973) – 4 sessions of 15 minutes during second hour of hypoxia exposure	Christensen, Gliner, Horvath *et al*, 1977

Table 1-4 Cognitive and psychomotor effects of hypoxia related to visual tasks

Of particular interest and relevance, the effect of hypoxia to slow RT interacts with light intensity (Fowler, Banner and Pogue, 1993). This study simulated degraded natural lighting, including mesopic conditions, by manipulating display luminance and background illumination. Hypoxia was induced breathing gas mixtures of 8% or 13% O_2. The task employed a contrast discrimination element and the effect of hypoxia appeared due to slowed visual processing rather than degradation of more central functions. A 'pre-processing' stage was implicated. A subsequent study using the same levels of hypoxia supported this proposition (Fowler and Nathoo, 1997). It was concluded that hypoxia slows neither stimulus identification nor feature extraction. It was proposed that the critical relationship was that between RT and stimulus luminance, such that hypoxia shifts the relationship to cause disproportionately greater slowing of responses to progressively less intense stimuli. Hence, RT depends on stimulus intensity, hypoxia reduces the apparent brightness of visual stimuli, and hypoxia slows reaction time. Thus, in order to avoid confounding measurement of functional visual sensitivity, tests that employ performance-related metrics that have cognitive, psychomotor or response/reaction time components should be avoided.

A variety of other studies, often with conflicting results, have been considered. They are summarised briefly in Table 1-4, with significant negative findings highlighted in italics. It is worth noting that many of the studies involved respiratory challenges that were controlled by monitoring S_aO_2, often with no mention of whether secondary hyperventilation might have been a confounding factor.

Conclusions

More complex and demanding visual tasks may exhibit effects of mild hypoxia, while simple, clearly visualised tasks appear resistant even to severe hypoxia. Tests of visual sensitivity should be simple, quick to learn and easily performed to avoid issues of task complexity that may make them vulnerable to hypoxia. Vigilance may be compromised by hypoxia, so tests of visual sensitivity should ensure that subjects are kept occupied such that their attention does not wander. Learning effects must be avoided during experimental exposures, as task learning itself might be compounded by hypoxia, so training on the vision test must be completed beforehand. Tests that measure response time or reaction time should be excluded as effects of hypoxia on these may confound interpretation of effects on visual sensitivity. When measures of stimulus duration are made, as the dependent variable, these should relate to variation in presentation time and be independent of subject response times.

2 Rationale

2.1 Background

The processes supporting retinal phototransduction require considerable metabolic energy, for rhodopsin phosphorylation, synthesis of cyclic guanosine monophosphate, chromophore transport and to support enzyme activity (Picaud, 2003). In the dark, rod photoreceptor O_2 uptake increases dramatically to support the ion pumps that maintain the 'dark current', consuming more O_2 than any function of any other cell (Steinberg, 1987; Arden, Sidman, Arap *et al*, 2005). In consequence, the retinal PO_2 falls to remarkably low levels near the photoreceptor inner segments and may even compromise oxidative phosphorylation in inner segment mitochondria (Stefansson, Wolbarsht and Landers, 1983; Linsenmeier, 1986; Ahmed, Braun, Dunn and Linsenmeier, 1993; Yu and Cringle, 2002; Wangsa-Wirawan and Linsenmeier, 2003). Hypoxia compromises ion pump function (Steinberg, 1987) and also delays regeneration of rhodopsin *in vitro* (Ostroy, Gaitatzes and Friedmann, 1993). By implication, outer retinal function may be increasingly vulnerable to exogenous hypoxia when rod QO_2 increases as light levels fall.

It is well established that mild hypoxia elevates scotopic thresholds during dark adaptation and that 'night vision', relying only on rods, is vulnerable to mild hypoxia, equivalent to breathing air at altitudes well below 10,000 ft (McFarland and Evans, 1939; Hecht, Hendley, Frank and Haig, 1946). On the other hand, more severe hypoxia is required to impair photopic visual performance, which has yet to be demonstrated convincingly at an equivalent altitude below 12,000 ft. However, the effect of hypoxia on mesopic vision has not been studied in any detail. If the outer retina becomes increasingly vulnerable to exogenous hypoxia as light levels fall then one might expect to witness a progressively more significant visual impairment under a fixed hypoxic challenge with progression from the photopic into the mesopic range. To be meaningful, mesopic effects of hypoxia should be evident at equivalent altitudes below 12,000 ft. In aviation, supplementary O_2 is generally only recommended at altitudes above about 10,000 ft, so this offers a relevant mild level of hypoxia against which to test visual performance. Also, the presence of functionally significant, endogenous, rod-driven

hypoxia might be revealed by using 100% O_2 to demonstrate a visual performance benefit, relative to normoxia, for given states of adaptation to dim light levels.

When flying at night, aircraft cockpits and flight decks are illuminated typically in the upper mesopic range. Thus, effects of mild hypoxic hypoxia to compromise mesopic vision are relevant to aircrew flying at night at actual or equivalent (cabin) altitudes that do not mandate the use of supplementary O_2 on respiratory grounds. Hypoxia has also been implicated in the aetiology of various ocular pathologies, including diabetes mellitus, glaucoma, macular disease, photoreceptor degeneration and vascular disorders (Wangsa-Wirawan and Linsenmeier, 2003). Thus, demonstrable psychophysical effects of mild hypoxia on the healthy retina may have wider clinical implications.

When examining possible effects of altered PO_2 consideration must be given also to any effects of changes in PCO_2, which may or may not co-exist and have the potential to confound the results. In particular, effects of hypocapnia on sensitivity to light and flicker must be considered, and respiratory challenges and adaptation states must be controlled with care, within and between subjects. However, when considering visual performance at low light levels the state of visual adaptation must also be controlled meticulously, within and between subjects, and any effect of ongoing light or dark adaptation assessed or, preferably, eliminated.

Thus, the overall programme of work falls into three discrete phases. First, the effects of respiratory disturbance (hypoxia, hyperoxia and hypocapnia) on the process of dark adaptation (threshold sensitivity to light) must be determined and a procedure for establishing stable mesopic adaptation states established. Second, any confounding influence of hypocapnia on mesopic flicker sensitivity must be assessed. Finally, metrics of visual performance can be assessed under respiratory disturbance across a range of low photopic to mid-mesopic adaptation states. This work encompassed assessment of foveal spatial contrast sensitivity, foveal chromatic sensitivity, visual processing speed, temporal contrast sensitivity across the central visual field, and low contrast acuity to ± 5° of visual angle from fixation.

2.2 *Hypotheses to be tested*

Underpinning hypothesis

Progressive, endogenous, rod-driven, outer retinal hypoxia may render visual perception increasingly vulnerable to exogenous hypoxia as the available light decreases through the mesopic range.

Dark adaptation

During dark adaptation, the outer retina is likely to be highly susceptible to exogenous hypoxia, delaying the time to achievement of scotopic sensitivity.

Supplementary O_2 might hasten rod adaptation to dark and achievement of scotopic sensitivity.

Hypocapnia might influence dark adaptation rate.

Flicker sensitivity

Increasingly severe hypocapnia may be associated with increasing sensitivity to flicker.

Spatial contrast sensitivity

Progressive rod-driven retinal hypoxia might increase the susceptibility of foveal spatial contrast sensitivity to exogenous hypoxia as luminance is reduced from photopic to upper and mid-mesopic levels.

Supplementary O_2 might optimise foveal spatial contrast sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Binocular summation of luminance contrast sensitivity might interact with respiratory disturbance.

Chromatic sensitivity

Progressive rod-driven retinal hypoxia might increase the susceptibility of foveal chromatic sensitivity to exogenous hypoxia as luminance is reduced from photopic to upper and mid-mesopic levels.

Supplementary O_2 should optimise foveal chromatic sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Binocular summation of chromatic threshold sensitivity might interact with respiratory disturbance.

Visual processing speed

Mild hypoxia may compromise the speed at which visual information is processed and thereby require longer stimulus presentation times to maintain cognitive performance.

An effect of hypoxia may become manifest when viewing dimmer stimuli.

Visual tasks of greater complexity may be more susceptible to hypoxic impairment.

Supplementary O_2 may optimise visual processing efficiency.

Temporal contrast sensitivity

Mild hypoxia might compromise temporal contrast sensitivity in a manner that suggests retinotopic susceptibility, particularly at lower light levels.

Supplementary O_2 might optimise temporal contrast sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Hypocapnia might enhance temporal contrast sensitivity, particularly at photopic luminance.

Contrast acuity

Mild hypoxia might compromise low contrast acuity progressively with increasing retinal eccentricity and decreasing light level.

Supplementary O_2 might enhance contrast acuity, particularly beyond the fovea and at dimmer light levels.

2.3 Experimental progression

Experiment 1 – Dark Adaptation

This experiment sought to establish the time course of dark adaptation, particularly the time to cone rod inflection, using a non-standard Friedmann Visual Field Analyser Mk II visual stimulus, under a variety of hypoxic, hyperoxic and hypocapnic challenges imposed in a hypobaric (decompression) chamber. The experiment was intended to highlight variations in late mesopic and early scotopic sensitivity; to determine a

procedure of visual adaptation that would enable stable mesopic adaptation states to be achieved in later experiments; and to quantify the physiological responses to low pressures for later comparison with those obtained during experiments using breathing gas mixtures. Five subjects each undertook five experiments. On each occasion a normoxic control dark adaptation, with the chamber held at GL, was compared with a dark adaptation conducted during either decompression to a simulated altitude of 10,000 ft; decompression to a simulated altitude of 15,000 ft; voluntary hyperventilation at GL; hyperoxia breathing 100% O2 at GL; or experimentally-induced hyperventilation at 15,000 ft.

Experiment 2 – Flicker Sensitivity

This experiment sought to establish whether the known effect of hyperventilation to increase sensitivity to flicker might be likely to interfere with subsequent work employing temporally modulated stimuli in the mesopic range, since this could confound interpretation of the effects of respiratory disturbance. Mild to moderate hypocapnia was imposed in a stepwise fashion through voluntary hyperventilation controlled using breath-by-breath feedback from respiratory mass spectrometry. The Geedev flickermeter was used to present a simple mesopic stimulus and ascending and descending measurements of CFF were made by 11 subjects.

Experiment 3 – Spatial Contrast Sensitivity

Sensitivity to spatial fluctuations in luminance contrast is a fundamental visual attribute. This experiment assessed its vulnerability to respiratory disturbance at reduced light levels. Following a carefully controlled procedure of retinal bleach, dark adaptation and light adaptation to either a low photopic, upper mesopic or mid-mesopic background field, the foveal CSF of 12 subjects was measured under normoxic, hypoxic and hyperoxic conditions. Stimuli comprised Gaussian spatial contrast stimuli (Gabor patches) presenting vertical sine wave gratings ranging in *sf* from 0.5 to 16 cpd. Experiments were conducted binocularly and monocularly (dominant eye) viewing a display directly or through ND 1.0 or 2.0 filters to achieve mesopic levels. The respiratory conditions were imposed, and masked from the subjects, by breathing air, 100% O_2 or a hypoxic gas mixture from dedicated pressure-demand breathing gas regulators.

Experiment 4 – Chromatic Sensitivity

Sensitivity to colour is another a fundamental visual attribute. This experiment assessed the vulnerability of threshold chromatic sensitivity to respiratory disturbance at low light levels. Binocular and monocular (dominant eye) vision tests were conducted following visual adaptation to low photopic, upper mesopic or mid-mesopic levels. Threshold chromatic sensitivity was measured along two Y-B and two R-G colour confusion axes using foveal, motion-defined chromatic stimuli presented against dynamic luminance contrast 'noise'. Experiments were conducted by 12 subjects viewing the test display directly or through ND 1.0 or 2.0 filters to achieve mesopic levels. Normoxic, hypoxic and hyperoxic respiratory conditions were imposed, and masked from the subjects, as in Experiment 3.

Experiment 5 – Visual Processing Speed

Evidence suggests that hypoxia delays the time taken to process visual information and that this delay occurs early in the visual system. This could have implications for aircrew flying at night and using modern display systems. This experiment explored the nature of the visual processing delay in relation to extraction of visual information from increasingly complex visual display scenes and decreasing stimulus luminance relative to a dark background field. Vision testing employed the Useful Field of View (UFOV®) test. Experiments were again conducted by 12 subjects viewing the test display directly or through ND 1.0 or 2.0 filters. Normoxic, hypoxic and hyperoxic respiratory conditions were imposed, and masked from the subjects, as in Experiments 3 and 4.

Experiment 6 – Temporal Contrast Sensitivity

Sensitivity to temporal fluctuations of luminance contrast is another fundamental visual attribute that is relevant particularly to the perception of motion. This experiment assessed the vulnerability of temporal contrast sensitivity to respiratory disturbance at low light levels. Vision testing was conducted monocularly (dominant eye) and employed a standard Frequency Doubling Technology (FDT) perimeter to generate 10° diameter temporal contrast gratings at 17 test locations across the central 40° of visual field. Tests were conducted by 12 subjects viewing, on separate occasions, either directly or through ND 1.0 or 2.0 filters. On each occasion, testing was performed while breathing air, 100% O_2 or 14.1% O_2, and some experiments were conducted with subjects hyperventilating to induce moderate hypocapnia. Normoxic, hypoxic and

hyperoxic respiratory conditions were randomised and balanced between subjects, who were unaware of the exposure order.

Experiment 7 – Contrast Acuity

The ability to resolve fine detail is a fundamental visual capability that is critically dependent on visual stimulus size, contrast and retinal location. This experiment assessed the threshold stimulus contrast required to discriminate the orientation of an alphanumeric character at a range of retinal eccentricities to ± 5° from fixation, for a range of light levels and respiratory conditions. Experiments were conducted by 12 subjects at low photopic, upper and mid-mesopic levels, viewing a test display directly or though ND 1.0 or 2.0 filters, while breathing air, 100% O_2, or 14.1% O2 to provide normoxic, hyperoxic and mildly hypoxic respiratory states. Respiratory exposure orders were again randomised, balanced and masked from the subjects. In addition, the study included assessment of the effects of the imposed respiratory disturbances on resting pupil diameter at each light level.

3 Materials and Methods

This section provides details of the materials and methods that recur repeatedly in a number of the experiments that follow to avoid undue repetition. Details of the vision tests employed are described in the relevant chapters.

3.1 Simulated Altitude Exposure

3.1.1 Background

Paul Bert demonstrated that a reduction in ambient PO_2, that is, the product of ambient pressure and fractional O_2 concentration, is responsible for the deleterious effects, and ultimately death, resulting from altitude exposure (Bert, 1878). Before their ill-fated, high altitude flight in the balloon *Zenith* in 1875, Tissandier, Sivel and Crocé-Spinelli experienced hypoxic visual disturbance during decompression to an equivalent altitude of ~7000 m in Bert's low pressure chamber (West, 1998, p55).

Subjects still undertake hypoxia experience training in decompression (hypobaric) chambers. These simply comprise a sealed compartment from which the air may be evacuated, decreasing ambient pressure and simulating altitude exposure. Occupants may breathe ambient chamber gas (usually air) at ambient pressure, or a gas mixture delivered through a breathing system, often under slight positive ('safety') pressure. Thus, when the breathing gas is air, hypobaric chamber exposures produce alveolar gas tensions that mimic those produced at an 'equivalent altitude' for the ambient pressure.

Hypobaric chamber studies are costly and chamber time is limited, precluding this method for extended studies. However, the relevant P_AO_2 for any given altitude can also be produced at sea level (or any other GL altitude above sea level) by reducing the F_IO_2 appropriately and increasing the balance of inert gas, usually N_2. A relatively brief initial study of visual sensitivity was conducted in a hypobaric chamber to assess the feasibility of using the hypobaric chamber in later studies, to validate a visual adaptation procedure at low ambient pressure (Chapter 4), and to provide baseline physiological data against which subsequent gas mixture studies could be compared.

3.1.2 The hypobaric chamber

A simplified schematic of the QinetiQ hypobaric chamber and major associated structures is shown in Figure 3.1. The vacuum pumps are capable of evacuating the various compartments of the chamber to an equivalent altitude in excess of 100,000 ft (ambient pressure of less than 10 mm Hg) at an initial rate of ascent of 40,000 ft min^{-1}. The chamber is manually controlled by trained Pressure Chamber Operators (PCO) working to well-established procedures and comprehensive checklists. In addition, a specific checklist is produced and reviewed for each experimental altitude profile.

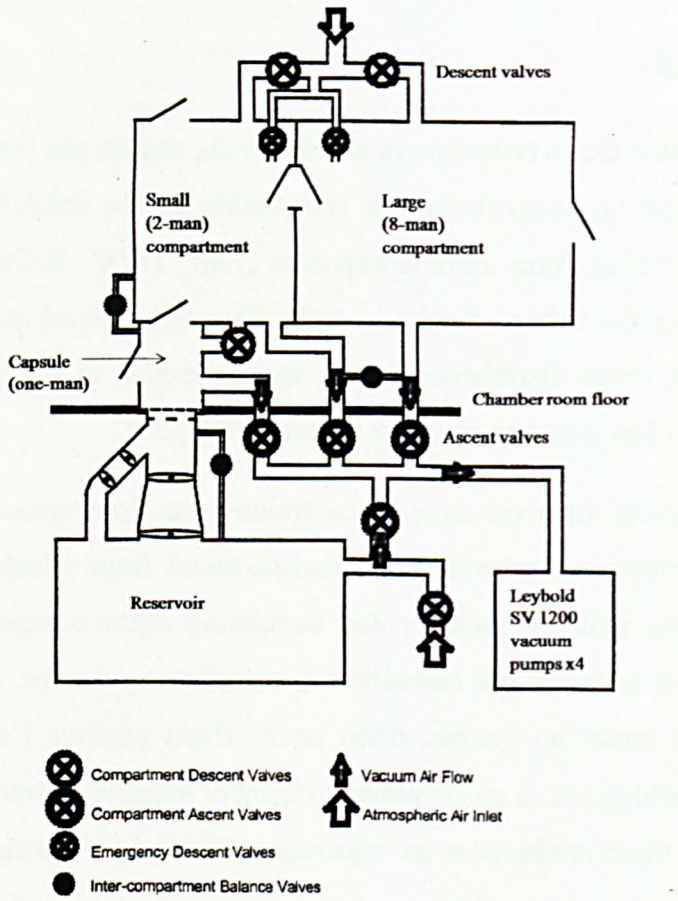


Figure 3.1 Simple schematic of the QinetiQ hypobaric chamber facility

The basic hypobaric chamber pressure instrumentation comprises calibrated pressure transducers in each compartment that measure absolute pressure, simulated altitude and rate of change of altitude. There are also digital displays of simulated altitude (ft) and absolute pressure (mm Hg) and analogue gauges showing simulated altitude (ft) and rate of change of altitude ($x10^3$ ft min^{-1}). A 'set point indicator' allows a specific altitude or pressure to be held constant with great accuracy, typically to within 0.5 mm Hg of a desired pressure setting in the hands of an experienced PCO.

The chamber has a permanent O_2 system installation supplied by 15-cylinder pallets of gaseous O_2 that feed a pressure-reducing manifold external to the building. The supply passes into the chamber room at a pressure of up to 120 bar (~1800 psig), to a further distribution manifold which feeds three 2250 L capacity O_2 bottles. These supply gas to the chamber O_2 distribution system via pressure-reducing regulators that reduce the supply pressure to ~300 psig. The supply then passes to the pressure-demand breathing gas regulators permanently mounted in each chamber compartment. Compressed air can also be supplied to breathing systems inside the chamber from gas cylinders or from the facility's permanent piped compressed air supplies. The vacuum pumps, air supplies, compressors and an uninterruptible power supply are located with the vacuum reservoir in a plant room directly beneath the chamber room. The pump inlet (vacuum) ports are isolated from the chamber and its ascent manifold pipe work by butterfly isolation valves that are opened by a low pressure compressed air system before the chamber can be decompressed.

The hypobaric chamber also has a 28 volt direct current power supply with distribution through electrical sockets on the walls of the chamber compartments. A permanent communications system is installed to allow experimenters outside to maintain constant contact with chamber occupants. Internal lighting comprises 240 volt fluorescent strip lights that are controlled externally and can be dimmed. There are numerous flanged instrumentation ports that allow cables, pipe work, gas supplies, probes and other experimental and monitoring devices to feed through the walls of the chamber. A video system provides visual and audio monitoring and recording of chamber activity. The large compartment is also fitted with air conditioning units. There are extensive safety and emergency alarm systems including sensors to monitor O_2 levels throughout the facility and associated laboratories.

3.1.3 Breathing gas mixtures

The alveolar air equation (Equation 1) may be used to derive a formula to calculate the appropriate F_IO_2 to produce any required P_AO_2. First, Equation 1 may be simplified by using appropriate numerical values for some of the terms. The laboratory facilities in which this work was conducted are located next to the airfield at Farnborough, Hampshire, at an elevation of 238 ft amsl (73 m) and latitude ~51°N. Thus, it is reasonable to approximate GL barometric pressure to the ICAO (1964) Standard Atmosphere sea level pressure of 760 mm Hg at 45°N, recognising that varying

meteorological conditions will also influence local barometric pressure. Substituting the saturated water vapour pressure at body temperature (47 mm Hg) and using a value of 0.845 for the respiratory exchange ratio (see section 1.3.1), Equation 1 simplifies to:

$$P_AO_2 = F_IO_2\,(760 - 47) - P_ACO_2\left(F_IO_2 + [\{1 - F_IO_2\}/0.845]\right)$$

If we further assume that the hypoxic exposures will be insufficiently severe to provoke secondary hyperventilation, and thus that normocapnia is maintained throughout, then we can substitute a normocapnia value of 40 mm Hg for P_ACO_2 (Lumb, 2000) and multiply out the correction factor:

$$P_AO_2 = F_IO_2\,(713) - F_IO_2\,(40) - \left(40 - 40\{F_IO_2\}/0.845\right)$$

Thus, dividing the correction factor elements by 0.845:

$$P_AO_2 = F_IO_2\,(713) - F_IO_2\,(40) - \left(47 - 47\{F_IO_2\}\right)$$

Removing the correction factor brackets:

$$P_AO_2 = F_IO_2\,(713) - F_IO_2\,(40) - (47) + 47(F_IO_2)$$

Summing the multiples of F_IO_2:

$$P_AO_2 = F_IO_2\,(713 - 40 + 47) - 47$$

Thus:

$$P_AO_2 = F_IO_2\,(720) - 47$$

Hence, expressing F_IO_2 as a function of P_AO_2:

$$F_IO_2 = (P_AO_2 + 47) / 720$$

Thus, the following expression enables determination of the approximate F_IO_2 required to induce a given P_AO_2 using a dry gas mixture at one atm (760 mm Hg):

$$F_IO_2 = (P_AO_2 / 720) + 0.065 \qquad \times 100\%$$

Equation 11 Expression for calculating the F_IO_2 that produces a given P_AO_2 at sea level

Hypoxic breathing gas mixtures typically contain a reduced O_2 fraction with a balance of N_2. In the work that follows, all gas mixture studies were conducted to simulate an altitude of 10,000 ft (3048 m). From the ICAO (1964) Standard Atmosphere, the ambient pressure at this altitude is 523 mm Hg. From Equation 1, adopting the same assumptions as previously, the P_AO_2 anticipated at this altitude should be ~55 mm Hg. From Equation 11, the F_IO_2 required to produce this degree of hypoxia at GL using a breathing gas mixture will be 14.1% O_2. Dry gas mixtures of 14.1% O_2 with a balance of N_2 were sourced from a commercial supplier and provided in 50L, 200 bar cylinders. Pressure-reducing regulators controlled the supply pressure from these cylinders.

3.2 Control of Respiratory Gases

3.2.1 Pressure-demand breathing gas delivery

All breathing gases were supplied through Normalair (now Honeywell Normalair-Garrett Ltd) Mk17F pressure-demand aircrew breathing gas regulators (Figure 3.2). The central gauge indicates the supply pressure of the gas feeding the high pressure inlet of the regulator. The normal working pressure for these regulators is between 200 and 400 psig. A breathing gas supply hose connects the regulator outlet to the subjects mask hose. If the 'airmix' lever (upper right in Figure 3.2) is selected to 'normal oxygen' then the regulator automatically dilutes the supply gas (usually 100% O_2) with gas from the ambient environment of the regulator (usually air) at a ratio determined by the ambient pressure. Thus, in operational use, 100% O_2 will be diluted appropriately to provide adequate protection against hypoxia at altitude whilst conserving the O_2 supply. However, if the 'airmix' lever is selected to '100% oxygen' then the gas delivered to the breathing gas hose has the same composition as the supply gas. Thus, any supplied gas mixture will be delivered at ambient pressure through this type of regulator, without air dilution, by selecting '100% oxygen' on the airmix lever, closing the air inlet shutter.

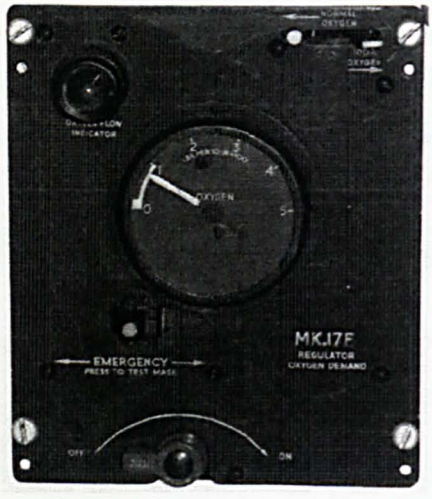


Figure 3.2 Front panel view of Mk17F pressure-demand breathing gas regulator

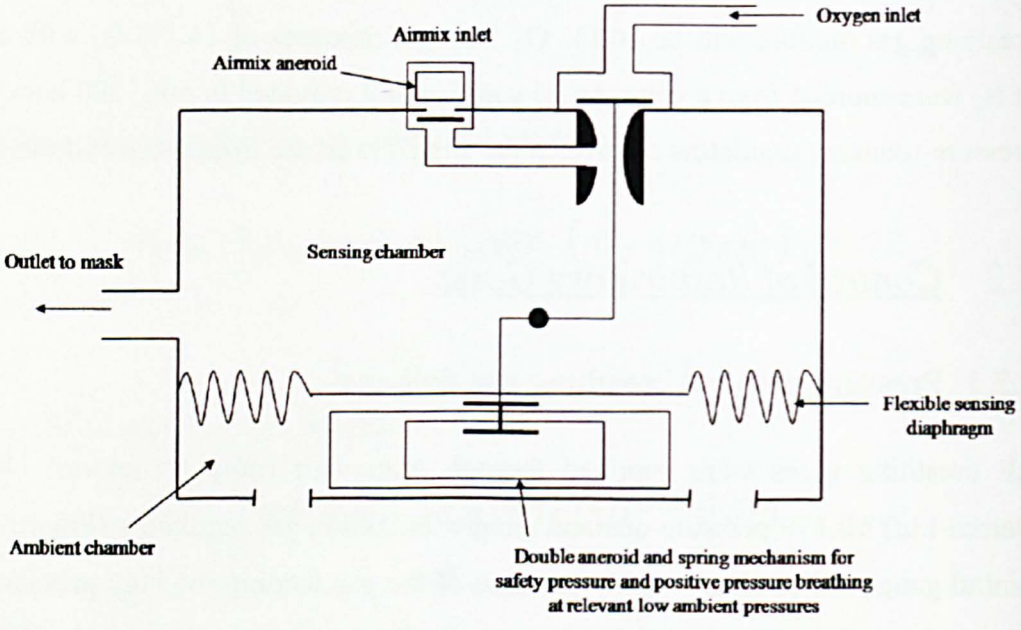


Figure 3.3 Schematic to show the principle of operation of a pressure-demand regulator

It is only when inspiratory demand is placed on the regulator that gas passes into the breathing hose. The principle of the pressure-demand operation of the regulator is illustrated in Figure 3.3. Turning the lowermost switch on the front panel to 'on' opens the shut off valve to the gas supply, charges the system, provides an indication of supply pressure on the central gauge and passes supply gas to a pressure reducer. Subsequent inspiratory demand will tend to decrease the pressure at the regulator outlet. This pressure drop is transmitted to the sensing chamber where it deflects the sensing diaphragm and moves the demand valve lever. The demand valve opens, allowing pressure-reduced supply gas to pass into a mixing tube. As a result of the Venturi effect,

if 'airmix' is selected a quantity of ambient air that is determined by ambient pressure is drawn in through the air inlet and mixes with the supply gas. This breathing gas mixture then passes into the breathing gas hose to supply the breathing mask. During expiration, the pressure in the regulator builds up as the supply gas flows into it and, as it is not demanded, the sensor diaphragm moves back, the demand valve closes and flow of gas through the regulator ceases.

When gas is flowing through the system, that is, during inspiration or if there is a leak, the blinker diaphragm will detect the flow and the 'dolls eye' O_2 flow indicator (top left in Figure 3.2) will show a white vertical bar. This indicator remains black when there is no flow through the system. A 'safety pressure' spring applies a force of about 2 mm Hg to the underside of the breathing diaphragm which opens the demand valve until an equal pressure within the system overcomes the spring. The pressure in the mask is thereby kept slightly above ambient throughout inspiration. The spring is prevented from acting on the breathing diaphragm by an aneroid until the ambient pressure exceeds the safety pressure altitude above which hypoxia may be a threat. This ensures that any mask leak is outboard, preventing inadvertent reduction of the F_IO_2 in the event of a poor face-mask seal. For the Mk17F regulator, safety pressure commences with ascent over about 10,000 to 12,000 ft. The pressure breathing aneroid only comes into play under inadvertent emergency exposures to very high altitude (over 38,000 ft) and is irrelevant to the current work. The F_IO_2 necessary to prevent hypoxia at different altitudes has been subject to detailed assessment over many years and is well documented elsewhere (Ernsting, 1963, 1965, 1978; Denison, 1981).

The emergency button is a facility that provides additional overpressure at any altitude at which it is selected, including GL. Once a subject's breathing mask has been fitted, the regulator's emergency facility can be used against a breath hold to check that there are no obvious leaks and thus that the face-mask seal is satisfactory. If there is a leak then the dolls eye will show 'steady white' to indicate gas flow through the system.

Different Mk17F regulators have very similar breathing characteristics. For the GL experiments conducted using different breathing gas mixtures, three Mk17F regulators were used, one dedicated to each breathing gas, and their pressure-flow characteristics were assessed in detail. Their supply gases were the laboratory's permanent piped supplies of high pressure air and 100% O_2 and a bottled supply (50 L, 200 bar) of 14.1% O_2 with a balance of N_2 (hypoxic gas mixture). Each gas passed through a pressure-reducing regulator and was delivered to its Mk17F regulator at a nominal supply

pressure of 350-400 psig. The regulators' airmix levers were set to 100% to ensure that the supply gas compositions were unchanged (that is, not mixed with room air) when delivered to the outlet hose.

The three regulators fed their gases to a 4-way tap (three inlets, one outlet) such that one at a time (or none) could be selected to feed a common mask tube hose. For the regulator assessments, this passed the regulator gas supplies to the inspiratory hose of a P/Q mask mounted on a backing plate connected to a computer-controlled mechanical breathing simulator that generated a selection of reproducible respiratory demands. A mask tapping allowed measurement of mask cavity pressures with a Celesco pressure transducer of appropriate range and flows were measured using an in-line Fleisch pneumotachograph in conjunction with a differential pressure transducer. Pressure and flow measurements were calibrated respectively using a Druck pressure gauge and KDG-Mobrey air rotameter of known performance.

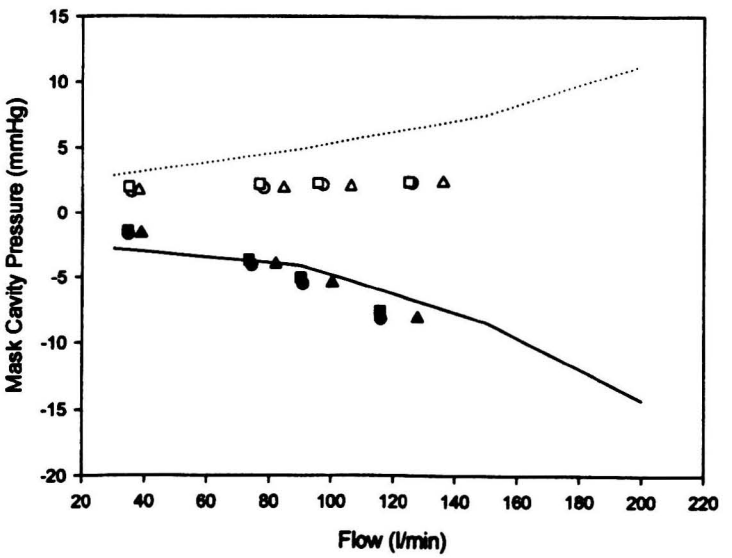


Figure 3.4 Pressure-flow characteristics of three Mk17F pressure-demand regulators

Figure 3.4 shows the minimum mask cavity pressures generated during inspiration (solid symbols) and maximum pressures during expiration (open symbols) for the regulators supplying air (circles), 14.1% O_2 (squares) and 100% O_2 (triangles) at four different demand flow rates. Mask cavity pressures were largely consistent with the normally accepted limits (solid and dotted lines), indicating adequate delivery of gas in response to demand, specifically at the low respiratory flow demands expected of a subject at rest. Mask cavity pressures fell marginally and transiently outside normal

limits under high inspiratory flow demands, with all three regulators performing similarly and acceptably.

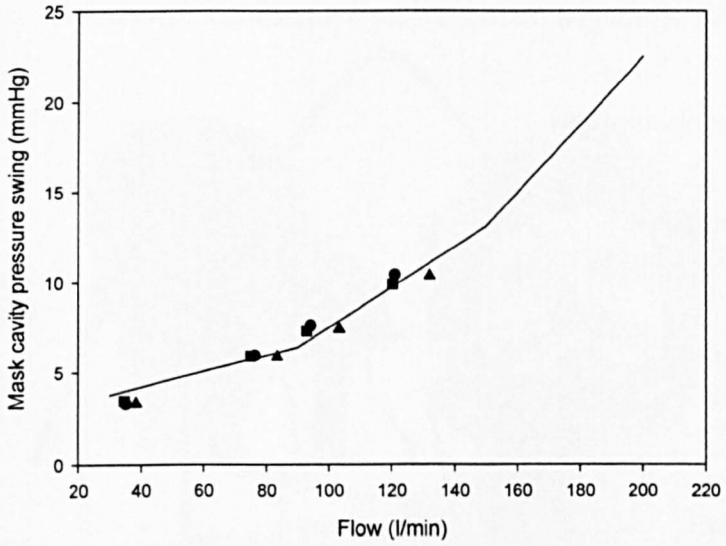


Figure 3.5 Mask cavity pressure swing characteristics of three Mk17F pressure-demand regulators

The mask cavity pressure swings generated by the different respiratory demands are shown for the regulators supplying air (circles), 14.1% O_2 (squares) and 100% O_2 (triangles) in Figure 3.5. Again, all performed similarly. The regulators were indistinguishable to the user and imposed minimal breathing resistance such that, at low flow rates associated with restful breathing patterns, they performed within accepted limits. Since the gases these regulators supplied were all odourless and tasteless, subjects were unable to tell which breathing gas was being supplied. Thus the respiratory conditions were masked from the subjects.

3.2.2 Breathing gas (oxygen) mask

In some experiments subjects wore standard Royal Air Force Type P/Q Series aircrew O_2 masks supported from Type 'G' cloth helmets, shown in Figure 3.6. The Type 'G' helmet is available in four sizes with two stud fastener settings for the adjustable O_2 mask suspension cables. The type P and Q masks are identical except for size, the latter being slightly smaller and more often providing a better fit for females. The use of pressure demand systems requires an O_2 mask that maintains a good face-mask seal, including with raised breathing pressures. The mask comprises a rigid fibreglass exoskeleton supporting a soft moulded rubber facepiece with a reflected edge that helps to maintain a mask seal when pressure builds up in the mask cavity. A metal strip is

moulded into the bridge of the nose and can be shaped to improve the fit for different subjects. The mask is supported from the helmet using a chain toggle harness attached to the exoskeleton. The combination of sizing, fitting and adjustment options allows good helmet and mask fits to be achieved for the vast majority of subjects, allowing continuous wear for prolonged periods without significant discomfort.



Figure 3.6 Type 'G' cloth helmet and P/Q O_2 mask *in situ* (with consent)

A schematic diagram showing the design principles of the inspiratory and expiratory valves is shown at Figure 3.7. The inspiratory valve is a soft rubber diaphragm that acts as a simple non-return valve, allowing gas to be drawn from the mask hose into the mask cavity during inspiration, but preventing expired gas from passing back through the mask inlet. The non-return expiratory valve is mounted at the bottom of the mask and allows drainage of moisture from the mask cavity. It is protected by a flexible rubber outlet snout. The valve plate is a thin metal disc held against a metal seat by a light spring which is easily overcome on expiration. It would also be overcome by the slight safety pressure generated by the demand regulator were it not for the compensation tube, which balances the pressure in the inlet port against a diaphragm and piston on the reverse side of the expiratory valve, thereby preventing the continuous escape of gas through the expiratory valve when it is supplied under positive pressure. A second spring is then required to prevent the valve opening inadvertently if the pressure in the inlet port falls. This arrangement is termed a 'split compensated expiratory valve'. The presence of the compensation tube means that any failure of the inspiratory valve will result in the expiratory valve being held shut during expiration, as the increase in mask cavity pressure will be transmitted via the inlet port and

compensation tube to the reverse side of the expiratory valve. In addition, there is a third (anti-suffocation) valve that allows ambient gas to enter the mask cavity if the supply hose is obstructed, once sufficient negative pressure is produced in the mask cavity on inspiration.

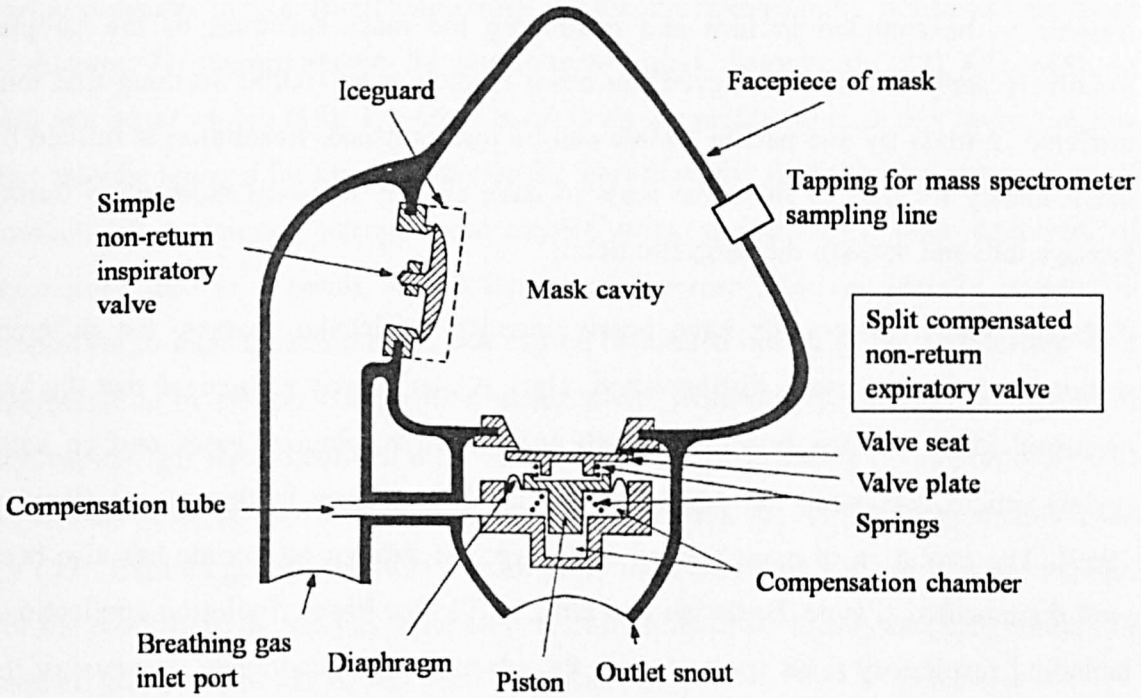


Figure 3.7 Schematic of the P/Q mask inspiratory and expiratory valve assemblies

A miniature dynamic microphone and switch assembly is mounted on the front of the mask and connects to the helmet communications system via a microphone lead. The helmet communications (pigtail) lead passes from the back of the helmet and connects to the hypobaric chamber communications system. The masks were modified to provide an access port through the exoskeleton to the mask cavity for a mass spectrometer sampling probe. As a result, good face-mask seals ensure excellent breath-by-breath analysis of respired gas composition. During the experiments reported here, the use of these masks ensured that there was no possibility of subjects re-breathing expired gas.

3.2.3 Respiratory mass spectrometry

Mass spectrometry has long been established as a highly sensitive means of gathering qualitative and quantitative information on the chemical composition of a sample substance (Throck Watson, 1976). The principle behind mass spectrometry is simple. A vaporised sample is ionised by electron bombardment and accelerated across a potential difference into a magnetic field. This takes place in a vacuum so that the influence of

molecular collisions is negligible and the accelerated ions all have the same energy as they enter the magnetic field. Heavier ions will then follow different trajectories to a detection device than those of lighter ions, where the arrival of each will generate a current proportional to that ion's beam intensity. The pattern of trajectories is adjusted by changing the strength of the magnetic field, allowing ions of different molecular weights to be sampled in turn and generating the mass spectrum of the sample. Relatively early machines achieved ion beam resolution of 10,000 meaning that ions differing in mass by one part in 10,000 can be distinguished. Resolution is limited by the tendency for ions of the same mass to have slightly different momentum during passage into and through the magnetic field.

Although many compounds have nearly identical molecular masses, the different respiratory gases are easily distinguished. Thus, it was quickly recognised that the tool promised utility for the breath-by-breath analysis of respiratory gases and an early review article documents its development and shortcomings in this regard (Fowler, 1969). The evolution of mass spectrometry over the subsequent decade has also been well documented (Payne, Bushman and Hill, 1979). For lower resolution applications, including respiratory mass spectrometry, the advent of the quadropole, in place of the magnet, offered advantages in terms of selective ion filtering, adjustable resolution, linear mass scale, tolerance to variations in initial ion energy and rapid scanning. The quadropole comprises four parallel rods, with circular cross-sections, mounted in a square array and electrically connected in diagonally opposing pairs. Specific electric fields are generated using a combination of alternating (alternating current) and constant (direct current) electric fields, sweeping through a range of voltages. This produces a mass filtering action as ions of specific mass/charge ratio will have a stable path through a specific field created by the potentials on the quadropole rods, while those with other mass/charge ratios will tend to scatter. Those with a stable path are sampled.

To maintain an extremely low operating pressure, a high vacuum 'turbomolecular' pump is employed which cannot operate against atmospheric pressure. Accordingly a 'low' vacuum pump operates first to reduce the internal pressure to a level at which the high vacuum pump can operate. In 'standby' mode these pumps remain operational. Switching to 'operate' activates a third mechanical pump which draws the sample, from ambient (atmospheric) pressure, into the inlet system, causing a pressure drop to approximately 0.5 mm Hg, with flow rate dependent primarily upon sampling line diameter. Respiratory samples can be drawn from a breathing mask and the gas content

analysed continuously to reveal the inspiratory and expiratory gas compositions characteristic of the normal breathing pattern.

The analysis is specific for gases of different molecular weights, requires tiny gas flows, and several gases can be analysed simultaneously. When considering the analysis of gas partial pressures in a 'normal' respiratory sample, the masses to be considered are those of diatomic N_2 (approximately 28 atomic mass units), diatomic O_2 (32), CO_2 (44), Ar (40) and water vapour (18). The other gases in air are present in such tiny quantities that they may be ignored for the purposes of the current study. Additionally, although small amounts of monatomic nitrogen and oxygen often appear in the mass spectrum of respiratory gas as a result of the ionisation process, they are usually present in proportion to their diatomic peers and can be calibrated out so as not to interfere with interpretation of the gas composition under study. Accordingly, the mass spectrum of dry inspired gas should contain well resolved peaks corresponding to the proportions of N_2 and O_2 in its composition, with a much smaller peak for Ar and even smaller peak for CO_2. Expired gas will contain a more prominent CO_2 peak and some water vapour. For the purpose of the studies reported here, which focus on the O_2 and CO_2 content of respired gas, the inert gas Ar has been treated as N_2.

The variations in PO_2 and PCO_2 during inspiration and expiration are of primary interest. The respiratory intrinsic variables, that is, the respiratory physiological properties that are being estimated when using mass spectrometry, are the P_AO_2 and P_ACO_2. The corresponding extrinsic variables that are measured are the end-tidal partial pressures of those gases, $P_{ET}O_2$ and $P_{ET}CO_2$. Lag times (to the start of detection of a change in sampled gas composition) are largely dependent upon sampling line length and are generally only a second or two in duration. Lag time was not critical in these experiments. For continuous analysis during expiration, satisfactorily brief response times (less than one second) are necessary to capture the changing partial pressures of O_2, N_2 and CO_2 and have been adequate in this regard for many years. The quadropole machines used in this series of experiments were capable of sampling up to 8 channels (gases) in 20 ms.

A schematic of the experimental arrangement of the mass spectrometer is shown in Figure 3.8. For experiments conducted in the hypobaric chamber the sampling capillary and the common delivery line for the calibration gases pass through the wall of the chamber. Accordingly, the mass spectrometer will sample gases from the tip of the sampling probe relative to the ambient pressure in the chamber and the quantity of each

gas in the sample may be presented in either 'normalised' or 'absolute' mode. In 'normalised' (summing) mode, the mass spectrometer presents the content of each gas measured as a proportion of the total gas sample, that is, its fractional concentration, assuming that only the gases being measured are present in the sample. Alternatively, and for the purposes of the current studies, the mass spectrometer can be calibrated to present only the O_2, N_2 and CO_2 data in 'absolute' mode, as an absolute partial pressure of each gas in mm Hg, calibrated against a selection of dry gas mixtures of known composition at each ambient pressure (equivalent altitude) at which experimental sampling is to occur. In this way the breath-by-breath end-expiratory gas tensions of O_2 and CO_2 will directly reflect the alveolar and arterial partial pressures.

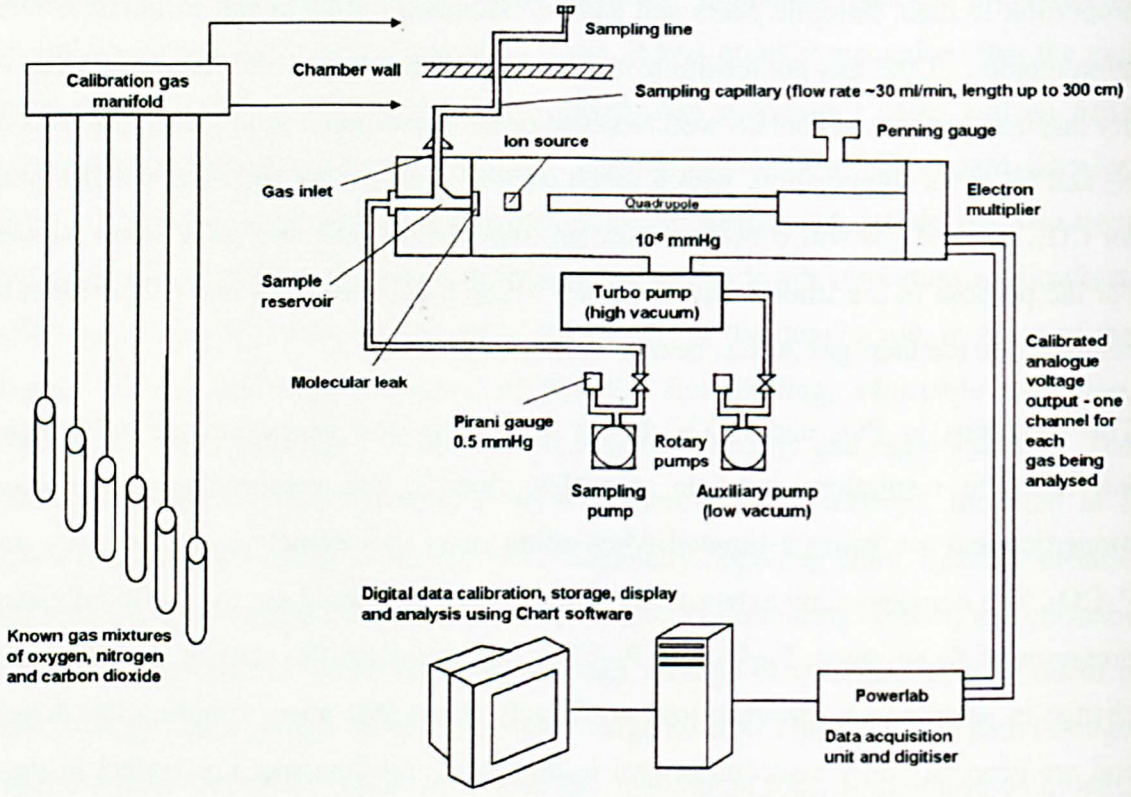


Figure 3.8 Schematic of the basic respiratory mass spectrometer experimental arrangement

For stable mass spectrometer analysis, the ion source must attain a physical and chemical equilibrium. Clearly, this is impossible when analysing respiratory gases which are constantly changing with the ventilatory cycle. Respiratory gas composition, temperature and water vapour content change from inspiration to expiration and back again. In addition, the different viscosity of component gases, their differing physical interaction with sample line materials, and chemical interactions with the filaments may all have an influence. Thus, when different inspired gases are to be used or when

analysis is undertaken over a prolonged period, repeated calibration is necessary to allow an assessment of 'drift' in source sensitivity.

Measuring and calibrating for water vapour content presents practical difficulties as water vapour condenses as it passes along sampling capillaries and moves at a different rate to the other gases in a sampled 'wave front'. Although heated steel capillaries and gas inlets can help to overcome these issues, water molecules tend to adsorb upon the unheated interior of metal vacuum systems and residual water vapour in the mass spectrometer can interfere with measurement. Knowledge of the partial pressure of water vapour in the expired gas is irrelevant to the work reported here as alveolar gas is known to be saturated with water vapour at body temperature and ambient pressure. Thus, water vapour was not measured in these experiments. Accordingly, measuring in 'normalised' mode would require a correction to be made to the results for the other expired gases to account for water vapour content. To avoid this unnecessary additional imposition all mass spectrometry measurements were made in 'absolute' mode.

Reactions occur at the heated surface of the ion-source filament as a function of the filament surface temperature and the partial pressure of O_2. The main products of this are oxides of the filament surface and carbon impurities in the filament give rise to carbon monoxide and CO_2. Significant changes in sampled O_2 concentrations therefore lead to delays in achieving steady state conditions of ion measurement, while the choice of filament current presents a balance between minimum unwanted reaction levels and maximum sensitivity. Furthermore, any increase in sensitivity adjustment made by increasing filament current is followed by a period of hysteresis during which sensitivity starts high and declines gradually over a number of hours, leading to further 'drift' which must be calibrated out immediately before and after each experiment. Nonetheless, under stable and calibrated conditions, measurements of $P_{ET}O_2$ and $P_{ET}CO_2$ are subject to a measurement error, that is physical and operator-induced error in instrument performance, of less than 1% across the physiological range ($P_{ET}O_2 < 1$ mm Hg; $P_{ET}CO_2 < 0.5$ mm Hg). When breathing high concentrations of O_2 mass spectrometer measurements of PO_2 become non-linear and are subject to greater error, but discrimination between the breathing gases used in the experiments that follow was invariably unequivocal.

During the course of these experiments, three different designs of mass spectrometer were employed (Innovision Amis 2000; Airspec MGA2000; Airspec QP9000) but the principles of their daily operation were the same. They were tuned to look for peaks of

O_2, N_2 and CO_2 and the optimal resolution setting for the mass spectrum 'peaks' was established before each series of experiments, as were the gain (signal amplification) settings for each channel (gas). The machines were routinely left switched on in 'standby' mode to maintain a high vacuum. On each experimental day the machine would be switched to 'operate' early in the morning to begin drawing an ambient air sample through the sampling probe and the sensitivity (filament current) would be adjusted to an appropriate setting. The sampling line flow rate was checked to be 30 ml min^{-1} and the ability of the machine to extract all the gas from an occluded sample line was checked to ensure that a high vacuum was being maintained with no inboard leaks. The machine would then be left to stabilise for at least an hour.

	Composition (%)			
Gas	N_2	O_2	CO_2	Ar
1	100	-	-	-
2	95.7	2	0.8	1.5
3	91	6	1.5	1.5
4	81	12	3.5	3.5
5	70	18	5.5	6.5
6	58.5	24	8.5	9
7	-	95	5	-
8	-	100	-	-

Table 3-1 Typical compositions of mass spectrometer calibration gases (ATPD)

The flow rate of each calibration gas through the common manifold was confirmed to be ~250 ml min^{-1} to ensure that an appropriate calibration gas 'envelope' would surround the sampling probe when set in a vented calibration gas delivery tube. This also ensured that the sampling probe, line and spectrometer inlet would not be over-pressurised. Once stabilised, the mass spectrometer could be 'initialised' using a known calibration gas, typically 70% N_2, 18% O_2 and 5.5% CO_2 with a balance of Ar, delivered at ambient temperature and pressure, dry (ATPD). The analogue data output voltages were then set to 'absolute'.

Immediately before each experiment a selection of calibration gases of known composition (Table 3-1) could then be passed through the machine and the analogue outputs recorded and calibrated onto a PC-based digital data recording and analysis system (ADInstruments). This calibration was effected on the basis that the 'absolute' analogue output value from the mass spectrometer reflected the partial pressure of each component of the dry calibration gas as a percentage of ambient barometric pressure in proportion to its fractional concentration in the calibration gas.

For each calibration, absolute barometric pressure was measured using a calibrated Druck DPI 740 'precision pressure indicator' or, for the hypobaric chamber experiments, the indicated barometric pressure on the hypobaric chamber instrument panels. For the hypobaric exposures, mass spectrometer calibration was conducted at GL and at each equivalent altitude under study. None of these 'altitude' assessments were conducted at sufficiently low ambient pressure to cause problems with reduced mass flow of gas through the sampling capillary, which might otherwise compromise the mass spectrometer signal. In addition to including at least four different $O_2/N_2/CO_2$ gas mixtures, calibrations also included the use of 100% N_2 to provide a 'zero' for O_2 and CO_2, and 100% O_2 to provide an indication (relative to barometric pressure) of the accuracy of the $P_{ET}O_2$ measurements when using 100% O_2 as the breathing gas. As well as calibrating again at the end, when experiments were prolonged beyond 90 minutes, additional calibrations were undertaken between conditions to allow further assessment of any mass spectrometer 'drift'.

3.3 *Contemporaneous Visual and Respiratory Adaptation*

The conduct of experiments intended to examine mesopic visual performance under a variety of respiratory challenges presents a number of technical difficulties. There is a requirement to ensure that assessment of visual performance between subjects is undertaken under identical, stable and reproducible visual and respiratory adaptation states. This demands careful control of both respired gas composition and the light reaching the subject's eyes. The former necessitates a breathing mask of some sort, maintenance of an excellent face-mask seal, and continuous monitoring of mask cavity gas composition. The latter requires objective and unambiguous physical control of the light viewed by the subject at all times.

To ensure that stable mesopic adaptation states were achieved, some of the experiments were conducted following periods of retinal bleach and dark adaptation, requiring the exclusion of all light during dark adaptation but enabling prior retinal bleach and subsequent light adaptation to a mesopic light level. Ambient light levels in the laboratory could be minimised but not reduced to complete darkness, for example due to dim light entering via the roof space, the requirement to view dimmed displays to monitor the face-mask seal, a red glow from the pulse oximeter finger probe, light emitting diodes on various items of equipment including emergency alarms, and, most

obviously by far, the vision test displays when primed for testing. Furthermore, there was a requirement for the investigator to move around the laboratory, for example to save data, switch breathing gases, set up the next vision test, assist the subject and so on. Thus, a minimum ambient light level was preferred to complete darkness during these experiments.

Using simple eyelid closure to exclude light was regarded as insufficiently objective and rigorous, at least for the early experiments. It was also desirable to avoid any patch that might put pressure on an eye if subsequent testing with that eye was required. Logically, these demands indicated the use of some form of subject visor, goggle or shroud to control the light reaching the subject. Various simple devices were assessed and modified and some would have been useful were it not for the requirement to integrate the visual apparatus with the respiratory mask and its supporting cloth helmet, which proved impractical.

The solution identified was to modify an Aircrew Respirator Mk 5 (AR5). The AR5 is an obsolescent respirator designed to protect aircrew skin, eyes and respiratory tract from the effects of particulate, droplet and gaseous contamination. In essence, it comprises a P/Q-type aircrew mask mounted on an expanded exoskeleton attached to a transparent polycarbonate visor and incorporating an inlet for a blown air supply and a drinking facility. The exoskeleton and visor are attached to a butyl rubber hood that envelops the head and covers the shoulders, with a relatively narrow neck and, normally, an integral rubber neck seal.

A number of modifications were made and are illustrated in Figure 3.9. The visors were modified by cutting away the bulk of the polycarbonate in front of each eye, creating viewing ports that allowed both eyes to have unobstructed central visual fields and eliminating any requirement to consider the cleanliness of the visor and the optical characteristics of the polycarbonate. The residual polycarbonate was painted matt black and its external surface was covered with Velcro. The neck seals were removed to enhance subject comfort during prolonged wear and a mass spectrometer tapping provided a port for direct access to the mask cavity using a sampling probe. Two respirators were modified in this way, one with the larger P-size mask (suitable for most males) and one with a Q-size mask (suitable for most females). These were found to provide good face-mask seals during the study procedures without the need for external support using a cloth helmet. Accordingly, much of the mask suspension apparatus was also removed. The anti-suffocation valve was not required and was sealed shut to

exclude light but the microphone was retained for the same reason. The drinking facility was retained as it did not admit light and enabled subjects to drink water through a flexible plastic straw during prolonged exposures, when not undertaking vision testing.

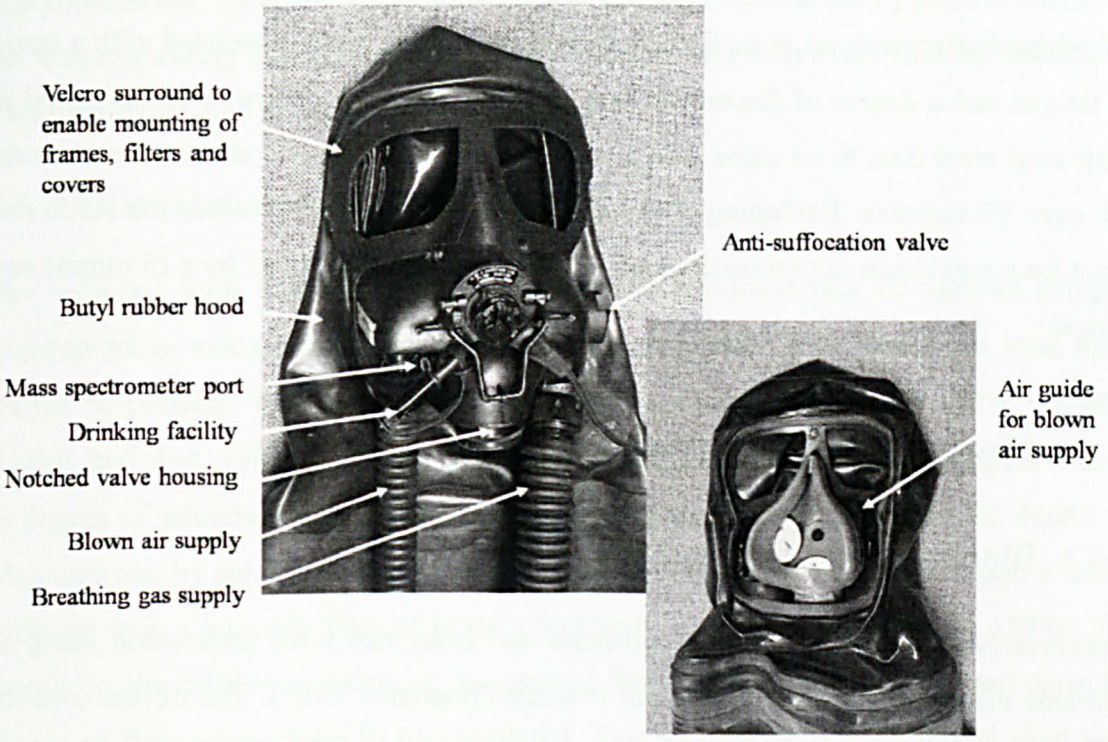


Figure 3.9 Aircrew Respirator Mk 5 modified to facilitate visual adaptation

Other AR5 polycarbonate visors were modified in the same way as the respirator visor to provide matching polycarbonate frames. These were also painted matt black and used as scaffolds upon which to mount 0.1 mm-thick Kodak Wratten neutral density (ND) gelatine filters of differing OD 1.0 and 2.0. Both sides of the polycarbonate filter frames were also covered with Velcro. The convex surface then permitted secure attachment to the respirator Velcro and was found to exclude light perfectly. The concave external surface allowed shaped Velcro covers to be applied to occlude one or both eyes.

As anticipated, when the filters were *in situ* their facial aspect was prone to early misting, rapidly obscuring vision. However, the respirator can be supplied with a gently blown air drawn from ambient and this was effective at preventing misting bilaterally. The air guide on the internal aspect of the respirator deflects the blown air supply away from the eyes and across the inner aspect of the visor. Eye irritation obliged a single experiment to be abandoned but this was subsequently repeated without incident. No other experiments were lost or confounded due either to eye irritation or filter misting.

Nonetheless, use of the AR5 demanded a high level of subject motivation. It can be uncomfortable to wear for protracted periods due to the firmness of the breathing mask, and it imposes a thermal load that promotes sweating across the head and wherever skin is in contact with the butyl rubber. The blown air supply assists with cooling and feels refreshing but continuous wear for 90 minutes or more is often associated with a sense of fatigue and a degree of discomfort that is distracting. Experiments were designed to limit total wear time to no more than 3 h, with rest periods to prevent continuous wear for over 90 minutes. Excluding donning, doffing and instrumentation, the AR5 was worn for a maximum of two sessions of ~75 minutes each, separated by a 15 minute rest period.

3.4 Physiological Monitoring

3.4.1 Blood pressure and heart rate

Non-invasive monitoring of blood pressure and heart rate were undertaken using an Ohmeda Finapres 2300 blood pressure monitor (Boehmer, 1987). The device uses the Peñaz photoplethysmographic method to measure beat-to-beat blood pressure in a finger wrapped in an inflatable cuff (Peñaz, 1973). The cuff volume is held constant by varying its pneumatic inflation pressure as controlled by a photoelectric signal from the cuff. This varies with the tendency of the digital arteries to change in size and is sensed by measuring the corresponding tendency for the arterial blood to absorb different amounts of light using a light source and sensor contained in the cuff. The device thereby generates a pressure waveform that has been shown to correlate closely with continuous intra-arterial measurements and has been validated against such measurements during hypobaric experimentation (Gradwell, 1993).

The pressure measured in the finger varies with the hydrostatic pressure gradient imposed by having the finger cuff at a different level from the heart. This can be corrected for but the pressure being measured in the finger will still differ from the absolute pressure that is normally measured at a more proximal (brachial) site in the arterial tree. However, in these experiments measurements of absolute pressure were not required. Instead the recordings were used to establish any differences in blood pressure that resulted from the imposed respiratory condition by ensuring that subjects kept their Finapres hand in the same position for both control and condition exposures.

The transducer of the Finapres patient interface module was calibrated on a daily basis against an independently-calibrated Druck DPI 701 pressure gauge, at approximately 50 mm Hg intervals, across a pressure range from 0 to 200 mm Hg. During hypobaric experimentation, the main body of the device was kept outside the hypobaric chamber, the lead connecting it to the hand-mounted servo unit and finger cuff passing through an instrumentation port in the wall of the chamber.

3.4.2 Arterial oxygen saturation

The subjects' S_aO_2 was estimated and monitored non-invasively throughout using a Kontron pulse oximeter model 7840 with a finger probe. This model has long been known to perform well in terms of measurement precision and bias (Severinghaus, Naifeh, and Koh, 1988). The light absorption characteristics of Hb change according to its degree of saturation with O_2 and pulse oximetry uses this to determine the degree of Hb saturation by shining red and infrared light of known wavelengths through a tissue perfused with a pulsatile flow of blood (Wukitsch, Petterson, Tobler *et al*, 1988). The change in the relative amounts of transmitted light between the systolic and diastolic phases of flow allows S_aO_2 to be calculated. The performance of the model used here has been assessed under hypobaric conditions and was reported to be the most rapidly responding of a range of oximeters when exposed to step changes in P_AO_2 (Young, Jewkes, Spittal *et al*, 1992). It has now been in routine use for medical monitoring of subjects undergoing hypobaric exposures for many years and has proven to be extremely reliable. It is specified to provide an analogue signal output where 1000 mV represents 100% saturation and 0 mV represents 40% saturation with a linear response between these extremes. The monitor was calibrated daily by using the voltage output when the finger probe was not applied as the 'zero' (40%) signal and the voltage output when displaying a stable measurement of the author's resting S_aO_2 as the upper calibration data point (typically 97%). As with the Finapres, during hypobaric studies the main oximeter was kept outside the chamber with the lead from the finger probe passing through one of the instrumentation ports.

Analogue outputs from both the blood pressure and O_2 saturation monitors were routed, together with the mass spectrometer data, to the ADInstruments data sampling system and then calibrated and recorded on a PC running complementary data analysis software.

3.5 *Ancillary Visual Apparatus*

3.5.1 Light box

The same light box was used for all periods of light adaptation in the experiments that follow. On each occasion, light adaptation exposures lasted for 5 minutes and were timed from switching on the light box. Measurements of the intensity of this bleaching light, timed from switching on the light box, are shown in the scatter plot at Figure 3.10. A total of 42 sets of data were obtained. Measurements of light intensity were made perpendicular to the centre of the box, under dim ambient light conditions (1-2 lux), using a calibrated Minolta luminance photometer. Data were recorded for 6 minutes, initially at 15 s and 30 s following switch on, and then every 30 s.

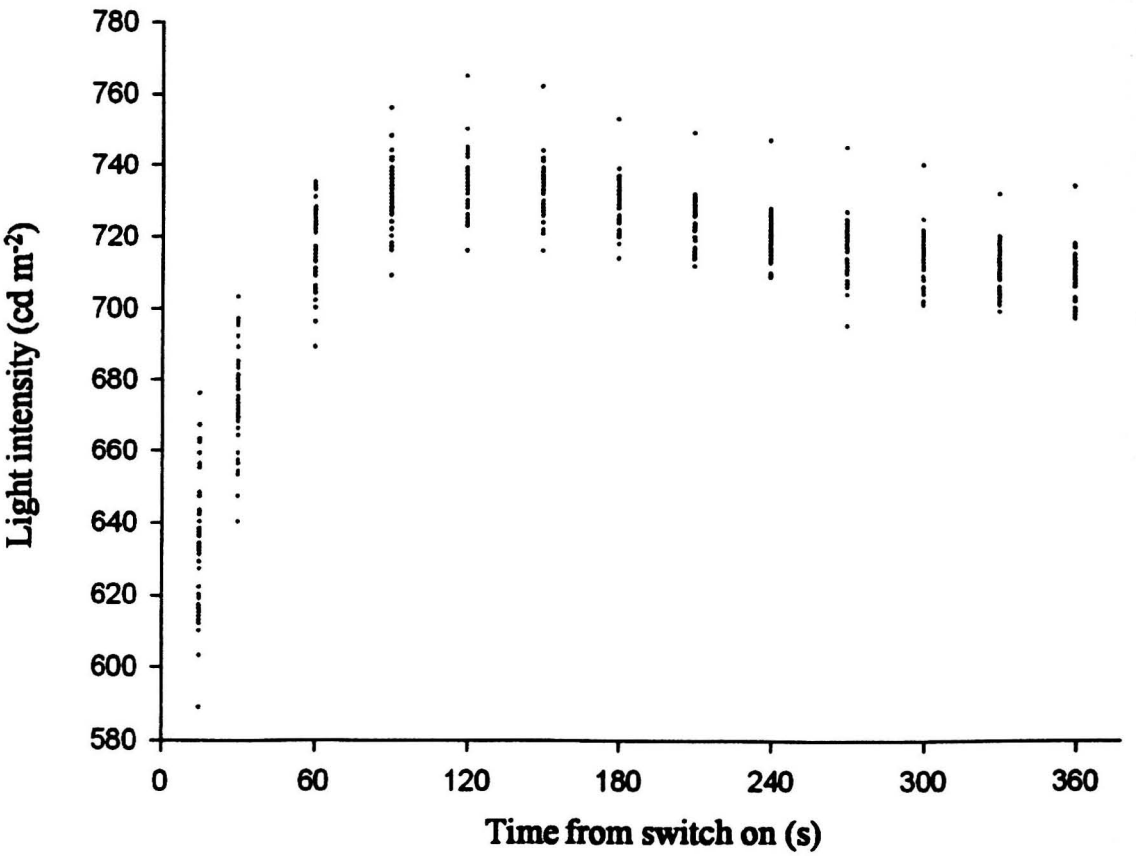


Figure 3.10 Scatter plot of light box intensity for six minutes from switch on

From Figure 3.10 it can be seen that light intensity almost invariably exceeded 600 cd m^{-2} by 15 s after switching on the box and was generally over 700 cd m^{-2} by 60 s. Light intensity then remained at 700-750 cd m^{-2} for the remainder of the 5 minute bleaching exposures. The data indicate a reasonably consistent light box response that is readily apparent when considering mean ± 1 and 2 SD plots in Figure 3.11.

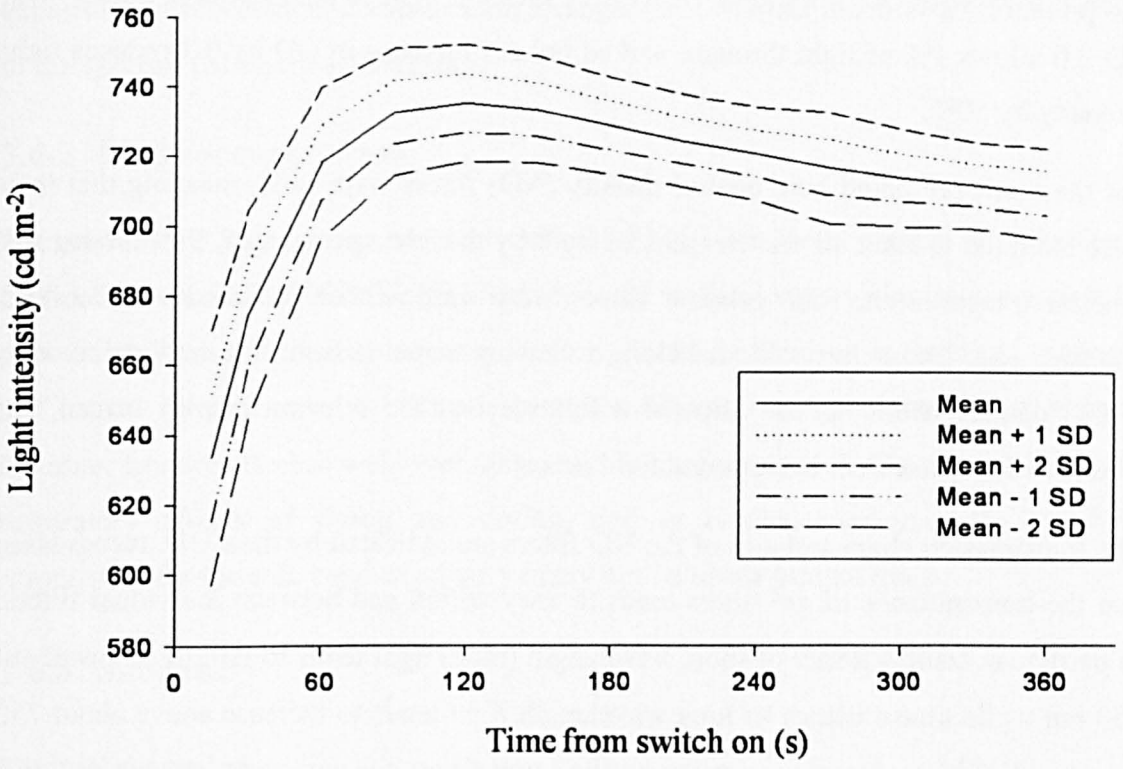


Figure 3.11 Mean (± 1 SD and ± 2 SD) light box intensity following switch on

Light box performance varied slightly between days, possibly in relation to ambient temperature, but was highly consistent within days. It is not considered that this will have confounded testing under the chosen mesopic adaptation states following subsequent dark and light adaptation. The illuminant had a colour temperature of 6500K.

3.5.2 Neutral density filters

The OD of a filter, D, is defined in terms of the amount of light that it allows to pass through or its transmittance, T, as shown in Equation 12.

$$D = \log_{10} (1/T)$$

Equation 12 Expression for the optical density of a filter

Alternatively, the transmittance may be expressed as shown in Equation 13.

$$T = 10^{-D}$$

Equation 13 Expression for the transmittance of a filter

Thus, a filter with OD 1.0 allows 10% of the incident light to pass through, a filter with OD 2.0 allows 1% of light through, and so on. An increase in OD by 0.3 reduces light intensity by 50%.

For the work presented here neutral density (ND) filters were used, meaning that they were intended to filter all wavelengths of light by the same proportion, thus having flat spectral transmission. Soft gelatine filters were used, either mounted on the AR5 polycarbonate frames or positioned along a viewing tunnel in front of a cathode ray tube (CRT) display. Both options allowed a full view of the relevant display screen and therefore determined net background field intensity.

The transmission characteristics of the ND filters are indicated by their OD, recognising that the transmittance of gel filters tends to vary within and between individual filters. In particular, transmittance of short wavelength (blue) light tends to fall off below about 450 nm while transmittance of long wavelength light tends to increase above about 750 nm. As all subject exposures employed the same filters for any given experiment, this should not have introduced any error when considering effects of respiratory condition, but the light level estimations will be approximate. However, specifically for the colour vision assessment of normal trichromats at reduced light levels under respiratory disturbance, the spectral transmission characteristics of the gel filters used with the AR5 are described in detail in Chapter 7.

3.6 Ethical Considerations

3.6.1 Ethical procedures and administration

All studies were conducted in accordance with experimental protocols that were approved in advance by an independent Local Research Ethics Committee. All studies adhered to the Declaration of Helsinki. Each subject was briefed in detail for each individual experiment and provided written informed consent before participating. Subjects were free to discontinue an experiment at any time and/or to withdraw their participation from further experiments without explanation. A Trial Dossier documented all supporting activity for each experiment including the relevant experimental protocol, Ethics Committee approval, laboratory and experimental risk assessments, subject information sheets, consent forms, medical decision forms, logs of subject exposures,

experimental procedures, compensation arrangements, subject disposal and final reports to the sponsor (Ministry of Defence).

3.6.2 Medical supervision

The nominated Supervising Medical Officer (SMO) for these studies was Professor David Denison PhD FRCP. He fulfilled the local medical supervision requirements for potentially 'high risk' human experimentation. Professor Denison is Emeritus Professor of Clinical Physiology at the Royal Brompton Hospital and National Heart & Lung Institute, London. He has well over 40 years of experience of full time research into the respiratory effects of flying and diving, and in clinical medicine. He has been responsible for the safe conduct of very many high altitude human trials.

3.6.3 Subjects

Medical screening

All subjects were interviewed and examined by independent and experienced medical examiners. The interview was based on a medical screening questionnaire designed to assess fitness for environmental exposures and modified to include suitability for hypobaric (altitude chamber), respiratory and visual studies, including exposure to confined spaces and dark. The questionnaire is reproduced at Appendix 2. The general medical examination included assessment of the cardiovascular and respiratory systems, abdominal examination, and assessment of ears, nose and throat for hypobaric exposures. Screening investigations included dipstick urinalysis and electrocardiogram to exclude underlying conditions that might be aggravated by hypoxia exposure, such as diabetes mellitus and ischaemic heart disease. Ongoing medical fitness was confirmed before each experiment.

Ophthalmic screening

The same examiners also performed comprehensive ophthalmic and visual assessments. A detailed ophthalmic history included specific enquiry into any history of visual disturbance, loss of vision, squint, eye infection or inflammation, painful or itchy eyes, eye trauma or injury, eye surgery, wearing of glasses or contact lenses, family history of eye disease and any personal history of oral or topical eye treatments. As a minimum, ophthalmic examination included assessment of near and distant (Snellen) acuity, accommodation, convergence, visual fields (by confrontation), ocular movements and

alignment, pupillary reactions, external inspection and ophthalmoscopy. Colour vision was assessed using Ishihara's pseudoisochromatic plates (PIPs), requiring all of the first 17 plates to be identified correctly. Additionally, subjects undertaking colour vision experiments were assessed using the Type I Nagel anomaloscope.

Subject exclusion

Generally, subjects were required to demonstrate a level of well being and fitness commensurate with UK military aircrew. Subjects were aged between 18 and 40 y and any long-term medical conditions or ongoing requirement for significant medication were generally disqualifying.

The following exclusion criteria were applied:

- Any history or examination findings suggestive of a condition that may be aggravated by hypoxia or voluntary hypocapnia.
- Any significant cardiovascular or respiratory condition.
- Any regular ocular medication including eye drops.
- Any requirement for spectacles or contact lenses unless correcting to 6/6 or better.
- Any significant past or current history of eye disease, eye surgery, eye injury, family history of eye disease, ocular abnormality or obvious visual deficiency including early presbyopia.
- Any fear, phobia or dislike of the dark or confined spaces.
- Any dental procedures performed less than 48 h prior to altitude exposure.
- Any significant neurological disease including migraine and epilepsy.
- Any ear, nose or throat surgery within the previous 6 months.
- Any use of drugs or medication (prescribed or non-prescribed, with the exception of paracetamol and oral hormonal contraception) in the 24 h prior to altitude exposure, except at the discretion of the medical examiner.
- Any other exposure to hypobaric or hyperbaric environments from 48 h prior to the start of the study until the study was complete.
- Any intercurrent illness.
- Any other finding or condition considered exclusive by the supervising medical officer.

Additionally, female subjects were required to demonstrate a negative urine pregnancy test before each experimental session.

Two healthy subjects were allowed to continue with experimentation while taking medication. One male was finishing a prolonged course of flucloxacillin for a resolving soft tissue infection. The other, a female, was well-established on long-term isotretinoin therapy for acne rosacea. Neither subjects' data nor their responses to respiratory challenge suggested a confounding effect of their medication. Female subjects using oral hormonal contraception were accepted for these studies and details of the preparations being used were noted.

Withdrawal criteria

Experiments were to be curtailed if any of the following criteria were met:

- At the subject's request.
- At the request of the principal investigator, supervising medical officer or any experimenter.
- Upon failure of any safety-related monitoring (as opposed to physiological data gathering) equipment.
- If the subject demonstrated any signs of distress or impending syncope.
- If the $P_{ET}O_2$ fell to/below 40 mm Hg for consecutive breaths.
- If the $P_{ET}CO_2$ fell consistently below 20 mm Hg.
- If the subject's S_aO_2 fell below 70% at any time.
- If the subject complained of anything other than trivial symptoms of hypoxia.
- If the subject complained of anything other than trivial symptoms of hypocapnia.
- If the subject complained of significant headache, abdominal discomfort, symptoms suggesting decompression illness or any other significant symptoms.
- If the subject or any experimenter expressed any anxiety due to darkness or claustrophobia.
- If there was any failure of safety-related communications equipment.
- If the ambient O_2 concentration in the laboratory/chamber exceeded normal levels at any time (indicating a potentially hazardous leak of O_2).

Training

The hypobaric chamber study employed experienced altitude subjects who were familiar with breathing through aircrew masks from pressure-demand regulators and who were familiar with the hypobaric chamber environment. All had first-hand practical experience of severe hypobaric hypoxia during training exposures at an equivalent altitude of 25,000 ft, a local requirement for hypobaric chamber experiments involving

exposure to altitudes in excess of 10,000 ft. The subjects were also trained in the technique of voluntary hyperventilation employed in the study, controlling their rate and depth of ventilation in accordance with guidance from experimenters monitoring $P_{ET}CO_2$ breath by breath using mass spectrometry.

For the GL breathing gas mixture studies, subjects were familiarised with breathing from a demand regulator through a mask until they were comfortable with the procedure. Subjects undertaking the hyperventilation exposures reported in Chapter 5 were to be trained in the technique to ensure they could tolerate the respiratory effort required and any mild symptoms that might ensue. In practice, this proved unnecessary.

All subjects were familiarised and trained, as required, on the various vision tests.

Inconvenience payment

The wearing of 'G' helmets and masks for protracted periods can be uncomfortable. This was minimised by careful fitting and was generally well tolerated during the relatively brief exposures conducted for this study. However, the same is not true of the AR5. Its use is generally regarded as mildly unpleasant and associated with a degree of discomfort from the enclosing butyl rubber hood and the firm face mask. In the most demanding experiments, subjects wore the AR5 for two periods of ~75 minutes, separated by a 10-15 minute break. In view of this imposition, a subject inconvenience payment of £25 was approved for each session requiring use of the AR5.

Respiratory exposure limits

Exposure to hypoxia must be constrained in terms of duration and severity, limited in these experiments to no more than one hour of acute exposure to a maximum equivalent altitude of 15,000 ft. In practice, exposures were either much shorter or much milder than this. Symptoms of hypocapnia generally begin when P_ACO_2 falls below about 25 mm Hg but only become severe below 20 mm Hg, with hyperventilation-induced loss of consciousness generally only occurring below about 15 mm Hg. Accordingly, a lower $P_{ET}CO_2$ limit of 20 mm Hg was established. On the few occasions that $P_{ET}CO_2$ fell transiently below this level, subjects were asked to breathe normally until $P_{ET}CO_2$ rose above this level. Under normobaric and hypobaric conditions, as opposed to hyperbaric pressures, healthy adults may be exposed to 100% O_2 for many hours without adverse effect.

Subject requirements and compliance

Both smoking (carbon monoxide exposure) and alcohol have been associated with enhanced effects of hypoxia in relation to visual and cognitive impairment (McFarland, 1971). The subjects in all the experiments reported here were non-smokers. They were also asked to avoid alcohol for 24 h before a morning experiment and to limit consumption to two units during the evening before an afternoon experiment. In addition, they were asked to avoid caffeine-containing drinks on the day of an experiment. Otherwise subjects were asked to maintain their normal lifestyle and to eat normally before each experiment. They were also asked to ensure a good night's sleep before a study day and to attend in good time and well rested before the start of each exposure. Two experiments were either postponed or discontinued and rearranged as a result of subject fatigue following sleep loss the previous night.

Before each experimental session involving altered breathing gas mixtures, subjects completed a lifestyle questionnaire, reproduced at Appendix 3, to enable assessment of compliance with the subject requirements and identification of any potentially confounding factors.

4 Dark Adaptation

4.1 Abstract

Purpose. This study was intended primarily to inform a procedure of visual adaptation for use in subsequent mesopic work by demonstrating, under conditions of hypoxia, hyperoxia and hypocapnia, the delay time to achievement of scotopic sensitivity (cone rod inflection) during dark adaptation. Secondary aims were to assess the hypothesis that cone rod inflection time might be affected by respiratory disturbance; to provide baseline physiological data for comparison with subsequent normobaric experiments; and to assess the possible influence of these respiratory disturbances on the night vision of contemporary aircrew.

Methods. Five male subjects with normal vision (aged 22 to 35 y) were exposed to mild hypoxia (equivalent to breathing air at 10,000 ft and 15,000 ft); moderate hypocapnia ($P_{ET}CO_2$ of 25 mm Hg at GL and 15,000 ft); and hyperoxia (breathing 100% O_2 at GL) in a hypobaric chamber. Respiratory status was monitored continuously by respiratory mass spectrometry. Dark adaptations were conducted by measuring detection time for progressively dimmer, green, spot flash stimuli, presented every 5 s using a Friedmann Visual Field Analyser Mk II. Stimulus intensity was decreased in 0.2 $\log_{10}$ unit steps following detection. Normoxic control exposures were conducted breathing air at GL. Variability in normoxic dark adaptation within and between subjects was examined using analysis of variance (ANOVA). Stimulus detection time displacements were assessed relative to normoxic control exposures using ANOVA and paired *t* tests.

Results. Significant effects on the delay time to achievement and subsequent early progression of scotopic sensitivity were seen with all three respiratory disturbances. Progressive hypobaric hypoxia delays scotopic sensitivity in a 'dose dependent' fashion. Supplementary O_2 and hypocapnia both hasten scotopic sensitivity. The magnitude of the effect of hypocapnia is sufficient to overcome that of hypoxia at 15,000 ft.

Conclusions. The effect of hyperoxia to hasten cone rod inflection at GL implies that rod photoreceptors are functionally hypoxic in the dark under normal respiratory conditions. The effects of hypoxia are progressive with increasing altitude and include horizontal shifts of early scotopic sensitivity as well as vertical shifts of late rod sensitivity. Hence hypoxia has both photosensitivity and neural 'gain' effects. The delay of cone rod inflection suggests that hypoxia impairs regeneration of rhodopsin *in vivo*. Temporal shifts of early scotopic sensitivity appear dependent on both PO_2 and pH, the latter implied by the effect of changing PCO_2, suggesting a continuum of response to both gases. The effects of hypoxia on rod function imply that it should influence mesopic visual sensitivity, such that increasing rod QO_2 may compromise visual performance progressively with decreasing light. Aircrew vision in dim light may be compromised by mild hypoxia and enhanced with supplementary O_2, even at GL.

Citation. The work reported in this chapter has been subject to peer review and is published at **Connolly, D. M., and Hosking, S. L. (2006) Aviation-related respiratory gas disturbances affect dark adaptation: A reappraisal.** ***Vision Research*****, 46: 1784-1793**. The paper is reproduced at Appendix 9.

4.2 *Introduction*

4.2.1 Background

In order to investigate effects of respiratory disturbance on mesopic visual performance, subjects must establish stable states of mesopic adaptation that can be reproduced reliably within and between individuals. The requirement to adapt also to a variety of imposed respiratory conditions means that the effects of those respiratory conditions must be shown not to compromise achievement of stable mesopic adaptation. Cone adaptation rapidly enables early sensitivity to stimuli in the mesopic range but rod adaptation is slower. If mesopic vision testing is undertaken before rod adaptation is complete then the results may be confounded. However, mesopic adaptation may be achieved by undertaking a process of dark adaptation followed by light adaptation to the desired mesopic level, illustrated in Figure 4.1. If the phase of rapid light adaptation, that is, exposure to a mesopic background field, is known to follow the point of cone rod inflection during dark adaptation, then a stable state of mesopic adaptation will be established readily, reliably and repeatably, within and between subjects.

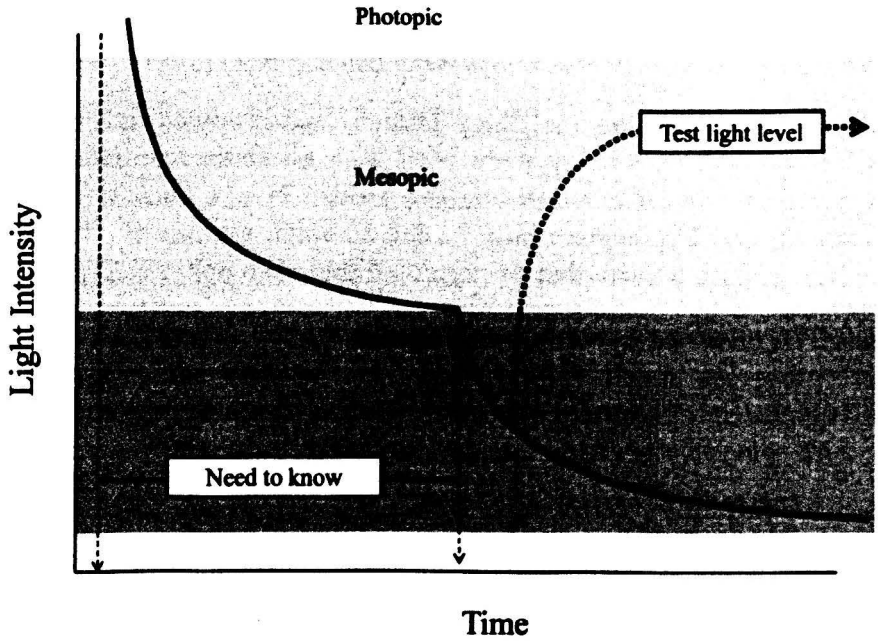


Figure 4.1 Principle of mesopic adaptation demanding knowledge of cone rod inflection time

This method can only be established if an identifiable level of rod adaptation to dark can be measured and timed under the various respiratory conditions to be imposed. The

obvious feature to identify is the cone rod inflection point, which marks attainment of scotopic sensitivity. Knowledge of the effect of respiratory challenge on the time to cone rod inflection will allow a duration of initial dark adaptation to be determined that ensures that subsequent exposure to a mesopic luminance results in light adaptation of both cones and rods (dotted line in Figure 4.1). It may then be assumed that a stable mesopic adaptation state will be achieved rapidly by both cones and rods, regardless of respiratory status.

Past studies indicate that hypoxia compromises cone and rod threshold sensitivity during adaptation to dark as well as the absolute sensitivity that cones and rods can achieve (see section 1.6.1). The effect is generally accepted as displacing the mixed dark adaptation curve vertically without affecting the timing of cone rod inflection. However, studies have not focused attention specifically on the time to the cone rod break while many have tended to mask the actual inflection point. Furthermore, the effects of hyperoxia and hypocapnia on the process of dark adaptation have not been reported. Accordingly, the time from the start of dark adaptation to cone rod inflection, identified as the 'need to know' time in Figure 4.1, must be established under these three respiratory disturbances. It should then be possible to devise an appropriate and readily reproducible process of visual adaptation that ensures unequivocal and stable mesopic adaptation, following a known retinal light history, regardless of respiratory condition.

4.2.2 Hypotheses

During dark adaptation, the outer retina is likely to be highly vulnerable to exogenous hypoxia, delaying the time to achievement of scotopic sensitivity.

Supplementary O_2 might hasten rod adaptation to dark and achievement of scotopic sensitivity.

Hypocapnia might influence dark adaptation rate..

4.2.3 Aims

First and foremost, the dark adaptation experiments were intended to establish the time to cone rod inflection under normal respiratory conditions and under hypoxia, hyperoxia and hypocapnia, so enabling determination of an unambiguous procedure for mesopic adaptation under respiratory challenge (section 4.5.5).

Second, the experiments were conducted in the hypobaric chamber to generate cardio-respiratory physiological data representative of exposure to low ambient pressures. This will provide baseline data to compare with the physiological responses to breathing gas mixtures used in later experiments (section 4.3.6).

Third, the underlying hypotheses were to be investigated and assessed (section 4.5.7).

Fourth, it was intended to use respiratory challenges of relevance in aviation to enable applied data to be derived and interpreted in relation to aircrew visual performance during flight (section 11.2.1).

4.2.4 Experimental design

Limited hypobaric chamber time was available, including time to modify the chamber for dark adaptation and set up the experiment. This limited the number of respiratory conditions and subjects that could be studied, with any increase in one obliging reduction of the other. Five respiratory conditions were considered necessary to meet the aims of the study. These were mild and moderate hypoxia, moderate hyperventilation, moderate hypoxia plus moderate hyperventilation, and hyperoxia. The decision to assess five conditions limited the number of subjects to six. This was considered acceptable as the primary aim of the study was to measure time to cone rod inflection rather than to demonstrate statistically significant differences between the respiratory conditions, which were not anticipated. Nonetheless, considerable variability in dark adaptation rate between subjects was expected. Thus, the small number of subjects required that data should be paired within subjects to identify any meaningful trends of respiratory conditions to influence the time to the inflection point. Accordingly, a within-subjects, repeated measures design was adopted, with each dark adaptation under an imposed respiratory condition being preceded by a control dark adaptation breathing air (normoxia) at GL, using identical procedures. Thus, each subject acted as his own control.

The normoxic control exposure was always conducted first to avoid any delayed effect of an imposed respiratory condition confounding a subsequent control, that is, to avoid post-exposure respiratory 'noise' affecting control data. The small number of subjects and the lack of balance in the order respiratory control and condition exposures are obvious limitations of the study. To mitigate these, the order in which the subjects undertook the various respiratory experiments was semi-randomised and, at all times,

subjects were masked as to whether they were breathing air or 100% O_2, both for the conditions and for the control exposures. One benefit of the consistent use of an initial control run was to allow the control dark adaptations to be assessed as a group to determine variability in dark adaptation, within and between subjects, under normoxic conditions.

Vision testing employed an unconventional method of measuring the increase in visual sensitivity during dark adaptation, intended to highlight the timing of the inflection point during mixed cone and rod adaptation. Traditionally, threshold sensitivity to a stimulus has been measured at given time intervals following offset of a bleaching light, represented in graph A of Figure 4.2, and showing how threshold sensitivity at the moment of cone rod inflection may be missed or misinterpreted. Instead of using this 'bottom up' method, the curve can be accessed from 'left to right' by presenting progressively dimmer stimuli in a 'stepwise' manner, flashing each at regular intervals until seen, as illustrated in graph B.

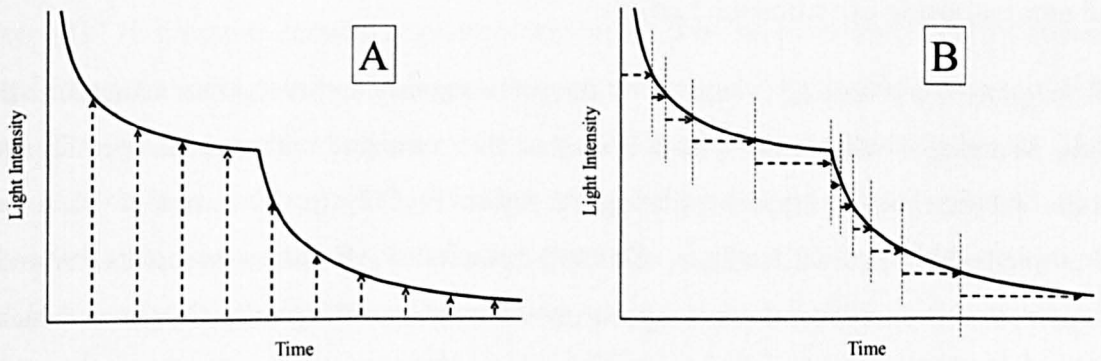


Figure 4.2 Alternative methods of establishing threshold sensitivity during dark adaptation

When using fixed stimulus intensities, following detection of the first stimulus, the time lag between successive stimuli should increase as the cone curve flattens towards its asymptote. The lag between stimuli should then be reduced abruptly immediately after the inflection point, when rod sensitivity rapidly increases, before extending once more as absolute sensitivity is approached. From graph B it can be seen that rapid detection of successive early rod stimuli should provide an indication of the likely timing of cone rod inflection as long as the relative intensities of the stimuli are known.

Whereas the traditional method highlights vertical shifts in sensitivity, the left-to-right method adopted here was intended to emphasise any lateral shifts of the initial ('early') phase of scotopic sensitivity under respiratory challenge. This necessitates dark

adaptation from a sufficient state of retinal bleach to generate an inflection point and the use of a stimulus to which both cones and rods are sensitive.

4.3 Materials and Methods

4.3.1 Subjects

Six healthy male volunteers were recruited for the study from a pool of experienced hypobaric chamber subjects in order to minimise hypobaric familiarisation and training requirements. One subject became unavailable at short notice for reasons unrelated to the study. The mean age of the five remaining participants was 28 y (range 22 to 35 y). All were familiar and comfortable with breathing through aircrew masks from pressure-demand breathing regulators, with the conduct of hypobaric chamber exposures, and with the subjective nature of decompression and recompression. Accordingly, it was judged that attempts to disguise GL exposures as hypobaric would be unlikely to deceive the subjects and it was impractical to do so without unnecessarily complicating and compromising experimental timings.

All subjects were trained in the technique of voluntary hyperventilation used in the study. Wearing a 'G' helmet with a P/Q mask instrumented with a mass spectrometer probe, subjects began hyperventilating to lower $P_{ET}CO_2$ to 25 mm Hg. This was accomplished with verbal feedback of breath-by-breath $P_{ET}CO_2$ measurements that were monitored constantly by a mass spectrometer operator. Target $P_{ET}CO_2$ was usually achieved within a minute or so whereupon the rate and depth of ventilation could be relaxed, maintaining the target level comfortably with relatively gentle effort. Once established at the target $P_{ET}CO_2$ minor adjustment of breathing rate and/or depth was prompted if the $P_{ET}CO_2$ drifted away from 25 mm Hg by more than one mm Hg. In this way, stable hypocapnia could be maintained easily for 45 minutes or more. Each subject required only a single training session to feel comfortable with the technique.

4.3.2 Equipment

Hypobaric chamber

Only the large compartment of the hypobaric chamber was used and the internal door to the small compartment was therefore sealed to exclude external light (Figure 3.1). The vacuum pumps were used to decompress the large compartment, controlled using the

appropriate ascent valve to limit the rate of 'ascent' to no more than an equivalent of 5000 ft min^{-1} and holding the chamber at the required target pressure (to within ± 0.5 mm Hg). Following the experiment, recompression was effected using the large compartment descent valve, allowing ambient air to enter the chamber at a controlled descent rate of no more than 5000 ft min^{-1}. To exclude external light during these dark adaptation experiments, the windows of the large compartment were occluded using opaque black cloth sandwiched between the two window layers. The chamber door seals were effective at excluding light both during decompression and when held closed during exposures undertaken at GL. Additionally, external lighting levels were minimised to reduce the possibility of stray light gaining access to the chamber. An inside observer (IO) was present for all exposures to operate the vision test apparatus and to act as 'safety person'. A torch and a night vision device were provided for use by the IO in the event of an emergency. Internal chamber lighting was controlled from outside the chamber; when on, mean illumination at subject eye level was ~1100 lux.

Breathing gas supply

Two Mk17F pressure-demand regulators were used. The first was one of the permanent, wall-mounted regulators in the large compartment, supplied with high pressure 100% O_2. The second was installed in the large compartment specifically for these experiments and was supplied with high pressure air from a source external to the chamber. The airmix lever was selected to '100% oxygen' on both regulators so that the first only delivered 100% O_2 and the second only delivered air from its high pressure supply, that is to say, neither supply gas was diluted with ambient air at any stage. Subjects wore a carefully fitted 'G' helmet and P/Q mask. The breathing gas hoses from both regulators supplied a common mask tube hose via a Douglas tap that enabled selection of the required breathing gas without the subject knowing which gas was being chosen. In this way the selected gas was supplied to the subject's breathing mask at the ambient pressure of the large compartment, except that an additional slight 'safety pressure' will have been present during exposures over 10,000 ft.

Physiological monitoring and data recording

Respired gases were analysed continuously using an Innovision A/S Amis 2000 mass spectrometer. Non-invasive monitoring of S_aO_2, heart rate and blood pressure were undertaken using the Kontron pulse oximeter with finger probe and the Ohmeda Finapres blood pressure monitor with finger cuff. Analogue outputs were calibrated and

recorded in real time together with chamber altitude. Mass spectrometer calibrations were conducted at each experimental altitude under consideration.

4.3.3 Respiratory conditions

The chosen respiratory conditions were intended to have practical physiological relevance in aviation and also to encompass the likely spectrum of respiratory disturbances envisaged for subsequent studies.

Hypobaric hypoxia was generated by breathing air at ambient chamber pressure equivalent to altitudes of 10,000 ft (69.7 kPa; 523 mm Hg) and 15,000 ft (57.2 kPa; 429 mm Hg). The former was chosen because the mild hypoxia accompanying ascent to altitudes up to 10,000 ft is generally regarded as innocuous to healthy aircrew, regulatory authorities do not usually mandate the use of supplementary O_2 until this altitude is exceeded, and it is an equivalent altitude that was likely to feature in subsequent mesopic studies, not least because significant effects at this altitude could have implications for the use of supplementary O_2 by aircrew. The higher altitude was chosen to impose moderate hypoxia to a degree that should be associated with clear effects on threshold sensitivity during dark adaptation (Figure 1.24). Using experienced hypobaric subjects at rest, it was anticipated that this level of hypoxia would be reasonably well tolerated and probably not severe enough to provoke, and therefore be confounded by, undue secondary hypocapnia. Thus, the absence of any detectable effect of hypoxia at 15,000 ft would have called into question the method of vision testing used in the study. Furthermore, the two different severities of hypoxia were expected to exert differential effects on the delay to cone rod inflection, should an effect be present.

The influence of hypocapnia was examined at GL in the hypobaric chamber by invoking moderate voluntary hyperventilation, breathing air, sufficient to drop the subjects' $P_{ET}CO_2$ to 25 mm Hg. Normocapnic control exposures were achieved by breathing air normally and thus were identical to normoxic controls. The same degree of hypocapnia was also examined in combination with moderate hypoxia by conducting voluntary hyperventilation while breathing air at an equivalent altitude of 15,000 ft.

The effect of hyperoxia alone was examined while breathing 100% O_2 at GL, with reference to control exposures breathing air at GL.

Each experimental session involved normoxic dark adaptation followed by dark adaptation under an imposed respiratory condition. Thus, there were 25 normoxic

control dark adaptations, five for each subject, that allowed assessment of the within and between subject variability in dark adaptation from day to day.

No subject undertook more than one experimental session on any given day and each subject undertook all his experimental sessions at approximately the same time of day. The order in which each subject undertook each of the five different experiments was semi-randomised such that different subjects did not undertake the same condition on the same day.

4.3.4 Vision testing

The vision testing equipment used in this study was a standard Friedmann Visual Field Analyser Mk II (Friedmann, 1980). The Analyser has a clinical dark adaptation mode that uses an achromatic wide-field visual stimulus. However, this was not used in the current study, as repeatedly stimulating the central retina with a wide field stimulus, even at sub-threshold intensities, was considered potentially to interfere with the dark adaptation process. To avoid repeatedly stimulating the same areas of retina, it was decided to present a few small 'spot' stimuli with each flash but without using a fixation point, so that the stimuli from successive flashes would be unlikely to fall on exactly the same retinal locations each time. The Analyser's black stimulus plate was retained and the q3 stimulus pattern was employed throughout all adaptations, providing a tetrad of spot flash stimuli. One of these was located at the centre of the stimulus plate with three peripheral stimuli located at approximately 4, 7 and 11 o'clock and subtending a visual angle of approximately 15° relative to the central stimulus. All dark adaptations were conducted monocularly, with subjects asked to position their O_2 mask against the Analyser chin rest and to maintain a forward gaze perpendicular to the centre of the stimulus plate. The seating height and chin rest position were adjusted accordingly for each subject before the start of experiment.

The Analyser's integral green filter was used throughout to ensure a stimulus to which both cones and rods would be sensitive, so highlighting the cone rod inflection point on the mixed dark adaptation curve. An established xenon flash tube provided a consistent visual stimulus with successive flashes which were presented every 5 s. The flash characteristics measured through the green filter are shown in Figure 4.3.

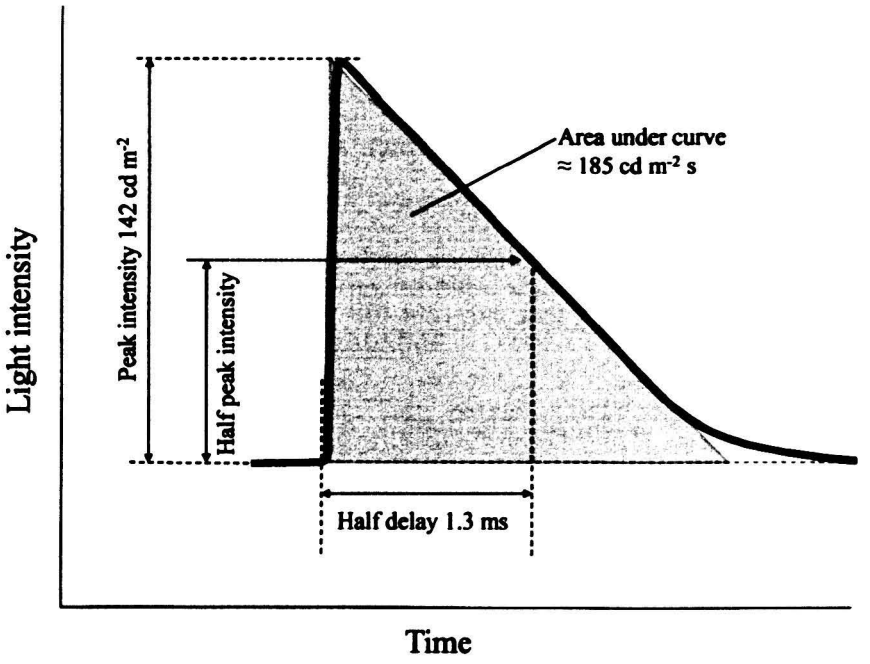


Figure 4.3 Friedmann Visual Field Analyser Mk II xenon flash stimulus characteristics

The Analyser's internal ND filters were used to control stimulus intensity, starting at the 3.0 ($\log_{10}$) setting. Progressively dimmer stimuli were achieved by increasing filter strength in 0.2 $\log_{10}$ unit steps until the maximum 4.6 setting was achieved.

Trial runs using this vision testing method suggested that it would highlight the timing of cone rod inflection and the subsequent initial rapid progression of rod sensitivity as intended. The essential nature of the dark adaptation curve produced in this way has been exaggerated in Figure 4.4 to illustrate how the method allows estimation of the timing of the cone rod inflection point. The time lag between detection of successive stimuli becomes very brief following detection of the 3.8 and 4.0 stimuli, indicating rapid adaptation to dark and that scotopic sensitivity is achieved at some stage between detection of the 3.6 and 3.8 stimuli. Following detection of the 3.6 stimulus, the progression of visual sensitivity must follow a path bounded (approximately) by lines A and B. Assuming that the initial rate of progression of scotopic sensitivity, from detection of the 3.8 stimulus to detection of the 4.0 stimulus, can be regressed by at least the same rate (line C), then the earliest possible inflection time, D, is given by the time at which lines A and C intersect, and the latest possible inflection time, E, is given by the intersection of B and C, which coincides with detection of the 3.8 stimulus. The steeper the initial rod threshold curve, then the closer together D and E must be and the narrower is the possible inflection time 'window'. If this 'window' is sufficiently and

consistently narrow, then the time to detection of the 3.8 stimulus becomes a surrogate marker of effects of respiratory disturbance on cone rod inflection time.

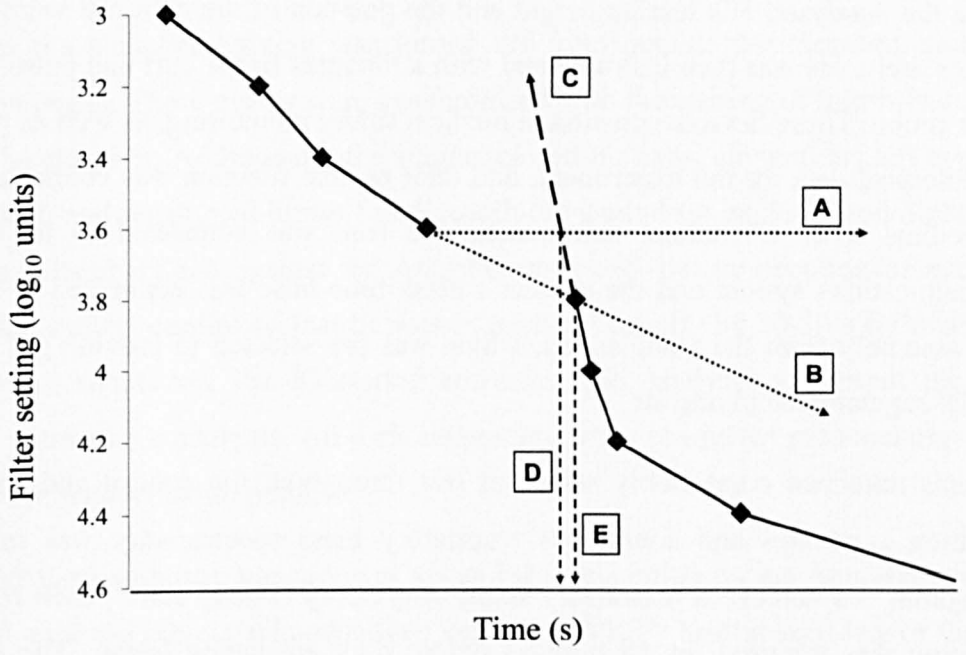


Figure 4.4 Extrapolation of cone rod inflection time using Friedmann Visual Field Analyser Mk II

Opaque matt black material was used to envelope the Analyser from the stimulus plate to the viewing port. A shroud of the same material was used to cover the subject whilst undertaking dark adaptation. In this way the faint stray light from elsewhere in the chamber, including from the dimmed Analyser control panel, was prevented from reaching the subject's eyes.

4.3.5 Experimental procedure

All experiments were conducted using exactly the same procedures with the subjects in the large end of the hypobaric chamber, with the chamber doors shut, regardless of whether any particular exposure involved decompression. Mass spectrometer data calibration was conducted carefully before each control run at GL and before and after each condition exposure, including at altitude when appropriate, using the various calibration gases. GL calibrations were made against the prevailing barometric pressure each day, which ranged from 98.75 to 101 kPa (740 to 758 mm Hg) during the study. Analogue outputs from the mass spectrometer, Finapres blood pressure meter and Kontron pulse oximeter were calibrated through the Powerlab system and recorded on Chart software (ADInstruments).

The subject was confirmed to be well, rested and fit to undertake the experiment. A cloth 'G' helmet and P/Q mask were carefully fitted to the subject to ensure a good face-mask seal and he was seated in the large compartment of the hypobaric chamber, facing the Analyser. His seating height and the position of the chin rest were adjusted appropriately. He was then instrumented with a Finapres finger cuff and pulse oximeter finger probe. These devices constituted medical safety monitoring, as well as providing physiological data for the experiment, and their normal function was confirmed before proceeding. The 'G' helmet communications lead was connected to the chamber communications system and the subject's mask tube hose was connected to the mask hose assembly from the Douglas tap, which was pre-selected to provide gas from the Mk17F regulator supplying air.

Subjects remained comfortably seated at rest throughout the control and subsequent condition exposures and continuous respiratory mass spectrometry was maintained throughout. To achieve a reasonably stable respiratory 'steady state', each respiratory condition was imposed for 15 minutes before dark adaptation began. The normoxic control exposures also included this 'adaptation' period of 15 minutes. The timing of hypoxic adaptation began when the ambient chamber pressure reached the destination altitude. All hypobaric decompressions and subsequent recompressions following dark adaptation were conducted over 3 minutes at no more 5000 ft min^{-1}. Hypocapnic adaptation time began as soon as voluntary hyperventilation commenced. This coincided with arrival at the destination altitude for the condition involving mixed hypocapnia with hypoxia at 15,000 ft. For the hyperoxia exposures, the adaptation time began when the 100% O_2 supply was selected using the Douglas tap.

An IO was present in the chamber for all experiments and wore a 'G' helmet and P/Q mask for the hypobaric exposures. The IO was supplied with O_2-enriched breathing gas from a standard Mk17F chamber regulator selected to 'airmix', so preventing hypoxia during altitude exposures. He or she also assisted with the experimental procedures and was trained to operate the Analyser. The bright chamber lighting is reflected from the white internal walls of the chamber and subjects had generally been exposed to this level of ambient illumination for 15-20 minutes by the time 10 minutes of respiratory adaptation were complete, providing plenty of time to establish a stable state of light adaptation and relatively well-bleached retina. However, to ensure that the region of retinal field under test during dark adaptation was uniformly light adapted, the last 5 minutes of respiratory adaptation coincided with controlled central retinal illumination

viewing the light box, with luminance of 785 cd m^{-2} (SD ± 30) as viewed with the chamber lighting on.

Following a countdown, at the end of light adaptation the light box was turned off by the IO as the chamber lighting was turned off from outside the chamber, and dark adaptation began. These events were synchronised with the starting of timers inside and outside the chamber. Additionally the subject closed his eyes, covered his left eye with an eye patch and positioned himself underneath the shroud (to exclude stray light from within the chamber) and against the Analyser chin rest, before opening his eyes. As soon as the subject confirmed that he was in position, usually by 20-30 s following the start of dark adaptation, the IO started activating the Analyser to present the flash stimulus at timed 5 s intervals. All dark adaptations were conducted monocularly, using the right eye with a natural pupil.

The initial flash stimulus intensity was set at 3.0 (log_{10} units) on the Analyser and was presented until the subject acknowledged verbally ("YES") having seen any of the four points of light. The time was recorded from a synchronised timer outside the chamber and the IO immediately decreased the intensity of the visual stimulus by 0.2 log_{10} units (increasing the filter setting to 3.2), continuing to trigger the flash every 5 s until seen again. This process continued for at least 20 minutes or until the subject perceived the dimmest stimulus that could be presented by the Analyser. Subjects had no cues for stimulus presentation. At the end of the normoxic control dark adaptation breathing air, chamber lighting was restored and the subject was allowed to rest for 10 minutes before repeating the procedure under the imposed respiratory condition. In this way, the second dark adaptation under the experimental respiratory condition usually commenced approximately 45-50 minutes after the start of the preceding normoxic dark adaptation.

4.3.6 Physiological parameters

Recording and measurement

Figure 4.5 illustrates a brief segment of cardio-respiratory data recorded using Chart software, and showing two full breaths over about 12 s recorded from a subject breathing air at an equivalent altitude of 15,000 ft. The upper two traces show the mask cavity PO_2 and PCO_2 measured by mass spectrometry, highlighting the end-expiratory phase of ventilation from which $P_{ET}O_2$ and $P_{ET}CO_2$ are derived, and the end-inspiratory phase from which measurements of P_IO_2 are taken. The PO_2 is highest during late

inspiration and indicates a P_IO_2 of ~97 mm Hg, reflecting the PO_2 of the inspired gas (air) at 15,000 ft (compare Figure 1.11). The PCO_2 is near zero during inspiration and rises during expiration. The $P_{ET}CO_2$ appears steady at marginally under 40 mm Hg reflecting normocapnia and, thus, that this experienced subject was not hyperventilating at 15,000 ft (compare Figure 1.12). The $P_{ET}O_2$ of approximately 45 mm Hg reflects the anticipated P_AO_2 at this altitude (Figure 1.12).

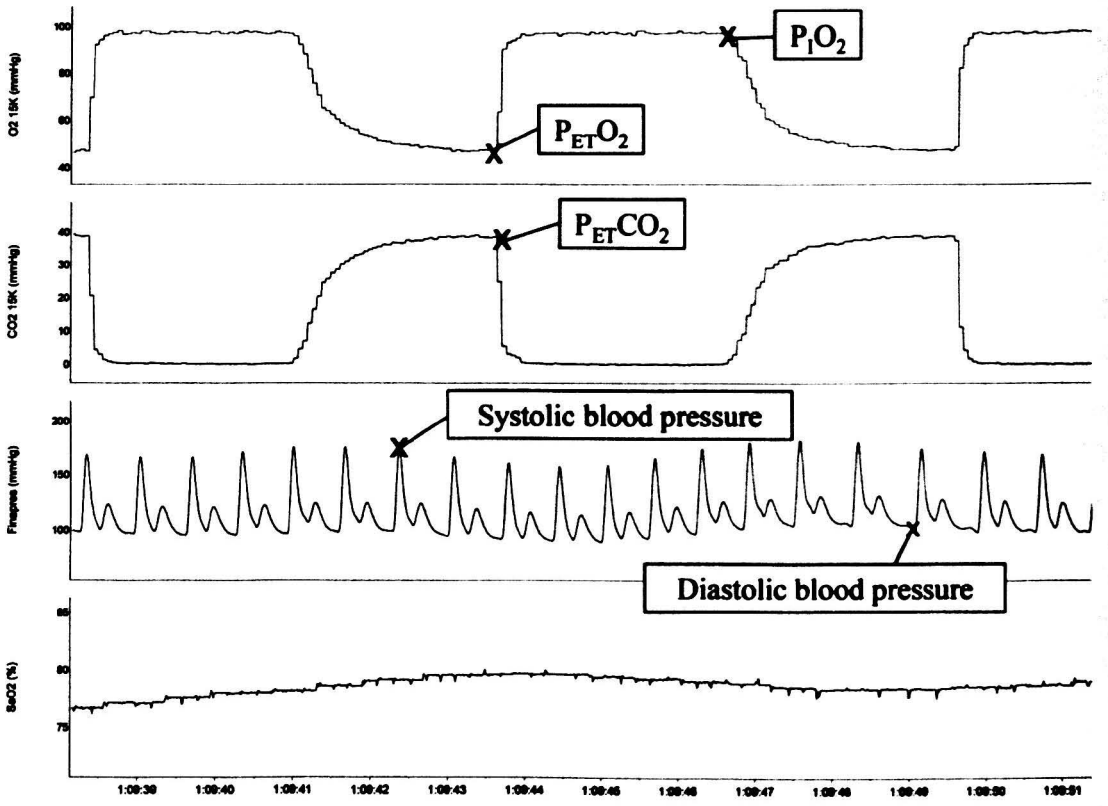


Figure 4.5 Segment of cardio-respiratory data during dark adaptation at 15,000 ft

Systolic and diastolic measurements are made from the peripheral pressure pulse at the appropriate point in the cardiac cycle, as in the third trace at Figure 4.5 which shows the pressure waveform in the Finapres finger cuff. The values from this trace are higher than the normal arterial blood pressure, recorded at the level of the aortic root, due to the hydrostatic effect of recording from a hand being held below the level of the heart. However, comparison of the effect of the respiratory condition upon mean blood pressure remains valid as subjects were asked to keep the Finapres hand in the same position for each experiment. Even under stable respiratory conditions S_aO_2 tends to oscillate gently over time and this becomes exaggerated under hypoxia.

The segment of Chart trace in Figure 4.5 does not show all the data channels recorded, which included ambient (large compartment) hypobaric chamber pressure, PN_2 and

heart rate, the latter recorded from the analogue output of the Finapres algorithm used to calculate it. The channels for PO_2, PN_2 and PCO_2 were reproduced and calibrated for each altitude at which testing occurred, that is, GL, 10,000 ft and 15,000 ft.

Heart rate data were extracted by taking six measurements at intervals of 5 s from the end of minutes 2, 7, 12 and 17 from the start of dark adaptation. Thus, 24 measurements were taken during each dark adaptation and the mean and SD calculated. For each respiratory condition, each subject's mean heart rate during normoxic dark adaptation was subtracted from his mean heart rate under respiratory disturbance and the results for the group were then subject to paired *t* test to assess the significance of any difference.

Blood pressure was sampled similarly with mean pressure calculated from each of the first six 'representative' pressure waveforms sampled after 2, 7, 12 and 17 minutes of dark adaptation. Mean blood pressure was calculated as one third of systolic pressure plus two thirds of diastolic pressure. Differences in mean blood pressure under normoxia and respiratory disturbance were also analysed using the paired *t* test.

The $P_{ET}O_2$, $P_{ET}CO_2$ and S_aO_2 data were sampled similarly after 2, 7, 12 and 17 minutes of dark adaptation and were also analysed using paired *t* tests.

Cardio-respiratory responses

There were no statistically significant effects on mean blood pressure of any of the respiratory challenges. Furthermore, only the exposure to 15,000 ft exerted a statistically significant effect on heart rate, which increased by a mean of 10 min^{-1} and was consistent with expectation for this equivalent altitude (Table 4-1).

Subject	Normoxia Mean (SD) (a)	15,000 ft Mean (SD) (b)	Difference in means (b – a)
1	71.9 (2.2)	82.8 (2.1)	10.9
2	69.6 (3.7)	80.4 (2.4)	10.8
3	86.1 (6.5)	97.5 (4.4)	11.3
4	61.1 (8.3)	63.5 (8.0)	2.4
5	71.7 (5.1)	86.2 (5.6)	14.5
		Mean difference	10.0
		SD	4.5
		SE	2.01
		t	4.97
		p	0.008

Table 4-1 Effect on mean heart rate (min^{-1}) of hypoxia at 15,000 ft equivalent altitude

Examination of the individual traces showed that the imposed respiratory conditions remained stable throughout dark adaptation. All subjects exhibited some minor

variability in normoxic $P_{ET}O_2$ from day to day, as indicated by the SD of within-subject data. This is attributable to variations in barometric pressure during the study. Table 4-2 presents the mean data across all five subjects for the (N = 25) normoxic dark adaptations and the (N = 5) dark adaptations conducted under each imposed respiratory challenge. Notwithstanding the fluctuations in barometric pressure, the normoxic data show that consistent P_AO_2 were produced between-subjects, as evidenced by the low SD of between subject data, indicating that they were responding similarly to fluctuations in ambient pressure from day to day. The $P_{ET}CO_2$ across the group remained steady and within normal limits, indicating that normocapnia was maintained throughout and attesting to the familiarity of the subjects with hypobaric studies and with breathing through aircrew masks from pressure demand regulators.

	$P_{ET}O_2$ (mm Hg) Mean (SD)	$P_{ET}CO_2$ (mm Hg) Mean (SD)	S_aO_2 (%) Mean (SD)
Normoxia (N=25)	112.2 (1.3)	41.0 (2.0)	97.6 (0.8)
Hypoxia 15,000 ft	44.2 (4.9)	40.1 (8.5)	77.7 (7.5)
Hypoxia 10,000 ft	63.5 (5.7)	42.7 (3.8)	89.8 (1.9)
Hypocapnia at GL	133.2 (2.3)	24.8 (1.3)	98.1 (1.3)
Hypocapnia with hypoxia at 15,000 ft	61.7 (5.6)	24.5 (0.5)	94.0 (1.7)
Hyperoxia at GL	706.6 (12.9)	37.4 (2.8)	99.1 (1.0)

Table 4-2 Mean (SD) $P_{ET}O_2$, $P_{ET}CO_2$ and S_aO_2 during dark adaptation

Gentle hyperventilation is a normal secondary response to hypoxia at 15,000 ft and would be expected to result in mild hypocapnia (Figure 1.12). In this study one subject developed noteworthy hypocapnia at this equivalent altitude, his $P_{ET}CO_2$ averaging 29 mm Hg. There was no significant hypocapnia at 10,000 ft. The $P_{ET}O_2$ achieved breathing air at 15,000 ft and 10,000 ft were broadly as anticipated (Figure 1.12), although $P_{ET}O_2$ were slightly higher than reported elsewhere (Gradwell, 2006). The S_aO_2 at these two altitudes were as anticipated, with those at 10,000 ft being representative of S_aO_2 measured in commercial aircrew (Cottrell, Lebovitz, Fennel *et al*, 1995).

During the exposures involving voluntary hyperventilation at GL and at 15,000 ft, the mean and SD values for $P_{ET}CO_2$ indicate excellent control at the target level of 25 mm Hg throughout the dark adaptations. Hyperventilation resulted in secondary elevation of $P_{ET}O_2$ both at GL and at 15,000 ft, as anticipated. However, due to the sigmoid nature of the Hb O_2 dissociation curve (Figure 1.10), the elevation of $P_{ET}O_2$ at 15,000 ft, from ~44 mm Hg without hyperventilation to ~62 mm Hg with hyperventilation has resulted in substantial elevation of S_aO_2, from ~78% to ~94%.

The GL hyperoxia condition resulted in elevation of S_aO_2 to in excess of 99%, as would be anticipated. The condition is associated with a slight reduction in $P_{ET}CO_2$ that was not considered meaningful initially but which, in the light of subsequent experiments, clearly is. It is attributable to the Haldane effect and is discussed further in Chapter 6. The results indicate that all 5 conditions produced the intended respiratory challenges.

4.3.7 Analysis of dark adaptation

Each individual experiment involved an initial dark adaptation under control conditions, providing 25 normoxic dark adaptations with which to assess the variability in dark adaptation within and between subjects. The variability of stimulus detection time within and between subjects was analysed for statistical significance using one-way ANOVA, established as an appropriate statistical method for data analysis (Armstrong, Eperjesi and Gilmartin, 2002). For each dark adaptation conducted under respiratory disturbance, the detection time displacement was determined for each stimulus intensity relative to its paired normoxic (control) detection time. Negative (leftward) displacement (decreased detection time) represents a hastening of adaptation to dark (or increased light sensitivity for a given time in darkness) while positive (rightward) displacement (increased detection time) represents delayed adaptation and reduced visual sensitivity. At each stimulus intensity, the displacements for the five subjects were analysed for statistical significance ($\alpha = 0.05$) using the paired *t* test, against the null hypothesis that a sample population experiencing no effect from the imposed respiratory condition would have a mean displacement of zero.

When conducting multiple *t* tests there is an increased likelihood of finding statistically significant results occurring by chance alone when no true effect exists, producing false positive results or Type I error. On the other hand, with only five subjects a single rogue value may oblige loss of significance at a 95% confidence level, even when a true effect exists, resulting in Type II error or a false negative outcome. Given the variability in response to respiratory disturbance, particularly hypoxia, within and between subjects, and the variability of visual performance between subjects, it was considered that the data presented here would be more vulnerable to Type II error than to Type I error. Indeed, the primary and secondary aims of this experiment did not encompass testing for significant effects of respiratory disturbance and it was considered highly unlikely that statistically significant effects would be seen with such a small pool of subjects. Thus, it wasn't considered that the data might overstate the impact of respiratory

disturbance. Accordingly, Bonferroni correction was considered but was not applied to the data. Applying Bonferroni correction on the basis that five independent studies have been conducted using the same subjects, then results presented here with $p < 0.01$ would still retain statistical significance to a 95% CI.

For convenience, the data are presented against a logarithmic stimulus intensity scale that is measured in Friedmann Analyser ND filter settings. As a representational baseline, some graphs show the mean control curve derived from all 25 normoxic control adaptations. For each stimulus intensity each subject's displacement under respiratory disturbance, relative to his own matched control detection time, is represented relative to the mean normoxic control curve. The mean displacement curve then represents the net effect of the condition across all five subjects.

4.4 Results

4.4.1 Normoxia

The five normoxic dark adaptations of each subject are shown in Figure 4.6 and exhibit a consistent pattern of stimulus detection over time. Following a highly variable period until detection of the first (3.0) stimulus, the next four stimuli at the 3.2 to 3.8 filter settings were seen at discrete intervals, typically a minute or two apart, sometimes with a longer interval to detection of the 3.8 stimulus. In contrast, following detection of the 3.8 stimulus, the 4.0 and 4.2 stimuli were seen in rapid succession, each separated from its predecessor by only 10 or 15 s. The 4.4 stimulus was then seen within a minute or two followed by a much longer and highly variable delay for the 4.6 stimulus. This same general pattern of response was preserved under respiratory disturbance.

Figure 4.6 illustrates the variability in dark adaptation rate within and between subjects from day to day. Each subject undertook all his experiments at the same time of day, starting with subject 1 at ~08.00 h and finishing with subject 5 at ~16.00 h, so diurnal variation does not explain the variability within or between subjects. In Figure 4.6, the order in which each subject's normoxic dark adaptations were undertaken are represented, in turn, by diamonds, squares, triangles, white circles and black circles.

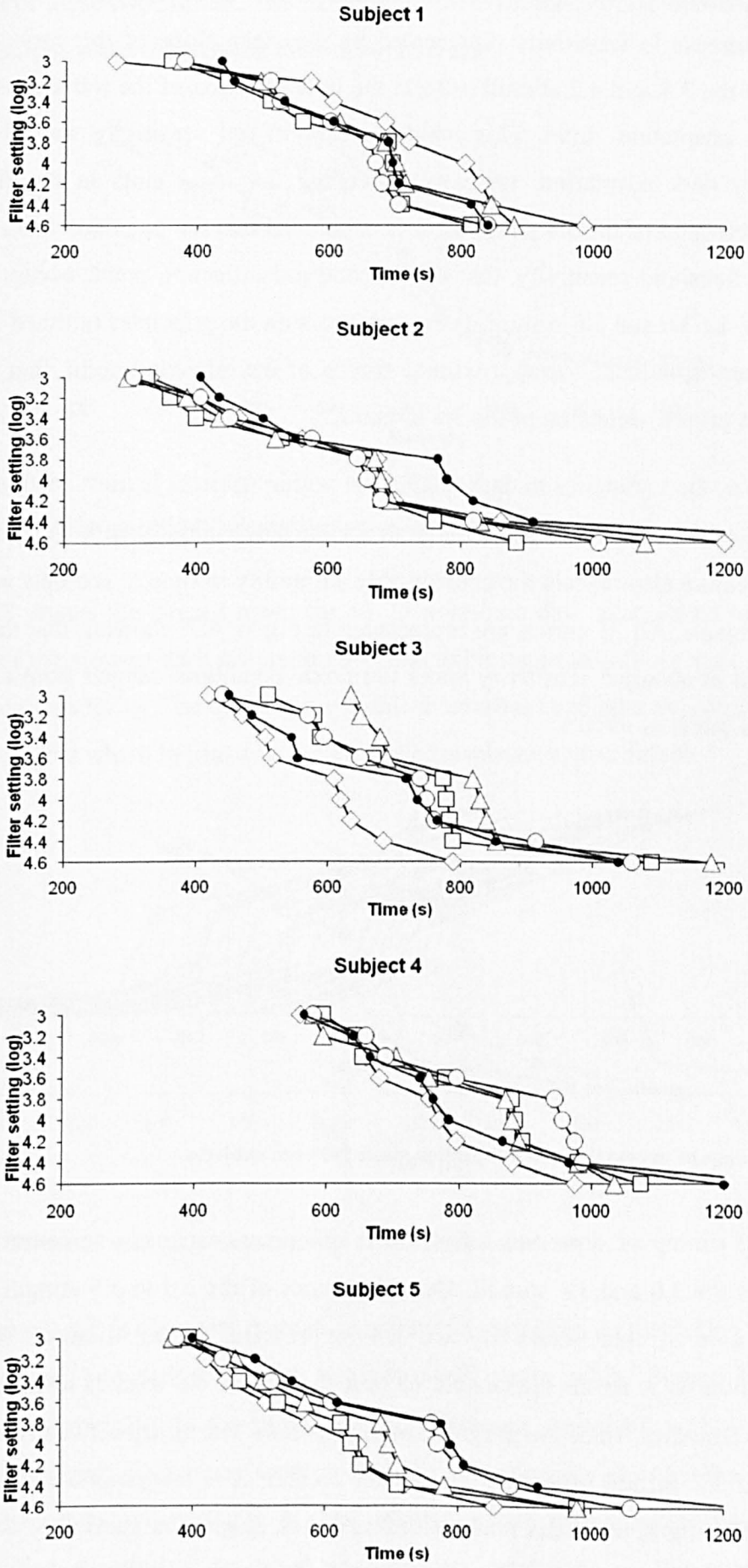


Figure 4.6 Variability in normoxic dark adaptation rate within and between subjects

From consideration of the appearance of the curves in Figure 4.6, it is concluded that the sharp increase in sensitivity represented by the steep slope of the curve between detection of the 3.8 and 4.2 stimuli reflects the initial portion of the rod element of the mixed dark adaptation curve. This rapid increase in rod sensitivity was obvious in nearly every dark adaptation, typically exceeding 0.4 $\log_{10}$ units in the first 30 s following detection of the 3.8 stimulus. It is concluded that the transition from mesopic to scotopic threshold sensitivity, that is, the cone rod inflection point, occurs between detection of the 3.6 and 3.8 stimuli. In accordance with the principles outlined in Figure 4.4, these curves indicate the approximate timing of the inflection point, that is, in the few seconds prior to detection of the 3.8 stimulus.

In Figure 4.6, the variability in dark adaptation within subjects is most obvious for the rod portion of the curve, and particularly so for the steep initial gain in rod sensitivity. The same feature also reveals the considerable variability in time to scotopic sensitivity between subjects. All 25 curves are represented in Figure 4.7, showing that the time to achievement of scotopic sensitivity under normoxic conditions ranged from as little as ~600 s to as much as ~950 s.

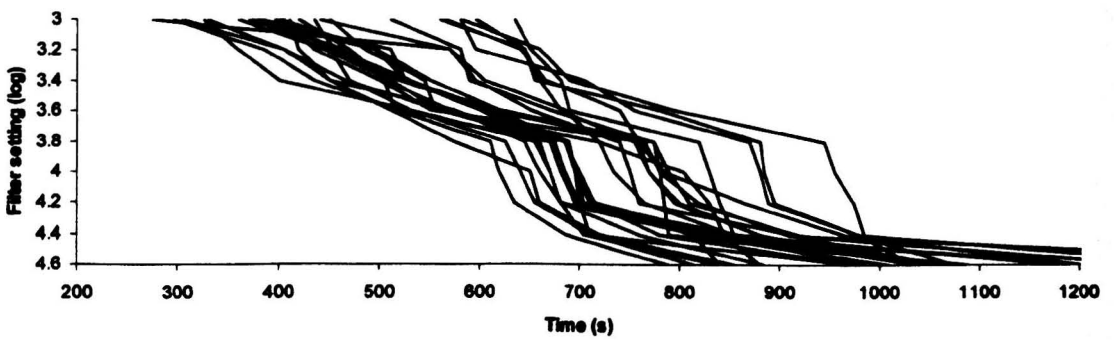


Figure 4.7 Range of normoxic dark adaptation times between subjects

The precise timing of cone rod inflection is unknown, occurring sometime between detection of the 3.6 and 3.8 stimuli. Detection times of the 3.0 to 3.6 stimuli represent the progression of cone sensitivity and it is appropriate to join these on the graphs shown. Similarly, it seems reasonable to join the 3.8 to 4.6 stimuli to represent rod sensitivity. However, since the progression of threshold sensitivity between detection of the 3.6 and 3.8 stimuli cannot be known for certain, it is inappropriate to join these points. Accordingly, the mean normoxic mixed dark adaptation curves for each of the five subjects are represented in Figure 4.8 with a discontinuity between the markers for

detection of these two stimuli. The variation in dark adaptation rate between subjects is again apparent when considering their mean curves.

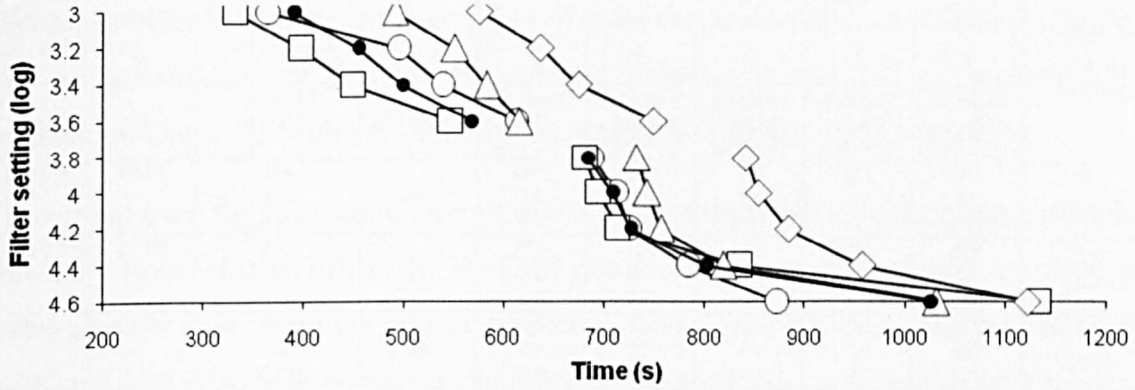


Figure 4.8 Mean normoxic dark adaptation curves for each of five subjects

Figure 4.9 shows the overall mean for all 25 normoxic dark adaptations together with outlines of the slowest dark adaptation and that exhibiting the earliest rapid progression of scotopic sensitivity. The mean curve in this diagram is used later as a representational baseline against which to illustrate the effects of respiratory disturbance.

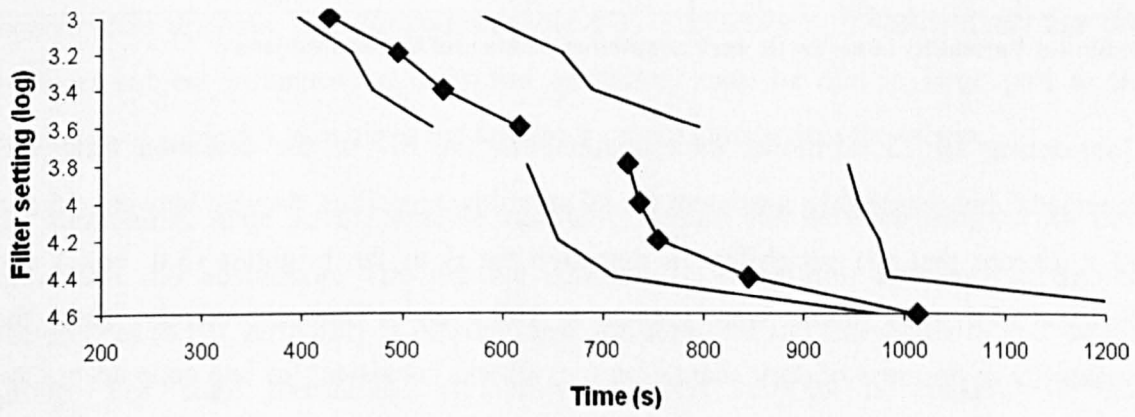


Figure 4.9 Normoxic dark adaptations: group mean and scotopic range

From Figure 4.8 it is clear that there is considerable variability in the timing of late cone sensitivity and late rod sensitivity between individuals, while Figure 4.6 suggests considerable variability in the timing of early scotopic sensitivity within individuals, noting that subject 4 achieves scotopic sensitivity noticeably slower than the rest. In view of the substantial variation in dark adaptation rate within and between subjects, more detailed descriptive statistical analysis was conducted on the normoxic data,

summarised in Table 4-3, to appreciate the variability of the normal responses to this vision testing procedure in this sample population.

Analyser filter setting	Subject (5 adaptations each)					Mean SD (s)	Group (N = 25 adaptations)	One-way ANOVA (α = 0.05)	
	1	2	3	4	5				
	Mean (s) SD (s) CoV (%)	Mean (s) SD (s) CoV (%)	Mean (s) SD (s) CoV (%)	Mean (s) SD (s) CoV (%)	Mean (s) SD (s) CoV (%)		Mean (s) SD (s) CoV (%)	F	p
3.0 (cone)	365 58 15.8	332 43 13.0	491 87 17.8	576 14 2.4	390 17 4.3	44	431 103 24.0	19.03	< 0.001
3.2 (cone)	495 50 10.1	398 28 7.0	551 74 13.4	637 25 3.9	455 28 6.1	37	507 93 18.4	20.75	< 0.001
3.4 (cone)	539 38 7.0	450 37 8.2	583 65 11.2	675 22 3.2	499 40 8.0	40	549 87 16.0	20.20	< 0.001
3.6 (cone)	613 45 7.4	547 22 4.1	616 77 12.4	750 45 6.0	568 48 8.5	47	619 86 13.9	12.27	< 0.001
3.8 (rod)	689 21 3.1	682 48 7.0	733 79 10.7	842 82 9.8	684 86 12.5	63	726 88 12.1	5.03	0.006
4.0 (rod)	713 53 7.4	695 48 7.0	743 78 10.5	855 77 9.0	709 68 9.6	65	743 85 11.4	4.85	0.007
4.2 (rod)	726 60 8.2	715 59 8.3	757 75 10.0	884 65 7.3	727 74 10.2	67	762 89 11.6	5.47	0.004
4.4 (rod)	784 71 9.0	836 55 6.6	820 87 10.7	958 44 4.6	802 94 11.8	70	840 92 10.9	4.45	0.01
4.6 (rod)	872 67 7.7	1135 233 20.5	1032 145 14.1	1123 142 12.7	1027 149 14.5	147	1038 172 16.6	2.26	0.099

Table 4-3 Variability in normoxic dark adaptation within and between subjects

Considering all 25 normoxic dark adaptations, the SD of the detection time was substantial but reasonably consistent for all stimulus intensities, varying between 85 and 93 s, except that the variability in detection times to the brightest (3.0, cone) and dimmest (4.6, rod) stimuli were greater, at 103 s and 172 s respectively. Thus, the variability in progress of dark adaptation overall was consistent for late cone adaptation and early scotopic (rod) adaptation, while variability was greater for detection of the first stimulus and much greater still for detection of the dimmest stimulus, as the progress of rod adaptation begins to asymptote.

However, the relative influence of within- and between-subject differences varies for the cone and rod phases of dark adaptation in this subject sample. For the cone stimuli, the within-subject SD detection times were consistently and often substantially less than the corresponding group SD. This is reflected by the greater group coefficients of variation (CoV) than those of individual subjects. The CoV provides a measure of dispersion and is calculated by dividing the SD by the associated mean and expressing

the result as a percentage. For rod stimuli, within-subject SDs were comparable to those of the group, as were CoV, suggesting that within-subject differences contributed more to group variability in rod adaptation than they did to variability in cone adaptation. Within-subject SDs averaged from 37 to 47 s for the cone stimuli and from 63 to 70 s for rod stimuli, except that for the dimmest stimulus it was 147 s, supporting the contention that within-subject variability is greater for rod than cone adaptation.

The group CoV for cone stimuli are substantial. They diminish with decreasing stimulus intensity from 24.0% (3.0) to 13.3% (3.6) primarily because later stimuli are detected after a longer delay from the start of dark adaptation. However, analysing the data using one-way ANOVA, with subject as the independent variable, confirms the heterogeneity of cone adaptation between subjects in this sample group, producing highly statistically significant differences for all cone stimulus intensities ($p < 0.001$). This supports the discrete appearance of the mean cone curves between subjects in Figure 4.8.

The SD and CoV for the first four rod stimuli (3.8 to 4.4) were reasonably consistent within subjects and within the group as a whole, supporting the contention that detection times for these stimuli are related. Between-subject variability remained a clearly statistically significant influence on early rod thresholds ($p \leq 0.01$), although this appears less dramatic than for cones (Table 4-3). Furthermore, it seems likely that this between-subject influence on early rod sensitivity may be due in large part to the tendency of subject 4 to achieve rod sensitivity more slowly than the others.

To summarise, there is considerable variability within and between subjects for both cone and rod adaptation. The relative contribution of within- and between-subject differences to net variability is not the same for cone and rod thresholds in this sample group. For cone thresholds, variability between subjects is considerable and substantially exceeds that of within-subject variability. Within-subject variability is greater for rod thresholds than it is for cones, but variability between subjects remains significant for the early rapid phase of rod adaptation after the cone rod break.

Any meaningful effect of respiratory disturbance to shift inflection time should be reflected by unambiguous displacement of the initial, rapidly-adapting portion of the rod curve. It is apparent from the foregoing that this part of the curve is subject to considerable variability within and also between subjects. Accordingly, it is highly unlikely that subtle effects of respiratory challenge would be detected in such a small sample group even when using data paired within subjects.

4.4.2 Hypoxia

The effect upon dark adaptation of hypoxia at 15,000 ft is shown by subject in Figure 4.10. Normoxic dark adaptation curves are represented with black triangles and the subsequent hypoxic adaptations with white triangles. It is clear that, for the most part, the time to detection by each subject of each stimulus was greater under hypoxia than under normoxia. Two subjects failed to achieve sensitivity to the 4.6 stimulus under hypoxia despite continuing with dark adaptation well beyond 20 minutes.

The apparently consistent effect of hypoxia to delay stimulus detection appears easily to overcome any within-subject variability that might be expected to disguise the effect. However, while individual subjects' curves are shifted to the right (with the single exception of the early cone curve of Subject 4), the effect is insufficient to overcome the between-subject variability in dark adaptation under normoxia. Some subjects still adapted faster when hypoxic than others did when normoxic.

Accordingly, for each dark adaptation conducted under hypoxia, the detection time displacement relative to the normoxic (control) detection time was determined for all stimulus intensities. The displacements for the five subjects were analysed for statistical significance ($\alpha = 0.05$) using the paired *t* test against the null hypothesis that a sample population experiencing no effect from hypoxia would have a mean displacement of zero. The results of the paired *t* tests are shown in Table 4-4, using two-tailed probability points of the *t* distribution against four degrees of freedom (*df*).

The paired *t* tests suggest a significant effect of hypoxia at 15,000 ft to delay the onset and progression of scotopic (rod) sensitivity and also suggests an effect to delay late cone adaptation (that is, the time to detection of the stimuli at the 3.4 and 3.6 filter settings but not the earlier cone stimuli). This is shown more effectively by plotting each subject's detection time displacement for each stimulus (b – a in Table 4-4) against a common representational baseline. This has been done using the group mean normoxic dark adaptation curve from Figure 4.9 and the resulting graph is shown in Figure 4.11, which also shows the mean displacement curve, obtained from the 'mean difference' values in Table 4-4, to illustrate the net effect of the respiratory condition. The graph suggests a progressive effect of hypoxia to compromise late, but not early, cone adaptation. The scotopic curves indicate a substantial effect of hypoxia to delay cone rod inflection and to compromise the subsequent progression of scotopic adaptation.

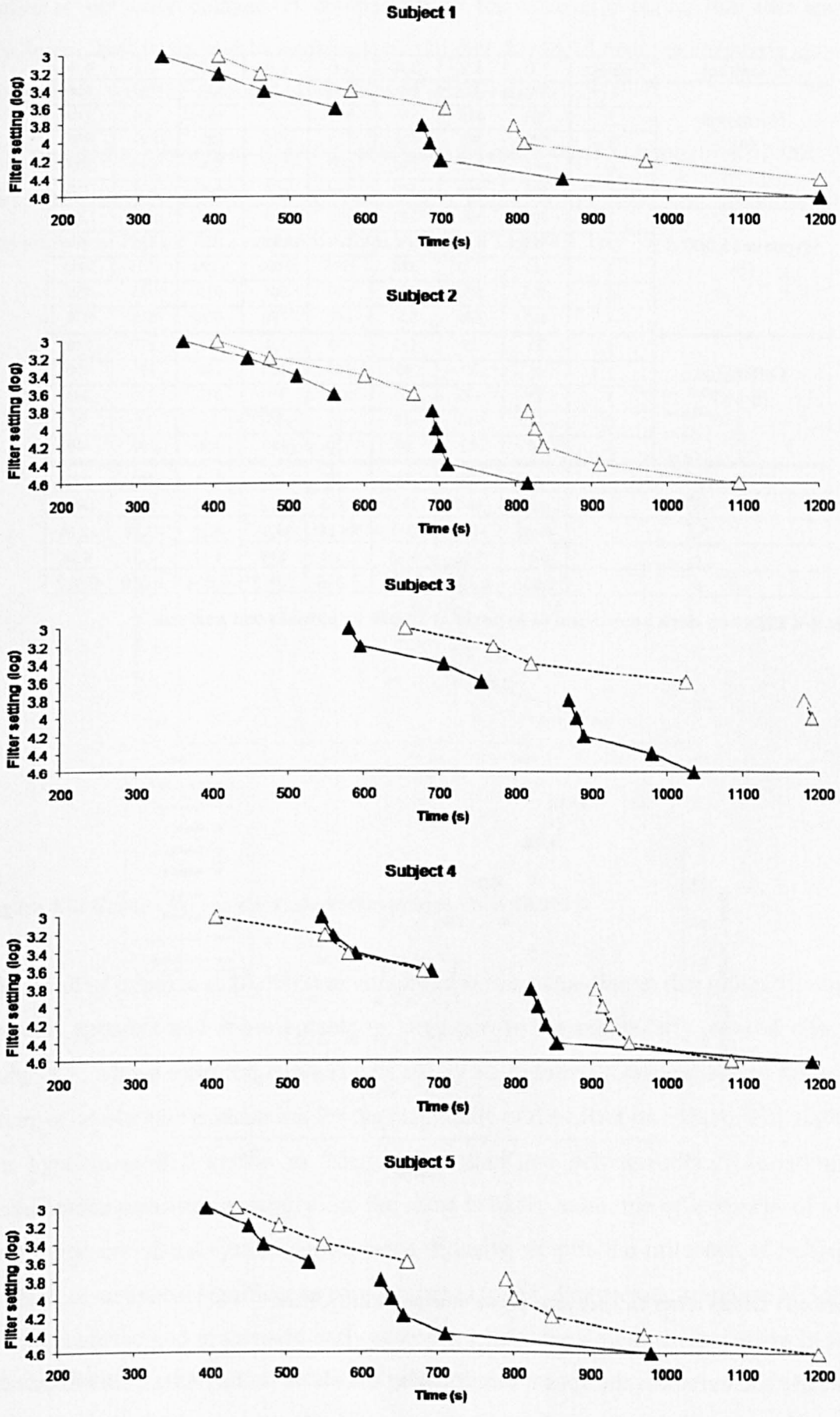


Figure 4.10 Subjects' dark adaptation curves under normoxia (▲) and hypoxia at 15,000 ft (Δ)

Condition	Subject	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4
Normoxia (a)	1	330	405	465	560	675	685	700	860
	2	360	445	510	560	690	695	700	710
	3	580	595	705	755	870	880	890	980
	4	545	560	590	690	820	830	840	855
	5	395	450	470	530	625	640	655	710
Hypoxia 15,000 ft (b)	1	405	460	580	705	795	810	970	1200
	2	405	475	600	665	815	825	835	910
	3	655	770	820	1025	1180	1190	1215	1445
	4	405	550	580	680	905	915	925	950
	5	435	490	550	660	790	800	850	970
Difference (b – a)	1	75	55	115	145	120	125	270	340
	2	45	30	90	105	125	130	135	200
	3	75	175	115	270	310	310	325	465
	4	-140	-10	-10	-10	85	85	85	95
	5	40	40	80	130	165	160	195	-60
Mean difference		19	58	78	128	161	162	202	272
SD		90.4	69.7	51.6	100.0	88.0	86.9	97.4	140.1
SE		40.42	31.17	23.05	44.74	39.35	38.88	43.58	62.66
t		0.47	1.86	3.38	2.86	4.09	4.17	4.64	4.34
p		0.663	0.136	0.028	0.046	0.015	0.014	0.010	0.012

Table 4-4 Effect on dark adaptation of hypoxia at 15,000 ft: paired *t* test analysis

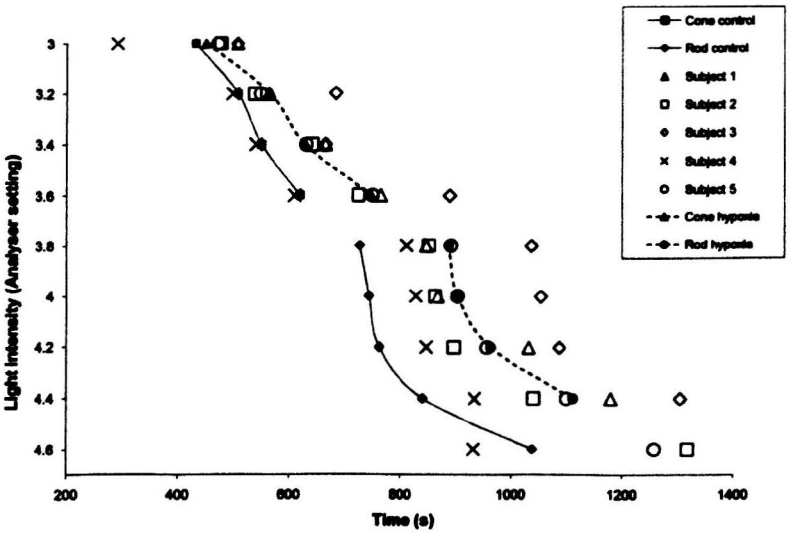


Figure 4.11 Group effect on dark adaptation of hypoxia at 15,000 ft

The early rod curve has the appearance of being translated to the right. Two subjects did not achieve sensitivity to the dimmest stimulus despite prolonged adaptation to well beyond 20 minutes. Hence, while not apparent from Figure 4.11, the hypoxic late rod

curve is not only delayed in comparison to the normoxic curve, but also appears shallower, that is, to tend to asymptote at a higher threshold than the normoxic curve, in accordance with the findings of McFarland and Evans (1939).

The results for hypoxia at 10,000 ft exhibited a similar trend to hypoxia at 15,000 ft but did not achieve statistical significance at any stimulus intensity. The group responses are shown in Figure 4.12 and individual curves in Figure 4.13.

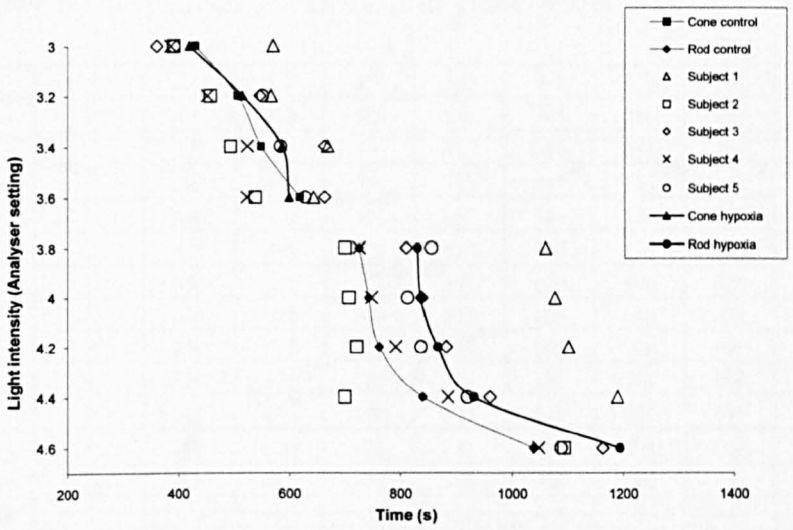


Figure 4.12 Group effect on dark adaptation of hypoxia at 10,000 ft

The trend of hypoxia at 10,000 ft to compromise rod adaptation in this group of subjects remains apparent and is attributable in large part to the particularly marked effect on Subject 1, whose early rod curve is delayed by ~5 minutes in comparison to normoxia. There is no obvious explanation for the magnitude of the effect on Subject 1 at 10,000 ft but hypoxia is well known to exaggerate within and between-subject variability in performance measures generally and the same is likely to be true of measures of visual sensitivity and visual system performance. Equally, despite the influence of Subject 1, the lack of statistical significance may attributed to the idiosyncratic response of Subject 2 who achieved and progressed early scotopic sensitivity slightly sooner when hypoxic than he did during his normoxic control adaptation.

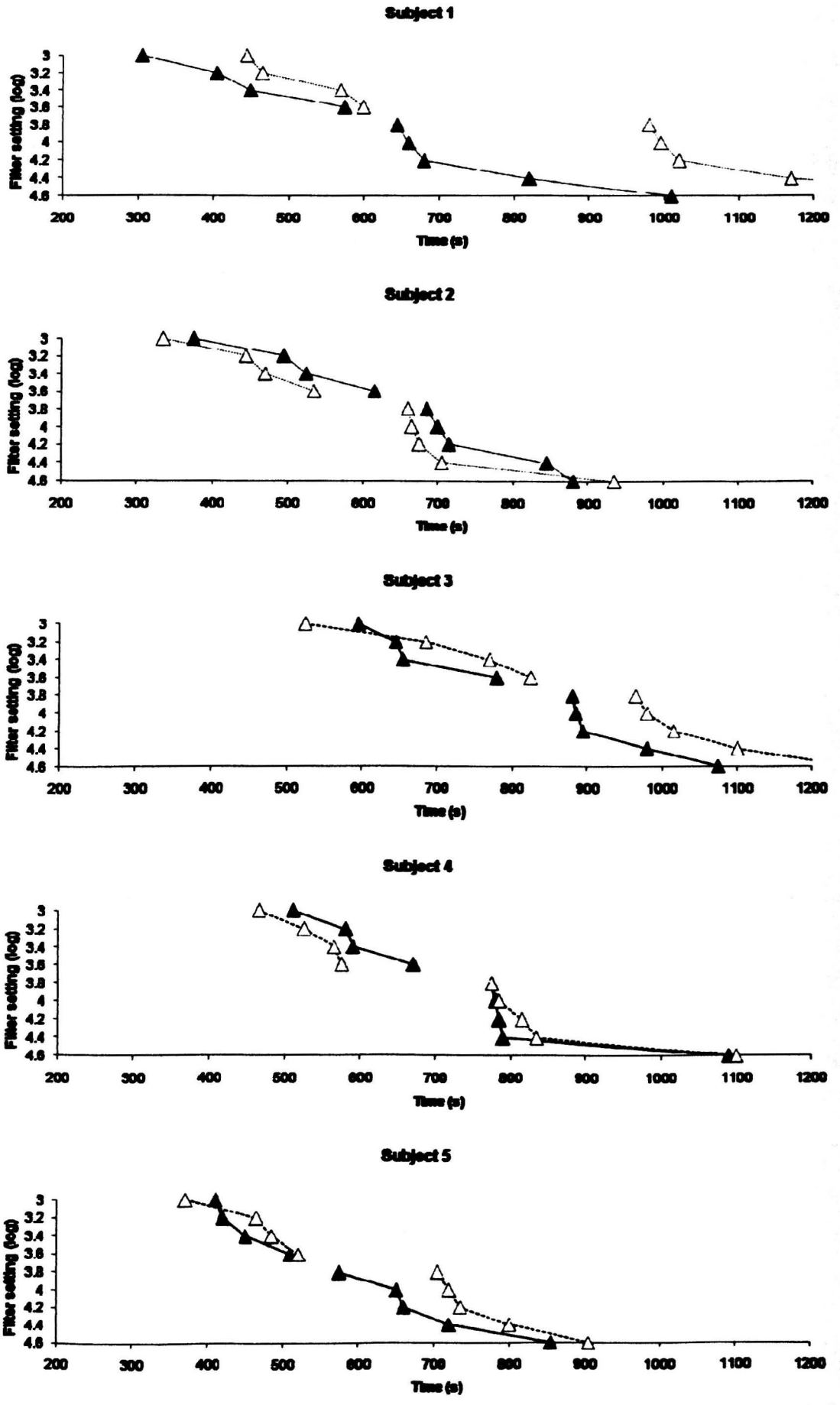


Figure 4.13 Subjects' dark adaptation curves under normoxia (▲) and hypoxia at 10,000 ft (Δ)

4.4.3 Hyperoxia

Hyperoxia was studied by examining the effect upon dark adaptation of breathing 100% O_2 at GL in comparison to control adaptations breathing air normally at GL. The results suggest a marked effect of hyperoxia to hasten the early stages of rod adaptation. This is supported by the appearance of the individual dark adaptation curves (Figure 4.14) as well as the group mean displacement times plotted against the representational normoxic baseline (Figure 4.15). In particular, the effect of hyperoxia to hasten scotopic sensitivity is highly statistically significant ($p < 0.01$) for three of the first four rod stimuli, as shown by the paired *t* test analysis in Table 4-5.

Condition	Subject	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
Normoxia (a)	1	405	435	500	550	765	780	820	970	1500
	2	435	455	530	605	690	695	705	815	840
	3	580	660	690	795	945	955	975	985	1330
	4	440	570	595	650	740	750	760	915	1060
	5	375	445	520	610	760	770	800	880	1060
Hyperoxia 100% O_2 (b)	1	335	415	475	555	630	640	685	780	970
	2	335	430	500	535	600	610	625	635	815
	3	500	575	660	765	890	900	910	925	995
	4	320	475	600	675	695	705	800	810	1225
	5	390	440	520	555	670	680	695	745	940
Difference (b – a)	1	–70	–20	–25	5	–135	–140	–135	–190	–530
	2	–100	–25	–30	–70	–90	–85	–80	–180	–25
	3	–80	–85	–30	–30	–55	–55	–65	–60	–335
	4	–120	–95	5	25	–45	–45	40	–105	165
	5	15	–5	0	–55	–90	–90	–105	–135	–120
Mean difference		–71	–46	–16	–25	–83	–83	–69	–134	–169
SD		51.8	41.0	17.1	39.8	35.5	37.2	66.5	53.8	270.4
SE		23.15	18.33	7.65	17.82	15.86	16.63	29.72	24.05	120.9
t		–3.07	–2.51	–2.09	–1.40	–5.23	–4.99	–2.32	–5.57	–1.40
p		0.037	0.066	0.105	0.233	0.007	0.008	0.081	0.005	0.235

Table 4-5 Effect on dark adaptation of hyperoxia breathing 100% O2: paired t test analysis

That the detection time displacement for the 4.2 stimulus did not achieve statistical significance appears due to the single delayed 'rogue' value of Subject 4. This stands in marked contrast to all the other early rod stimuli (3.8 to 4.4 filter settings) which were hastened in all subjects relative to the respective normoxic control values. It seems likely that the delay in detection of this single stimulus is attributable to reduced attention, as the subject detected the subsequent (4.4) stimulus almost immediately. This loss of statistical significance as the result of a single anomalous measurement provides a good example of the susceptibility of these data to Type II error as a result of using so few subjects. Nonetheless, the effect of 100% O_2 to hasten scotopic sensitivity, and, by implication, the timing of cone rod inflection, appears unequivocal.

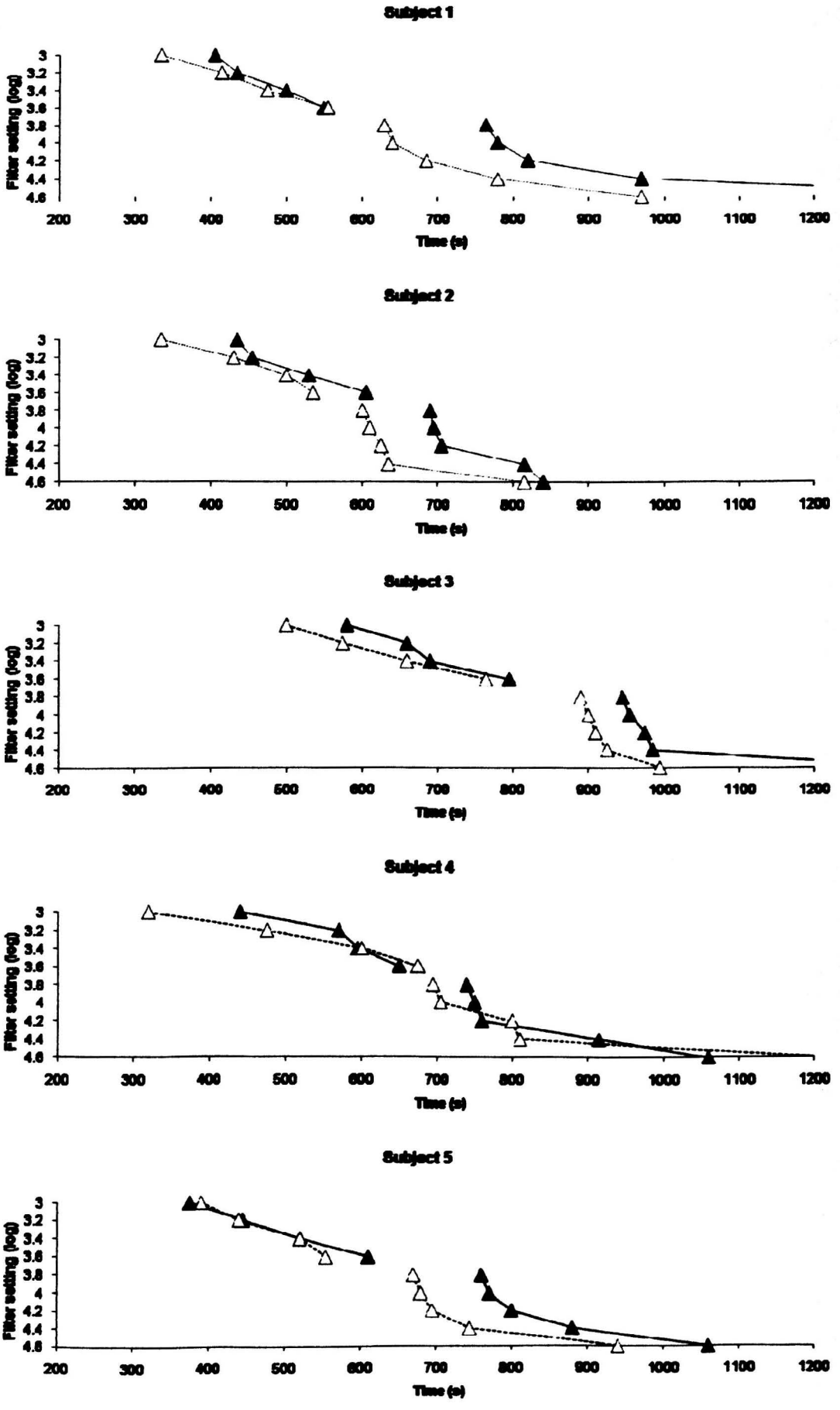


Figure 4.14 Subjects' dark adaptation curves under normoxia (▲) and hyperoxia (Δ)

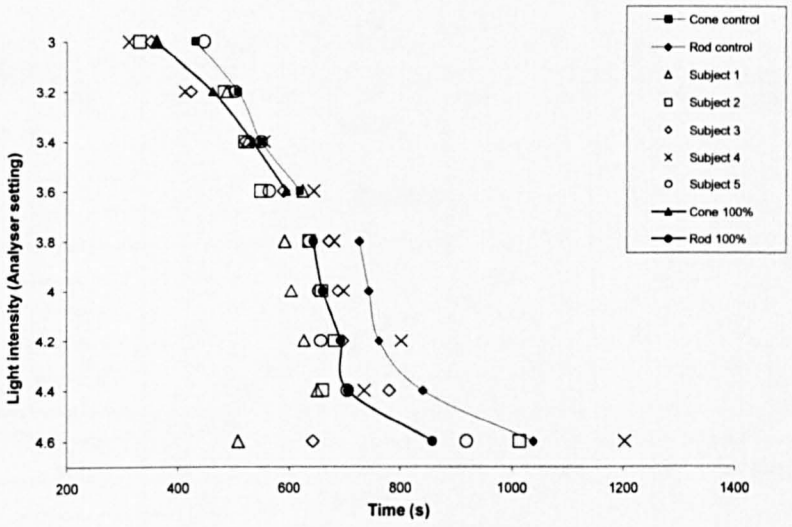


Figure 4.15 Group effect on dark adaptation of hyperoxia breathing 100% O_2

4.4.4 Hyperventilation

Individual curves during hyperventilation sufficient to lower the $P_{ET}CO_2$ to 25 mm Hg are shown relative to normoxic (and normocapnic) dark adaptation in Figure 4.16. They indicate that hyperventilation hastens sensitivity to dim stimuli during dark adaptation. The results of paired *t* test analysis are shown in Table 4-6.

Comparison of the individual curves in Figure 4.16 with the results of the paired *t* test in Table 4-6 suggest that there have been occasions when marginal results in one subject or another may have obliged loss of statistical significance ($\alpha = 0.05$). Nonetheless, statistically significant results were achieved at three stimulus intensities and were close to being achieved at three more. Hyperventilation at GL appears to hasten both cone and rod adaptation to dark, reducing the time taken to achieve scotopic sensitivity.

The effects of GL hyperventilation on group mean displacement times are shown with reference to the normoxic (and hence normocapnic) baseline for the group data in Figure 4.17.

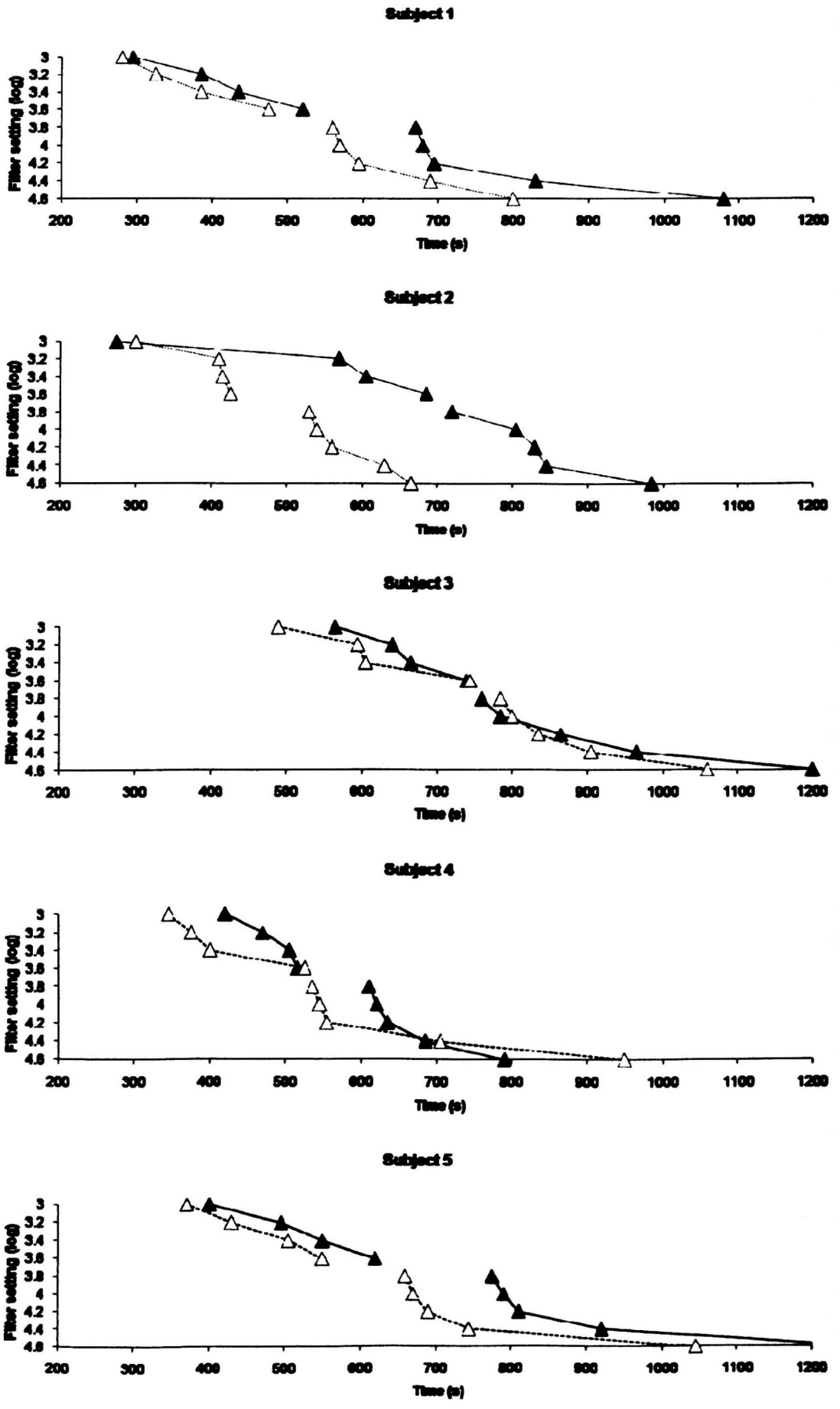


Figure 4.16 Subjects' dark adaptation curves under normoxia (▲) and hyperventilation (Δ)

Condition	Subject	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
Normoxia (a)	1	295	385	435	520	670	680	695	830	1080
	2	275	570	605	685	720	805	830	845	985
	3	565	640	665	740	760	785	865	965	1200
	4	420	470	505	515	610	620	635	685	790
	5	400	495	550	620	775	790	810	920	1260
Hyperventilation (b)	1	280	325	385	475	560	570	595	690	800
	2	300	410	415	425	530	540	560	630	665
	3	490	595	605	745	785	800	835	905	1060
	4	345	375	400	525	535	545	555	705	950
	5	370	430	505	550	660	670	690	745	1045
Difference (b – a)	1	–15	–60	–50	–45	–110	–110	–100	–140	–280
	2	25	–160	–190	–260	–190	–265	–270	–215	–320
	3	–75	–45	–60	5	25	15	–30	–60	–140
	4	–75	–95	–105	10	–75	–75	–80	20	160
	5	–30	–65	–45	–70	–115	–120	–120	–175	–215
Mean difference		–34	–85	–90	–72	–93	–111	–120	–114	–159
SD		42.5	45.7	60.7	110.4	78.2	101.2	90.3	94.2	190.9
SE		19.00	20.43	27.16	49.36	34.95	45.26	40.37	42.11	85.39
t		–1.79	–4.16	–3.31	–1.46	–2.66	–2.45	–2.97	–2.71	–1.86
P		0.148	0.014	0.030	0.218	0.056	0.070	0.041	0.054	0.136

Table 4-6 Effect on dark adaptation of hyperventilation at GL: paired *t* test analysis

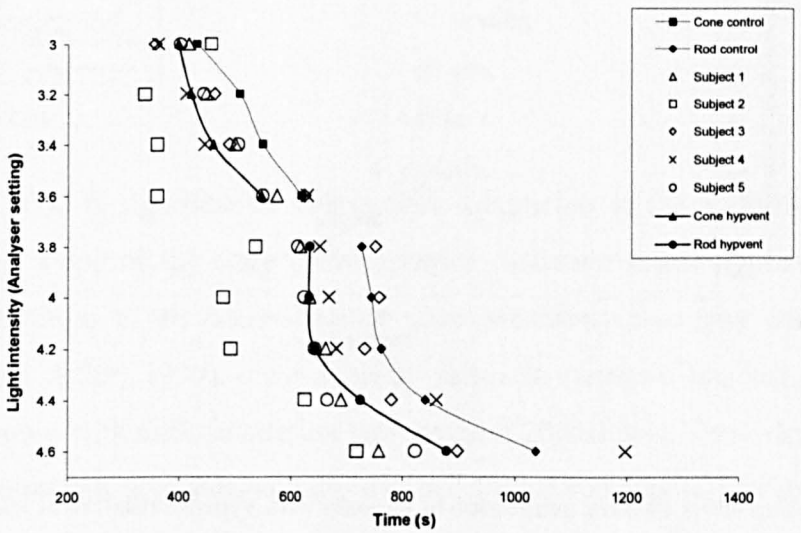


Figure 4.17 Group effect on dark adaptation of moderate hyperventilation at GL

Hyperventilation at 15,000 ft elicited a similar response to that at GL. The results of paired *t* test analysis are shown in Table 4-7 and group mean displacement times are represented in Figure 4.18. Figure 4.18 shows that the tendency for hypoxia at 15,000 ft

to delay dark adaptation when breathing normally is completely overwhelmed by the effect of moderate hyperventilation, which still hastens the earlier stages of both cone and rod adaptation relative to the control exposure. However, the effect of hyperventilation at 15,000 ft to hasten dark adaptation does appear somewhat 'blunted' by the concomitant hypoxia in comparison to its effect at GL, particularly for the later stages of cone and rod adaptation. Nonetheless, statistical significance is still achieved at two cone stimulus intensities.

Stimulus	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
Mean difference	–81	–83	–56	–17	–55	–55	–50	–12	9
SD	81.7	60.4	39.6	39.6	52.0	55.6	73.7	116.1	194.9
SE	36.52	27.00	17.71	17.72	23.24	24.85	32.94	51.90	87.16
t	–2.22	–3.07	–3.16	–0.96	–2.37	–2.21	–1.52	–0.23	0.10
p	0.091	0.037	0.034	0.392	0.077	0.091	0.204	0.829	0.923

Table 4-7 Effect of hyperventilation at 15,000 ft relative to GL orthopnoea: paired t test analysis

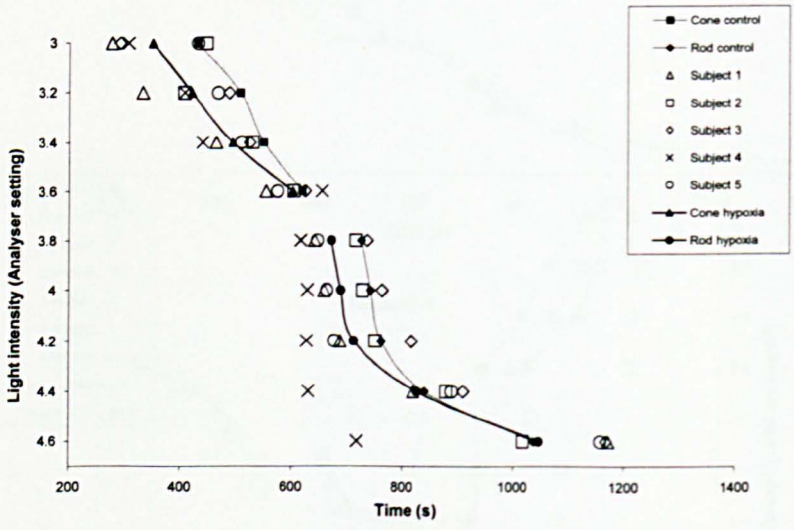


Figure 4.18 Group effect on dark adaptation of hypoxia with hyperventilation at 15,000 ft

4.5 Discussion

4.5.1 General observations

The chosen visual stimulus and study method highlights well the early progression of scotopic sensitivity and, by inference, the timing of cone rod inflection. The normoxic dark adaptations show substantial within-subject variability in the rate of dark

adaptation from day to day, especially for rods. However, there is even greater variability between subjects, particularly for the cone-dominated portion of the mixed dark adaptation curve. Even so, the imposed respiratory disturbances readily demonstrate effects on the time course of dark adaptation when analysing paired control/condition data, despite using only five subjects. Since a single rogue value is sufficient to oblige loss of statistical significance, making these analyses extremely vulnerable to Type II error, it seems reasonable to retain $\alpha = 0.05$ when considering the level of statistical significance for the *t* test analysis. A total of 45 two-tailed *t* tests have been conducted which would be expected to result, purely by chance, with only one or two statistically significant results, in each direction, at this α level. Instead, there are 15 statistically significant results, all suggesting either that hypoxia delays or that hypocapnia and hyperoxia hasten dark adaptation. That is to say, they are consistent within each respiratory condition. Furthermore, some of these results are highly statistically significant ($p < 0.01$) while others are close to achieving significance at this α level. Given the clear vulnerability to Type II error when using only five subjects, it seems unlikely that these results overstate the impact of respiratory disturbance on the temporal process of dark adaptation. Furthermore, the magnitudes of some of the temporal displacements are substantial. In sum, the findings indicate gross effects of respiratory disturbance.

4.5.2 Hypoxia

Hypoxia at 15,000 ft significantly delays dark adaptation at the majority of stimulus intensities. The slope of the cone curve appears shallower under hypoxia, consistent with the expectation of elevated absolute cone sensitivity noted by early observers (McDonald and Adler, 1939). Two subjects failed to perceive the last rod stimulus despite continuing with dark adaptation well beyond 20 minutes. Thus, detection of the dimmest stimulus was also substantially delayed for all subjects and is consistent with the expectation of elevated absolute rod sensitivity (McFarland and Evans, 1939). However, it is the effect on early rod adaptation that is particularly pronounced, such that the onset (cone rod inflection) and rapid early progression (first 30 s) of scotopic sensitivity are delayed by several minutes under hypoxia. This portion of the rod curve appears translated to the right under hypoxia. This lateral temporal displacement of early scotopic sensitivity does not appear to have been reported previously (Connolly and Hosking, 2006).

Mean detection time displacement curves under hyperoxia (circles) and hypoxia at 15,000 ft (triangles) and 10,000 ft (squares) are represented against the global normoxic baseline curve in Figure 4.19 to compare the effects of the various conditions of altered PO_2 on the time course of dark adaptation. The trend of hypoxia to delay rod adaptation remains apparent at 10,000 ft but the effect is less marked than at 15,000 ft and did not achieve statistical significance at any stimulus intensity. This reflects the idiosyncratic responses of one subject and the lack of statistical power generally when using as few as five subjects. Nonetheless, the appearance of the rod curves in Figure 4.19 suggests a relationship between PO_2 and the temporal shift of early scotopic sensitivity, with the prevailing PO_2 determining when rapid progression of scotopic sensitivity can occur. In consequence, the effect of hypoxia to delay rod adaptation appears progressive with increasing equivalent altitude.

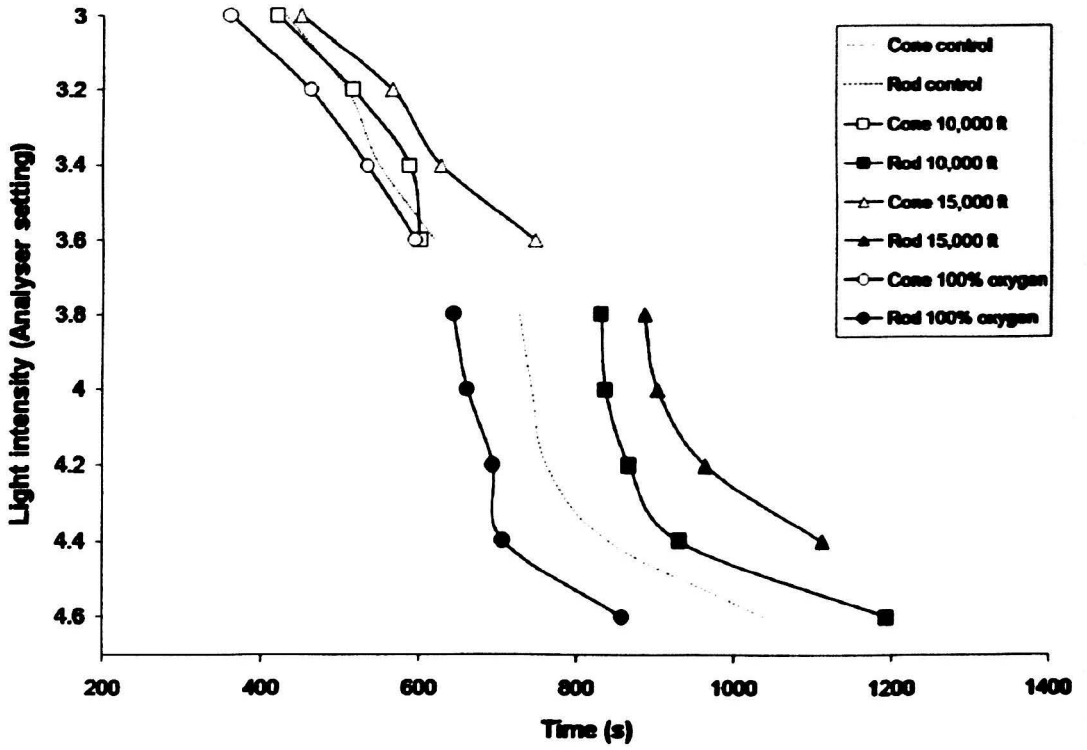


Figure 4.19 Temporal shifts of early scotopic adaptation curves under altered PO_2

Early studies of dark adaptation under hypoxia involved relatively slow and infrequent measurements of threshold stimulus intensity which would be unlikely to capture a data point close to the moment of cone rod inflection (McFarland and Evans, 1939). The prominence of this feature would then be lost when taking mean curves over a number of exposures. In many studies the inflection point is barely apparent or has been inferred in the absence of appropriate data points (McFarland and Evans, 1939; Kobrick and

Appleton, 1970). The current study highlighted the rapid progression of early scotopic threshold sensitivity using fixed stimulus intensities and measuring time as the dependent variable. Contrary to earlier studies, hypoxia does appear to affect the timing of cone rod inflection and early scotopic adaptation, shifting the initial rod curve to the right. This also means that past studies are likely to have averaged mixtures of pre- and post-inflection thresholds, further camouflaging the true inflection point and masking effects on the early rod curve. The appearance of the lateral shift of the early rod curve is quite distinct from the hypoxic elevation of later threshold sensitivity that was clearly demonstrated by McFarland and Evans (1939). Hence, hypoxia must have both horizontal (temporal) and vertical (sensitivity) effects that may be distinguished at the beginning and end, respectively, of the rod adaptation curve.

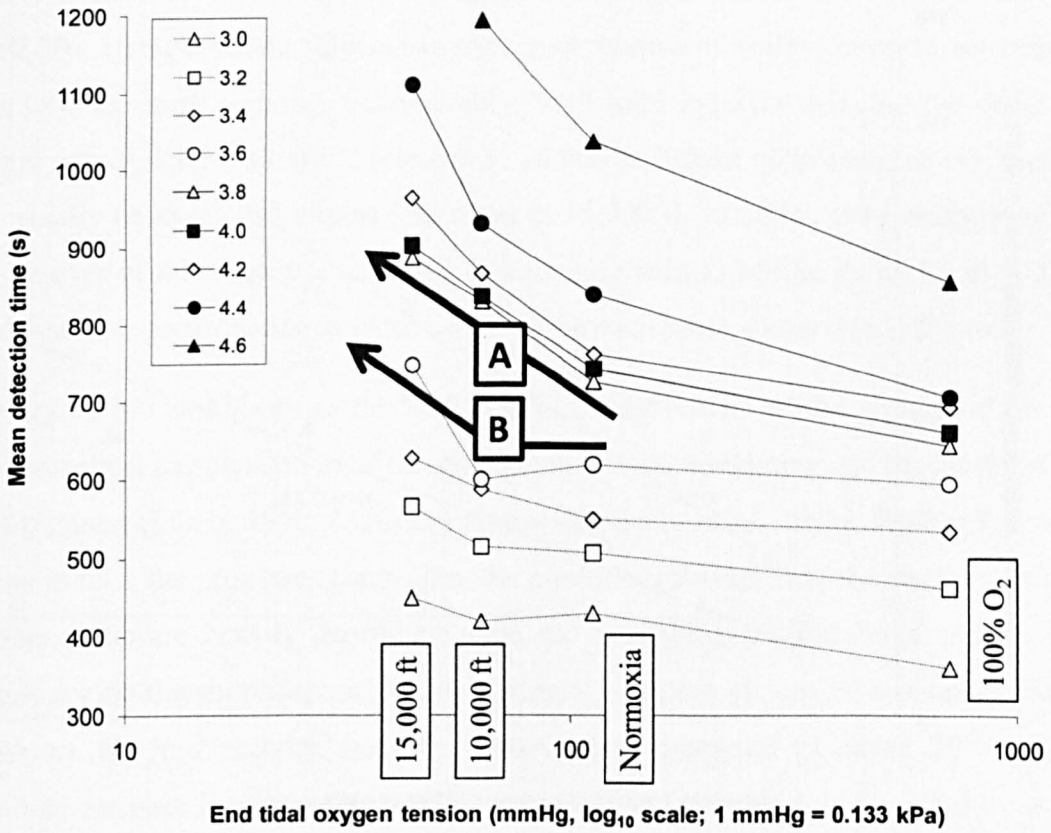


Figure 4.20 Relationship of stimulus detection time to PO_2: effects of hypoxia

The relationship between $P_{ET}O_2$ and the mean detection time for each of the nine stimuli is explored further in Figure 4.20. Cone stimuli are shown with dashed lines and rod stimuli with solid lines. The appearance of the rod curves suggests that the effect of hypoxia to delay scotopic sensitivity is rapidly progressive once PO_2 falls below ~100

mm Hg, that is, with any reduction below normal (Arrow A). In contrast, the cone curves remain flat until PO_2 drops below that associated with an equivalent altitude of 10,000 ft (Arrow B). However, by the time PO_2 has fallen to that equivalent to breathing air at 15,000 ft the cone curves are also subject to a temporal delay.

On the basis that cone rod inflection occurs in the few seconds before detection of the 3.8 stimulus, the appearance of the 3.8 stimulus curve in Figure 4.20 may be considered to represent the effect of altered PO_2 on the timing of cone rod inflection. Clearly, the results presented here suggest that hypoxia progressively delays the timing of cone rod inflection. The proximity of the curves for the 3.8, 4.0 and 4.2 stimuli is unaffected by changes in PO_2, suggesting that following cone rod inflection the rapid progression of scotopic sensitivity proceeds at a rate that is independent of PO_2.

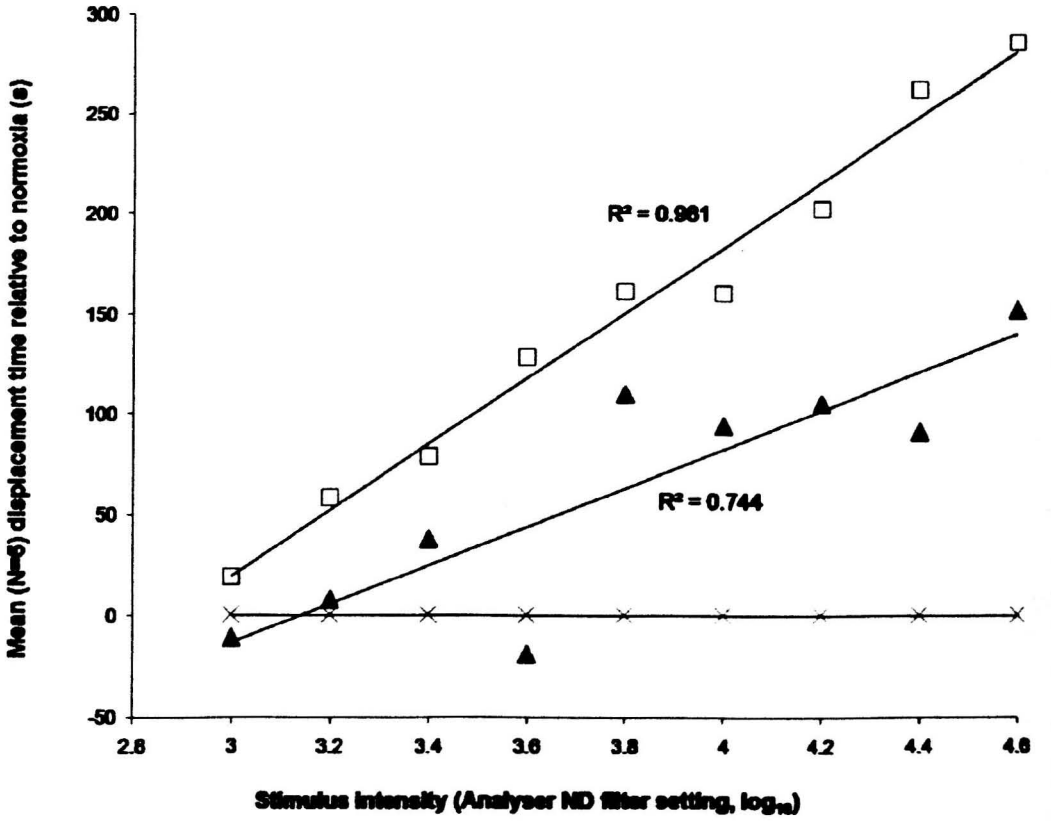


Figure 4.21 Nature of the detection time delay at 10,000 ft (▲) and 15,000 ft (□)

In Figure 4.21 the mean detection time displacements for each stimulus under hypoxia at 15,000 ft and 10,000 ft have been plotted in relation to a normalised normoxic baseline. The graphs indicate that the effect of hypoxia to delay adaptation is steadily progressive with decreasing stimulus intensity. Given that an inflection point 'notch' exists in these data, the strength of the linear correlation between detection time delay

and $\log_{10}$ stimulus intensity under hypoxia at 15,000 ft is surprising. Extension of this line towards the normoxic baseline would seem to indicate that this degree of hypoxia would not compromise detection time to stimuli much brighter than the first one used in this study. This implies that the processes involved in early cone adaptation should be unaffected by this degree of hypoxia. Although the data for hypoxia at 10,000 ft appear less well correlated, the correlation coefficient $r = 0.863$ remains statistically significant for nine observations ($p = 0.003$).

Only one other study appears to have used a comparable method of assessing dark adaptation by fixing stimulus intensity until detected (Jackson, Owsley and McGwin, 1999). That study assessed the effect of advancing age to impair the progress of dark adaptation, which exhibited a similar appearance to the effect of hypobaric hypoxia. Subjects in their 70s achieved mean inflection times ~2.5 minutes slower than subjects in their 20s. Using a young subject sample representative of healthy aircrew, the results of the current study compare unfavourably, with mild hypoxia delaying the onset of scotopic sensitivity by almost 2 minutes at 10,000 ft (where supplementary O_2 would not normally be used) and almost 3 minutes at 15,000 ft. In effect, the performance of younger eyes in this regard is impaired in a manner akin to ageing by up to 50 y. The dark adaptation performance of older eyes may be even more vulnerable to hypoxia.

Recovery of rod sensitivity in the dark requires deactivation of the photoproducts of light absorption, depolarisation of the photoreceptor cell membrane and regeneration of visual pigment (Lamb, 1990; McBee, Palczewski, Baehr *et al*, 2001). Examining each of these in turn, the processes controlling the phototransduction cascade reactions in rod outer segments are heavily dependent upon the availability of metabolic energy, for example for rhodopsin phosphorylation, synthesis of cyclic guanosine monophosphate, to support enzyme activity and for chromophore transport (Picaud, 2003). The substantial increase in photoreceptor ion pump activity in the dark is impaired by even mild hypoxia (Steinberg, 1987), suggesting that the availability of metabolic energy in the photoreceptor is critically dependent upon an ongoing supply of O_2. Finally, hypoxia impairs regeneration of rhodopsin in vitro (Ostroy, Gaitatzes and Friedmann, 1993). A recent review of dark adaptation kinetics suggested that the impact of hypoxia may be explained by effects on photoreceptor circulating current and/or slowing of 11-cis retinal synthesis in the pigment epithelium (Lamb and Pugh, 2004). The current study supports that hypoxia may impair scotopic sensitivity *in vivo*, at least in part, by delaying photoproduct deactivation and/or regeneration of photochemical sensitivity.

4.5.3 Hyperoxia

In comparison to normoxic dark adaptation, hyperoxia hastens cone rod inflection and causes a leftward temporal shift of the early scotopic sensitivity curve, significantly hastening detection of early rod stimuli in this study ($p < 0.01$). Figure 4.20 has been reproduced at Figure 4.22 to highlight the effects of hyperoxia.

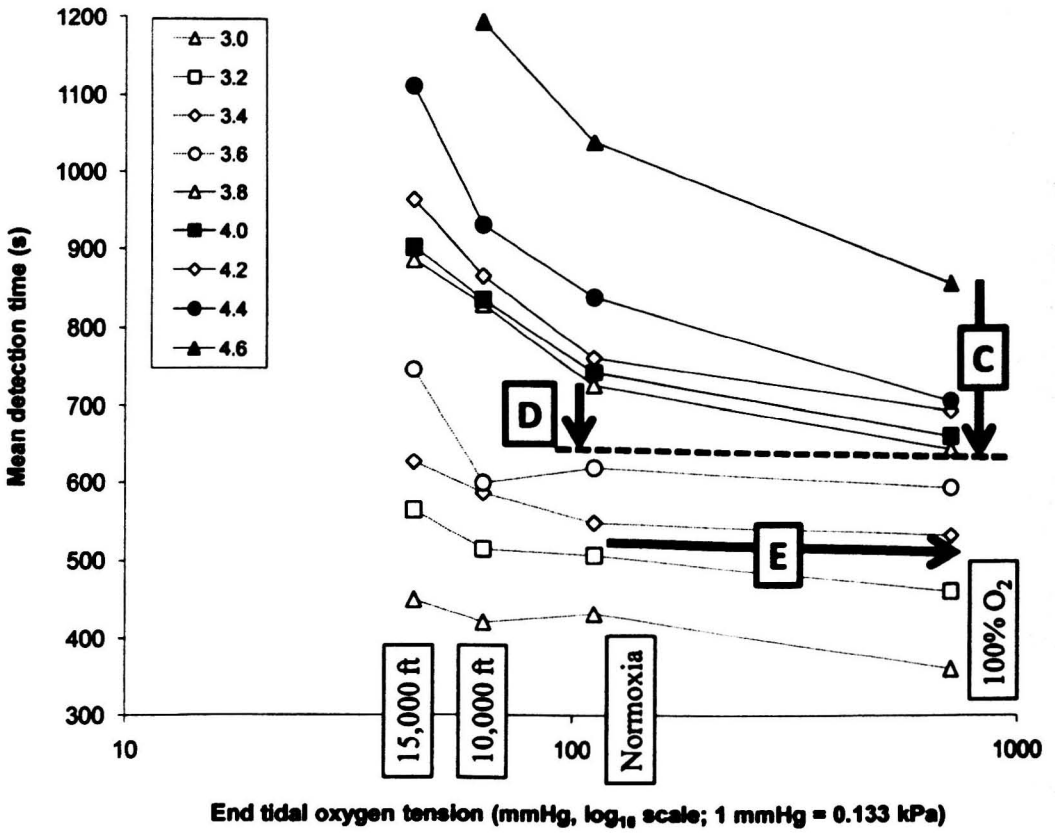


Figure 4.22 Relationship of stimulus detection time to PO_2: effects of hyperoxia

Most obviously in Figure 4.22, hyperoxia shortens the duration of dark adaptation by compressing the time course of rod adaptation over the range of stimulus intensities tested (Arrow C). Hyperoxia also shortens the time to the start of scotopic sensitivity (cone rod inflection) relative to normoxia (shown by Arrow D). In contrast, the 'flat' cone curves suggest that hyperoxia has little influence on the time course of late cone adaptation (Arrow E), although the time to detection of the first (3.0) cone stimulus was significantly reduced under hyperoxia ($p = 0.037$) so perhaps hyperoxia may benefit early cone adaptation.

This last observation is supported by the appearance of the early cone curve under hyperoxia plotted against a normalised normoxic baseline in Figure 4.23. This graph also shows the hypoxia data at 15,000 ft. Solid symbols represent statistically significant data points ($p < 0.05$). In this instance, polynomial trend lines have been fitted to the hyperoxia and 15,000 ft data. Those for the 15,000 ft hypoxia data continue to suggest a near linear relationship between detection time displacement and log stimulus intensity, but the hyperoxia data clearly cannot fit a linear correlation. The cone curve suggests that while hyperoxia might possibly hasten the early stage of cone adaptation, there is a marginal benefit to detection of later cone stimuli. However, the benefit to scotopic adaptation is substantial, is greater for dimmer stimuli and appears to increase disproportionately as rod adaptation progresses.

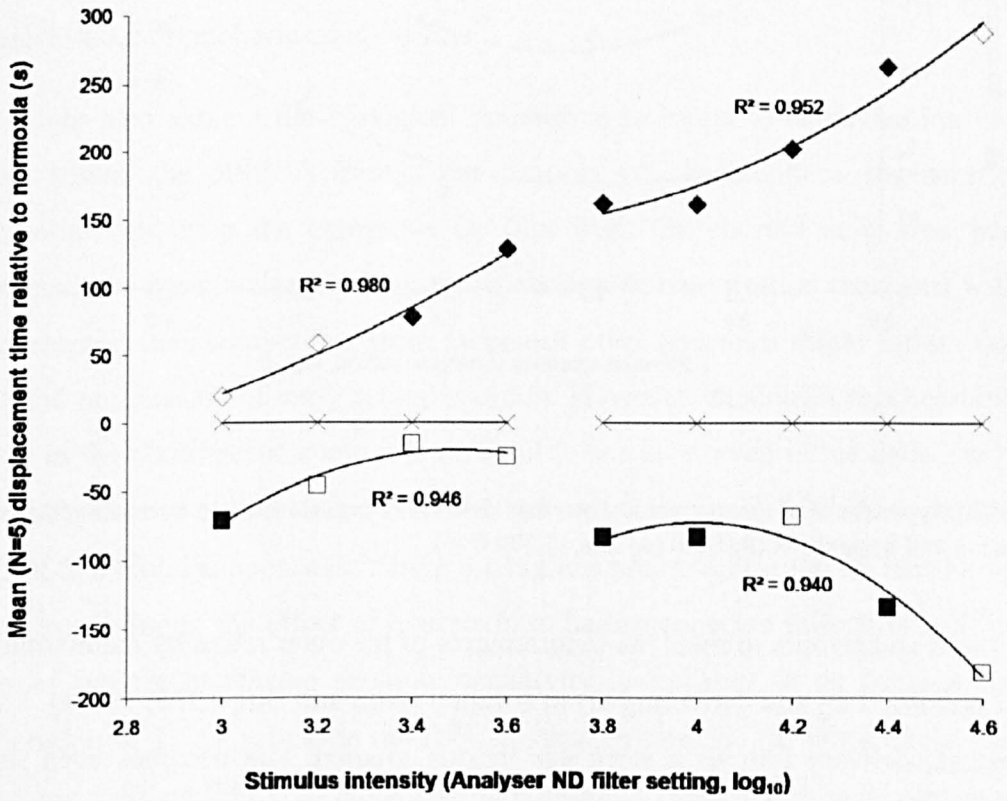


Figure 4.23 Hyperoxic (□) detection time relative to normoxia (x) and hypoxia (◊)

If one assumes that optimal dark adaptation is achieved when breathing 100% O_2, the normoxic and hypoxic data can both be represented against an 'optimal' hyperoxic baseline. This is shown in Figure 4.24 for just the rod data, fitted with polynomial trend lines, demonstrating that the course of normoxic rod adaptation is similar in appearance to the hypoxic data at 10,000 ft and 15,000 ft when plotted against a normalised hyperoxic baseline. The progress of rod adaptation under normoxic conditions then

follows a similar pattern to that under hypoxic conditions. Thus, early scotopic adaptation appears functionally hypoxic under normal respiratory conditions, breathing air at rest at one atmosphere.

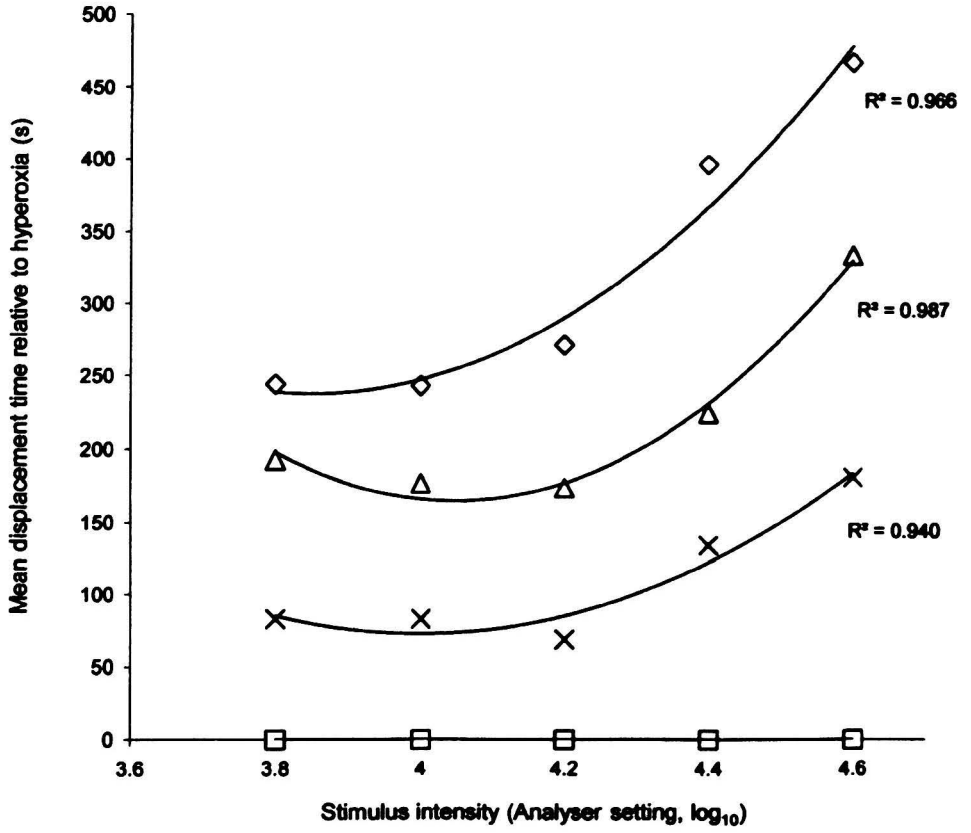


Figure 4.24 Appearance of the normoxic detection time curve (x) relative to a normalised hyperoxic baseline (□) and hypoxia at 10,000 ft (Δ) and 15,000 ft (◊)

The choroid endeavours to meet the requirements of the outer retina by maintaining the highest possible PO_2 and delivering O_2 in solution (Alm and Bill, 1970; 1972a). For the outer retina, delivery follows a steep gas tension gradient that becomes even steeper when rod QO_2 increases in darkness (Linsenmeier, 1986). Tissue PO_2 falls to extremely low levels near photoreceptor inner segments (Stefansson, Wolbarsht and Landers, 1983; Ahmed, Braun, Dunn *et al*, 1993; Yu and Cringle, 2002) and may compromise oxidative metabolism in inner segment mitochondria despite increased O_2 flux from the inner retina (Wangsa-Wirawan and Linsenmeier, 2003). Accordingly, the O_2 requirements of the dark-adapted eye appear vulnerable to reductions in choroidal PO_2 induced by decreasing choroidal flow (Yancey and Linsenmeier, 1989). Breathing 100% O_2 does not appear to affect choroidal blood flow in humans (Kergoat and Faucher, 1999). Hence, the increase in P_aO_2 when breathing normobaric 100% O_2, from

~100 mm Hg to ~700 mm Hg, should enhance the delivery of O_2 from the choroid to the photoreceptors and, if the photoreceptors are functionally hypoxic, result in an increase in outer retinal QO_2. In the cat, 100% O_2 does not appear to increase photoreceptor QO_2 by comparison with normoxia (Linsenmeier and Yancey, 1989), although the choroidal PO_2 reported in that study was lower than might be expected. On the other hand, hyperoxia causes slight alkalinization at the outer nuclear layer in the dark that suggests some suppression of rod photoreceptor glycolysis, predicting a slight increase in QO_2 in comparison to normoxia (Yamamoto and Steinberg, 1992). In that study, electroretinogram effects suggested that 'the tissue was hypoxic prior to the addition of O_2 even though the P_aO_2 before and after the hyperoxic episode was within the normoxic range'. This offers support to the contention that human rod photoreceptors might also be functionally hypoxic in the dark when breathing air normally under normobaric conditions.

This might also explain the biological imperative to locate re-isomerisation of 11-*cis* retinal within the RPE. Thereby, the process of chromophore regeneration has preferential call upon the enormous O_2 flux from the choroid at a time when the photoreceptors are functionally hypoxic. If rhodopsin regeneration remained within the photoreceptor, then competition from numerous other processes might further delay the onset and progression of early scotopic vision. However, rhodopsin regeneration is not located in the photoreceptor and it is difficult to see how, even in the dark, the process of chromophore re-isomerisation could be starved of O_2 in the RPE, lying immediately adjacent to a blood supply maintaining the highest possible PO_2 and O_2 flux to the outer retina. Accordingly, the effect of hyperoxia to hasten cone rod inflection and the early phase of rapidly increasing scotopic sensitivity is unlikely to be through hastened regeneration of 11-*cis* retinal in the RPE. Instead, it must be far more likely that these effects are mediated through enhanced photoproduct deactivation and reintegration of chromophore in photoreceptor outer segments.

4.5.4 Hypocapnia

Hypocapnia induced by voluntary hyperventilation, sufficient to lower the $P_{ET}CO_2$ to 25 mm Hg, hastens cone and rod adaptation to dark at GL and at an equivalent altitude of 15,000 ft. At 15,000 ft, moderate hyperventilation overwhelms the tendency of hypobaric hypoxia to delay dark adaptation at that altitude. Hyperventilation at 15,000 ft was associated with an increase in mean $P_{ET}O_2$ to 8.23 kPa (61.7 mm Hg) from 5.89

kPa (44.2 mm Hg) when breathing air normally at that altitude, thereby elevating mean S_aO_2 from 78% to 94%. This is expected as the fall in P_ACO_2 must be made up by a corresponding and proportional increase in the alveolar partial pressures of the gases in the breathing mixture. Nonetheless, it should not be assumed that net cerebral oxygenation is necessarily increased as considerable cerebral vasoconstriction will accompany the hypocapnia. This is generally associated with a decreased jugular venous O_2 content, reflecting poorer cerebral oxygenation.

However, the effect of hyperventilation on scotopic sensitivity should be independent of any concomitant change in $P_{ET}O_2$ (and hence also P_AO_2, P_aO_2 and choroidal PO_2) or S_aO_2. Firstly, the increase in PO_2 and S_aO_2 with hyperventilation might tend to normalise the dark adaptation curve, in comparison to the effect of hypoxia alone, but should not hasten it further. Secondly, the magnitude of the increase in $P_{ET}O_2$ (and S_aO_2) is relatively slight, giving values comparable to hypoxia at 10,000 ft, yet the adaptation curves are quite different. Thirdly, hyperventilation at GL also caused a slight increase in $P_{ET}O_2$ (albeit with a marginal effect on S_aO_2) yet its effect on dark adaptation was even more pronounced than hyperventilation at altitude. Finally, the effect of hyperventilation at GL is of a magnitude at least comparable to (if not greater than) that observed when breathing 100% O_2 at GL, yet it is hardly likely that the very slight increase in PO_2 and S_aO_2 associated with moderate hyperventilation could account for the effect of hyperoxia observed when breathing 100% O_2. The effect of hyperventilation to hasten dark adaptation must be due to the resulting hypocapnia and not to any associated slight increase in PO_2.

The influence of hyperventilation on modifying the dark adaptation response to hypoxia at 15,000 ft may be gauged by comparing the detection times measured at 15,000 ft with and without hyperventilation, as shown in Table 4-8. The detection time displacements under hypoxia alone are compared with those measured at 15,000 ft with accompanying voluntary hyperventilation. These have then been subject to a paired *t* test analysis which shows a highly statistically significant effect of hyperventilation to hasten adaptation at 15,000 ft, for the majority of stimulus intensities, in comparison to the result that might be expected with normocapnia at that altitude. It may be argued that pairing the data in this way is invalid as the experiments were conducted on different days against different normoxic baselines. Accordingly, one-way ANOVA was also conducted on the unpaired detection time displacement data and raw detection time data. The former support the paired *t* test results while the latter continue to suggest a

significant benefit on rod sensitivity of hyperventilation at 15,000 ft, with the cone results approaching statistical significance. It is noteworthy that the subject least affected by hypoxia at 15,000 ft was also the subject who exhibited secondary hypocapnia ($P_{ET}CO_2$ of 29 mm Hg).

Stimulus	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4
Paired *t* test analysis (hypoxia with hyperventilation minus without hyperventilation)								
Mean difference	-100	-145	-134	-145	-216	-217	-252	-222
SD	113.9	65.6	39.7	118.6	61.3	53.3	74.5	206.1
SE	50.92	29.33	17.78	53.03	27.4	23.85	33.34	92.18
t	-1.96	-4.94	-7.54	-2.73	-7.88	-9.1	-7.56	-2.41
p	0.121	0.008	0.002	0.052	0.001	0.001	0.002	0.074
One-way ANOVA (unpaired detection time displacement data relative to control)								
F	3.37	12.29	21.25	9.08	22.34	22.12	21.28	4.47
p	0.104	0.008	0.002	0.017	0.001	0.002	0.002	0.067
One-way ANOVA (unpaired raw detection time data)								
F	3.59	2.37	4.71	5.02	10.05	9.73	11.94	7.5
p	0.095	0.162	0.062	0.055	0.013	0.014	0.009	0.025

Table 4-8 Effect of accompanying hyperventilation relative to pure hypoxia at 15,000 ft

The mechanism whereby hypocapnia might affect visual sensitivity remains conjectural although the resulting respiratory alkalosis is most obviously implicated, as hypocapnia is well known to increase nerve cell excitability. Wald, Harper, Goodman *et al* (1942) found that the effect of hyperventilation to enhance sensitivity was lost when adding 2% CO_2 to the breathing gas. Also, artificially-induced metabolic alkalosis does not affect absolute visual sensitivity, in contrast to the effect of hypocapnia, further implying that it is the fall in P_ACO_2 that is significant (Alpern and Hendley, 1952). Charged acid-base species in blood are prevented from crossing into brain extracellular fluid by the BBB and the RPE similarly controls ion flux between the choroid and the SRS (Strauss, 2005). In contrast, CO_2 is highly soluble, diffuses rapidly and is the only acid-base species that freely crosses the BBB. Arterial hypocapnia may tend to reduce choroidal flow slightly (Samuel and Beaugié, 1974; Harris, Arend, Wolf *et al*, 1995) but should still be expected to generate a PCO_2 gradient promoting outer retinal alkalosis. In the brain, however, some evidence suggests that hypocapnia may not increase intracellular pH *in vivo* due to a compensatory increase in the production of metabolic acids and stimulation of glycolysis (Mellergård and Siesjö, 1998). A similar effect of hypocapnia on the outer retina may be of considerable benefit to photoreceptors during dark adaptation.

The retinal pH gradient and changes induced by light appear similar in mammals and lower vertebrates, while changes in pH are known to affect retinal cell function and

sensitivity (Barnes, 1998). Light induces outer retinal alkalinization superimposed on a gradient of decreasing pH from GC to photoreceptors. In the cat the most acidic pH was recorded in the dark near the outer nuclear layer, close to the site of photoreceptor mitochondrial activity and cytoplasmic glycolysis (Yamamoto, Borgula and Steinberg, 1992). At low levels of illumination, arterial hypoxaemia also acidifies the extracellular space at the outer nuclear layer, suggesting that rods are sensitive to mild hypoxia during dark adaptation, with a consequent increase in glycolytic activity (Yamamoto and Steinberg, 1992). Müller cells may also have a role in removing acid from this region through enhanced elimination of CO_2 (Barnes, 1998). Respiratory alkalosis may help to offset the acidification that occurs in the dark and with hypoxia, to the benefit of the underlying metabolic processes, through enhanced elimination of H^+ through the choroid via the bicarbonate buffer system. Notwithstanding the foregoing, the mechanism by which hypocapnia might exert a temporal effect to shorten the delay time to the onset of scotopic vision is unknown.

4.5.5 Lessons for subsequent studies

Subjects

More accurate control and monitoring of subject variables including sleep, diet, alcohol and coffee were considered worthwhile in an attempt to reduce the variability in visual performance within and between subjects that might result from extraneous factors. Compliance with pre-exposure requirements was reasonable but with lapses, leading to the introduction of a lifestyle questionnaire (Appendix 3) in an effort to identify and document potentially confounding factors in future experiments.

Experimental design

Practical constraints, in particular the small number of subjects, the need to repeat some experiments, and the limited availability of the hypobaric chamber, dictated that the order of the different experiments could not be randomised fully between subjects. Additionally, the order of control and condition exposures was invariant in each experiment. These deficiencies were addressed, as far as practicable, in subsequent gas mixture experiments. Also, it was impossible to fully mask subjects to the nature of their respiratory exposures in the decompression chamber and this was also addressed for the respiratory conditions imposed in later experiments.

Vision testing

Training effects on the Friedmann Analyser are likely to have been minimal but attentional effects of respiratory disturbance will almost certainly have influenced the results under dark adaptation, with prolonged periods of inactivity without sensory stimulation encouraging the subjects' attention to drift away from the task in hand. For the studies that followed, visual tasks were chosen that promoted maintained attention and repetitive forced responses. However, the ambient environment was controlled to avoid extraneous influences, especially noise, on psychological arousal level, recognising that these could confound interpretation of performance differences. Despite great effort, it was difficult to ensure a perfect blackout within the chamber throughout the course of the Study and the importance of reproducing exactly the same ambient lighting conditions was taken forward to the mesopic work.

Respiratory exposures

Experiments were lost on two occasions due to problems with the respiratory apparatus. A rare breathing gas regulator failure occurred when it began inappropriately to deliver breathing gas under positive pressure, resulting in increasing expiratory resistance that obliged cessation of the experiment. On another occasion a breathing mask inspiratory valve became stuck open, resulting in complete expiratory obstruction as the patent compensation tube transmitted expiratory mask cavity pressure to the compensation chamber of the expiratory valve, forcing it closed. In all subsequent work, the breathing equipment was regularly and carefully serviced and checked thoroughly prior to each use and the masks were cleaned immediately after each experiment. Three dedicated breathing gas regulators were used for all the subsequent work.

Visual adaptation

Using the procedure of retinal bleach and dark adaptation conducted here, under normoxic conditions cone rod inflection would be expected to occur within 900 s from the start of dark adaptation. During the adaptations conducted under hypoxia at 10,000 ft, four subjects achieved cone rod inflection within this time, with the fifth detecting the first rod stimulus at 905 s. Accordingly, it is reasonable to assume that a matching procedure of retinal bleach followed by 15 minutes of dark adaptation will bring subjects close to or beyond the time of cone rod inflection. In the mesopic experiments that follow, a period of time was then allowed for subjects to light adapt to controlled mesopic adaptation states that should be at least one order of magnitude brighter. This

ensured that all subjects underwent identical well-defined processes of visual adaptation and that all achieved stable mesopic adaptation states by the time mesopic vision testing began, regardless of respiratory condition. To assist with validating this, it is noted that the human contrast sensitivity function can be used to follow the process of adaptation to dark (Margrain, 1997). Accordingly, if the first vision tests conducted following contemporaneous visual and respiratory adaptation were tests of contrast sensitivity, then the general quality and reproducibility of the results should indicate whether stable mesopic adaptation states were achieved.

4.5.6 Statistical power

While it was necessary to use a limited number of subjects for the hypobaric chamber studies, a larger subject pool could be employed in subsequent laboratory studies using hypoxic gas mixtures. The hypobaric dark adaptation study produced statistically significant results at $\alpha = 0.05$ using only five subjects acting as their own controls. It was considered reasonable to use selected data from these experiments to inform the decision on an appropriate number of subjects for subsequent visual threshold tests in dim light under respiratory disturbance. Specifically, the displacement times for detection of the 3.8 stimulus under hypoxia at 10,000 ft and 15,000 ft were examined. For this stimulus the normoxic SD detection time is 88 s (Table 4-3). The mean delay to detection of this stimulus at 10,000 ft was 100 s and at 15,000 ft was 161 s. Using a two-sample *t* test on the data collected at 15,000 ft, detection of a statistically significant difference to $\alpha = 0.05$ with a minimum of 90% power would require 11 subjects. This would provide a very high degree of sensitivity to the effect of hypoxia at 15,000 ft to delay cone rod inflection. More usefully, a pool of 12 subjects would enable comparison of the effects of additional factors (such as gender and exposure order) by sub-group while retaining a balanced design to allow parametric statistical analysis using ANOVA. At 10,000 ft a mean displacement of 100 s was not statistically significant. However, a study achieving this difference when employing 12 subjects would still achieve in excess of 75% power to $\alpha = 0.05$.

4.5.7 Summary and future work

The results support the hypotheses. Hypoxia impairs visual sensitivity, compromises rod adaptation to dark and delays achievement of scotopic sensitivity while hypocapnia and hyperoxia enhance visual sensitivity, facilitate dark adaptation and hasten achievement of scotopic sensitivity. Thus, the delay time to the onset and rapid

progression of early scotopic sensitivity is affected by both PO_2 and PCO_2. The effects on the rod adaptation curve include distinct early (temporal) and later (sensitivity) components. Hyperoxia, breathing 100% O_2 at GL, hastens the onset and early progression of scotopic sensitivity, suggesting that rods are functionally hypoxic in the dark when breathing air normally. The mechanism whereby hypocapnia enhances visual sensitivity may be related to increased elimination of H^+ from near the photoreceptors. In practice, hyperventilation counters the effect of hypoxia, hastens scotopic vision and may be regarded as protective of visual sensitivity in dim light. Its effect at GL is comparable in magnitude to that of breathing 100% O_2. Recent reviews have emphasised the rate-limited kinetics of dark adaptation and retinoid metabolism (McBee, Palczewski, Baehr *et al*, 2001; Lamb and Pugh, 2004). The availability of O_2 and effects of pH may be fundamental determinants of dark adaptation rate in this context.

Further work would be required to define in detail the consequences of milder and more severe levels of hypoxia on early rod sensitivity following cone rod inflection. This could be achieved most readily using hypoxic gas mixtures rather than a hypobaric chamber. In view of the implication that rods are functionally hypoxic in the dark at GL, the benefit of supplementary O_2 to hasten dark adaptation in relation to normoxia should be confirmed in an independent study. The effects of altered PCO_2 upon the timing of early scotopic sensitivity are currently unexplained and merit further study, particularly in relation to retinal acid-base balance. Specifically, it may be predicted that hypercapnia should delay cone rod inflection and this could be examined using breathing gas mixtures containing small supplements of CO_2. The influence of age warrants consideration in relation to susceptibility of dark adaptation to hypoxic impairment.

5 Flicker Sensitivity

5.1 Abstract

Purpose. Hypocapnia is known to enhance flicker sensitivity but no attempt has been made to correlate the magnitude of the effect in relation to the severity of hypocapnia or use of mesopic stimuli. From the available data it was impossible, therefore, to assess the likelihood that any given severity of hyperventilation might confound interpretation of the effects of hypoxia on sensitivity to temporally-modulated stimuli in the mesopic range. This short study was intended to inform this question as well as to provide some indication of the likelihood that hyperventilation might interfere with acquisition of mesopic visual information by aircrew during flight, specifically from visual displays.

Methods. Eleven healthy subjects (aged 22 to 36 y) participated, all having normal vision. Repeated ascending (fusion) and descending (flicker) measurements were made while breathing normally and at four levels of progressive mild to moderate hypocapnia induced by voluntary hyperventilation. $P_{ET}CO_2$ was monitored breath by breath using respiratory mass spectrometry, with visual and verbal feedback to the subject for control of rate and depth of ventilation. The mesopic visual stimulus was a 2.6° Gaussian blob viewed through a 5.2 mm artificial pupil. The influences of individual variability and hypocapnia on mean CFF were assessed using two-way ANOVA and the relationship between group mean CFF and $P_{ET}CO_2$ was examined using Pearson correlation.

Results. Five discrete respiratory conditions were generated. Between-subject differences in CFF were greater than within-subject differences. Two-way ANOVA distinguished the relative influences on CFF of individual sensitivity to flicker ($F[8,36] = 46.13$, $p < 0.001$) and target level of hypocapnia ($F[4,40] = 4.63$, $p = 0.005$). The relationship between decreasing mean $P_{ET}CO_2$ and increasing mean CFF was consistent with a linear correlation (Pearson $R = -0.949$, $p = 0.013$).

Conclusions. The results support a close relationship between decreasing P_ACO_2 and increasing flicker sensitivity for this mesopic stimulus, although the magnitude of the effect of hypocapnia is small and unlikely to be relevant in aviation. However, sensitivity to flicker is greater for higher intensity stimuli and it is possible that the effect of hyperventilation is non-monotonic.

Citation. The work reported in this chapter has been subject to peer review and is published at **Connolly, D., and Hosking, S. (2007) Quantitative correlation of hyperventilation with flicker sensitivity.** ***Optometry and Vision Science*****, 84: 529-534.** The paper is reproduced at Appendix 9.

5.2 *Introduction*

5.2.1 Background

Using foveal stimuli, Granger and Ikeda (1961) undertook the most carefully controlled study of flicker sensitivity under hyperventilation to date. They examined flicker as a function of stimulus size and intensity under orthopnoea and hyperventilation. The authors made most of the observations themselves with hyperventilation controlled by matching respiratory rate to the beat of a metronome (36-40 min^{-1}) while attempting to maintain a constant depth of ventilation. Hyperventilation was imposed for only half a minute and measurements of flicker frequency were made before, during and after hyperventilation. This transient hyperventilation was associated with "highly reproducible changes" in flicker frequency that decayed to normal within about 2 minutes of stopping hyperventilation. Typically, hyperventilation increased flicker frequency by 2 to 3 cps, more so for progressively brighter and larger (> 1°) stimuli when flicker frequency could approach and even exceed 50 cps.

Before considering possible effects of altered P_AO_2 on temporally modulated visual stimuli, it was considered appropriate to examine the likely influence of hypocapnia as a potentially confounding factor, as well as being of interest in its own right in relation to flicker perception when viewing aircrew displays. No recent studies have examined the relationship between hyperventilation and flicker, none have correlated measurement of P_ACO_2 with CFF and none have investigated the effect of graduated hypocapnia. Although the effect of hyperventilation to increase flicker sensitivity is greater for brighter stimuli, its effect on mesopic stimuli has not been quantified. Besides being relevant to the current work in excluding potentially confounding effects of secondary hypocapnia during subsequent studies of hypoxia, the results should indicate the merit or otherwise of pursuing more detailed study of the influence of hyperventilation on perception of flicker.

5.2.2 Hypothesis

Increasingly severe hypocapnia may be associated with increasing sensitivity to flicker.

5.2.3 Aims

This experiment was intended primarily to assess the possibility that secondary hyperventilation might confound interpretation of the effects of hypoxia on sensitivity to temporally-modulated stimuli in the mesopic range (section 5.5.2).

Second, the underlying hypothesis was investigated (section 5.4).

Lastly, it was intended to assess the likelihood that hyperventilation might interfere with acquisition of visual information by aircrew during flight, specifically from visual displays (section 11.2.2).

5.2.4 Experimental design

The scope of this preliminary exploratory study was limited intentionally to a single visual stimulus and a single experimental exposure for each participating subject. Notwithstanding that Granger and Ikeda (1961) documented that the effect of hyperventilation to increase sensitivity to flicker was greater for brighter and larger stimuli, a relatively small mesopic stimulus was chosen for this study as the subsequent experiments were intended particularly to consider effects of respiratory disturbance on central visual sensitivity in the mesopic range.

The requirement to conduct each subject's experiment in a single session dictated that consideration be given to limiting the duration and severity of hyperventilation to that comfortably achievable by the subjects without undue fatigue or discomfort. In consequence, the hyperventilation conditions were not be randomised and the severity of hyperventilation was increased in a stepwise fashion, accepting that the severity of each condition was confounded by a different and proportionate duration of preceding milder hyperventilation. This avoided the need to allow protracted recovery times after bouts of more severe hyperventilation before subsequent milder exposures and also avoided the need for repeatedly hyperventilating through and beyond the less severe conditions. Nonetheless, if stable conditions of hypocapnia could be established at each 'step' then each should be associated with a discrete acid-base disturbance (respiratory alkalosis) that should allow the hypotheses to be tested and the aims of the experiment to be achieved.

5.3 *Materials and Methods*

5.3.1 Subjects

A total of 12 healthy subjects (6 male and 6 female) volunteered for the study but one female subject became unavailable at short notice due to relocation. The participants' mean age was 29.2 y (range 22 to 36 y). The mean age of the males was 31.3 y (24 to 36 y) and of the females was 26.6 y (22 to 34 y). Care was taken to exclude volunteers who might be unduly sensitive to flicker, specifically those with any past history of epilepsy or migraine. All subjects had some experience of breathing through aircrew masks from pressure-demand breathing regulators. In view of the ease with which subjects were able to maintain a steady level of moderate hyperventilation during the dark adaptation study, it was considered that prior training in the technique was unnecessary. Subjects were fitted with an appropriately-sized 'G' helmet and P/Q mask. Each conducted a single afternoon experiment involving progressive hypocapnia induced through voluntary hyperventilation. Before the experiment subjects were trained to undertake ascending (critical fusion frequency) and descending (critical flicker frequency) measurements using the selected vision test, with an emphasis on accuracy over speed.

5.3.2 Equipment

Experiments were conducted in a dimly illuminated physiology laboratory (2-3 lux) with a permanent, high pressure, compressed air supply. This was regulated to supply a Mk17F pressure-demand regulator at a nominal supply pressure approaching 400 psig. The airmix lever was selected to '100% oxygen' to ensure that only gas from its high pressure air supply was delivered to the subject, that is to say, no ambient air was supplied to the subject's mask and there was no possibility of re-breathing expired gas. The P/Q mask was modified to allow continuous mask cavity gas sampling using an Airspec QP9000 respiratory mass spectrometer. Pre- and post-experiment calibrations were separated by less than an hour and employed known gas mixtures specifically to ensure a linear response across the range of $P_{ET}CO_2$ under investigation. There was insignificant mass spectrometer 'drift' during the course of each exposure and a measurement error of <1% or 0.5 mm Hg was confirmed. Non-invasive 'safety' monitoring of S_aO_2, heart rate and blood pressure were undertaken using a Kontron pulse oximeter with finger probe and an Ohmeda Finapres blood pressure monitor.

5.3.3 Vision testing and pupil size

The Geedev flickermeter is a battery-operated device comprising a control box linked to a stimulus presentation tube with a soft rubber eye cup. When positioned against the viewing eye to exclude ambient light, the subject stares along the axis of the tube, through an exit aperture of internal diameter 5.2 mm, to view a dim foveal stimulus presented against a dark background. The stimulus is a yellow, Gaussian profile 'blob' with 1/e diameter subtending approximately 2.6° of visual angle. It flickers with a 1:1 ratio light:dark duty cycle, modulated sinusoidally, at a rate controlled in 0.1 cps steps using a dial on the control box.

The mean luminance of the stimulus was measured using a Spectra-Pritchard photometer. An additional aperture was used in front of the objective lens of the photometer to match the output aperture of the flickermeter and the input aperture of the photometer. As the aperture restricted the light reaching the photometer and thus made the photometer self-calibration void, the aperture-photometer combination was calibrated using a Gamma Scientific standard (calibrated) lamp. Thus, the fixed, time-averaged luminance of the Geedev flickermeter stimulus was measured at 3.8 cd m^{-2}.

Most subjects viewing a uniform field luminance of 3.8 cd m^{-2} have a natural pupil diameter exceeding that of the flickermeter exit aperture (Wyszecki and Stiles, 1982, p107-109; Boff and Lincoln, 1988, Section 1.233) and the Geedev flicker stimulus is presented against a dark background. Nonetheless, there is wide variation between subjects, with one formula predicting a pupil diameter of only 3.5 mm in response to a field luminance of 3.8 cd m^{-2} (De Groot and Gebhard, 1952). Accordingly, normocapnic pupil size was measured from images captured, at the moment of completion of simulated Geedev flicker measurements, using an infrared, digital video camera under representative ambient lighting. Ten measurements were made in each of nine participating subjects and the results are shown in Table 5-1.

Subject	A	B	C	D	E	F	G	H	I	Mean (n=90)	SD (n=90)
Mean pupil diameter (mm)	6.08	6.22	5.89	5.65	6.89	6.28	7.61	6.74	7.56	6.55	0.70
SD (mm)	0.37	0.10	0.20	0.36	0.11	0.20	0.22	0.26	0.13		

Table 5-1 Mean horizontal pupil diameter of nine subjects using the Geedev flickermeter

The data indicate that subjects' pupil diameters were consistently greater than the exit aperture of the flickermeter. Accordingly, measurements of flicker frequency were

made using the dominant eye and a natural pupil, viewing through the flickermeter exit aperture to control retinal illumination. Thus, it is assumed that any tendency of hyperventilation to dilate the pupil (Gavriysky, 1995) is highly unlikely to have confounded the results of vision testing.

5.3.4 Experimental procedure

The subject acclimatised to the dim light in the laboratory while being fitted with a cloth 'G' helmet and P/Q mask. All measurements were undertaken using the dominant eye and the non-dominant eye was kept closed during measurements. The mask hose was connected to the breathing gas supply hose and the subject was instrumented with the Finapres finger cuff and pulse oximeter finger probe. The mass spectrometer sampling probe was connected to the mask and data recording began.

Restful breathing was recorded for five minutes before the first flicker/fusion measurements were undertaken. First the flickermeter was set to flicker at 10 cps and the subject slowly increased the flicker frequency until fusion just occurred. The measurement was recorded and the flickermeter was adjusted by the investigator to flicker at a rate roughly 10 cps greater than that just measured. The subject then decreased the flicker frequency until flicker was just perceived and the measurement was again recorded. The flickermeter was again adjusted to flicker at approximately 10 cps below the measurement just made and the process was repeated. Alternating ascending (fusion) and descending (flicker) measurements continued until five of each were complete. A set of 10 measurements took about five minutes.

When these normocapnia data were complete, subjects adjusted their rate and depth of breathing to achieve a $P_{ET}CO_2$ of 35 mm Hg. This was maintained easily for five minutes with very gentle hyperventilation. Subjects were able to watch a computer screen showing a real time digital display of PCO_2 and a graphical display of each breath against a calibrated scale showing the target $P_{ET}CO_2$ required. All subjects were able to use these two cues, according to preference, to adjust their breathing to achieve a steady state of hypocapnia close to the target level. After five minutes of gentle hyperventilation the fusion and flicker measurements were repeated. Each individual measurement took about 30 s and between measurements subjects were able to look at the computer screen to make fine adjustments to breathing rate and depth, thus avoiding $P_{ET}CO_2$ 'drift' during the period of flicker measurements. In general, subjects readily maintained the breathing pattern established over the previous few minutes while they

undertook the vision tests. This process was then repeated for $P_{ET}CO_2$ of 30, 25 and 20 mm Hg. Subjects were able easily both to increase their respiratory effort to hit the next target $P_{ET}CO_2$ level and to maintain that level for 10 minutes at a time. Only the final level of hypocapnia required a degree of respiratory effort that some subjects found challenging. At this level of hypocapnia some experienced mild symptoms, typically slight tingling around the lips and in the fingers, or slight light-headedness.

Thus, after the first set of flicker measurements, subjects hyperventilated continuously and progressively for just over 40 minutes in total, a process that was accomplished satisfactorily by all. After the last measurements were taken, subjects were allowed to breathe normally and respiratory gas tensions were monitored until $P_{ET}CO_2$ rose above 30 mm Hg. Monitoring was then discontinued, the instrumentation was removed and the subjects were debriefed on the experiment. All symptoms were minor and resolved rapidly upon cessation of hyperventilation.

Subjects generally found it easier and quicker to achieve accurate ascending measurements, turning the control dial of the Geedev flickermeter clockwise until fusion just occurred. In contrast, some reported that descending measurements were more difficult and perhaps more prone to error, being associated with a degree of 'overshoot' and with difficulty anticipating the start of flicker.

5.3.5 Respiratory conditions

Mask cavity PCO_2 was recorded continuously when breathing normally at rest and throughout the four increasingly severe levels of voluntary hyperventilation, aiming for target $P_{ET}CO_2$ of 35, 30, 25 and 20 mm Hg. $P_{ET}CO_2$ was measured for all expirations during the five minutes prior to vision testing as well as for all expirations during the five minutes of testing. Mean $P_{ET}CO_2$ was calculated within and between subjects. This included comparison of $P_{ET}CO_2$ for each minute of respiratory data for the five minute sampling periods before and during vision testing, to allow assessment of any variation in $P_{ET}CO_2$ throughout the 10 minutes of hyperventilation. Specifically, this allowed comparison of the quality of control of $P_{ET}CO_2$ before and during vision testing. Mean $P_{ET}CO_2$ was then calculated within subjects for the five-minute periods before and during flicker measurement. These were subject to paired *t* test analysis to determine whether the levels of hypocapnia before and during measurement were significantly different. Finally, mean $P_{ET}CO_2$ was estimated for the complete 10 minute blocks of hyperventilation for each subject.

The mean $P_{ET}CO_2$ for each subject's 10 minutes of hyperventilation at each target level of hypocapnia is shown in Table 5-2. The data indicate that each subject achieved reasonable control of $P_{ET}CO_2$ throughout the 10 minutes at each hypocapnia condition, with mean $P_{ET}CO_2$ generally lying within the range -1 to +2 mm Hg with respect to the target level. Subject 9 began hyperventilating gently when wearing the P/Q mask at rest so orthopnoea data were not available. The normal resting P_ACO_2 is 40 mm Hg (SD ± 2 mm Hg). Thus the orthopnoea data available for 10 of the 11 subjects is representative of a normal physiological sample population. It is noted that the variance in $P_{ET}CO_2$ observed at each target level of hypocapnia is less than that occurring normally at rest. The subjects were generally able to maintain a stable $P_{ET}CO_2$ throughout each 10-minute period at each level of hyperventilation.

Target $P_{ET}CO_2$ (mm Hg)	Orthopnoea (normocapnia)	35	30	25	20
Subject 1	40.9	36.5	31.7	25.3	21.4
Subject 2	39.7	36.1	32.0	26.6	22.1
Subject 3	42.0	35.6	31.1	25.7	20.7
Subject 4	36.0	34.3	30.4	25.7	22.5
Subject 5	37.4	36.0	30.1	25.9	20.4
Subject 6	38.2	36.4	32.1	26.0	21.7
Subject 7	40.0	35.9	30.6	25.7	20.1
Subject 8	36.9	34.4	29.4	24.3	19.2
Subject 9	-	34.1	30.8	25.9	21.7
Subject 10	40.8	34.6	30.3	25.4	20.8
Subject 11	42.3	36.5	31.3	26.2	20.6
Mean	39.4	35.5	30.9	25.7	21.0
SD	2.2	1.0	0.8	0.6	1.0

Table 5-2 Mean $P_{ET}CO_2$ for each subject at each target level of hypocapnia

For each subject at each level of hypocapnia, mean $P_{ET}CO_2$ was calculated for the five minutes before testing and the five minutes during testing. A paired *t* test conducted on these data found no significant difference ($\alpha = 0.05$) at any level of hypocapnia. The mean $P_{ET}CO_2$ between subjects was calculated for each minute of hyperventilation before and during vision testing and also suggested stable control of hypocapnia at each target level. Again, the variation in $P_{ET}CO_2$ between subjects was less during hyperventilation than when breathing normally at rest. It is concluded that consistent and discrete respiratory disturbances were imposed throughout the 10 minutes at each target level of hypocapnia. These are represented in Figure 5.1, which shows that group mean $P_{ET}CO_2$ tended to float slightly above the target level of hypocapnia but was consistent for each level of hypocapnia. Target levels were within one SD of the mean $P_{ET}CO_2$ achieved. Variability in $P_{ET}CO_2$ across the group was greatest when breathing normally.

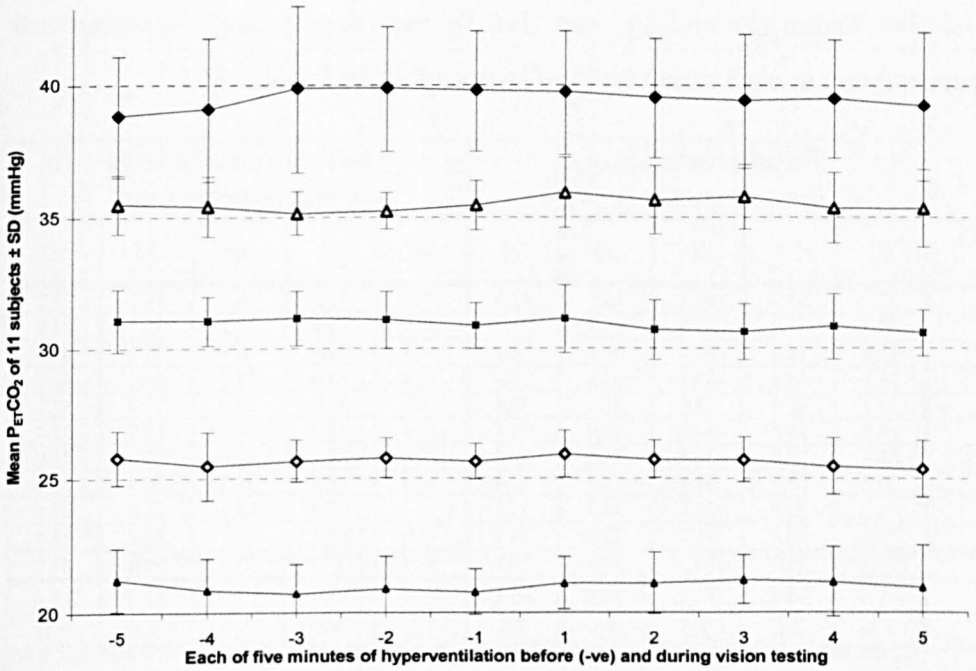


Figure 5.1 Mean (± SD) $P_{ET}CO_2$ between subjects at each target level of hypocapnia (N=11)

5.3.6 Analysis

The flicker and fusion data were first reviewed for idiosyncratic and inconsistent responses. As a result, selected data were excluded from further detailed analysis. The remaining flicker and fusion data were compared and then merged into a single data set. The distribution of the normocapnia flicker data was examined for normality and variability, and mean CFF was calculated from the 10 measurements for each subject, and across the sample group, for each respiratory condition. The influences on mean CFF of differences in individual sensitivity and of target levels of $P_{ET}CO_2$ were tested using two-way ANOVA. To assess the consistency of changes in CFF with decreasing $P_{ET}CO_2$ within, as opposed to between, subjects, the direction and statistical significance of differences in mean CFF, between all possible combinations of two levels of hypocapnia, were examined using *t* tests on data paired by individual subject. Finally, the relationship between grouped mean $P_{ET}CO_2$ and mean CFF was examined using Pearson's correlation.

5.4 *Results*

The mean of five fusion (ascending) and five flicker (descending) measurements is shown for each subject at each target level of hypocapnia in Table 5-3.

	Fusion (ascending) measurements (cps)					Flicker (descending) measurements (cps)				
Target $P_{ET}CO_2$ (mm Hg)	40	35	30	25	20	40	35	30	25	20
Subject 1	37.0	38.2	40.3	41.1	41.3	37.0	37.2	38.5	38.2	37.2
Subject 2	35.3	35.9	37.0	36.9	37.5	35.5	35.4	35.2	35.1	34.8
Subject 3	33.8	34.4	34.4	34.5	34.2	34.3	34.9	34.8	35.3	34.4
Subject 4	33.5	34.8	35.1	35.5	36.2	34.4	35.0	35.7	36.3	36.7
Subject 5	33.7	32.5	33.0	35.1	34.5	32.5	32.6	32.8	33.2	32.7
Subject 6	34.2	34.5	34.9	35.4	35.4	35.3	35.2	34.7	34.9	35.3
Subject 7	33.9	37.7	34.4	34.6	42.3	25.5	22.1	21.8	20.6	21.9
Subject 8	30.2	28.7	31.9	32.3	33.4	30.0	28.5	31.2	30.7	31.5
Subject 9	-	32.4	32.6	32.8	33.1	-	27.9	23.6	22.1	23.6
Subject 10	31.3	30.7	31.7	33.0	33.5	32.0	32.1	31.7	32.2	33.5
Subject 11	33.8	33.7	35.3	32.5	34.9	33.7	33.0	33.3	30.6	31.9
Mean	33.7	34.0	34.6	34.9	36.0	33.0	32.2	32.1	31.7	32.1
SD	1.9	2.8	2.5	2.5	3.1	3.3	4.4	5.1	5.6	5.0

Table 5-3 Mean critical flicker/fusion frequency for each subject at each target $P_{ET}CO_2$

The data suggest a tendency for CFF to increase as $P_{ET}CO_2$ falls. This is more obvious for more subjects when considering the fusion data than the flicker data. Scatter plots for these flicker and fusion data are shown in Figure 5.2. The plots are very similar but the slightly greater ordinate scale for the fusion data indicates that, overall, fusion measurements tended to be slightly greater than flicker measurements. Just two subjects (numbered 7 and 9 in the preceding table), represented by the open triangles and squares, contribute all the obvious outlying (low) data points on the flicker scatter plot, while there are no such obvious outlying data on the fusion scatter plot. Subject 7 (triangles in Figure 5.2) volunteered spontaneously at the end of the experiment that he had experienced considerable difficulty in making accurate flicker measurements while hyperventilating, feeling particularly rushed under the later conditions. This suggests a criterion shift in his reporting of flicker and, as a result, his data were excluded from further analysis. Subject 9 (squares in Figure 5.2) was the subject who exhibited involuntary hyperventilation upon donning the P/Q mask. Thus, no orthopnoea data are available for this subject. Additionally, she appeared uncomfortable during the experiment, also making rushed measurements, and her fusion frequency data are far more erratic than those of any other subject. Accordingly, her data have also been excluded from further analysis. In sum, the outlying CFF data in Figure 5.2 are attributed to measurement error.

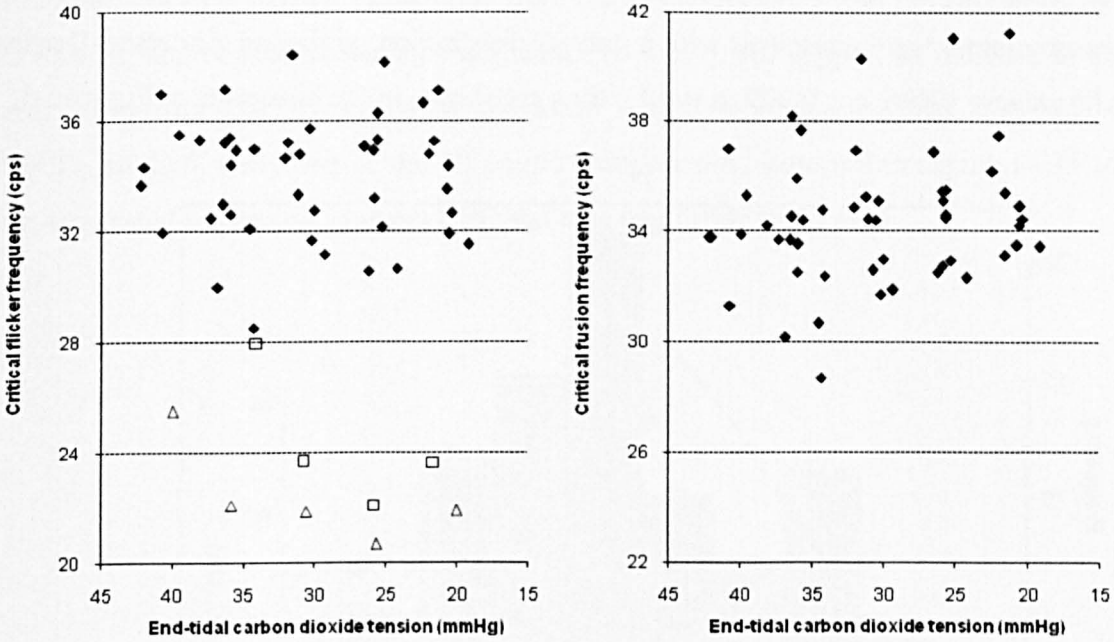


Figure 5.2 Scatter plots of subjects' mean critical flicker and fusion frequencies (N=11)

Disregarding those two subjects' data provides the revised mean critical fusion and critical flicker data shown in Table 5-4 (N=9). The variability of the flicker measurements now approximates the variability of the fusion measurements. The ascending data clearly suggest a trend of fusion threshold to increase with worsening hypocapnia, both within and between subjects, with no obvious trend to the descending data. However, both measurement methods were undertaken to measure the same phenomenon, that is, the threshold for the perception of flicker. Assuming this to be probabilistic in nature then both measurement methods should be valid ways of estimating this threshold. It could be argued that adaptation to the presence or absence of flicker could make the thresholds measured by the descending and ascending methods, respectively, different. Nonetheless, for the purposes of this experiment both methods were assumed to estimate the same threshold and both sets of data were combined. Thus, further analysis was conducted on the mixed data set of the nine remaining subjects.

	Fusion (ascending) measurements (cps)					Flicker (descending) measurements (cps)				
Target $P_{ET}CO_2$ (mm Hg)	40	35	30	25	20	40	35	30	25	20
Mean	33.6	33.7	34.8	35.1	35.7	33.9	33.8	34.2	34.1	34.2
SD	1.99	2.80	2.67	2.71	2.48	2.10	2.54	2.24	2.57	2.00

Table 5-4 Revised group mean critical fusion and critical flicker frequencies (N=9)

The normocapnia (orthopnoea) flicker data of the nine remaining subjects (90 measurements) were consistent with a normal distribution, giving an Anderson-Darling (AD) value = 0.496, p = 0.209 ($\alpha = 0.1$); they are shown in the histogram at Figure 5.3.

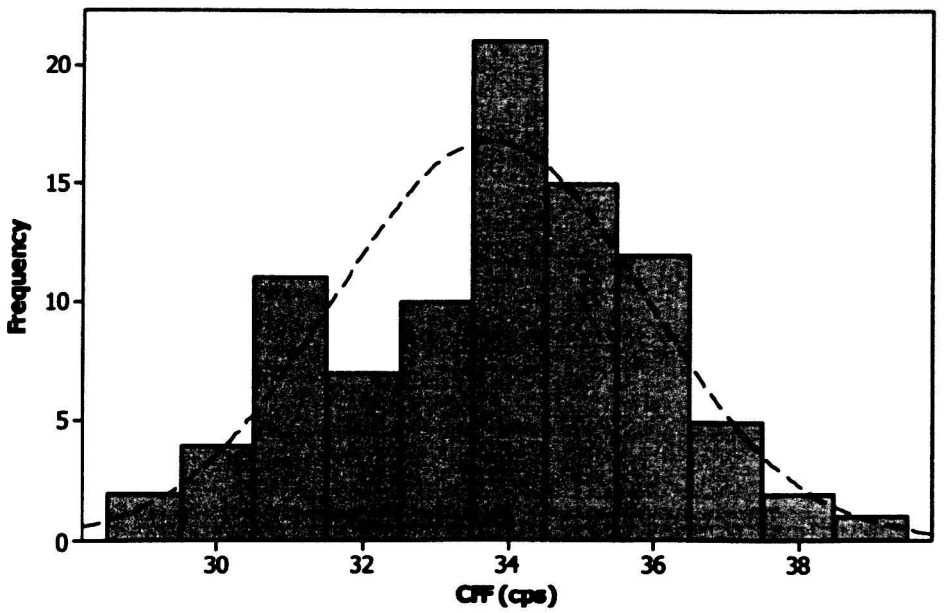


Figure 5.3 Histogram (with reference normal curve) of CFF under restful breathing (N=9 subjects)

For each set of 10 measurements, each subject's mean CFF is shown against his or her mean $P_{ET}CO_2$ during those measurements in Figure 5.4, suggesting a general trend, more pronounced in some subjects than others, for CFF to increase as $P_{ET}CO_2$ falls.

For eight of the nine sets of data, regression lines support a trend for CFF to increase with progressive hypocapnia. Between subjects, the mean increase in CFF between restful breathing and the most severe level of hypocapnia is only 1.2 cps. The variability of flicker sensitivity between subjects was far greater than within subjects, with each individual's results appearing to cluster at each level of hyperventilation when compared to the group results.

Two-way ANOVA confirmed the distinct influences on mean CFF of individuals' inherent sensitivity to flicker ($F[8,36] = 46.13$, $p < 0.001$) and the target level of hypocapnia ($F[4,40] = 4.63$, $p = 0.005$).

A total of 10 two-tailed *t* tests were conducted upon individuals' CFF data paired for all possible combinations of two levels of hypocapnia. Statistical significance ($p < 0.05$) was achieved for transitions from normocapnia to 20 mm Hg ($p = 0.01$); from normocapnia to 30 mm Hg ($p = 0.024$); from 35 to 30 mm Hg ($p = 0.043$); and from 35

to 20 mm Hg ($p = 0.024$). Transitions from normocapnia to 25 mm Hg and from 35 to 25 mm Hg approached statistical significance ($p < 0.1$). All support an increase in CFF with decreasing PCO_2, confirming the trend within individuals across the range of $P_{ET}CO_2$ studied. Only one of the 10 paired comparisons, from normocapnia to 35 mm Hg, suggested a decrease in mean CFF that was negligible (0.02 cps).

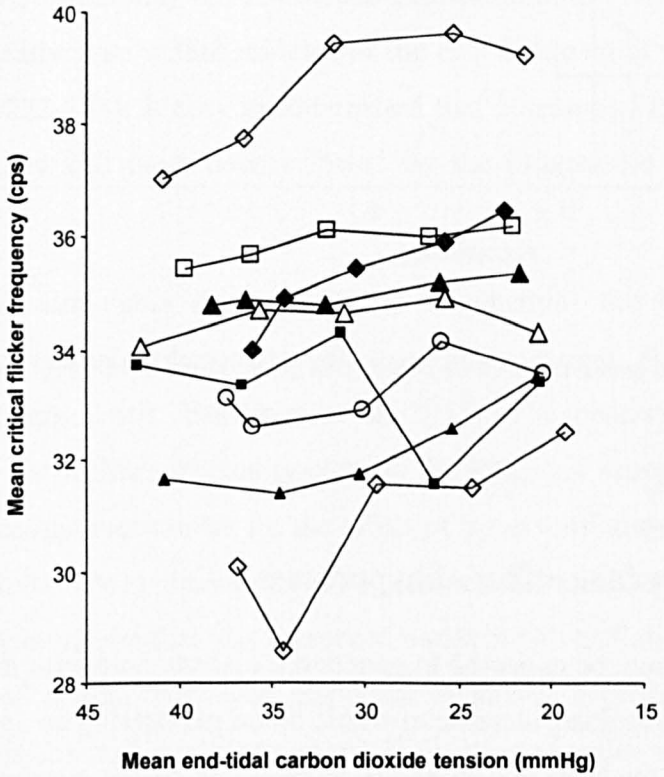


Figure 5.4 Mean CFF plotted against mean $P_{ET}CO_2$ for each of nine subjects

The results of two-way ANOVA and the consistency of the trend, within subjects, for CFF to increase with progressive hypocapnia together suggest that the net effect, between subjects, may be represented appropriately by plotting group mean CFF against group mean $P_{ET}CO_2$. This is shown in Figure 5.5. Horizontal error bars are SD of the mean $P_{ET}CO_2$ between subjects. Vertical error bars are SE of the mean of all 90 flicker and fusion measurements at each target level of hypocapnia, and reflect the wide variability between rather than within subjects. The Pearson correlation coefficient of the regression line for these data is $R = -0.949$ ($p = 0.013$), suggesting a linear relationship between decreasing group mean $P_{ET}CO_2$ and increasing group mean CFF.

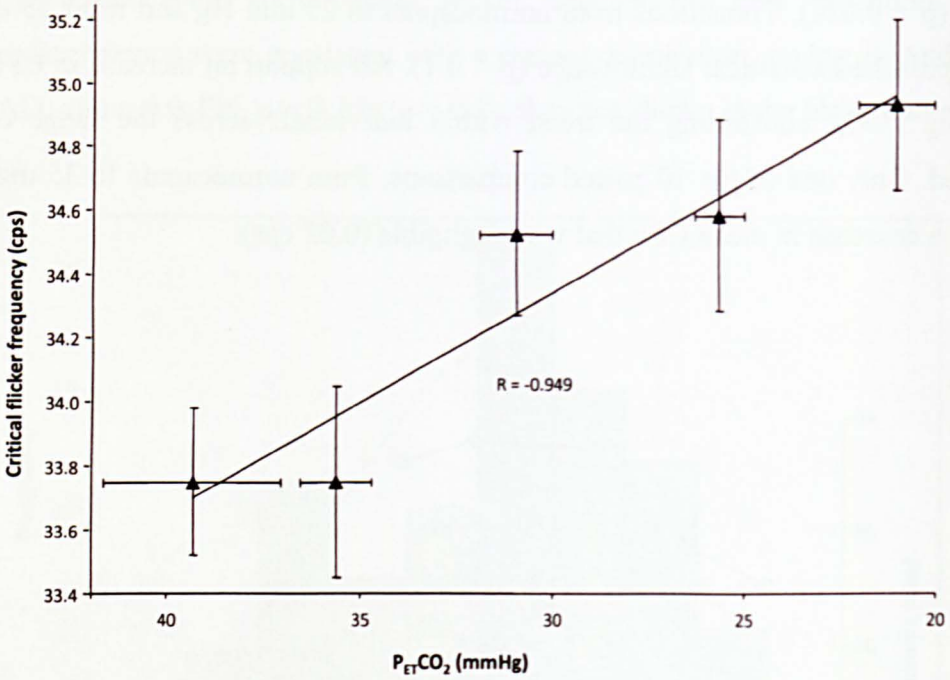


Figure 5.5 Correlation of group mean (± SE) CFF with group mean (± SD) $P_{ET}CO_2$

5.5 *Discussion*

5.5.1 Mechanism of the effect of hypocapnia

Each target $P_{ET}CO_2$ may be expected to generate a discrete acid-base disturbance, with the magnitude of the resulting increase in whole blood pH bearing an approximately log linear relationship to the reduction in PCO_2, as modified by the concomitant change in plasma bicarbonate concentration (Lumb, 2000). Each pH response during vision testing should be determined by the prevailing PCO_2 rather than the duration of any preceding milder hyperventilation. It is possible that fatigue and symptomatic distraction may have influenced later measurements, but the linear nature of the group mean data (Figure 5.5) suggests that any such effects are outweighed by the response to changes in ventilation.

Effects of hypoxia (breathing 14% O_2 with a balance of N_2) and hypercapnia (5% CO_2 with a balance of O_2) to lower the frequency at which flicker is perceived were long ago attributed to the resultant acidosis (Simonson and Winchell, 1951). Alpern and Hendley (1952) examined the effects upon flicker frequency of acid-base disturbances of both metabolic and respiratory origin. Metabolic acidosis (mean ΔpH of –0.11) and metabolic alkalosis (mean ΔpH of +0.11) were not associated with a statistically significant change in CFF. On the other hand, respiratory acidosis (mean ΔpH of –0.16),

induced by breathing 7% CO_2 with 93% O_2, was associated with a fall in CFF of 4 cps at both luminance levels tested ($p < 0.001$). Similarly, respiratory alkalosis (mean ΔpH of +0.20), induced through voluntary hyperventilation, was associated with a statistically significant rise in flicker frequency of 3 to 5 cps ($p < 0.001$ for the three higher luminance levels tested; $p < 0.02$ for the dimmest luminance level tested). The difference in response to metabolic and respiratory acid-base disturbances is explained by the impermeability of the BBB and BRB to H^+ and HCO_3^- ions but the free and rapid diffusion of CO_2, which may then influence extracellular and intracellular pH. Arterial pH reaches a steady state within minutes of the establishment of a new level of P_aCO_2 (Lumb, 2000, p222-248). It may be anticipated that corresponding cerebral and retinal tissue pH changes will have been effected by the progressive hypocapnia imposed during this study.

Hyperventilation diminishes the a-wave and exaggerates the b-wave of the ERG, reflecting outer retinal (photoreceptor) and inner retinal (bipolar cell) activity respectively (Alpern, Faris, Eskildsen et al, 1955). The observation that retinal GC firing rate loses its 'flickering' quality close to the ERG and sensory fusion frequencies also supports a retinal mechanism for the effect of hyperventilation on flicker sensitivity (Granger and Ikeda, 1961). Turner (1965) concluded that temporal adaptation to flicker was a central phenomenon that was preserved under hyperventilation, further supporting a retinal site of action. However, alkalosis enhances neuronal excitability, alters synaptic transmission and modifies nerve cell function (Somjen and Tombaugh, 1998; Chesler, 2003), while the rate at which the after-effects of hyperventilation on visual evoked responses resolve suggests an association with correcting acid-base balance (Gavriysky, 1991; Jensen, Hari and Kaila, 2002). Furthermore, recent evidence suggests that hypocapnia modulates intracortical inhibition and affects visual cortex excitability in a comparable fashion to motor cortex (Sparing, Dafotakis, Buelte *et al*, 2007). In sum, hypocapnia may be considered most likely to modulate net flicker sensitivity through a combination of both retinal and central mechanisms.

The possible confounding effect of altered P_AO_2 should be considered. Hypoxia is known to reduce sensitivity to flicker (Seitz, 1940; Birren, Fisher, Vollmer *et al*, 1945; Scow, Krasno and Ivy, 1950). The effect is apparent long before secondary hyperventilation occurs and is therefore independent of PCO_2, so any effect of hyperventilation to increase P_AO_2 should have the opposite effect, offsetting that of hypoxia. Hypocapnia is accompanied by an increase in P_AO_2 that, ignoring water

vapour, is approximated by a fractional proportion of the change in P_ACO_2 equivalent to the F_IO_2. Nonetheless, the magnitude of the effect when breathing air (20.95% O_2) at one atmosphere is negligible, being comparable to day-to-day fluctuations from changing meteorological conditions (barometric pressure). This cannot account for the observed effect of hyperventilation on flicker sensitivity as the resulting impact on P_aO_2, S_aO_2 and tissue O_2 delivery must be minimal.

5.5.2 Interpretation of the results

CFF measurements were unhurried and were taken with care by the subjects whose data were analysed, with none aware of any differences in judgement of CFF during the experiment. Even so, in adopting a method of limits approach, the possibility of a criterion shift in the reporting of CFF that may have been related to the degree of hypocapnia cannot be excluded. Nonetheless, this is unlikely and the data support a quantitative correlation between the severity of hypocapnia and the threshold for perception of flicker. The trend is apparent despite the small absolute magnitude of the effect and the substantial variability in underlying flicker sensitivity, particularly between subjects. However, for the mesopic stimulus used here, the scale of the effect is small and is unlikely to have relevance to the later mesopic studies, although further investigation may be warranted to quantify the relationship between hypocapnia and CFF at higher luminance (Alpern and Hendley, 1952; Granger and Ikeda, 1961).

Sensitivity to flicker increases with retinal illumination and may be expected to change with fluctuations in pupil diameter. A tendency to pupillary dilatation under hypocapnia could enhance flicker sensitivity by increasing retinal illumination (Gavriysky, 1991). From Table 5-1, this is unlikely to have confounded the current study. However, it remains possible that hypocapnic mydriasis might further enhance sensitivity to flicker under conditions where pupil size is uncontrolled, such that the current results may understate the impact of hyperventilation on net flicker sensitivity, particularly for brighter and larger stimuli.

5.5.3 Lessons for subsequent studies

Study design

There are clear limitations deriving from the simple design of this study, particularly that the different levels of hypocapnia were not randomised. The severity of each level of hypocapnia is confounded by the duration of preceding hyperventilation and both

fatigue and symptomatic distraction may have influenced later measurements. However, randomised hypocapnia conditions, balanced for exposure order and allowing adequate time for complete recovery between conditions, would necessitate a much larger and more extensive study that was out of keeping with the limited aim of the current one. An alternative that is likely to have been acceptable to the subjects could have been to conduct a second set of exposures in which the most severe hypocapnia condition was imposed first, with successive conditions tending to normocapnia as ventilation was gradually allowed to normalise.

Vision testing

The second major limitation relates to the nature of the vision test employed. A comprehensive assessment of the relationship between flicker and hyperventilation should be expected to encompass a wide variety of stimulus sizes and intensities, specifically including larger, photopic stimuli.

Such a study should employ a forced-choice technique with a staircase procedure to estimate flicker thresholds, rather than using a method of limits approach known to exhibit differences between ascending and descending techniques. In this study, the behaviour of the flicker and fusion data sets under hypocapnia was dissimilar. Two-way ANOVA was used to examine the relative influence of individual variation in flicker perception and target level of hypocapnia on subjects' discrete mean fusion and flicker data sets. Individual variability was highly statistically significant for both the flicker data ($F[8,36] = 40.05$, $p < 0.001$) and the fusion data ($F[8,36] = 41.01$, $p < 0.001$). However, while the influence of the target level of hypocapnia was highly statistically significant for the fusion data ($F[4,40] = 9.90$, $p < 0.001$), it was not significant for the flicker data ($F[4,40] = 0.63$, $p = 0.642$). Plotting the group mean fusion and flicker data against mean $P_{ET}CO_2$ between subjects, the Pearson correlation coefficient for fusion remains highly significant with $R = -0.976$ ($p = 0.004$), but there was no significant correlation with the flicker data. This suggests that the accuracy of the descending method of limits approach is compromised during hyperventilation, such that the fusion and flicker data might not reflect the same thresholds.

Subjects

In retrospect it would have been worthwhile spending longer with the subjects rehearsing both the hyperventilation and the vision testing before commencing data

gathering, as familiarity may have enhanced the quality of the results from the two subjects whose data were discounted.

5.5.4 Summary and future work

For a mesopic stimulus, increasingly severe hypocapnia elevates CFF progressively and in an apparently linear fashion, but the magnitude of the effect is slight and unlikely to be relevant in aviation. It is possible that the effect might be more pronounced for larger brighter stimuli, particularly if the photopic pupil tends to dilate with hyperventilation, and this may warrant further study. A comprehensive assessment of this phenomenon should encompass a more sophisticated vision testing methodology using a 4-AFC paradigm to assess a range of stimulus sizes and intensities at various eccentricities. Ideally, measurements should be conducted with and without control of retinal illumination and the effects of hypocapnia on pupil size should be documented fully. A breathing gas circuit would assist the subjects and allow them to concentrate more on the vision test, while putting the $P_{ET}CO_2$ under more direct control of the investigators. Finally, it would be worthwhile to document the acid-base status of the subjects during each 'plateau' level of hypocapnia, or perhaps immediately before and after each vision test.

6 Spatial Contrast Sensitivity

6.1 Abstract

PURPOSE. The discrimination of subtle differences in relative brightness of objects in the central visual field is a fundamental pre-requisite for their resolution and identification. Foveal spatial contrast sensitivity at mesopic field intensities is poorly documented under conditions of respiratory disturbance. This study examined the effects of mild hypoxia and hyperoxia, relative to normoxia, on the human CSF at low photopic luminance, viewing a display screen directly (28 cd m^{-2}), and at borderline upper mesopic and mid-mesopic luminance, using ND 1.0 and ND 2.0 filters, respectively.

METHODS. Twelve healthy subjects (6M, 6F) participated. Identical procedures of retinal bleach, dark adaptation and mesopic light adaptation were adopted for all subject exposures and subjects wore the modified AR5 respirator to enable close control of contemporaneous visual and respiratory adaptation (section 3.3). The first study compared the effect of hypoxia, equivalent to breathing air at 10,000 ft, with normoxic performance at all three light levels. A second study compared hyperoxic performance relative to hypoxic control exposures at the two mesopic light levels. Mild hypoxia was imposed breathing 14.1% O_2, hyperoxia was achieved breathing 100% O_2, and normoxic exposures were conducted breathing air, with all gases supplied through dedicated breathing gas regulators (section 3.2.1). Respiratory exposure order was balanced between males and females. The foveal CSF was examined binocularly and then monocularly (dominant eye) at seven *sf* ranging from 0.5 to 16 cpd using brief Gaussian Gabor patch stimuli subtending 5.4° of visual angle and centred on a fixation point. Gratings were modulated sinusoidally about a mean luminance equal to the background field. A 'seen or not seen' forced-choice technique was adopted with a staircase procedure involving 20 randomised presentations at each *sf*. Data were analysed using repeated measures, balanced ANOVA for main effects and interactions of light level, *sf*, number of viewing eyes, gender and breathing gas.

RESULTS. As expected, background field intensity was the primary determinant of contrast sensitivity, which varied with *sf* to give a typical 'inverted U' appearance to the CSF. A consistent and highly statistically significant effect of binocular summation to enhance contrast sensitivity, relative to monocular viewing, was evident for all *sf* at all light levels and under all respiratory conditions. There were no meaningful effects of gender. Neither mild hypoxia nor hyperoxia influenced contrast sensitivity to any meaningful extent.

CONCLUSIONS. The normal spatial CSF is well preserved at the fovea under the imposed respiratory challenges. Any effect of these respiratory disturbances to alter pupil size, and thereby retinal illumination, had no influence on net contrast sensitivity at the fovea.

6.2 *Introduction*

6.2.1 Background

Relatively severe hypoxia compromises differential brightness sensitivity in dim light (McFarland, Halperin and Niven, 1944). Hecht, Hendley, Frank *et al* (1946) identified a 'definite' effect of hypoxia to compromise brightness discrimination against very dim background fields at an equivalent altitude of only 8,000 ft. However, the relatively few recent attempts to document the effect of hypoxia on contrast sensitivity at functionally meaningful levels of photopic and upper to mid-mesopic luminance have been rather less convincing.

At low mesopic luminance, sensitivity to square wave gratings was impaired at high *sf* (7 and 14 cpd) but only at a minimum equivalent altitude of 13,000 ft (Leber, Roscoe and Southward, 1986), while photopic spatial and temporal contrast sensitivity were unaffected by hypoxia at 7,000 ft and 12,000 ft (Yap, Garner, Legg *et al*, 1995). On the other hand, acute hypobaric hypoxia has been reported to enhance sensitivity to sinusoidal contrast gratings at an equivalent altitude of ~18,000 ft in a decompression chamber (Benedek, Kéri, Grósz *et al*, 2002). However, the respiratory conditions were not well controlled in this last study and it is probable (if not highly likely) that the results may have been confounded by secondary hypocapnia. Most importantly, the proposition that hypoxia improves visual performance is counter-intuitive and cannot be explained by a logical physiological mechanism. Conversely, the possible link between hypoxia, visual impairment and vascular dysfunction in diabetic retinopathy has been investigated, demonstrating a significant benefit of isocapnic hyperoxia on the contrast sensitivity of the diabetic subjects (Harris, Arend, Danis *et al*, 1996).

Given the fundamental importance of contrast discrimination to central visual performance, there is a requirement to document unequivocally the response of the CSF under conditions of altered retinal oxygenation and at photopic and mesopic background intensities that are meaningful in contemporary aviation by day and night. As well as investigating the effect of hypoxia, the potential benefit of supplementary O_2 warrants consideration, both in terms of counteracting any deficit under hypoxia and of enhancing performance in its own right.

6.2.2 Hypotheses

Progressive rod-driven retinal hypoxia might increase the susceptibility of foveal spatial contrast sensitivity to exogenous hypoxia as luminance is reduced from photopic to upper and mid-mesopic levels.

Supplementary O_2 should optimise foveal spatial contrast sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Binocular summation of luminance contrast sensitivity might interact with respiratory disturbance.

6.2.3 Aims

The primary aims of these studies were to assess the hypotheses, documenting the responses of the foveal spatial CSF, at photopic and mesopic light levels, to conditions of mild hypoxia and hyperoxia of relevance in aviation (sections 6.5.3 and 11.2.3).

A subsidiary aim was to support or refute the possibility reported by Benedek, Kéri, Grósz *et al* (2002) that uncomplicated hypoxia might benefit contrast sensitivity (section 6.5.5).

A second subsidiary aim was to consider whether any effects on visual performance under conditions of altered oxygenation might be due to a confounding effect on pupil size, which could not be measured directly owing to the chosen experimental methodology. This is relevant to consideration of the results in the subsequent two chapters, those data being gathered during the same experimental sessions as the CSF data reported here (section 6.5.5).

6.2.4 Experimental design

This chapter and the two that follow document the results from an extended series of experiments that were conducted over a period approaching 6 months' duration. Each individual experiment investigated visual performance on three successive display-based vision tests under two respiratory conditions. Vision testing was preceded by identical processes of retinal bleach and dark adaptation followed by light adaptation to a known photopic, upper mesopic or mid-mesopic luminance. These were controlled by viewing the test displays either directly or through ND filters of OD 1.0 or 2.0. The principle behind the use of these filters is illustrated in Figure 6.1, showing how

transmittance of 10% and 1% respectively reduces the perceived luminance of a low photopic display to upper and mid-mesopic levels.

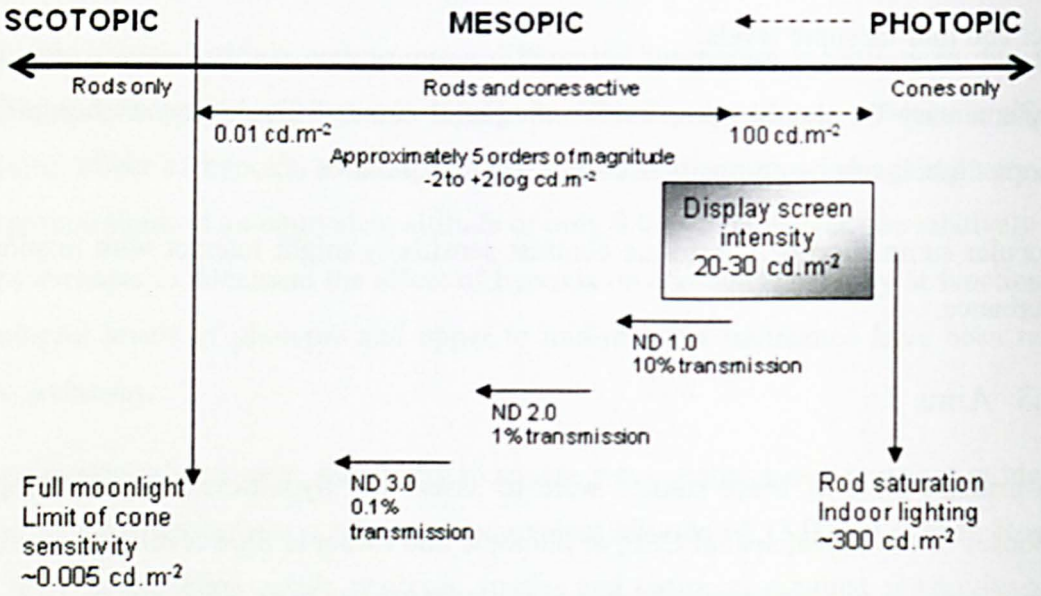


Figure 6.1 Principle of the use of ND filters to control upper and mid-mesopic luminance

The first three experimental series (Study 1) compared visual performance under conditions of normoxia and mild hypoxia. The final two series (Study 2) repeated the previous mesopic assessments to compare visual performance under the same level of mild hypoxia with that achieved when breathing 100% O_2, to identify whether or not supplementary O_2 might offer a performance benefit in relation to hypoxia that might not be apparent relative to normoxia. The experimental parameters are summarised in Table 6-1. The terms Study 1 and Study 2 retain these meanings throughout this and the next two chapters.

Study	Experiment	Viewing conditions	Respiratory Control	Respiratory Condition
1	1	Direct viewing	Normoxia (21% O_2)	Hypoxia (14.1% O_2)
	2	ND 1.0 filter	Normoxia (21% O_2)	Hypoxia (14.1% O_2)
	3	ND 2.0 filter	Normoxia (21% O_2)	Hypoxia (14.1% O_2)
2	4	ND 1.0 filter	Hypoxia (14.1% O_2)	Hyperoxia (100% O_2)
	5	ND 2.0 filter	Hypoxia (14.1% O_2)	Hyperoxia (100% O_2)

Table 6-1 Imposed conditions of background visual and respiratory adaptation

In order to establish unequivocal and identical states of mesopic adaptation between subjects, the principle of visual adaptation illustrated in Figure 4.1 was adopted. In Chapter 4, all subjects exposed to mild hypoxia (10,000 ft) achieved cone rod inflection within 905 s of dark adaptation, while the inflection point was achieved earlier under normoxic and hyperoxic conditions. In the current study, vision testing was conducted at background light intensities well above the cone plateau. Accordingly, the same procedure of retinal bleach (five minutes) followed by 15 minutes of dark adaptation, further followed by five minutes of adaptation to the background field luminance ensured a generous time allowance for subjects to achieve identical, stable states of visual adaptation before commencing vision testing, regardless of respiratory condition. Thus, the process of visual adaptation lasted 25 minutes in total.

At least 15 minutes of respiratory adaptation are required to achieve a reasonable steady state following an imposed respiratory challenge, enabling wash-in and wash-out of alveolar gases, and equilibration of circulating gas tensions and Hb O_2 saturations (Yap, Garner, Legg *et al*, 1995). Ethical clearance of the study protocol limited the maximum duration of hypoxia exposure to one hour (except to complete a vision test in progress). Thus, only 45 minutes of hypoxia exposure were available for vision testing, assuming that prior visual and respiratory adaptation were undertaken contemporaneously. This requirement drove the design for the timeline of each experimental exposure, shown at Figure 6.2. Thus, testing of contrast sensitivity began after 25 minutes of visual adaptation, the last 15 minutes of which were accompanied by respiratory adaptation.

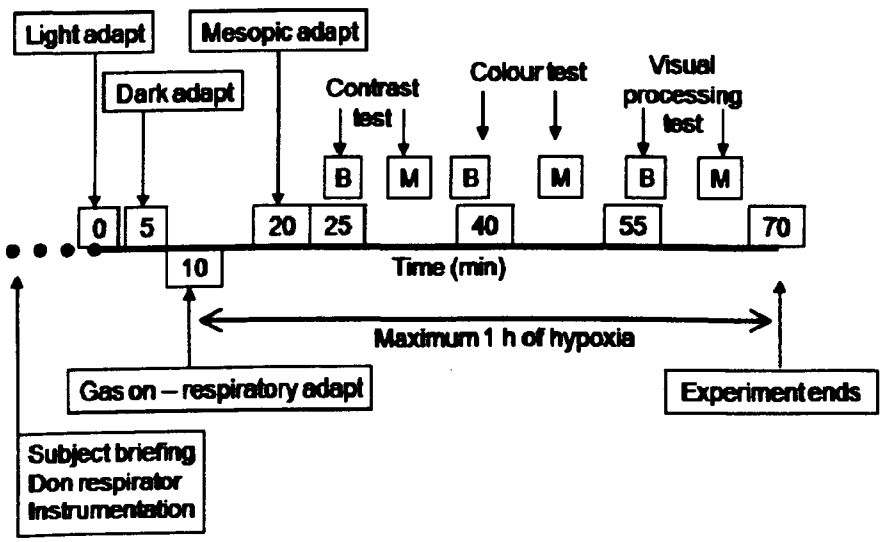


Figure 6.2 Experimental timeline for respiratory and visual adaptation and vision testing

Binocular summation is known to enhance contrast sensitivity in comparison to viewing monocularly (Pirenne, 1943; Home, 1978; Legge, 1984a; Rabin, 1995). Thus, the possibility exists that monocular and binocular measures of visual performance may be differentially susceptible to the effects of respiratory disturbance. Accordingly, it was considered that tests of contrast sensitivity should be conducted both binocularly (labelled 'B' in Figure 6.2) and monocularly ('M'). After careful consideration it was decided that the binocular test should always precede the monocular test, as it was not possible to randomise this factor as well as randomising for gender and respiratory exposure order sub-group while retaining balance for subsequent ANOVA. Also, the visual response to respiratory disturbance may vary during the first hour of exposure (Kobrick and Appleton, 1971). Thus, randomisation of the order of binocular and monocular testing between subjects would confound direct comparison of binocular or monocular results between respiratory conditions as a result of different durations of respiratory exposure. Since the primary purpose of these experiments is to consider the effects of respiratory disturbance, it was concluded that otherwise comparable data by viewing condition should be conducted under identical respiratory conditions, including the duration of the respiratory challenges. The same rationale was followed for the tests of chromatic sensitivity and visual processing speed that followed contrast sensitivity testing and that are reported in Chapters 7 and 8 respectively.

6.3 *Materials and Methods*

6.3.1 Subjects

A total of 12 subjects (six male and six female) completed each of the experimental series in Table 6-1. However, one female subject was lost from the subject pool due to relocation, part way through the study, and had to be substituted. Thus, a total of 13 subjects were used having a mean age of 28.7 y (range 22 to 39 y). These comprised seven females with a mean age of 26.1 y (22 to 34 y) and six males with a mean age of 31.7 y (24 to 39 y).

Background subject details and screening requirements are as detailed in Chapter 3, section 3.6.3. All subjects achieved Snellen VA of at least 6/6 in each eye, corrected if necessary using untinted spectacles (two female subjects) or contact lenses (one male subject). The ophthalmic prescriptions for these subjects were unchanged throughout and subjects used their normal correction for all experiments.

The male subjects tended to be more experienced at undertaking respiratory studies and breathing from aircrew O_2 systems. However, all were familiarised with breathing from pressure demand breathing gas regulators through aircrew O_2 masks. Subjects were trained in the contrast sensitivity test by undertaking it three times in succession, under 'shirtsleeve' conditions, to ensure that a consistent CSF was achieved. Before each experiment all subjects completed a lifestyle questionnaire, females undertook a urine test to exclude pregnancy and distant Snellen VA was re-checked to ensure that there had been no change in VA since medical screening.

Subjects acted as their own controls for all experiments but were masked to the exposure order of the two respiratory conditions. This was randomised equally between males and females, with subjects unknowingly self-allocating to one order or the other according to their availability for testing.

6.3.2 Equipment

Experiments were conducted in a dimly illuminated physiology laboratory with piped supplies of high pressure compressed air and 100% O_2. These and a bottled supply of hypoxic gas mixture fed three dedicated Mk17F pressure demand breathing gas regulators at a nominal supply pressure of just under 400 psig. The regulator selection levers were selected to 100% to deliver undiluted supply gas. The regulators had identical pressure/flow characteristics and imposed minimal breathing resistance, making them indistinguishable to the user, and their breathing gases were delivered, via a selection tap, to a common supply hose. This passed to the mask tube hose of a modified AR5 respirator worn by the subject. A port to the mask cavity of the AR5 enabled continuous analysis of breathing gas composition using an Airspec MGA 2000 mass spectrometer.

The same light box used in previous experiments was employed to provide the retinal bleach prior to dark adaptation. Kodak Wratten ND filters of OD 1.0 and 2.0 were mounted on polycarbonate scaffolds that matched the residual polycarbonate frame cut from the AR5 visor. When wearing the ND filters, ambient air was blown gently, and independently of the breathing gas supply, across the facial aspect of the filters to ensure that they remained free from misting. Hence, normal corneal oxygenation was preserved, regardless of respiratory condition, and subjects wore their normal corrective spectacles and lenses, as required. All vision test displays were viewed along matt black viewing tunnels.

Non-invasive monitoring of blood pressure, heart rate and O_2 saturation were undertaken using an Ohmeda Finapres 2300 blood pressure monitor and Kontron 7840 pulse oximeter with finger probe. Analogue outputs from both devices were calibrated and recorded, together with the mass spectrometer data, via an ADInstruments Powerlab 16S data recording and analysis system to a Dell Precision 610 personal computer (PC) running Powerlab/Chart software.

6.3.3 Respiratory conditions

It has generally been accepted that supplementary O_2 is unnecessary in aviation until an equivalent altitude of 10,000 ft is exceeded, assuming normal physiology, while lower pressures are allowed by some regulatory authorities if exposures are brief (McFarland, 1971; Ernsting, 1978). Thus, hypoxia equivalent to breathing air at 10,000 ft represents an appropriate initial challenge with which to assess the possibility of visual impairment under different viewing conditions. The intention to undertake a prolonged series of lengthy experiments using 12 subjects precluded the use of the hypobaric chamber. Instead, low O_2 breathing gas mixtures were used to simulate hypobaric hypoxia. From Figure 1.12, the anticipated P_AO_2 at 10,000 ft is approximately 55 to 60 mm Hg. From Equation 11, the dry inspired F_IO_2 required at GL to produce a P_AO_2 of 55 mm Hg is approximately 14.1%. This would be expected to maintain S_aO_2 around 90% and hence represents a relatively mild degree of hypoxia matching that achieved at an equivalent altitude of 10,000 ft in the hypobaric chamber (Table 4-2). Thus, Study 1 employed a hypoxic breathing gas mixture comprising 14.1% O_2, with a balance of N_2, with normoxic control exposures undertaken breathing air.

Hyperoxia is readily achieved by breathing 100% O_2 at GL, resulting in $P_{ET}O_2$ of over 650 mm Hg and S_aO_2 of virtually 100%. Any possible benefit of supplementary O_2 should be readily apparent using such marked hyperoxia, which may be anticipated to optimise retinal oxygenation under normobaric conditions. Thus, Study 2 examined visual performance breathing 100% O_2, this time with control exposures breathing the same hypoxic gas mixture as in Study 1, to demonstrate whether hyperoxia might confer any visual advantage over hypoxia equivalent to breathing air at 10,000 ft.

6.3.4 Vision testing

Vision testing employed vertical contrast gratings with sinusoidally-modulated spatial contrast profiles. These were presented as circular 'Gabor' patches with a Gaussian

contrast profile such that grating contrast fades and merges into mean background luminance at the periphery of the patch, thereby avoiding edge detection effects. Gabor target radii occupied 2.7° of visual angle when viewed from a distance of 70 cm, so the patches occupied ~5° of central visual field. Thus, the stimuli were sufficiently large to prevent target size confounding spatial resolution at the light levels tested (Pokorny, 1968). A central fixation point was provided in the form of a black cross with a radius of 0.2° of visual angle, providing a location cue to optimise contrast sensitivity and mitigating any impairment due to loss of attention under hypoxia (Pestilli and Carrasco, 2005). Stimuli were displayed for 1.5 s with attack and decay times of 0.1 s. Gratings were presented at seven *sf* comprising 0.5, 1, 2.06, 4.13, 5.5, 8.26 and 16.51 cpd, chosen to automatically to be compatible with the display characteristics. Maximum grating contrast ranged from peak display luminance to black, with the background field luminance set to the mean of these at 28 cd m^{-2}.

Criterion-free forced-choice tests for measuring contrast sensitivity thresholds on a CRT display take longer than 'method of limits' procedures but exhibit comparatively low within-subject, between-subject and test-retest variability (Nio, Jansonius, Lamers *et al*, 2005). The subjects for this experiment were known by the experimenter to be highly motivated and experienced in human studies. Since test duration was a concern in these experiments, a compromise between the 'forced-choice' and 'method of limits' approaches was adopted whereby subjects were obliged to respond either 'Yes' or 'No', using a button box, following each stimulus presentation, according to whether the grating was seen or not. A high stimulus-response rate was achieved, enabling 20 presentation sequences for each *sf* in a relatively brief test. A semi-automatic linear staircase technique was used to measure threshold contrast sensitivity with random step selection of *sf*. Contrast decrements were set to 8.0 dB while increments were set to 5.5 dB. Typically, 5 to 7 reversals were achieved and the resulting threshold was calculated from the last 5 of these. Trials of this test method indicated that consistent CSF curves could be obtained with limited practice on the test. The overall test duration depended on how quickly and confidently subjects responded to the total of 140 stimulus presentations, typically occupying between five and six minutes.

Each stimulus presentation was accompanied by a 'beep' and another acknowledged the subject's response. In the rare event that a response was not forthcoming within 5 s a timeout beep signalled to the subject that the next stimulus was about to be presented. The stimulus generating the missed response would then be randomised for subsequent

presentation again. The test method was designed intentionally to avoid measuring response time since contrast-dependent reaction times are known to be compromised under hypoxia (Fowler, Banner and Pogue, 1993).

Stimulus presentation was controlled using a Dell Optiplex GXi PC running Windows 98. The PC was fitted with a 4 Mb Video Signal Generator (VSG) 2/2 video card and ran VSG software version 5.1 controlled by Psycho for Windows version 2.36 (Cambridge Research Systems). This generated Gabor stimuli on a gamma-corrected ELO FlexScan T560i-T display monitor with 600 lines (line width 370 microns) at a frame rate of 100 Hz.

6.3.5 Experimental procedure

Upon arrival at the laboratory, each subject completed the lifestyle questionnaire at Appendix 3, fitness to participate was confirmed and, the subject drank water *ad libitum* to satisfy any apparent thirst. Female subjects undertook a urinary pregnancy test prior to each experiment and provided written confirmation that this was negative before proceeding. The subject's Snellen VA was re-checked, wearing correction if necessary, and the subject then entered the laboratory and sat for a few minutes to acclimatise to the dim ambient light level while they were briefed on the forthcoming experiment. The laboratory was dimly illuminated throughout using one standard 40W light bulb in a baffled anglepoise lamp placed behind and angled away from a diffusing curtain. The lamp was screened from direct view by the subject and ambient illumination at subject eye level was consistently in the range from 1 to 2 lux. The subsequent procedure was intended to ensure careful control of subject light levels and breathing gas alongside precise experimental timing to ensure that all subjects achieved identical states of contemporaneous visual and respiratory adaptation.

The subject donned the AR5 and adjusted it for a comfortable but snug mask fit. A cloth halter was placed over the head and to it were attached the supply hose from the four-way tap fed by the breathing gas regulators, and the electric blower to supply the blown air to demist the inner aspect of the respirator faceplate and filters. The breathing gas supply hose was not connected and, at this stage, the subject continued to breathe ambient (room) air through the mask inspiratory hose. The subject was instrumented with the Finapres finger cuff and its pressure servo and the pulse oximeter finger probe. The mass spectrometer probe was connected to the AR5 mask sampling port.

The subject was positioned, seated at rest, viewing the light box directly through the cutaway portions of the AR5 polycarbonate visor from a distance of approximately 45 cm. Seating height was adjusted to ensure comfortable viewing throughout the experiment. The light box was switched on to start each experiment (Time 0) and the subject stared through the centre of the light box for five minutes to complete a partial bilateral retinal bleach (Figure 6.3, first picture). After exactly five minutes (Time 5), the light box was switched off and subjects closed their eyes while a filter visor was applied by Velcro to the AR5 visor. This was itself masked with a cover comprising another layer of Velcro material. Once the filters and cover were *in situ*, subjects opened their eyes to undertake 15 minutes of dark adaptation (Figure 6.3, second picture). The Farnell Instruments power supply to the small electric blower was switched on to supply blown air to the AR5 and adjusted to maintain a quiet, steady flow. The subject's chair was gently repositioned in front of and facing the first display.



Figure 6.3 The process of concurrent visual and respiratory adaptation (with permission)

After three minutes of dark adaptation (Time 8) the subject was reminded to maintain a careful face-mask seal and this was checked on the mass spectrometer trace. Subjects were allowed to gently support the mask with a free hand before and between vision tests. The first breathing gas (air, 14.1% O_2 or 100% O_2) was selected using the 4-way tap. After 10 minutes of dark adaptation (Time 15) the breathing gas supply hose was connected to the mask inspiratory hose and the subject began 15 minutes of respiratory adaptation. From now until the end of the experiment, the mass spectrometer trace was monitored to ensure that a satisfactory face-mask seal was maintained throughout. Upon completion of dark adaptation (Time 20) the visor cover was removed and the subject undertook five minutes of light adaptation, viewing the test display through the

appropriate ND filters (Figure 6.3, third picture). In the first (photopic) experiment, the filters were also removed along with the visor cover, allowing direct viewing of the test display through the cutaways in the polycarbonate visor of the AR5 (as in Figure 6.3, first picture).

At this stage in all experiments, by far the brightest object in the subject's field of view was the test display screen, viewed along a matt black viewing tunnel to exclude stray light. After a few minutes the subject positioned a notch in the AR5 expiratory port against a 'chin' rest, thus establishing the correct viewing distance from the centre of the display screen (70 cm). This support from the chin rest, pushing against the expiratory manifold, helped to ensure a good face-mask seal during vision testing. Thus, by Time 25, the subject completed mesopic adaptation in parallel with 15 minutes of respiratory adaptation to the test breathing gas. At that point vision testing began. When testing binocularly, the entire test area of the display was within the field of view of each eye. All visual adaptation procedures and vision tests were conducted with natural pupils.

The first vision test was the binocular test of contrast sensitivity. Upon completion, the subject relaxed, the results were saved, the subject rested for a minute or two and a Velcro patch was applied to occlude the view of the non-dominant eye. When ready to continue, the subject re-positioned for the monocular test of contrast sensitivity. Upon completion of this test the subject again relaxed and the results were saved before moving on to the test of colour vision (Chapter 7). As each test of contrast sensitivity occupied about five to six minutes, contrast testing was completed between about Time 25 to 40 (15 to 30 minutes of respiratory exposure).

When all vision testing was complete (Time 70) the breathing gas supply hose was disconnected, the mass spectrometer probe was removed, the filter visor was removed and the air blower and monitoring were stopped. The instrumentation was removed and the AR5 was doffed. The subject was then allowed to rest for 10 to 15 minutes and the mass spectrometer was recalibrated. The entire procedure was then repeated with the second breathing gas. Upon completing all vision testing under the second respiratory exposure, the subject was debriefed and allowed to depart when ready. The mass spectrometer was recalibrated for a final time and the physiology and vision data files were saved and copied.

6.3.6 Physiological parameters

Cardio-respiratory parameters were recorded continuously throughout all exposures with markers to indicate where each vision test began and ended. Appropriately calibrated traces were analysed in detail to determine cardio-respiratory status during every vision test. In particular, blood pressure (systolic and diastolic), heart rate, S_aO_2, P_IO_2, $P_{ET}O_2$, and $P_{ET}CO_2$ were measured repeatedly for each vision test, generating over 5000 sets of data (7 physiological parameters x 6 vision tests x 2 respiratory conditions x 12 subjects x 5 experiments). Respiratory rate and mean and pulse pressures were also estimated. Measures of central tendency and dispersion were derived for each parameter and indicated that, within subjects, parameters remained stable during vision testing under each imposed respiratory condition. Mean within-subject data were then pooled to examine responses between subjects.

Study 1 – Comparing responses to hypoxia (14.1% O_2) with normoxic controls				
			Normoxia	Hypoxia
Direct viewing	Blood pressures (mm Hg)	Systolic	120 (3.9)	128 (7.1)
		Diastolic	68 (3.7)	70 (3.8)
		Mean	85 (3.6)	89 (4.7)
		Pulse	52 (2.9)	58 (4.6)
	Heart rate (min^{-1})		65 (1.8)	69 (2.1)
ND 1.0	Blood pressures (mm Hg)	Systolic	117 (2.9)	121 (2.9)
		Diastolic	69 (2.6)	70 (2.5)
		Mean	85 (2.5)	87 (2.3)
		Pulse	48 (2.5)	51 (2.4)
	Heart rate (min^{-1})		72 (2.9)	75 (3.2)
ND 2.0	Blood pressures (mm Hg)	Systolic	117 (4.0)	120 (4.5)
		Diastolic	66 (3.5)	65 (3.4)
		Mean	83 (3.5)	84 (3.6)
		Pulse	51 (2.5)	55 (2.7)
	Heart rate (min^{-1})		65 (2.8)	68 (2.9)

Study 2 – Comparing responses to hyperoxia (100% O_2) with hypoxic controls (14.1% O_2)				
			Hyperoxia	Hypoxia
ND 1.0	Blood pressures (mm Hg)	Systolic	116 (3.8)	118 (2.7)
		Diastolic	68 (3.0)	67 (2.6)
		Mean	84 (3.1)	84 (2.4)
		Pulse	47 (2.0)	51 (2.2)
	Heart rate (min^{-1})		63 (3.3)	72 (3.8)
ND 2.0	Blood pressures (mm Hg)	Systolic	123 (4.4)	132 (2.4)
		Diastolic	69 (4.5)	71(2.8)
		Mean	87 (4.3)	91 (2.4)
		Pulse	54 (2.1)	61 (2.5)
	Heart rate (min^{-1})		63 (2.7)	72 (3.2)

Table 6-2 Mean (±SE) values for cardiovascular parameters during Studies 1 and 2

Cardiovascular responses are summarised in Table 6-2. The slight increase in heart rate under hypoxia was statistically significant (paired *t* test) for direct viewing in Study 1 (p

< 0.01) and for both exposures in Study 2 ($p < 0.001$). The slight tendency for systolic and pulse pressures to rise with hypoxia was not statistically significant. Cardiovascular status remained essentially stable between respiratory conditions and the slight increase in heart rate at this level of hypoxia was consistent with expectation.

Study 1 – Comparing responses to hypoxia (14.1% O_2) with normoxic controls				
			Normoxia	Hypoxia
Direct viewing	Gas tension (mm Hg)	P_IO_2	156 (0.7)	108 (0.5)
		$P_{ET}O_2$	107 (1.2)	60 (0.9)
		$P_{ET}CO_2$	36.7 (0.5)	37.5 (0.5)
	S_aO_2 (%)		97.7 (0.3)	90.6 (0.4)
	Respiratory rate (min^{-1})		14.4 (0.8)	13.4 (1.0)
ND 1.0	Gas tension (mm Hg)	P_IO_2	157 (0.6)	107 (0.5)
		$P_{ET}O_2$	109 (1.1)	60 (1.1)
		$P_{ET}CO_2$	38.0 (0.7)	38.9 (0.6)
	S_aO_2 (%)		97.9 (0.3)	91.3 (0.4)
	Respiratory rate (min^{-1})		14.3 (0.8)	13.9 (0.7)
ND 2.0	Gas tension (mm Hg)	P_IO_2	155 (0.7)	105 (0.6)
		$P_{ET}O_2$	108 (1.2)	58 (1.0)
		$P_{ET}CO_2$	38.2 (1.1)	38.2 (1.1)
	S_aO_2 (%)		98.3 (0.3)	91.0 (0.6)
	Respiratory rate (min^{-1})		13.4 (0.7)	13.1 (0.6)

Study 2 – Comparing responses to hyperoxia (100% O_2) with hypoxic controls (14.1% O_2)				
			Hyperoxia	Hypoxia
ND 1.0	Gas tension (mm Hg)	P_IO_2	744 (4.8)	105 (0.6)
		$P_{ET}O_2$	681 (4.9)	58 (1.5)
		$P_{ET}CO_2$	35.0 (0.8)	39.1 (1.1)
	S_aO_2 (%)		99.7 (0.2)	91.5 (0.6)
	Respiratory rate (min^{-1})		15.4 (2.7)	13.8 (0.6)
ND 2.0	Gas tension (mm Hg)	P_IO_2	740 (5.4)	105 (0.7)
		$P_{ET}O_2$	681 (5.1)	59 (1.4)
		$P_{ET}CO_2$	33.2 (0.9)	38.0 (1.2)
	S_aO_2 (%)		99.8 (0.1)	91.8 (0.7)
	Respiratory rate (min^{-1})		14.8 (0.7)	13.6 (0.7)

Table 6-3 Mean (±SE) values for respiratory parameters during Studies 1 and 2

Respiratory rate, gas tension and S_aO_2 data are shown in Table 6-3 for each experiment. For the gas tension measurements, full calibration included reference to changes in absolute ambient (barometric) pressure during each respiratory exposure, measured using an independently calibrated Druck DPI 740 precision pressure indicator. The resulting gas tensions are subject to a measurement error of < 1% under normoxic and hypoxic conditions, equivalent to ± 1 mm Hg for estimations of PO_2 and ± 0.5 mm Hg for PCO_2 (when breathing air). Subjects generally maintained excellent mask seals throughout the experiments. Occasional mask leaks were quickly recognised and easily corrected by the subjects. One female subject experienced mask discomfort towards the end of each exposure but no contrast sensitivity data were lost because of this. The PO_2 and S_aO_2 data indicate that the intended respiratory conditions were successfully

imposed, closely controlled and remained stable throughout. The hypoxia exposures were highly reproducible.

There was no meaningful effect of mild hypoxia to induce secondary hyperventilation, with normocapnic values of $P_{ET}CO_2$ remaining stable under hypoxia. However, every exposure to hyperoxia was accompanied by a more or less immediate fall in $P_{ET}CO_2$, quickly settling to a new low plateau value. $P_{ET}CO_2$ then remained low until the end of the exposure and quickly corrected with offset of 100% O_2. The effect was so pronounced that it was initially attributed to a measurement artefact when using the mass spectrometer. Mass spectrometer calibrations tend to become non-linear in the presence of 100% O_2, inducing greater measurement error for high PO_2, although it was unclear how this might affect estimation of PCO_2. Careful recalibration excluded any obvious calibration artefact. The possibility of increased filament oxidation in the presence of high O_2 concentrations was considered but, logically, this would not result in a reduction in measured PCO_2. In order to exclude an effect of using 100% O_2 to compromise accurate measurement of PCO_2, carefully calibrated mass spectrometer responses were examined using a 95% O_2 and 5% CO_2 gas mixture, alternating with 100% O_2. The outcome is represented in Figure 6.4.

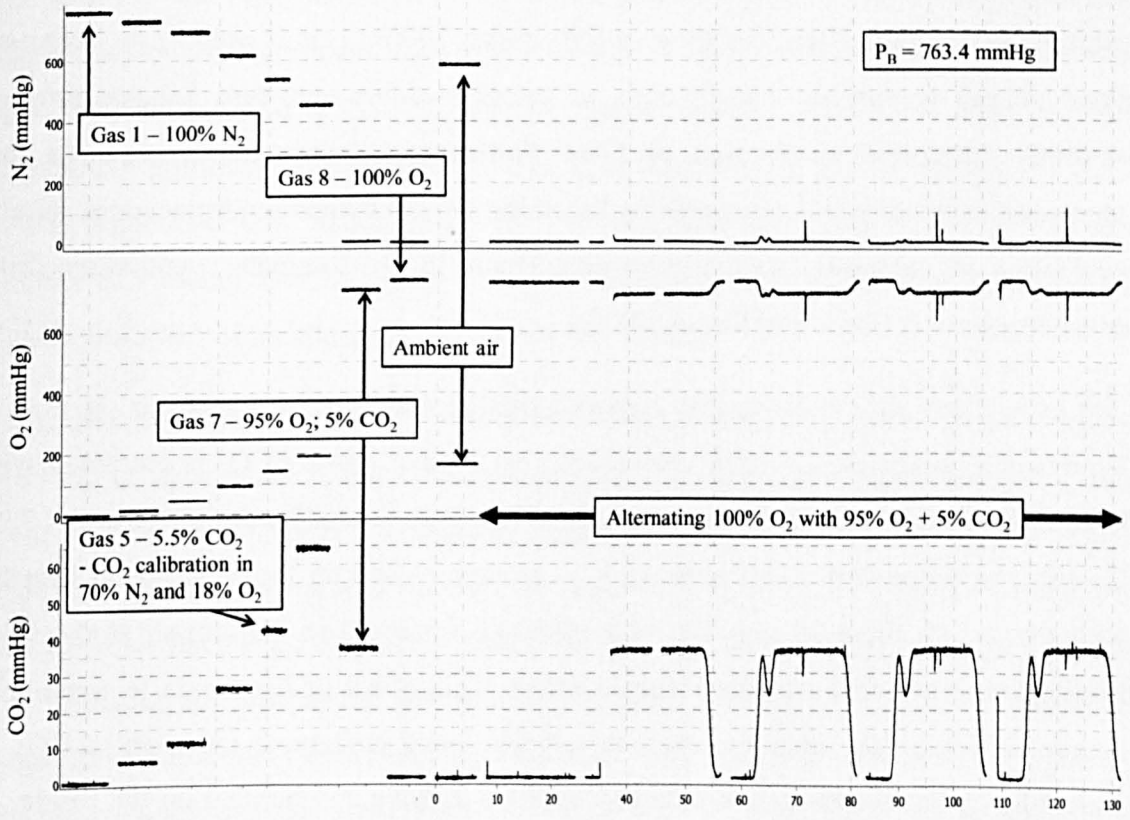


Figure 6.4 Accuracy of mass spectrometer PCO_2 measurement with high O_2 concentrations

Considering Figure 6.4, the first six calibration gases were used to confirm linear responses to PO_2 and PCO_2 in the physiological range. The two test gases are then documented. A prolonged spell of air sampling (not shown) was then followed by a spell sampling 100% O_2, to induce any disturbance in spectrometry that might result, before switching repeatedly between 100% O_2 and 95% O_2 with 5% CO_2. Under these conditions the measured PCO_2 remained highly accurate and reproducible in relation to the initial calibration. Thus, the hypocapnia that accompanied hyperoxia was not due to measurement error.

In considering the possible physiological responses that might result in hypocapnia, the obvious candidate is hyperventilation. A significant increase in respiratory rate was apparent in at least 7 of the subjects and was more obvious under hyperoxia than hypoxia. This was attentional, with subjects breathing faster and shallower while concentrating on the vision tests and relaxing in between, with slower, deeper breaths. However, the increase in respiratory rate was relatively slight and insufficient, of itself, to explain the fall in $P_{ET}CO_2$, which was apparent almost immediately upon switching to the hyperoxic gas mixture and preceded any increase in respiratory rate. Furthermore, ventilation decreases in response to prolonged isocapnic hyperoxia, so would not tend to promote hypocapnia (Ren, Fatemian and Robbins, 2000). This is only to be expected, since normal respiratory control aims to preserve stable acid-base balance through carefully controlled elimination of CO_2. Furthermore, when the hypocapnia that accompanies hyperoxia is prevented by breathing CO_2 to maintain normocapnia, minute ventilation is increased, so the prevailing tissue PCO_2 dominates ventilatory drive despite hyperoxia (Iscoe and Fisher, 2005).

However, there is evidence that hyperventilation induced by hyperoxia is due to hypercapnia in the central chemoreceptors, that is, that tissue PCO_2 is increased with hyperoxia (Becker, Polo, McNamara *et al*, 1996). In their study, administration of 75% O_2 was associated with a fall in $P_{ET}CO_2$ to around 37-38 mm Hg, which is in keeping with the fall to about 35 mm Hg with 100% O_2 observed in the current study. Both findings are attributable to the Haldane effect. This effect of hyperoxia to promote a degree of tissue CO_2 retention, through reduced blood carbamino transport of CO_2, is unavoidable, but the finding is believed unlikely to have any bearing on the results of the vision tests reported here. However, it is impossible to distinguish definitively

between the impact on visual performance of systemic hyperoxia and any accompanying mild tissue hypercapnia (and hence acidosis).

6.3.7 Analysis

The data for the Study 1 experiments comparing hypoxia with normoxia at low photopic (direct viewing), upper mesopic (ND 1.0) and mid-mesopic (ND 2.0) light levels were subject to repeated measures, balanced ANOVA to examine main effects and interactions of light level, number of viewing eyes, gender, breathing gas and *sf* (main effects $\alpha = 0.05$, interactions $\alpha = 0.01$). A similar analysis was undertaken on the Study 2 data, comparing responses under hypoxia with those obtained when breathing 100% O_2 at the two mesopic levels. A more stringent requirement was adopted for considering an interaction to be statistically significant to reduce the likelihood of Type I error. All analyses were conducted using Minitab 14 software. Data sets are shown in Appendix 4.

6.4 Results

6.4.1 Study 1

The detail of the balanced ANOVA is reproduced in Appendix 4. ANOVA indicated highly statistically significant main effects of light level ($p < 0.001$), number of viewing eyes ($p < 0.001$) and *sf* ($p < 0.001$) on spatial contrast sensitivity, together with statistically significant interactions between light level and number of viewing eyes ($p = 0.003$), light level and breathing gas ($p = 0.008$), light level and *sf* ($p < 0.001$), and number of viewing eyes and *sf* ($p = 0.004$). There was no suggestion of a statistically significant effect of breathing gas condition ($p = 0.998$).

All graphs that follow represent the spatial CSF using $\log_{10}/\log_{10}$ scales and the typical inverted 'U' appearance is self-evident throughout, illustrating the main effect of *sf*. The main effect of light level is illustrated in Figure 6.5 for binocular and monocular spatial CSF under both normoxic and hypoxic conditions. The results for viewing through the ND 1.0 filter have been displaced slightly to the right, along the abscissa, to facilitate discrimination of some of the data points. The graphs illustrate a consistent pattern of progressive impairment with reducing luminance that is more pronounced for higher *sf*. The interactions between light level and number of viewing eyes and between light level and *sf* are also self-evident from this Figure. However, the similarity of the hypoxic curves to their normoxic counterparts, both binocular and monocular, is

obvious and the nature of the statistically significant interaction between light level and breathing gas is unclear from these graphs.

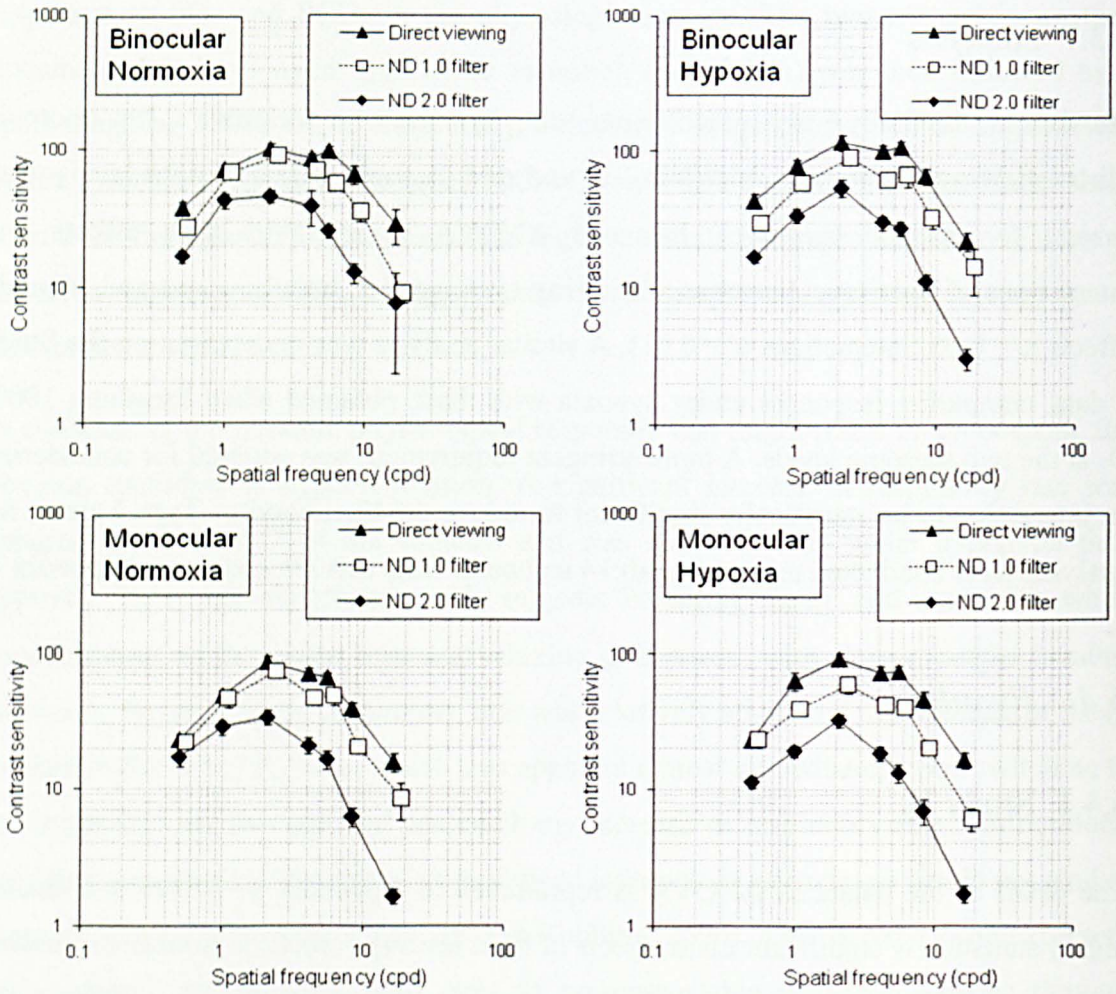


Figure 6.5 Effect of light level on mean (± SE) spatial contrast sensitivity

The main effect of viewing monocularly in comparison to viewing binocularly is shown in Figure 6.6 for each light level and respiratory condition. Monocular viewing almost invariably incurs an impairment of contrast sensitivity at all *sf*, irrespective of light level or respiratory status. Furthermore, the interaction between number of viewing eyes and *sf* is self-evident from this Figure.

The effect of mild hypoxia in comparison to viewing under normoxia is shown for all light levels and viewing conditions in Figure 6.7. No consistent effect of hypoxia to influence contrast sensitivity at any *sf* is apparent at any light level when viewing either binocularly or monocularly. The consistency of the results between respiratory conditions is noteworthy. Ignoring sporadic outlier values that have exaggerated the SE values for isolated data points, there is a single exception to this observation. When

viewing monocularly at the lowest light level, that is, through the ND 2.0 filter, a possible trend for hypoxia to compromise contrast sensitivity is suggested for the two lowest *sf*, that is, at 0.5 and 1.0 cpd. However, this is far from unequivocal.

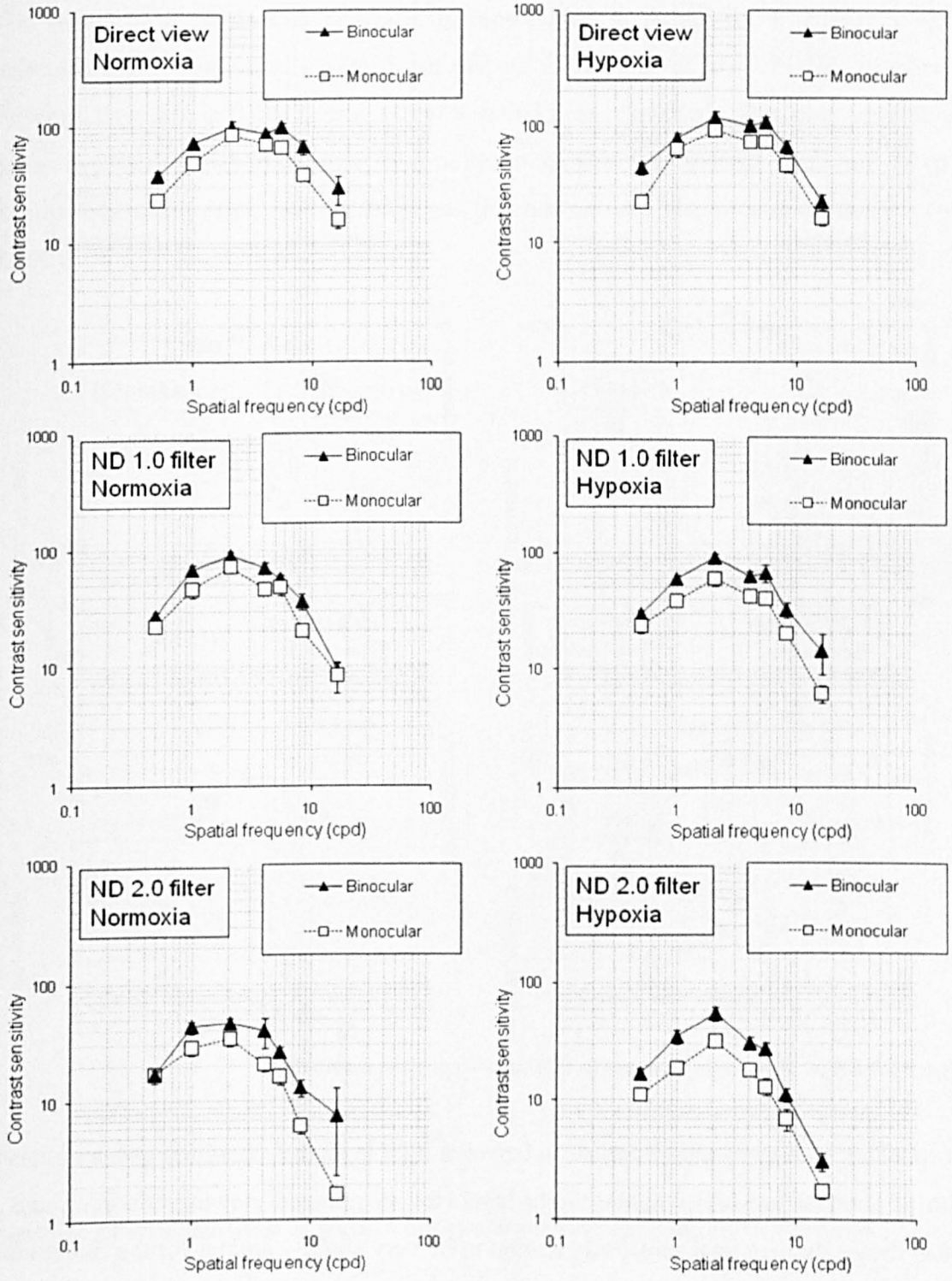


Figure 6.6 Effect of monocularity on mean (± SE) spatial contrast sensitivity

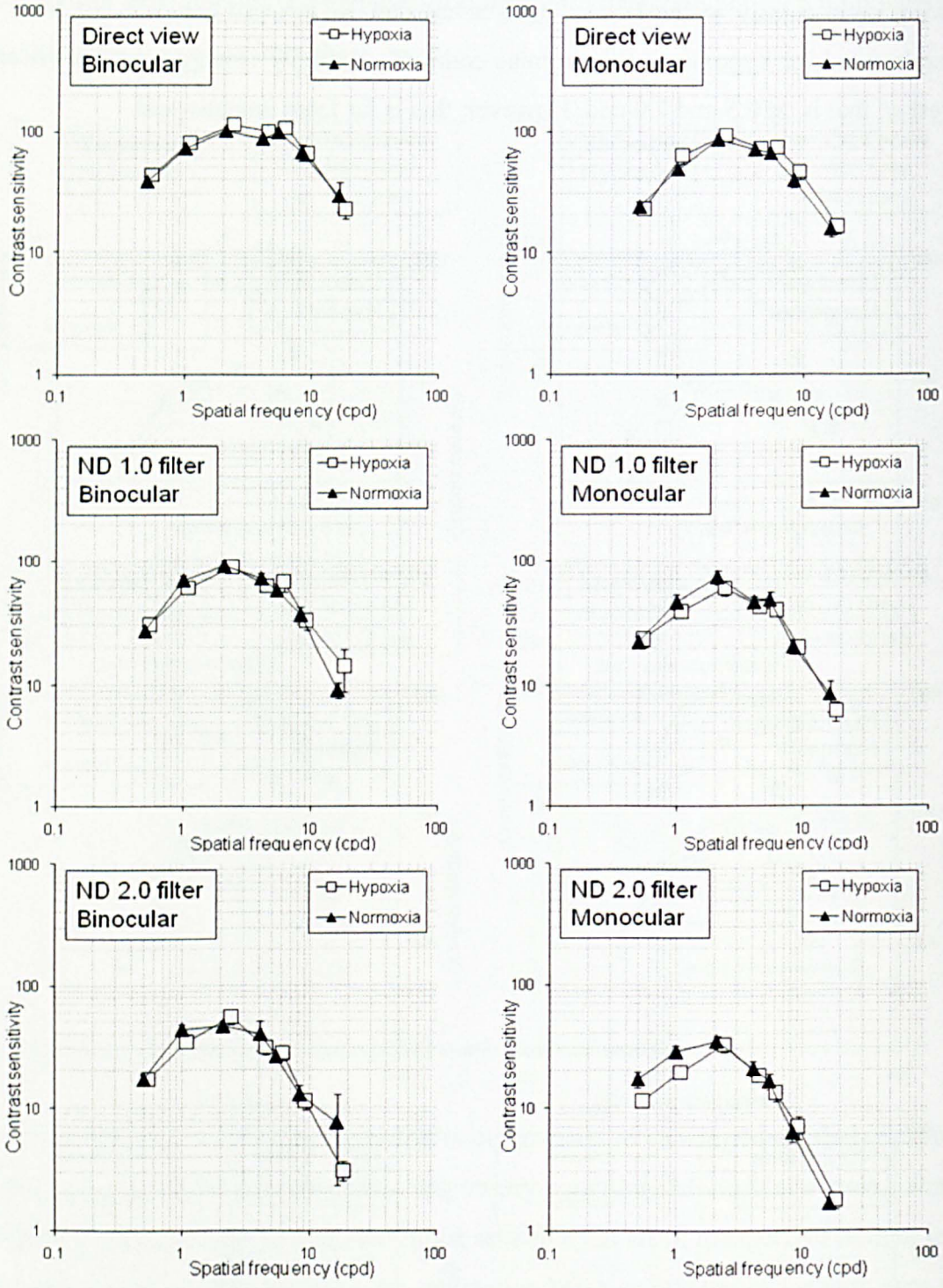


Figure 6.7 Effect of hypoxia on mean (± SE) spatial contrast sensitivity

In considering the apparent interaction between light level and breathing gas, an interval plot of contrast sensitivity against light level (for all grouped viewing condition and *sf* data) shows that contrast sensitivity tended to be very slightly greater for the direct view condition (28 cd m^{-2}) when hypoxic than when breathing air, while the reverse was true for the two mesopic viewing conditions. This appearance of the grouped data almost completely disappeared when considering the data by *sf*, and as evident in Figure 6.5,

there is no apparent effect of respiratory condition varying by light level when accounting for number of viewing eyes and *sf*. The magnitude of this apparent interaction is slight and it is not considered further.

6.4.2 Study 2

The detail of the balanced ANOVA is reproduced in Appendix 4. ANOVA again indicates highly statistically significant effects of light level ($p < 0.001$), number of viewing eyes ($p < 0.001$), and *sf* ($p < 0.001$), as expected. The only interaction achieving statistical significance was between number of viewing eyes and *sf* ($p = 0.002$). The main effect of breathing gas did not achieve statistical significance ($p = 0.149$).

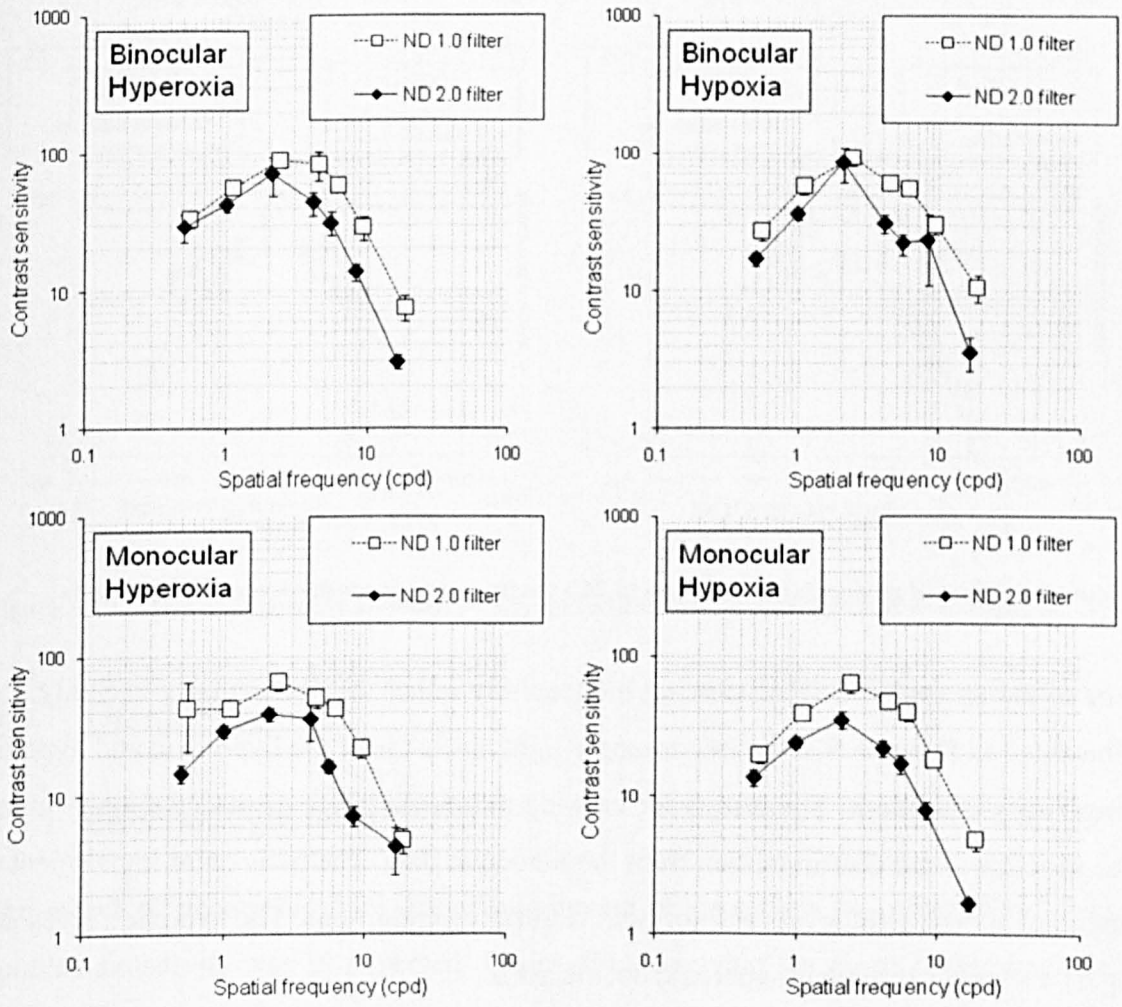


Figure 6.8 Effect of light level on mean (± SE) spatial contrast sensitivity under hyperoxia

The effect of light level in Study 2 is illustrated in Figure 6.8 for binocular and monocular spatial CSF under hyperoxic and hypoxic conditions. Again, the results for viewing through the ND 1.0 filter are displaced slightly along the abscissa to aid

discrimination from the ND 2.0 data. The impairment with reduced luminance remains apparent under hyperoxia and, as previously, is more pronounced at higher *sf*. The similarity of the hypoxic curves to their hyperoxic counterparts remains apparent.

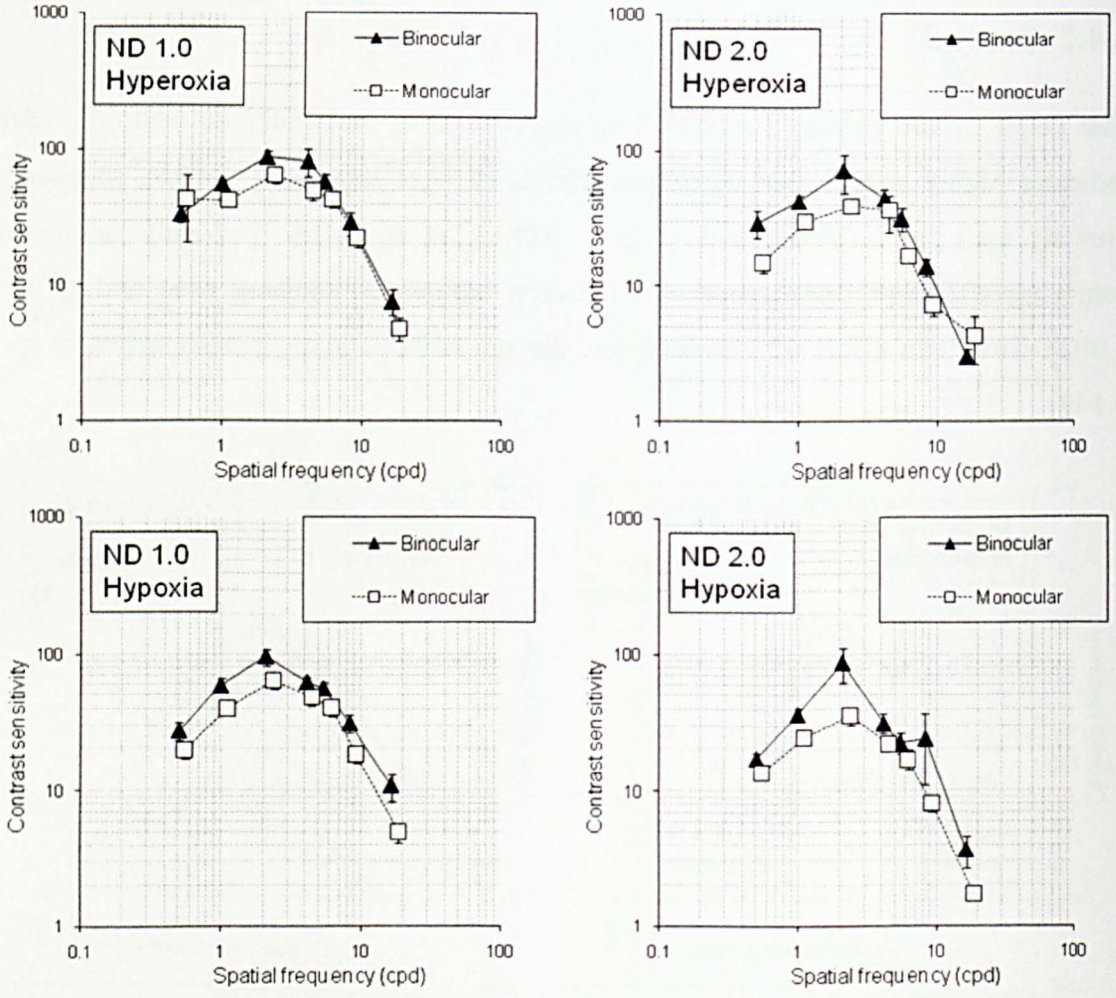


Figure 6.9 Effect of monocularity on mean (± SE) spatial contrast sensitivity under hyperoxia

The effect on spatial CSF of viewing monocularly rather than binocularly in Study 2 is illustrated in Figure 6.9 at the two mesopic light levels under the hyperoxic and hypoxic respiratory conditions. The results for viewing monocularly are displaced slightly along the abscissa to aid discrimination from the binocular data. The monocular impairment is apparent at both light levels and for both respiratory conditions. The hypoxic curves are again very similar to their hyperoxic counterparts.

In Figure 6.10 the results under hyperoxia are compared to those under hypoxia, exhibiting no meaningful difference in contrast sensitivity at either light level. There is no obvious benefit of hyperoxia. Compared to Study 1, the results between respiratory conditions appear slightly more variable but there are no consistent differences between the breathing gases. There are sporadic outlier values that have exaggerated the

magnitude of the SE for isolated data points but with no consistent pattern in relation to *sf*. There is no clear trend for hypoxia to compromise contrast sensitivity at low *sf* with decreasing light level when compared to hyperoxic performance.

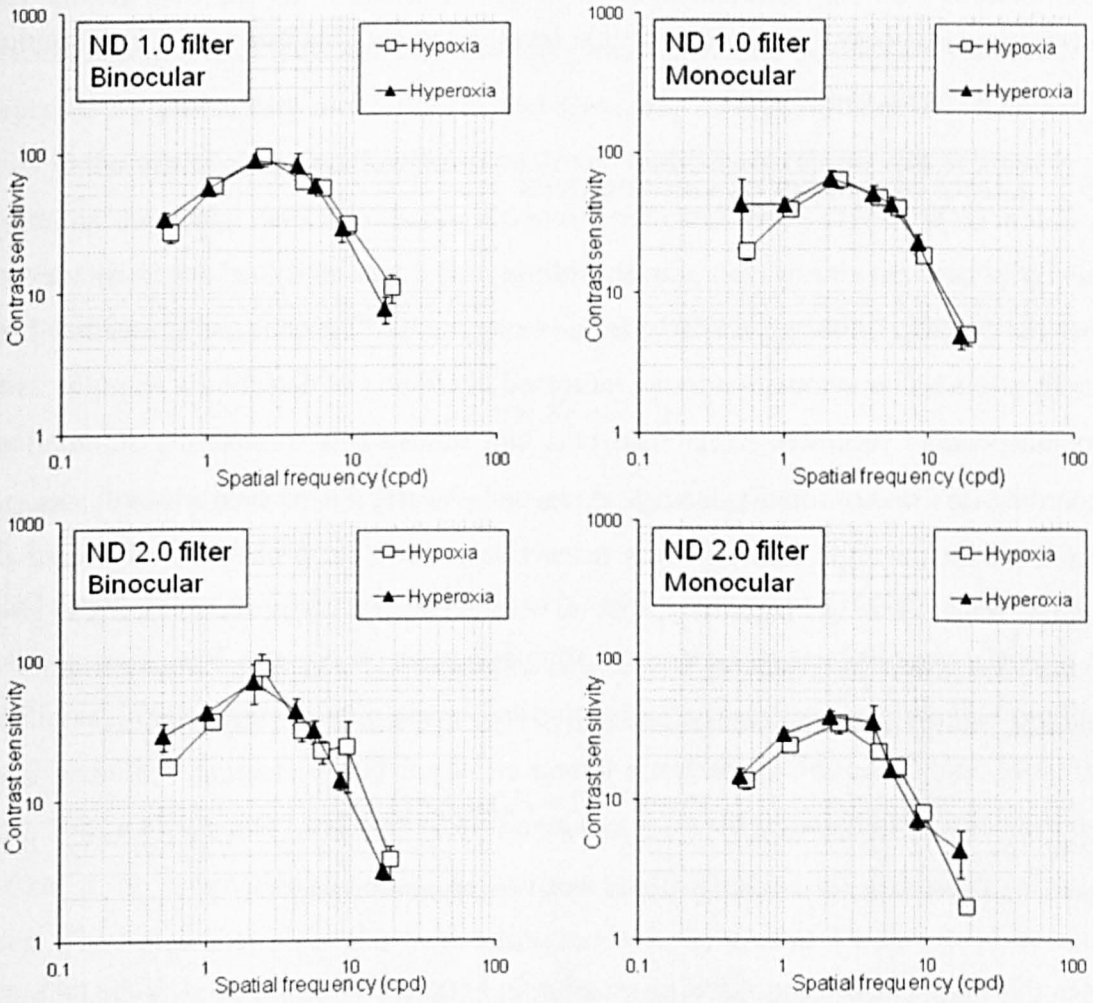


Figure 6.10 Effect of hyperoxia on mean (± SE) spatial contrast sensitivity relative to hypoxia

6.5 *Discussion*

6.5.1 Effect of light level

The effect of decreasing background luminance progressively to compromise spatial contrast sensitivity was as expected. Under direct viewing the mean background field intensity was approximately 28 cd m^{-2} and at the lower end of the photopic range. When viewing through the ND 1.0 filter the mean field luminance would be expected to fall to around 10% of this value or ~2.8 cd m^{-2}, decreasing further to around 1% of this value or ~0.28 cd m^{-2} when using the ND 2.0 filter. A more detailed description of filter transmission characteristics is given in relation to the study of chromatic sensitivity in section 7.3.4. The boundary between photopic and mesopic vision is blurred, varying

with luminance and retinal eccentricity. At the fovea, the detection of stimuli presented at a mean luminance of 2.8 cd m^{-2} will remain cone dominant and may be regarded as borderline low photopic/upper mesopic in nature. From Figure 6.5, the spatial CSF was reasonably well preserved at this luminance. However, a more obvious loss of contrast sensitivity was apparent at the lowest light level tested and this was particularly marked for higher *sf*, again as expected.

6.5.2 Effect of number of viewing eyes

Sensitivity to these sine wave contrast gratings was consistently enhanced by viewing binocularly rather than monocularly in all experiments. Pirenne (1943) attributed the benefit of binocular summation to the increased likelihood of detecting a stimulus using two independent receptors rather than one, and termed this 'probability summation'. According to the probability theory, if P_R and P_L are the probabilities of seeing a stimulus with the right and left eyes respectively, then the probability of seeing the stimulus when viewing binocularly, that is, with either or both of two eyes, P_T, is given by the expression in Equation 15. In this expression, P_T is defined as certainty of detection (unity) minus the probability that neither eye will detect the stimulus.

$$P_T = 1 - (1 - P_R)(1 - P_L)$$

Equation 14 Probability of stimulus detection with two independent sensors

By multiplying out, the simplified expression in Equation 15 is obtained. If the two eyes are identical, with detection probability P_E, then the expression simplifies further to that shown in Equation 16. A binocular summation factor of 1.2 is typical of probability summation but will vary with the slope of the psychometric function for monocular detection (Jiménez, Valero, Anera *et al*, 2003; Simmons and Kingdom, 1998).

$$P_T = (P_R + P_L) - (P_R \times P_L)$$

Equation 15 Simplified probability summation expression for binocular stimulus detection

$$P_T = 2P_E - P_E^2$$

Equation 16 Binocular detection probability for two identical eyes (probability summation)

The benefit of binocular viewing has also been attributed to a process of neural summation whereby the visual signal provided from two eyes is more readily distinguished from background visual noise during higher order processing (Campbell and Green, 1965a). In support, it has been demonstrated that the stimulation of corresponding retinal locations can result in greater binocular summation than would be expected by probability alone (Thorn and Boynton, 1974). Furthermore, many nerve cells in the visual cortex are known to be driven binocularly (Hubel and Wiesel, 1968). Based on the signal detection theory work of Green and Swets (1966), if S_R and S_L are the sensitivities of the right and left eyes respectively, then binocular sensitivity, S_B, is predicted by the quadratic summation model as shown in Equation 17 (Legge, 1984b).

$$S_B = \sqrt{(S_R^2 + S_L^2)}$$

Equation 17 Binocular sensitivity prediction according to the quadratic summation model

While probability summation explains the observed binocular benefit measured using some stimuli, and may do so even for quite complex visual challenges (Meese and Williams, 2000), many studies show that binocular performance exceeds that predicted by probability summation and implicate neural summation instead (Blake, Sloan and Fox, 1981). Frisén and Lindblom (1988) note that reported summation factors vary from 1 (that is, no summation) to about 2 (that is, a doubling of sensitivity) when using two eyes. They note that binocular summation tends to be greater for threshold detection tasks than for suprathreshold discrimination or resolution tasks, while tasks of greater complexity generally yield lower summation factors than simpler challenges. Accordingly, they propose a hierarchic model of binocular summation whereby the level of summation varies according to the nature and complexity of the visual challenge. Where the stimulus excites cortical cells that are almost exclusively driven monocularly, the level of summation would be expected to be high, as each eye would then act as an independent sensor. On the other hand, complex resolution tasks involving a greater proportion of higher order, binocularly driven cells should benefit less from binocular viewing. While other theories of binocular enhancement have been advanced, it remains likely that the level of benefit and mechanisms involved depend fundamentally on the nature of the task.

If the two eyes in question are identical, then the sensitivity resulting from neural summation, shown in Equation 17, should, in theory, be enhanced by a factor that simplifies to $\sqrt{2}$, or approximately 1.41. From past reports, the summation factor for

sine wave gratings typically approximates $\sqrt{2}$ (Campbell and Green, 1965a), supporting neural summation as the likely mechanism responsible for increased binocular sensitivity in relation to threshold detection of simple contrast gratings. Thus, a similar binocular summation factor may be anticipated in the experiments reported here. Furthermore, based on past reports, this factor should be independent of *sf* (Holopigian, Blake and Greenwald, 1986). With these points in mind, the binocular summation factors from the foregoing experiments have been analysed in further detail.

The mean between-subject sensitivities (N=12) at each *sf* were calculated for each light level and respiratory condition for each pair of binocular and monocular tests. The binocular to monocular sensitivity ratio was calculated as the index of binocular summation for each experimental condition. As there was no statistically significant effect of respiratory challenge, the means were calculated, from the results for both Study 1 and Study 2, by light level and *sf* but without regard for respiratory condition. The results are shown in Table 6-4 and represented in Figure 6.11. The results generally support the contention that binocular threshold sensitivity to sine wave contrast gratings is enhanced over monocular sensitivity as a result of neural summation, that is, by a factor approximating $\sqrt{2}$.

	Light level			
sf	Direct viewing	ND 1.0	ND 2.0	Mean
0.5	1.79	1.17	1.44	1.46
1.0	1.37	1.46	1.56	1.46
2.1	1.22	1.40	1.85	1.49
4.1	1.31	1.49	1.56	1.45
5.5	1.49	1.41	1.73	1.54
8.3	1.59	1.60	2.14	1.78
16.5	1.62	1.80	2.31	1.91
Mean	1.49	1.48	1.80	

Table 6-4 Mean luminance contrast binocular summation factor by light level and *sf*

However, two trends are suggested. Firstly, the summation factor tends to increase at the lowest light level, that is, the only level at which rods are likely to make a meaningful contribution to threshold sensitivity. Secondly, the summation factor also tends to increase progressively with increasing *sf* above 4.1 cpd, and this may be promoted particularly by the results at the lower light levels. To investigate the significance of these apparent trends, the data in Table 6-4 were subject to a two-way ANOVA ($\alpha = 0.05$) to examine possible main effects of light level and *sf*. The results are shown in Table 6-5 and indicate a statistically significant effect of light level. The effect of *sf* approaches but does not achieve statistical significance, although the striking

appearance of the mean data in Figure 6.11 suggests that the possibility of an increased summation factor at high *sf* should not be discarded too readily.

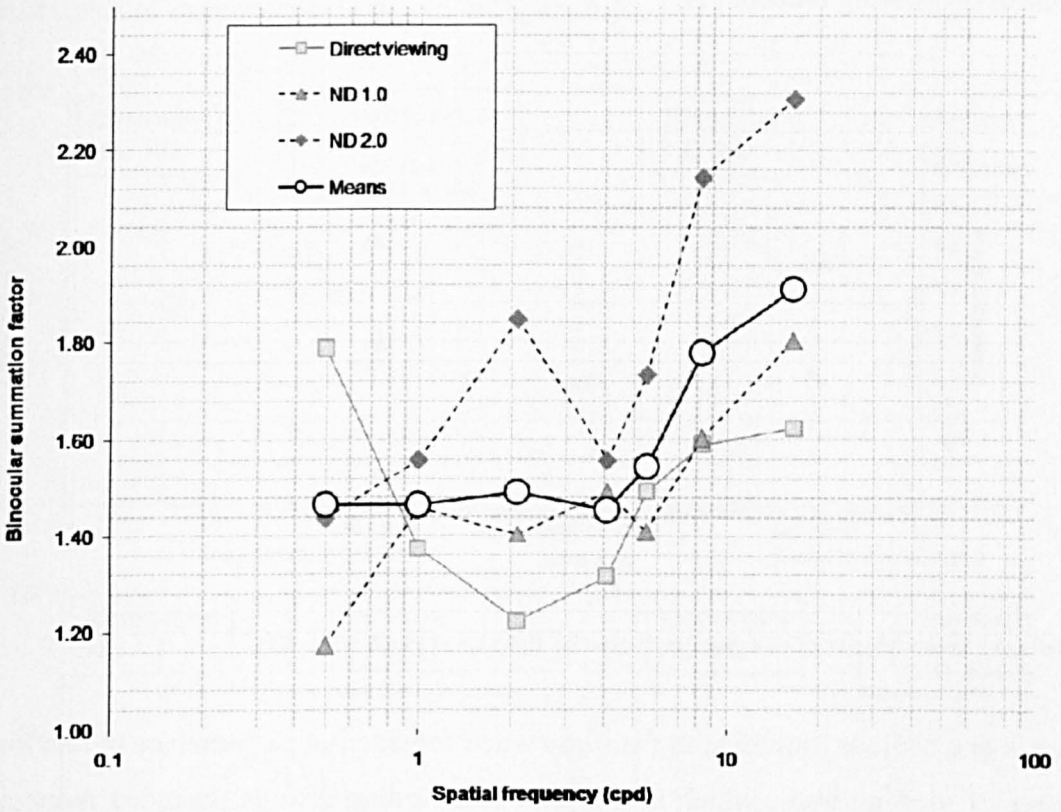


Figure 6.11 Binocular summation factor for contrast sensitivity at each *sf* by light level

Factor	Degrees of Freedom	SS	MS	F	P
sf	6	0.59776	0.099627	2.43	0.089
Light level	2	0.47387	0.236933	5.79	0.017
Error	12	0.49107	0.040922		
Total	20	1.56270			

S = 0.2023 R-Sq = 68.58% R-Sq(adj) = 47.63%

Table 6-5 Two-way ANOVA to assess effects of *sf* and light level on binocular summation

The main effect of light level is illustrated further in Figure 6.12 using 95% CI for the mean values across all *sf*, supporting an effect of mesopic viewing to increase the binocular summation factor.

In sum, the data and analysis support the contention that neural summation explains binocular summation for simple spatial contrast gratings viewed at the fovea at photopic luminance. They also support the contention that this is independent of *sf*, although

suggesting a possible trend for summation factor to increase at high *sf* that has been reported elsewhere (Simpson and Manahilov, 2005). The data and analysis also support a statistically significant effect of mesopic viewing to increase the summation factor, implying that the rod contribution to foveal contrast sensitivity is more obviously enhanced by viewing binocularly.

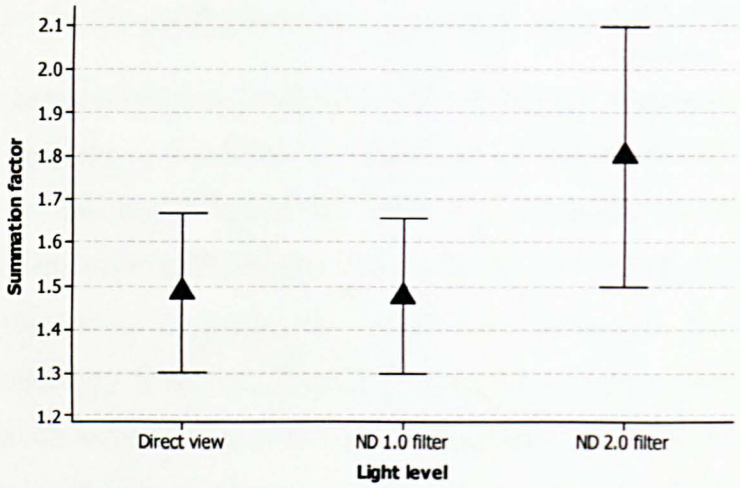


Figure 6.12 Interval plot of summation factor by light level (with 95% CI)

There is one obvious limitation to the study when considering performance in relation to number of viewing eyes, which is that binocular testing always preceded monocular testing. It is possible that considerations such as fatigue, inattention and boredom may have compromised the second bout of contrast testing in each sequence. However, the reproducibility of the results and their concordance with past reports suggests that this is unlikely. Furthermore, given the extended nature of testing under each pair of respiratory conditions, and that these were randomised, any residual net order effects should be trivial. Additionally, since contrast testing followed 25 minutes of visual and respiratory adaptation, during which the subjects were essentially unoccupied, all appeared alert and eager during the subsequent, relatively brief, tests of binocular and monocular contrast sensitivity. It is considered exceptionally unlikely that the order of binocular and monocular testing will have confounded the results.

6.5.3 Effect of respiratory condition

The hypotheses are refuted. No effect of respiratory challenge is evident in either Study 1 or Study 2. The former indicates that the normal spatial CSF is unaffected by hypoxia equivalent to breathing air at 10,000 ft, the latter that it is optimised when breathing air

at one atm. The latter conclusion assumes that performance under hypoxia was the same in both studies. This was assessed using one-way ANOVA ($\alpha = 0.05$) to examine the hypoxic mesopic data sets from the two studies and confirmed no statistically significant difference between them ($p = 0.79$). The similarity of the hypoxia data from the two sets of experiments is shown in Figure 6.13.

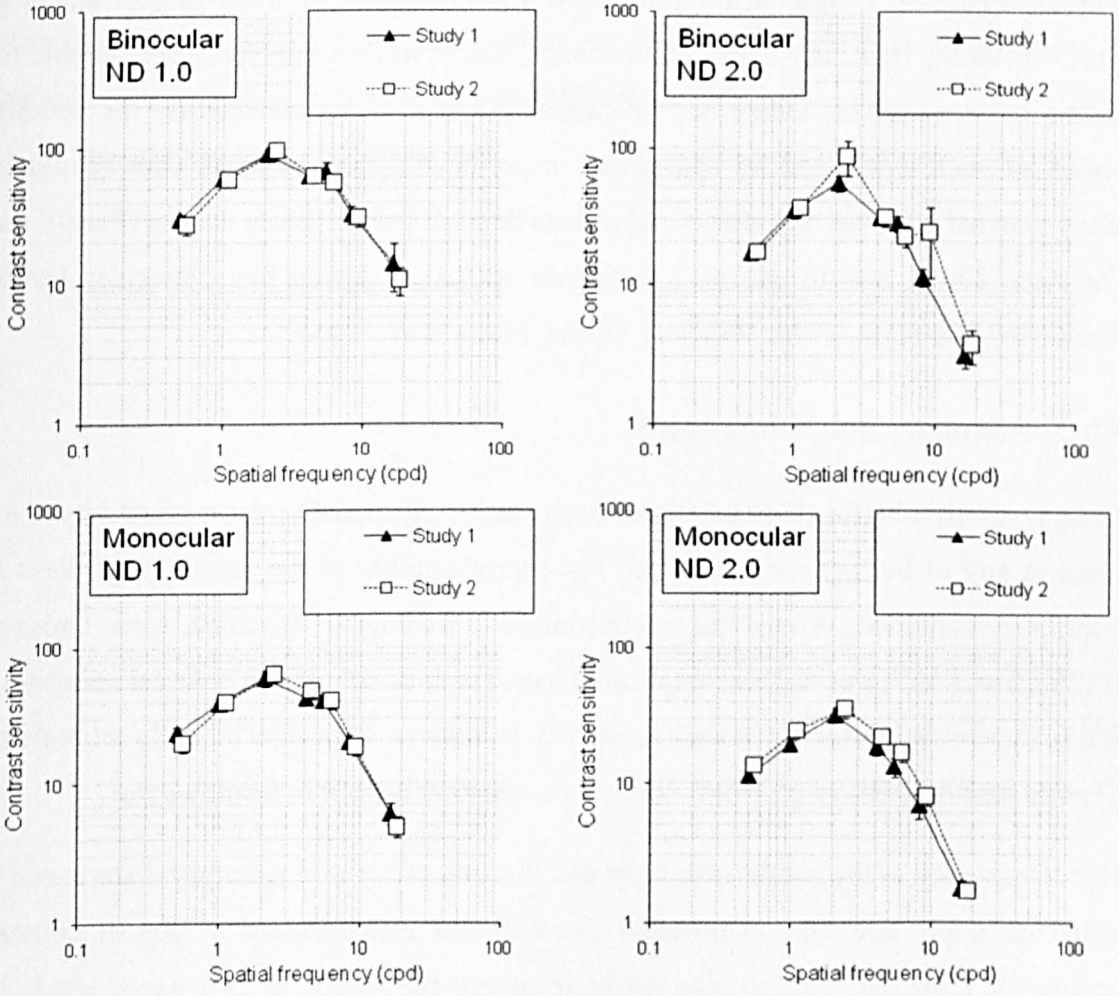


Figure 6.13 Comparison of hypoxic contrast sensitivity functions achieved in Studies 1 and 2

6.5.4 Lessons for subsequent studies

Vision testing

The chosen test method has demonstrated convincingly that the imposed respiratory conditions have no bearing on the foveal CSF while consistent effects of *sf*, light level and monocular viewing were elicited as anticipated. Accordingly, the test method appears to have been successful. However, in retrospect, it might have been more informative to have examined spatial contrast sensitivity to sine wave gratings at a selection of eccentricities, as well as at fixation, on the basis that greater rod density

with eccentricity might exaggerate any sensitivity to local tissue hypoxia at lower light levels.

Although the chosen test was adequate in this instance, it would be preferable in future studies to employ a true 'forced choice' technique, perhaps using a 2-AFC (if testing bilaterally along the horizontal meridian) or 4-AFC paradigm (if testing by quadrant). A criterion-free 2-AFC approach exhibits low within-subject, between-subject and test-retest variability (Nio, Jansonius, Lamers *et al*, 2005), while 4-AFC tests are suitable for testing naïve observers (Jäkel and Wichmann, 2006). When considering the detailed design of such a test and an appropriate experimental methodology, care should be taken to avoid confounding attentional effects through inappropriate cueing (Pestilli and Carrasco, 2005), and to use an appropriate occlusion device for monocular testing (Smith and Harwerth, 1979; Wildsoet, Wood, Maag *et al*, 1998).

6.5.5 Summary and future work

There is no meaningful effect of altered oxygenation on spatial contrast sensitivity at the fovea at any of the light levels tested. The reproducibility of the results, regardless of respiratory condition, refutes the counter-intuitive findings of Benedek, Kéri, Grósz *et al* (2002) and indicates that uncomplicated hypoxia does not benefit contrast sensitivity. While hyperoxia enhances contrast sensitivity in diabetic subjects with early retinopathy (Harris, Arend, Danis *et al*, 1996), there is no apparent benefit in healthy eyes.

The consistency of the results also indicates that any effect of respiratory disturbance to influence pupil size was insufficient to confound measurement of foveal contrast sensitivity. Changes in pupil size might influence the results in two ways, either by altering retinal illumination or by altering the influence of optical aberrations (Campbell and Green, 1965b). At lower light levels when the pupil is more dilated, any tendency of hypoxia to constrict the pupil, for example, would tend to reduce retinal illumination but also decrease optical aberrations, compromising and enhancing contrast sensitivity respectively, particularly for gratings of higher *sf*. In any event, the consistency of the results suggests that the net effect of any influence of respiratory disturbance on pupil size is negligible in this instance. Furthermore, the consistency of the data indicates that the process of visual adaptation was successful in promoting stable and reproducible adaptation states within and between individuals.

In addition to examining contrast sensitivity away from fixation, it would be worthwhile to assess older subjects under hypoxic challenge, as contrast sensitivity has been shown to diminish with age in normal observers with healthy eyes, for example to static gratings of high *sf* when over about 40 to 50 y of age (Owsley, Sekuler and Siemsen, 1983; Sloane, Owsley and Jackson, 1988; McKendrick, Sampson, Walland *et al*, 2007). In addition, the process of binocular summation, assessed using sine-wave gratings, is affected by age at modest eccentricities from the fovea, and might also be influenced by changes in oxygenation state (Pardhan and Whitaker, 2003).

7 Chromatic Sensitivity

7.1 Abstract

PURPOSE. The effect of mild hypoxia on chromatic sensitivity in the mesopic range is poorly documented. This study examined the effects of mild hypoxia and hyperoxia on R-G and Y-B chromatic sensitivity thresholds at low photopic (22.3 cd m^{-2}), borderline upper mesopic (1.67 cd m^{-2}) and mid-mesopic (0.21 cd m^{-2}) luminance.

METHODS. The City University Colour Assessment and Diagnosis (CAD) test was used to measure binocular and monocular R-G and Y-B chromatic sensitivity, with dynamic luminance contrast noise to isolate the use of colour signals. Colour vision testing followed the tests of contrast sensitivity reported in the previous chapter and therefore followed the identical method of visual and respiratory adaptation. The test display screen was viewed either directly or through ND 1.0 or 2.0 filters. Mild hypoxia was imposed breathing 14.1% O_2 and investigated relative to control exposures breathing air (normoxia) at each light level. Subsequently, hyperoxia, breathing 100% O_2, was assessed relative to hypoxia under the two mesopic conditions. The repeated measures design allowed assessment of main effects and interactions of light level, number of viewing eyes, gender, breathing gas and colour axis using balanced ANOVA.

RESULTS. Light level, number of viewing eyes, and oxygenation state were significant determinants of both Y-B and R-G chromatic sensitivity. Binocular summation of the chromatic signal was consistent under all experimental conditions and is independent of the luminance channel; monocular viewing increases Y-B and R-G chromatic thresholds by a factor of ~1.26. Hypoxia, equivalent to breathing air at 10,000 ft, elevates Y-B and R-G chromatic thresholds to a greater extent with decreasing light in the mesopic range. Both monocular viewing and hypoxia exaggerate the tritan nature of mesopic colour ellipses. Supplementary O_2 conferred no advantage over breathing air. One male and one female introduced orthogonal sources of gender bias; the male was diagnosed with minimal deuteranomaly (R-G deficient) using the CAD test, and the only female using hormonal contraception exhibited loss of Y-B sensitivity.

CONCLUSIONS. In the mesopic range, mild hypoxia impairs chromatic sensitivity progressively with reducing luminance, supporting the contention that increasing QO_2 with decreasing light renders outer retinal function vulnerable to exogenous hypoxia in the mesopic range. However, supplementary O_2 confers no benefit over breathing air in relation to cone function under such circumstances. The binocular summation of chromatic signals is consistent and approximates probability summation. The CAD test is highly sensitive to mild congenital and acquired colour vision deficiencies while conventional colour vision screening techniques fail to identify colour deficiencies that may become manifest only in the mesopic range.

CITATION. The work reported in this chapter has been subject to peer review and is published at **Connolly, D. M., Barbur, J. L., Hosking, S. L., and Moorhead, I. R. (2008). Mild hypoxia impairs chromatic sensitivity in the mesopic range.** ***Investigative Ophthalmology and Visual Science*****, 49: 820-827.** The paper is reproduced at Appendix 9.

7.2 *Introduction*

7.2.1 Background

In two studies mild hypoxia has been reported to compromise photopic colour vision at an equivalent altitude of as low as 10,000 ft (Brandl and Lachenmayr, 1994; Karakucuk, Oner, Goktas *et al*, 2004). However, both studies are open to methodological criticism (section 1.6.4). On the other hand, moderate hypoxia compromises mesopic colour sensitivity progressively in the periphery of the visual field (Kobrick, 1968), and tritan defects have been demonstrated at low photopic luminance when hypoxia is severe (Smith, Ernest and Pokorny, 1976). These and other studies suggest that moderate hypoxia produces a general impairment of colour sensitivity and discrimination, perhaps with a more obvious effect on the Y-B axis, that may be worse at lower luminance and in the retinal periphery. However, the effect of hypoxia on chromatic thresholds is poorly documented in relation to decreasing mesopic luminance.

At mesopic levels, rod signals influence colour perception, for example by changing the perception of hue (Buck, Knight, Fowler *et al*, 1998; Lythgoe, 1931) and affecting colour matches (Trezona, 1970) and wavelength discrimination (Stabell and Stabell, 1977). Rod intrusion compromises near-threshold R-G discrimination (Nagy and Doyal, 1993), impairs S-cone mediated (tritan) chromatic discrimination progressively with decreasing retinal illumination (Knight, Buck, Fowler *et al*, 1998), and desaturates cone-mediated colours, even at high mesopic light levels (Stabell and Stabell, 1996). An effect of hypoxia to compromise the rod contribution to colour perception might reasonably be considered unlikely to have practical significance, particularly since the chromatic processes of rods and cones tend to suppress each other, with cones dominant at higher mesopic levels (Stabell and Stabell, 1998). However, as the available light decreases and rod sensitivity and QO_2 increase, progressive rod-driven retinal hypoxia might compromise cone oxygenation also, rendering cone-mediated colour sensitivity vulnerable to exogenous hypoxia. Further reduction in luminance might be expected to exaggerate this impairment if the level of hypoxia is fixed.

Such an effect would be of direct relevance to the physiological colour vision of aircrew at night, when aircraft cockpits and flight decks are illuminated typically in the mesopic range. However, hypoxia is implicated increasingly as an aetiological or contributory factor in various ocular pathologies, such as glaucoma and diabetes (Dean, Arden and

Dornhurst, 1997; Drasdo, Chiti, Owens *et al*, 2002; Mattiello, Maneiro and Gastelú, 2001), so effects of hypoxia on normal colour vision have wider relevance.

Mesopic colour perception varies in relation to changes in the density and distribution of the various types of photoreceptor, differences in their spatial summation properties, varying relative contributions of rod and cone signals with changing luminance, and other complex interactions between rod and cone signals that are not fully understood. However, the Colour Assessment and Diagnosis (CAD) test is a sensitive tool for measuring threshold chromatic sensitivity in the mesopic range and possible effects of imposed experimental conditions (Barbur, Rodriguez-Carmona and Harlow, 2006; Rodriguez-Carmona, Harlow, Walker *et al*, 2005; Walkey, Barbur, Harlow *et al*, 2001).

Binocular summation has been reported in relation to colour discrimination tested using the FM100 (Verriest, Van Laetham and Uvuls, 1982; Newman, Wolfe, Stewart *et al*, 1991). Binocular summation has also been reported for colour contrast sensitivity, although the magnitude of the effect appears less than for luminance contrast (Simmons and Kingdom, 1998; Jiménez, Medina, Jiménez del Barco *et al*, 2002; Jiménez, Valero, Anera *et al*, 2003; Forte, 2005). However, binocular summation is not always evident (Costa, Ventura, Perazzolo *et* al, 2006) and complex binocular interactions may occur in relation to colour signal processing (Erkelens and van Ee, 2002; Medina, 2006; Medina and Mullen, 2007). The CAD test exploits dynamic luminance contrast masking to isolate sensitivity to the chromatic signal (Barbur, Harlow and Plant, 1994). Thus, the test offers the opportunity to investigate further the relationship between binocular and monocular thresholds for detection of colour. The current study afforded an opportunity to examine these also with respect to light level and respiratory state.

7.2.2 Hypotheses

Progressive rod-driven retinal hypoxia might increase the susceptibility of foveal chromatic sensitivity to exogenous hypoxia as luminance is reduced from photopic to upper and mid-mesopic levels.

Supplementary O_2 should optimise foveal chromatic sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Binocular summation of chromatic threshold sensitivity might interact with respiratory disturbance.

7.2.3 Aims

The primary aim of these colour vision studies was to assess the hypotheses in relation to effects of altered oxygenation state on foveal chromatic sensitivity with decreasing luminance (sections 7.5.4).

The secondary aim was to assess any effect of binocular summation to benefit chromatic sensitivity in comparison to monocular performance (section 7.5.2).

A subsidiary aim was to confirm or refute any effect of hypoxia, equivalent to breathing air at 10,000 ft, to compromise colour sensitivity at photopic luminance (Brandl and Lachenmayr, 1994; Karakucuk, Oner, Goktas *et al*, 2004) (section 7.5.4).

Lastly, it was intended to interpret the findings in relation to aircrew visual performance during flight and with respect to the use of current and future aircrew colour displays (section 11.2.4).

7.2.4 Experimental design

The design for these experiments was outlined in section 6.2.4. Briefly, vision testing was preceded by identical processes of retinal bleach and dark adaptation followed by light adaptation to a known photopic, upper mesopic or mid-mesopic luminance. These were controlled by viewing the test display either directly or through ND filters of OD 1.0 or 2.0. The rationale for the process of visual adaptation employed was illustrated in Figure 4.1. The principle behind the use of ND 1.0 and 2.0 filters was illustrated in Figure 6.1. The first three experimental series (Study 1) compared visual performance under conditions of normoxia and mild hypoxia. The final two series (Study 2) repeated the previous mesopic assessments to compare visual performance under the same level of mild hypoxia with that achieved when breathing 100% O_2, to identify whether or not supplementary O_2 might offer a performance benefit in relation to hypoxia. The experimental parameters are summarised in Table 6-1. The terms Study 1 and Study 2 retain the same meanings as in Chapter 6.

The timeline of each experimental exposure was illustrated at Figure 6.2. Testing of chromatic sensitivity followed that of contrast sensitivity and occupied the period from about 30 to 50 minutes of exposure to each respiratory condition, varying slightly between subjects according to the time taken to complete the preceding tests of contrast sensitivity. Binocular testing of chromatic sensitivity preceded monocular testing in

accordance with the same rationale outlined for the assessment of contrast sensitivity, that is, to preserve the validity of direct comparison between respiratory conditions such that duration of respiratory exposure was the same for each viewing condition.

7.3 *Materials and Methods*

7.3.1 Subjects

Background subject details and screening requirements are as detailed in Chapter 3, section 3.6.3. Specific subject details for the experiments in this chapter are detailed in section 6.3.1. Subjects comprised six males and six females for each experiment, with one female substituted during the course of the study. All had healthy eyes and achieved Snellen VA of at least 6/6 in each eye, corrected if necessary using untinted spectacles (two females) or untinted contact lenses (one male).

Subject	Yellow				Red:Green			
	1	2	3	Means	1	2	3	Means
F1	13.5	12	17	14.17	47.5	46	43	45.5
F2	14	20	15	16.33	44	41	36	40.33
F3	17	15	13.5	15.17	49	48	47	48
F4*	13.5	13.5	13.5	13.5	44	43	44	43.67
F5	14	13	13.5	13.5	45	46	46	45.67
F6	13	12	15	13.33	46	48	48	47.33
F7	12	15.5	16	14.5	44	41.5	45	43.5
M1	20	19	13	17.33	42	45	47	44.67
M2	13.5	14	16.5	14.67	41	42	42.5	41.83
M3*	13.5	13	13	13.17	45	46	48.5	46.5
M4	12	14	13	13	46	47	44	45.67
M5	13	15	16	14.67	41	42	41	41.33
M6	22	16	15	17.67	40	47	44	43.67
Means	Yellow			14.69	Red:Green			44.43
SD				1.55				2.33

An * indicates subjects subsequently identified as having anomalous colour vision

Table 7-1 Nagel anomaloscope screening data (3 matches) for male (M) and female (F) subjects

The subjects were screened to exclude congenital colour deficiency, that is, to verify normal trichromacy. Initial screening required subjects to correctly identify the first 17 of Ishihara's pseudoisochromatic plates. This assessment was undertaken by an experienced aircrew medical examiner as part of the medical and ophthalmic clinical examination for study participation. One male volunteer failed Ishihara screening due to an obvious R-G colour deficiency. Those passing the Ishihara test were assessed using

the Nagel Type I anomaloscope as the 'gold standard' for clinical assessment of R-G colour vision, with each subject required to achieve a mean Rayleigh match, from three attempts, within the normal range, and to have a normal matching range. Nagel testing was conducted by a post-doctoral vision scientist. Neither examiner was involved in the conduct of the subsequent study. The results of anomaloscope screening for the 13 participating subjects are shown in Table 7-1 and histograms of these data sets are shown for the yellow and R-G components of the colour matches, respectively, in Figure 7.1 and Figure 7.2.

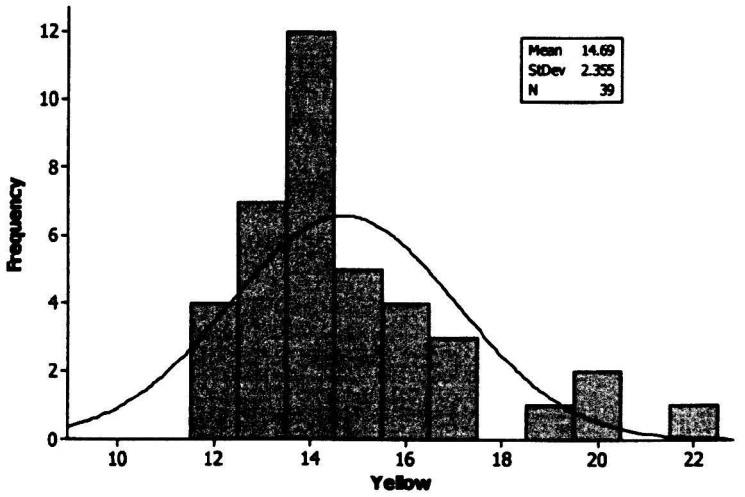


Figure 7.1 Histogram (with fitted probability distribution) for Nagel 'yellow' screening data

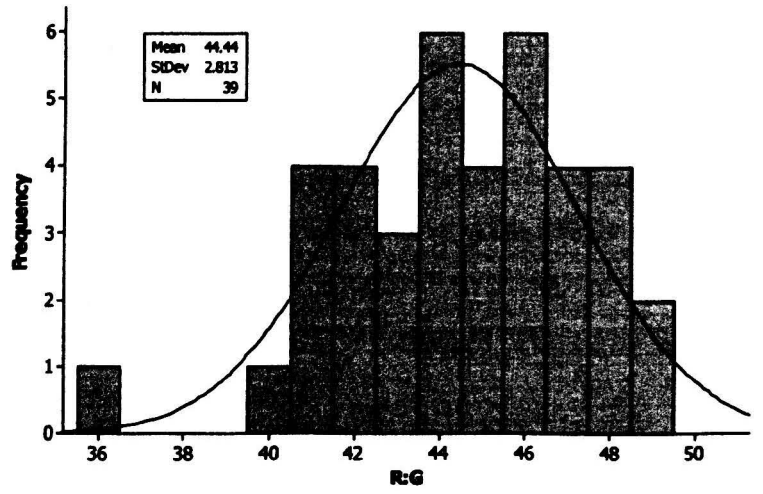


Figure 7.2 Histogram (with fitted probability distribution) for Nagel 'R-G' screening data

The mean value of the R-G element of the Rayleigh match for normal trichromats using the Type I Nagel anomaloscope is 44 ± 4 (2 SD), giving a normal range for ~95% of the

population of between 40-48 (Birch, 2003, p23). This is consonant with the mean and SD of the subject group shown in Table 7-1 and with the fitted probability distribution shown in Figure 7.2. Thus, the colour vision of the subject pool is representative of a population of normal trichromats, at least with respect to Rayleigh matches.

As with the tests of spatial contrast sensitivity, subjects acted as their own controls for all experiments but were masked to the exposure order of the two respiratory conditions. This was randomised equally between males and females, with subjects unknowingly self-allocating to one order or the other according to their availability for testing.

7.3.2 Equipment

Equipment details are given in section 6.3.2. It is necessary to consider further the transmission characteristics of the ND filters used in the study and these are detailed appropriately in section 7.3.4.

7.3.3 Respiratory conditions

The respiratory conditions are detailed in section 6.3.3.

7.3.4 Vision testing

Chromatic sensitivity thresholds were measured using a modified version of the City University Colour Assessment and Diagnosis (CAD) test, which employs dynamic luminance contrast 'noise' to isolate the detection of colour signals (Barbur, Harlow and Plant, 1994; Rodriguez-Carmona, Harlow, Walker *et al*, 2005). The test stimulus was generated in the centre of a large, uniform, achromatic background field (30° x 24°) and comprised a square array of 15 x 15 achromatic chequers subtending a horizontal visual angle of ~3.3° and presented for 824 ms. The uniform background provides a steady state of light adaptation equivalent to a D65 average daylight source, with (x, y) chromaticity of 0.305, 0.323, shown as a solid triangle in Figure 7.3, which represents the Commission Internationale d'Éclairage (CIE) 1931 – (x, y) chromaticity chart. The luminance of each chequer scintillates rapidly, independently and randomly, above and below background, to generate achromatic, luminance contrast 'noise', masking the detection of residual luminance contrast signals in the "isoluminant" coloured stimulus that might otherwise cue stimulus motion. The 'noise' does not affect the threshold for detection of colour signals (Barbur, 2004b). The sensitivity, specificity and repeatability

of a web-based version of the CAD test have been assessed independently with a favourable outcome (Seshadri, Christensen and Lakshminarayanan, 2005).

The colour-defined stimulus comprises a chromatic displacement step, occupying 5 x 5 scintillating chequers (~1.1° square), that traverses the chequerboard diagonally from one corner to its opposite, along one of four possible directions. The chromatic stimulus is present for 674 ms and is preceded and followed by 75 ms of luminance contrast noise alone. The subject's task is to indicate the direction of colour stimulus motion by pressing one of four buttons arrayed in a square, with each button corresponding to a destination corner on the chequerboard. The chance probability of a correct response is reduced to 1/16 by requiring the subject to report the correct direction of stimulus motion twice before the strength of the colour signal is reduced. Thus, the test is a true 4-AFC procedure suited to psychophysical testing (Jäkel and Wichmann, 2006).

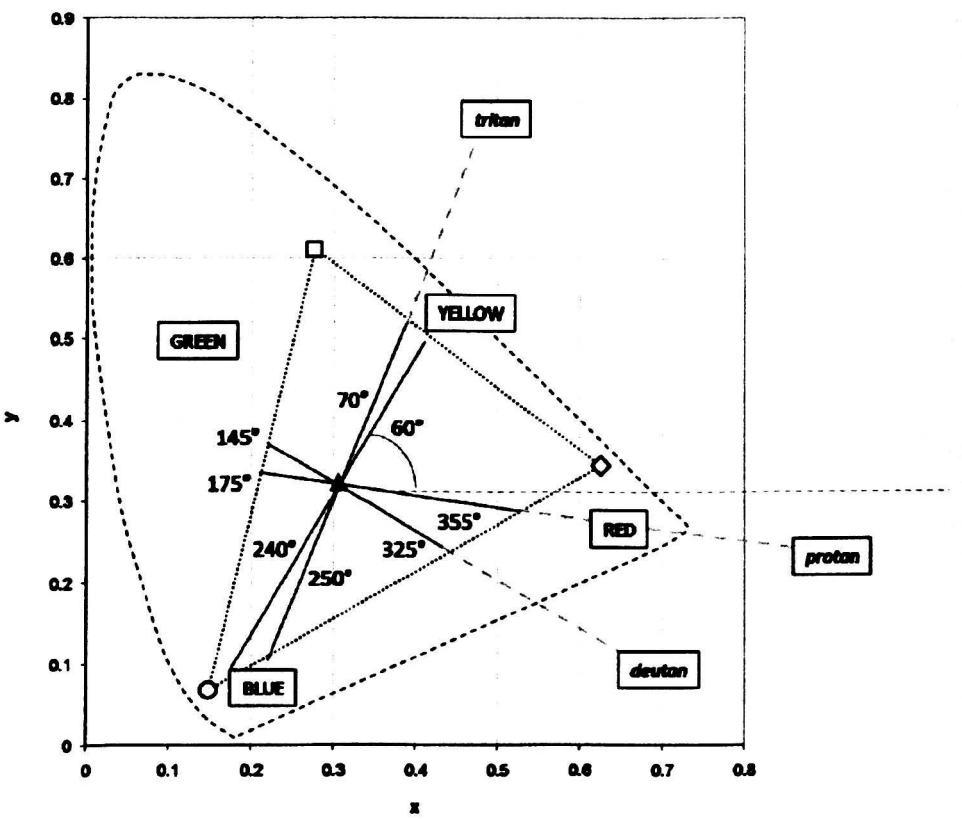


Figure 7.3 Colour test directions and phosphor coordinates

Randomly interleaved staircases were used with variable step sizes to measure threshold chromatic sensitivity along eight colour directions in the CIE 1931 – (x, y) chromaticity

chart (Figure 7.3). The angle of each direction shown is measured anticlockwise with reference to a horizontal meridian through background chromaticity illustrated for the yellow 60° stimulus. Each staircase continued until eight reversals were achieved, with the first two being ignored for determination of chromatic thresholds. Step size increments in axis length, that is, in distance on the (x, y) chromaticity chart from background chromaticity, started at 0.01 and finished at 0.002.

The eight colour directions correspond to four colour confusion axes, of which two are R-G and two are Y-B (Figure 7.3). These include the protan, deutan and tritan colour confusion axes shown that pass through the mean dichromatic convergence points at chromaticity coordinates (0.75, 0.25), (1.40, -0.40) and (0.17, 0) respectively (Birch, 2003). Testing could have been limited to a single R-G and a single, orthogonal Y-B axis, in an attempt to estimate the semi-major and semi-minor axes of the corresponding colour discrimination ellipses (MacAdam, 1942). This would aim to maximise the output of either the R-G or the Y-B colour channels, that is, the cone-contrast signal generated by the test stimuli in S-cones would be approximately zero along the R-G axis whilst the M- and L-cone contrast signals would be approximately zero along the Y-B axis. However, doubling the number of chromatic stimuli offers several advantages, besides approximating this aim. Firstly, R-G testing can be conducted along the protan and deutan R-G colour confusion axes, providing information that has the potential to be compared directly with the responses of subjects with congenital colour deficiency in future studies. Secondly, any confounding influence of individual anomalous or idiosyncratic responses is lessened. Lastly, the likelihood of drawing erroneous conclusions due to tilting of the ellipse axes at reduced luminance may be reduced (Walkey, Barbur, Harlow *et al*, 2001). Hence, the directions of R-G chromatic displacement away from background chromaticity were chosen to follow protan (175°-355°) and deutan (145°-325°) colour confusion axes. The directions of Y-B chromatic displacement were chosen to follow the tritan colour confusion axis (70°-250°) and a second Y-B axis that was offset slightly (60°-240°) while retaining a clearly 'yellow' hue to the Y stimulus. The semi-major and semi-minor axes could then be approximated by deriving mean R-G and Y-B axis lengths, if necessary.

Test stimuli were generated on a Sony Trinitron Multiscan G520 CRT display monitor. Display chromaticity calibration (Figure 7.4) was undertaken using a Minolta CS1000 telespectroradiometer. The display phosphor chromaticity coordinates are red (x = 0.625, y = 0.344), green (x = 0.276, y = 0.610) and blue (x = 0.148, y = 0.069),

represented by the open diamond, square and circle symbols that bound the triangular colour gamut of the display monitor in Figure 7.3.

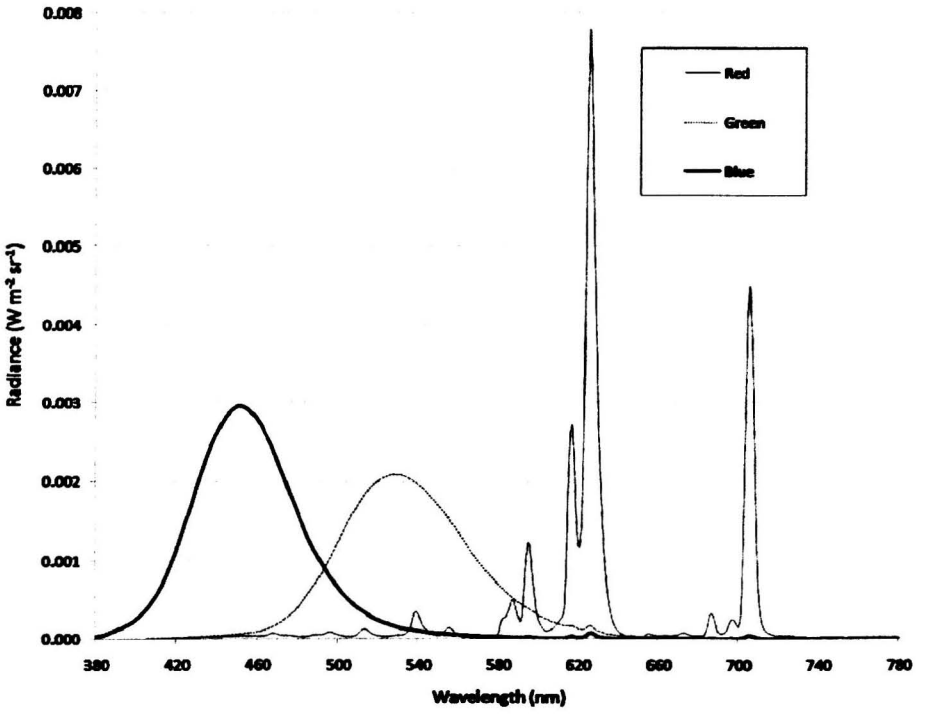


Figure 7.4 Test display phosphor calibration

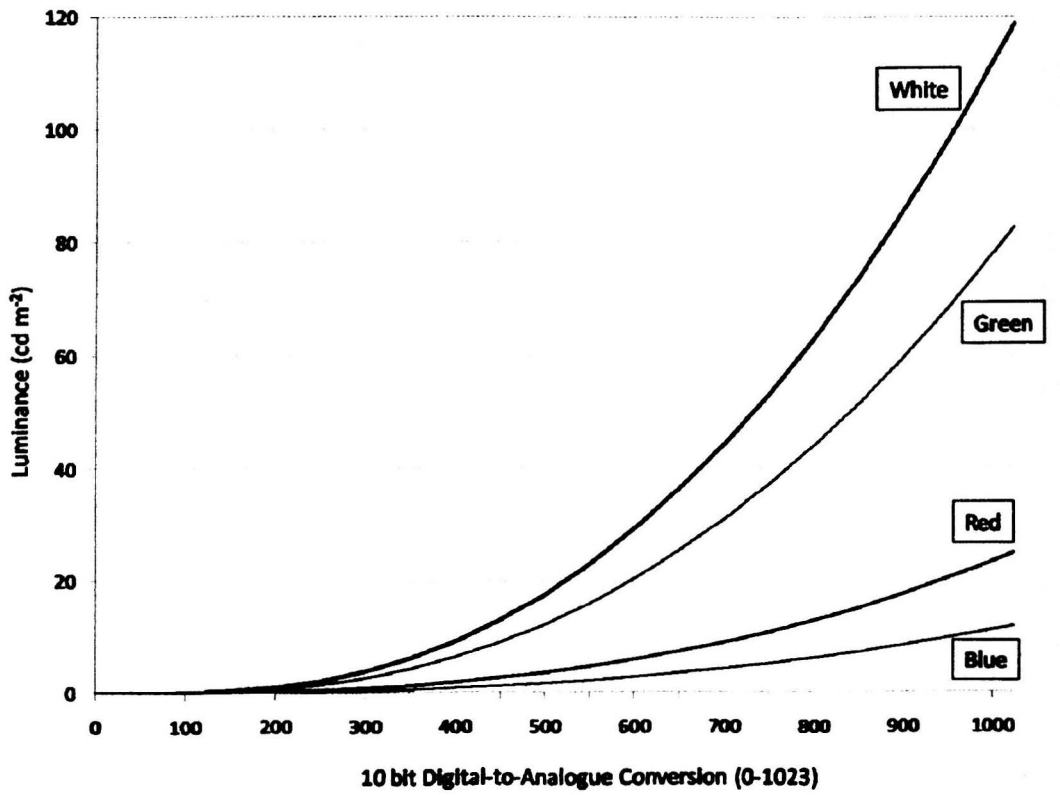


Figure 7.5 Graphic card / display gun luminance calibration

The display luminance calibration was conducted using a Lichtmesstechnik (LMT) luminance photometer Model 1009 and is shown in Figure 7.5. The Kodak Wratten gelatine ND 1.0 and 2.0 filter transmission characteristics are shown in Figure 7.6. The performance of the ND 1.0 filters used over right and left eyes was identical, allowing a reasonably flat 8% transmission over the range of wavelengths from 500 to 700 nm. The ND 2.0 filter over the left eye consistently allowed slightly less light to pass than that over the right eye. However, both allowed approximately 1% transmission from 500 to 700 nm.

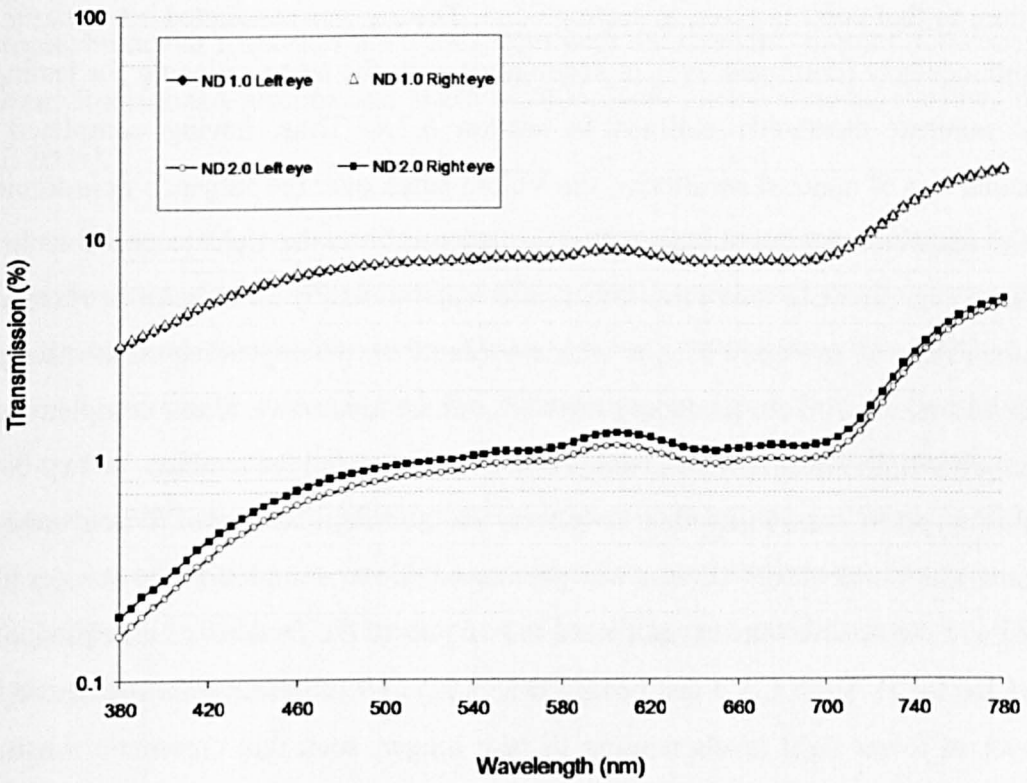


Figure 7.6 Neutral density filter transmission characteristics

The nominal background adapting field luminance and mean stimulus masking luminance for the CAD test was set at 24 cd m^{-2} in the software recipes of all tests. However, direct measurements of display screen luminance taken after a minimum warming up period of one hour indicated that central display luminance was consistently of the order of 22.3 cd m^{-2} during testing. The corresponding luminance when viewing through the ND 1.0 filter was 1.67 cd m^{-2} while that viewing through the ND 2.0 filter was 0.21 cd m^{-2}.

All subjects were familiarised with the vision test and 4-AFC procedure before starting the study. To undertake the test, the subject positioned a notch in the expiratory port of the P/Q mask against a chin rest, viewing the display screen along a matt black viewing tunnel from a distance of 70 cm. A small, white, circular, central fixation point was provided. Emphasis was placed on maintaining fixation at the centre of the test area to optimise sensitivity to moving chromatic stimuli passing rapidly through fixation.

7.3.5 Experimental procedure

The tests of chromatic sensitivity followed those of contrast sensitivity and the detailed procedure to that point is given in section 6.3.5. Testing was conducted binocularly and then monocularly (dominant eye) in accordance with the same rationale for testing of spatial contrast thresholds outlined in section 6.2.4. Thus, having completed the monocular test of contrast sensitivity, the Velcro patch over the subject's non-dominant eye was removed and the subject's chair rolled gently to the right to position for the CAD test. The binocular test commenced after approximately 30 minutes of respiratory exposure (Time 40 in Figure 6.2), to within a minute or two depending on how long the subject had taken with the preceding tests of contrast sensitivity. Upon completion, the subject relaxed, the results were saved and the subject rested for a minute or two before the Velcro patch was re-applied to cover the non-dominant eye. When ready to continue, the monocular CAD test was performed. Upon completion the subject again relaxed and the results were saved before moving on to the final set of unrelated vision tests (Chapter 8). Each CAD test occupied about 7 to 9 minutes, with monocular tests and tests at lower light levels tending to take longer, such that chromatic sensitivity testing occupied ~20 minutes in total and was complete by ~50 minutes of respiratory exposure (Time 60).

7.3.6 Physiological parameters

Physiological responses to these exposures are detailed in section 6.3.6.

7.3.7 Analysis

The output from a single CAD test provided the chromatic threshold along each of the eight colour directions expressed both as coordinates on the CIE 1931 – (x, y) chromaticity chart and in distance from background chromaticity. A fitted ellipse was also provided for each set of data, together with calculations of the semi-major and

semi-minor axis lengths and ellipse orientation (tilt angle). The data for the Study 1 experiments, comparing hypoxia with normoxia at low photopic (direct viewing), borderline upper mesopic (ND 1.0) and mid-mesopic (ND 2.0) light levels, comprised 144 sets of chromatic threshold data (12 subjects x 3 light levels x 2 viewing conditions x 2 breathing gases). The Study 2 data, comparing responses under hypoxia with breathing 100% O_2 at the two mesopic levels, comprised 96 data sets.

The distances of threshold colour sensitivity from background chromaticity, along each of the eight colour directions (Figure 7.3), were taken as the raw data for statistical analysis. An initial descriptive analysis was undertaken to confirm that the normoxic, photopic, binocular responses were consistent with the standard 'normal' CAD observer (Barbur, Rodriguez-Carmona and Harlow, 2006; Rodriguez-Carmona, Harlow, Walker *et al*, 2005).

The data for each study were interrogated using repeated measures, balanced ANOVA to examine main effects and interactions of light level, number of viewing eyes, gender, breathing gas and colour axis (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$). A more stringent requirement was adopted for considering an interaction to be statistically significant to reduce the risk of Type I error for the very many interactions under consideration. Where statistically significant effects of breathing gas were identified the ANOVA was repeated to include consideration of respiratory exposure order, specifically to exclude an interaction with breathing gas condition ($\alpha = 0.05$). All analyses were conducted using Minitab 14 software.

7.4 Results

7.4.1 Study 1

The summary data set for Study 1 is reproduced in Appendix 5. First, the group mean binocular, normoxic results when viewing the display directly were compared to the results of the City University 'standard CAD observer', estimated from testing 240 normal trichromats. In Figure 7.7, the calculated 95% response range for the standard CAD observer (N = 240) is bounded by the solid and dotted grey ellipses and the median ellipse for this data is shown with a solid black line. The responses of this subject sample are slightly positively skewed along both R-G and Y-B axes. The distance on the CIE 1931 – (x, y) chromaticity chart from background chromaticity

(0.305, 0.323) to the median red and green thresholds is 0.00436 and to the median Y-B thresholds is 0.01115. These distances constitute one CAD unit along the orthogonal R-G and Y-B axes respectively.

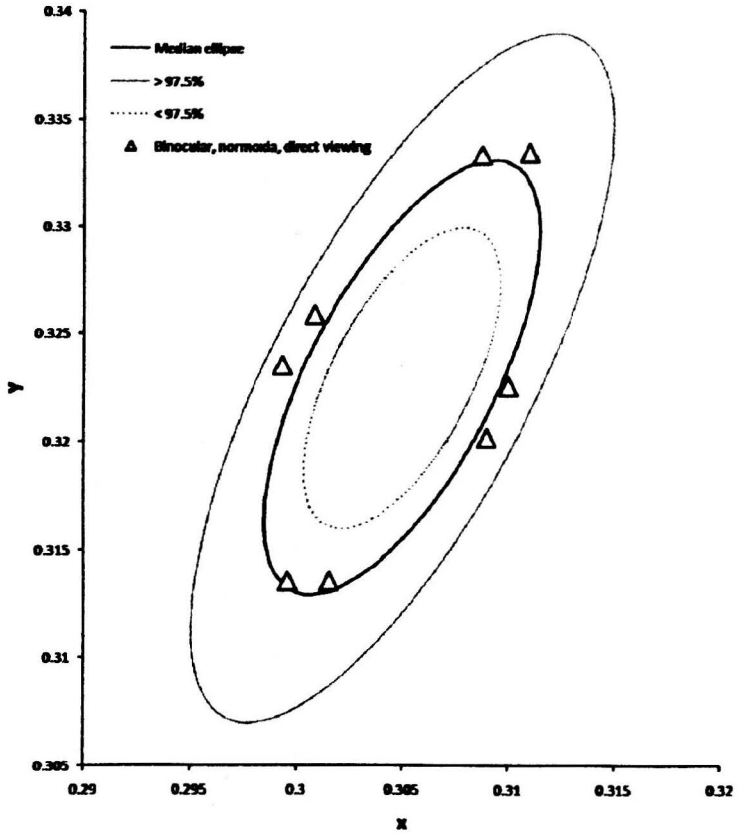


Figure 7.7 Group mean responses (Δ) relative to the 'standard normal CAD observer'

From the current experiment, the 12 subjects' group mean (x, y) responses for each colour test direction, viewing directly with both eyes under normoxic conditions, are also shown in Figure 7.7. They are close to the expected median values and well within the respective 95% ranges. The raw data for all four R-G and all four Y-B colour test directions were grouped and averaged giving a mean R-G threshold of 1.19 ± (SD) 0.28 CAD units and a mean Y-B threshold of 0.98 ± 0.23 CAD units. Thus, although the net R-G thresholds tended to be slightly greater than expected, it was concluded initially that the subject pool was representative of the standard normal CAD observer and therefore of normal trichromatic subjects.

The detailed results of the initial balanced ANOVA are reproduced in Appendix 5. Unsurprisingly, given the obvious loss of colour sense in dim light and the elliptical nature of the data, both light level and colour axis were highly statistically significant determinants of chromatic sensitivity measured as distance from the D65 reference

point ($p \leq 0.001$). The number of viewing eyes was also highly statistically significant ($p < 0.001$). Unexpectedly, a main effect of gender ($p = 0.011$) achieved statistical significance. A main effect of breathing gas was also evident ($p = 0.024$). Statistically significant interactions were identified between light level and number of viewing eyes ($p < 0.001$), light level and colour axis ($p < 0.001$), and, importantly, between light level and breathing gas condition ($p = 0.008$). Interactions that almost achieved statistical significance and warrant further consideration were between light level and gender ($p = 0.011$), number of eyes and colour axis ($p = 0.019$), and gender and colour axis ($p = 0.026$).

The results for all 12 subjects when viewing binocularly under normoxic conditions are shown for all three light levels in the scatterplot at Figure 7.8. It is apparent that each drop in light level is associated with a substantial reduction in both R-G and Y-B sensitivity, while responses between individuals become much more variable as light level decreases. These effects appear more pronounced for Y-B thresholds than for R-G thresholds. The variability in response at each light level is such that the extremes of each range overlap. Main effects of light level and colour axis are self-evident.

Chromatic sensitivity appears to deteriorate rapidly as the available light falls from upper to mid-mesopic levels, and particularly for Y-B stimuli. Furthermore, sensitivity along the Y-B axis appears to be compromised more for yellow stimuli than for blue at the lowest light level tested. This is more readily apparent when considering group mean sensitivities for each colour direction, shown in Figure 7.9. The graph shows the interaction between light level and colour axis, with decreasing light promoting the normal loss of tritan sensitivity. The data also exhibit a clearly asymmetric loss of Y-B sensitivity, at the expense of yellow, supporting observations that have been made previously in this regard (Walkey, Barbur, Harlow *et al*, 2001; Barbur, Rodriguez-Carmona and Harlow, 2006).

Monocular and binocular thresholds are compared for the data at 22.3 cd m^{-2} in Figure 7.10, at 1.67 cd m^{-2} in Figure 7.11, and at 0.21 cd m^{-2} in Figure 7.12. The main effect of number of viewing eyes is a consistent reduction in chromatic sensitivity when viewing monocularly. The effect is unambiguous in males and females at all light levels and under both respiratory conditions.

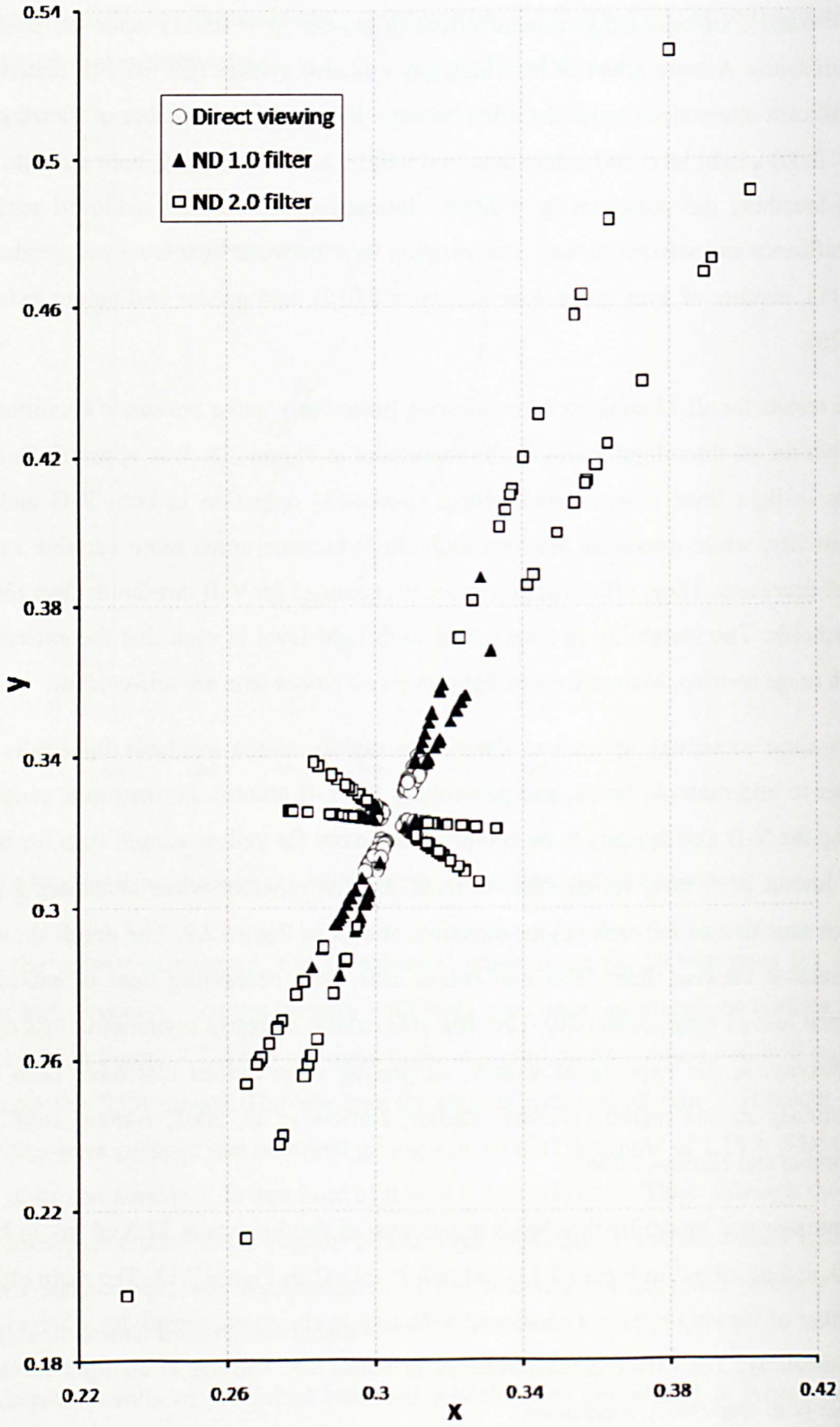


Figure 7.8 Chromaticity diagram scatterplot - normoxic binocular data at each light level (N = 12)

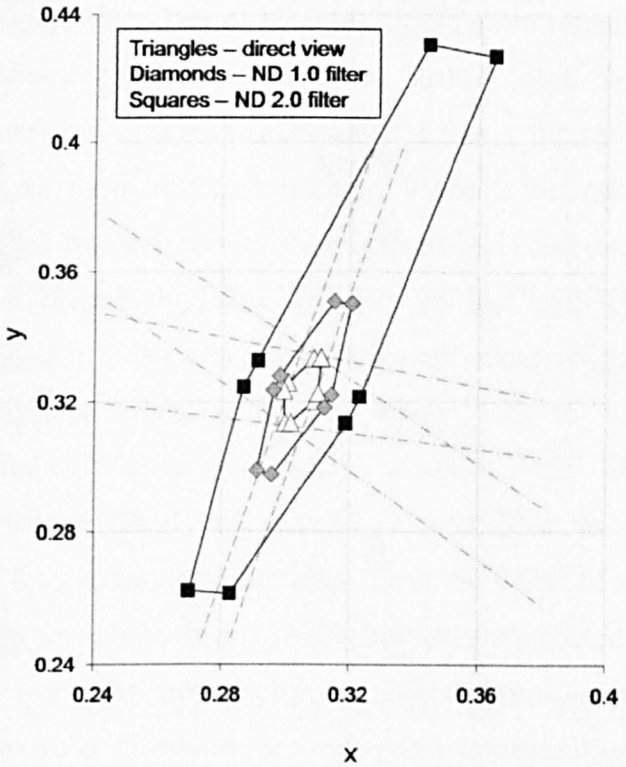


Figure 7.9 Group mean chromatic sensitivities for each viewing condition (N = 12)

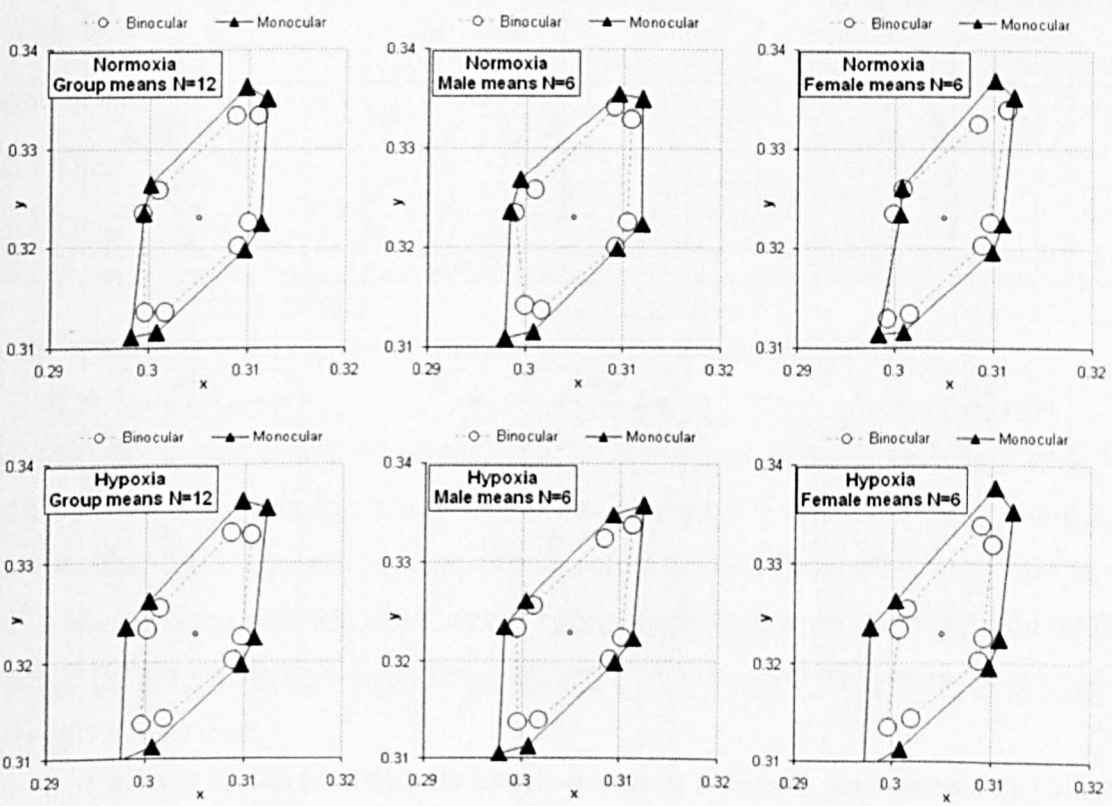


Figure 7.10 Comparison of binocular and monocular thresholds viewing directly (22.3 cd m^{-2})

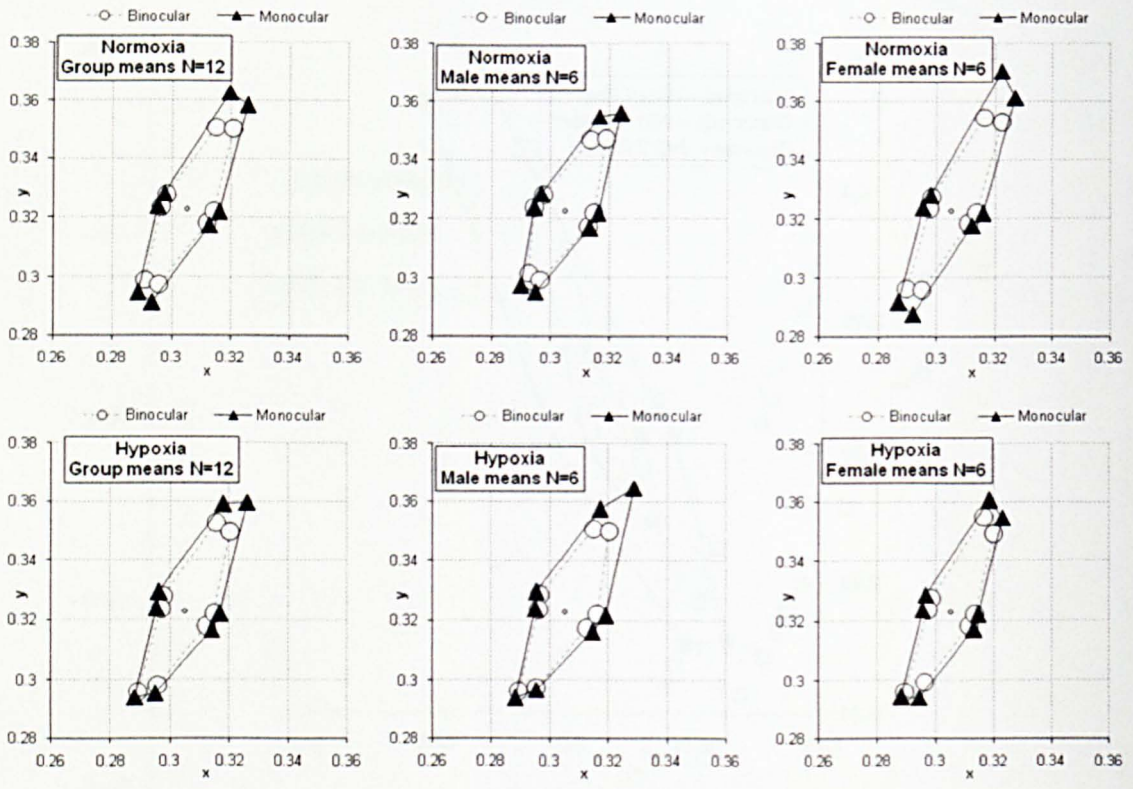


Figure 7.11 Binocular and monocular thresholds viewing via the ND 1.0 filter (1.67 cd m^{-2})

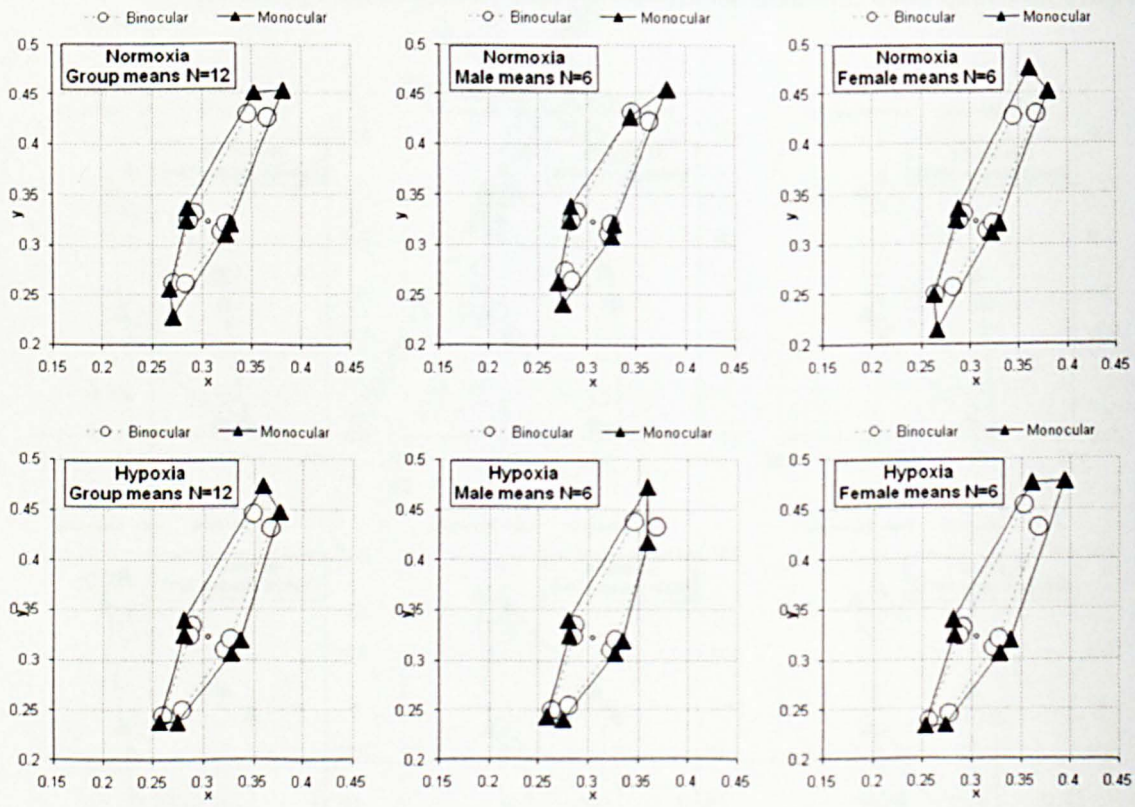


Figure 7.12 Binocular and monocular thresholds viewing via the ND 2.0 filter (0.21 cd m^{-2})

The highly significant interaction between light level and number of viewing eyes reflects preservation of an effect of binocular summation regardless of light level. The nature of this interaction has been considered further. Mean thresholds (N = 12) were calculated for each colour axis at each light level and for each respiratory condition when viewing binocularly and monocularly. From these, mean R-G and Y-B axis lengths were derived for each combination of light level and respiratory condition. The differences in absolute lengths between monocular and binocular colour axes and the monocular to binocular mean axis length ratios are compared in Table 7-2. The ratios remain reasonably consistent, within the range 1.13 to 1.42, with no obvious relationship to light level or respiratory condition. The absolute distance from background chromaticity clearly increases to a greater extent with monocular viewing at lower light levels than it does at higher ones. Thus, the effect of viewing monocularly to compromise colour sensitivity is progressive with reducing luminance, as expressed by absolute distance from background chromaticity, while monocular thresholds may be predicted from binocular thresholds according to a consistent ratio that averages 1.25. This is somewhat less than might be predicted by neural summation and is only slightly greater than would be expected with probability summation.

		R-G thresholds		Y-B thresholds	
Light level	Respiratory condition	Mean Difference (Mon–Bin)	Mean Ratio (Mon/Bin)	Mean Difference (Mon–Bin)	Mean Ratio (Mon/Bin)
Direct viewing	Normoxia	0.00075	1.15	0.00249	1.23
	Hypoxia	0.00141	1.30	0.00348	1.33
ND 1.0 filter	Normoxia	0.00113	1.13	0.00858	1.29
	Hypoxia	0.00198	1.22	0.00589	1.19
ND 2.0 filter	Normoxia	0.00506	1.29	0.02424	1.28
	Hypoxia	0.00839	1.42	0.01739	1.16
	Mean ratios		1.25		1.25

Table 7-2 Effect of monocular viewing on mean R-G and Y-B thresholds (N=12)

For each colour axis tested, the mean (± SE) thresholds are shown at each light level for both viewing and both respiratory conditions in Figure 7.13. The graphs indicate a consistent effect of hypoxia to compromise colour sensitivity at the lowest light level and a tendency towards this effect at the intermediate light level. Notwithstanding the scale of the graphs, there is no apparent difference between the respiratory conditions at the highest light level.

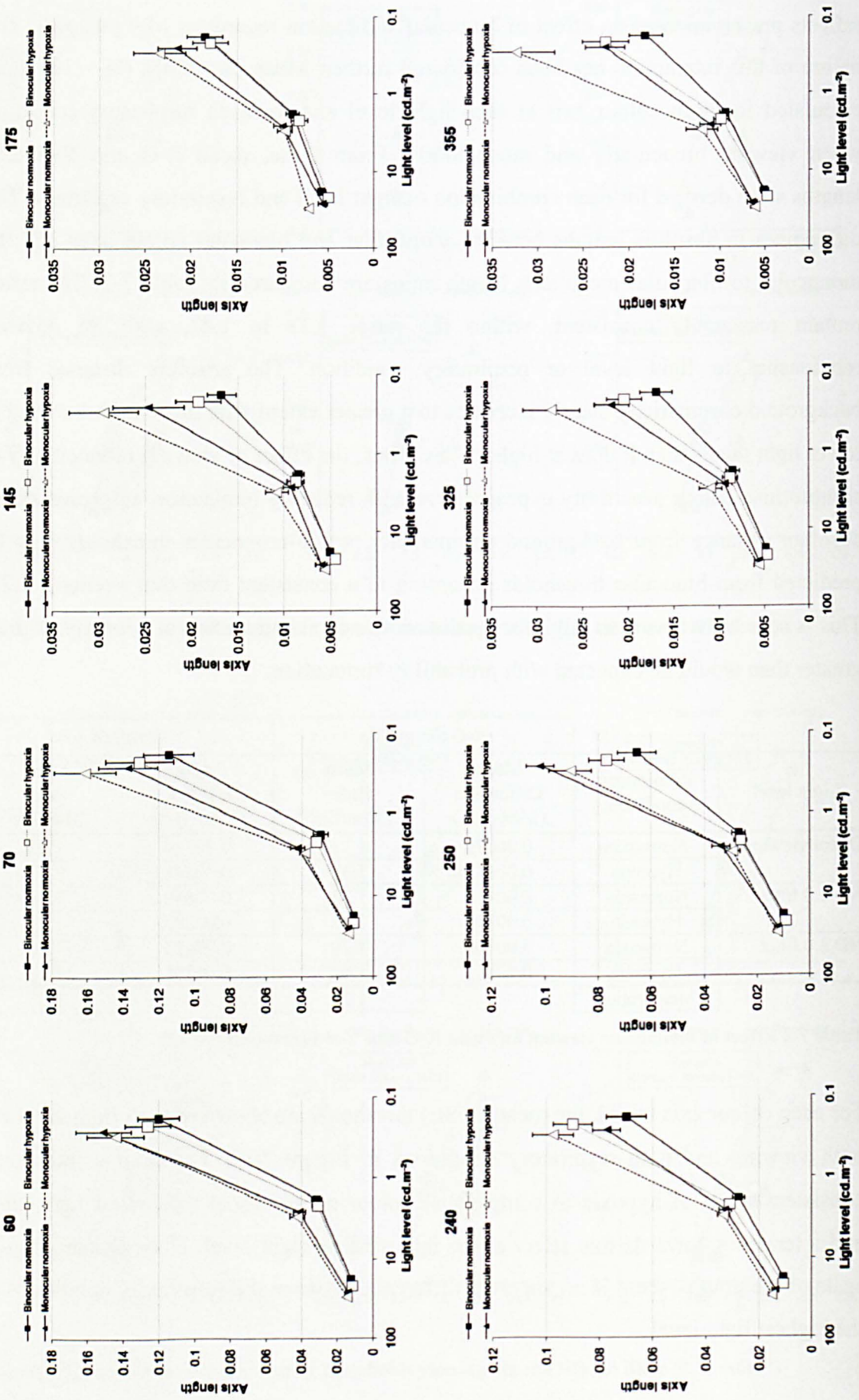


Figure 7.13 Group mean (± SE) chromatic thresholds for each colour axis in Study 1

To assist interpretation of the effect of respiratory condition, the data were expressed as multiples of the standard CAD observer's binocular, photopic thresholds. Mean thresholds were estimated from the four Y-B and four R-G colour axes, approximating the orthogonal semimajor (Y-B) and semiminor (R-G) colour ellipse axes for each test. Group means were derived and divided by the median thresholds of the 'standard CAD observer'. Thus, sensitivity matching that of the standard observer would equal unity. The results, shown in Figure 7.14, indicate progressive loss of colour sensitivity with decreasing luminance, consistently enhanced colour sensitivity when viewing binocularly, and an effect of hypoxia to impair sensitivity that is absent under direct viewing (DV), is well established at mid-mesopic luminance (ND 2.0) and is suggested at upper mesopic luminance (ND 1.0). Thus, the interaction between breathing gas and light level would appear to be a progressive effect of hypoxia as light level falls. The influences on chromatic sensitivity of decreasing light, monocular viewing and hypoxia appear to be additive.

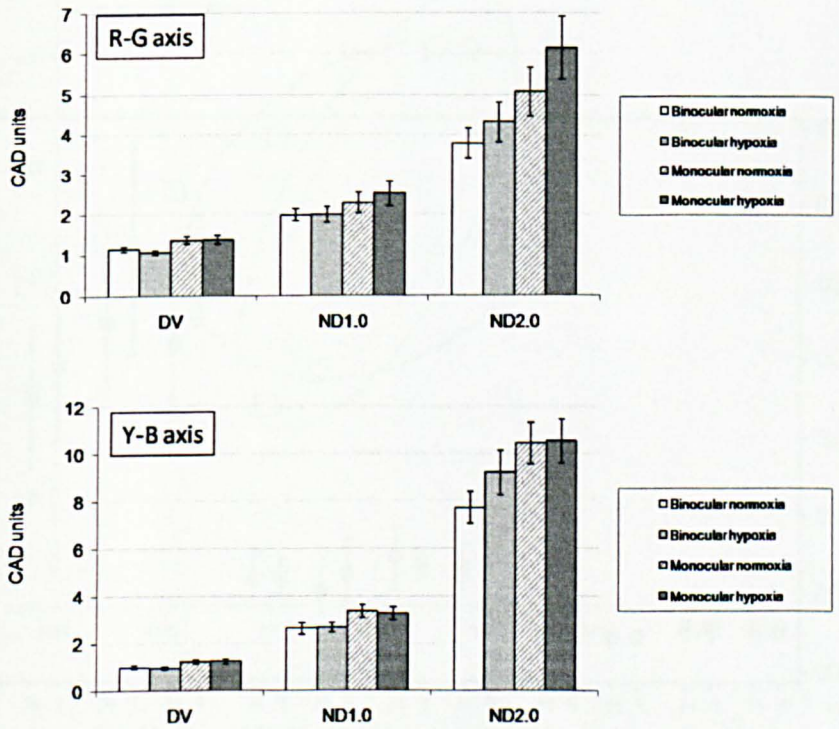


Figure 7.14 Semimajor (Y-B) and semiminor (R-G) ellipse axes in Study 1 (N = 10)

The results in Figure 7.14 exclude the data of two subjects found to have mild colour deficiency when investigating the origin of the statistically significant main effect of gender.

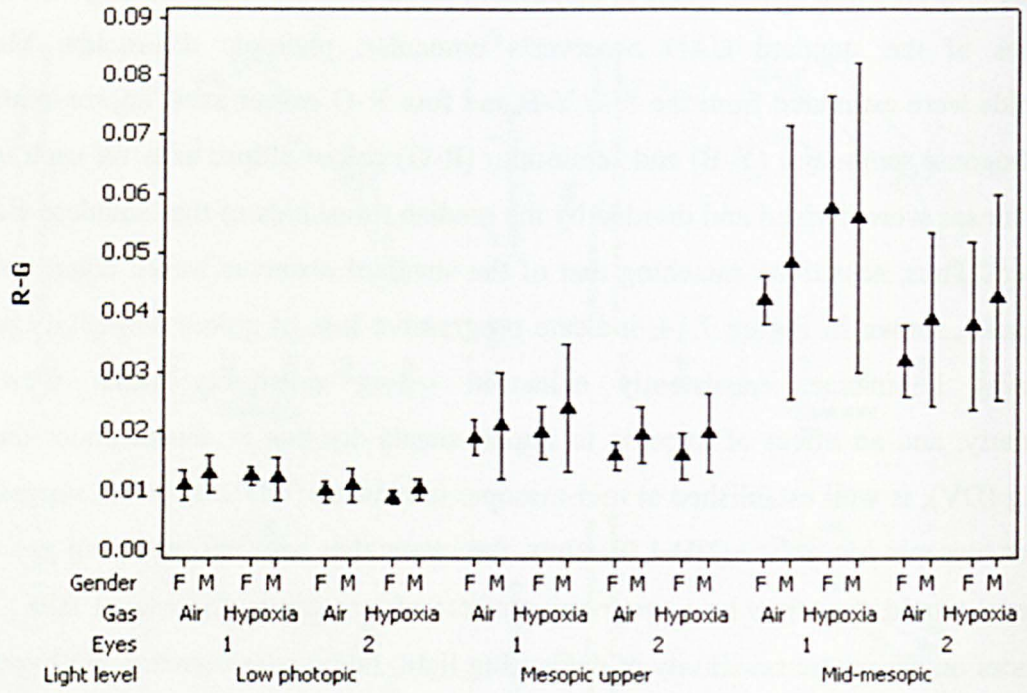


Figure 7.15 Tendency of mean R-G axis length (CIE 1931) to be greater in males (95% CI)

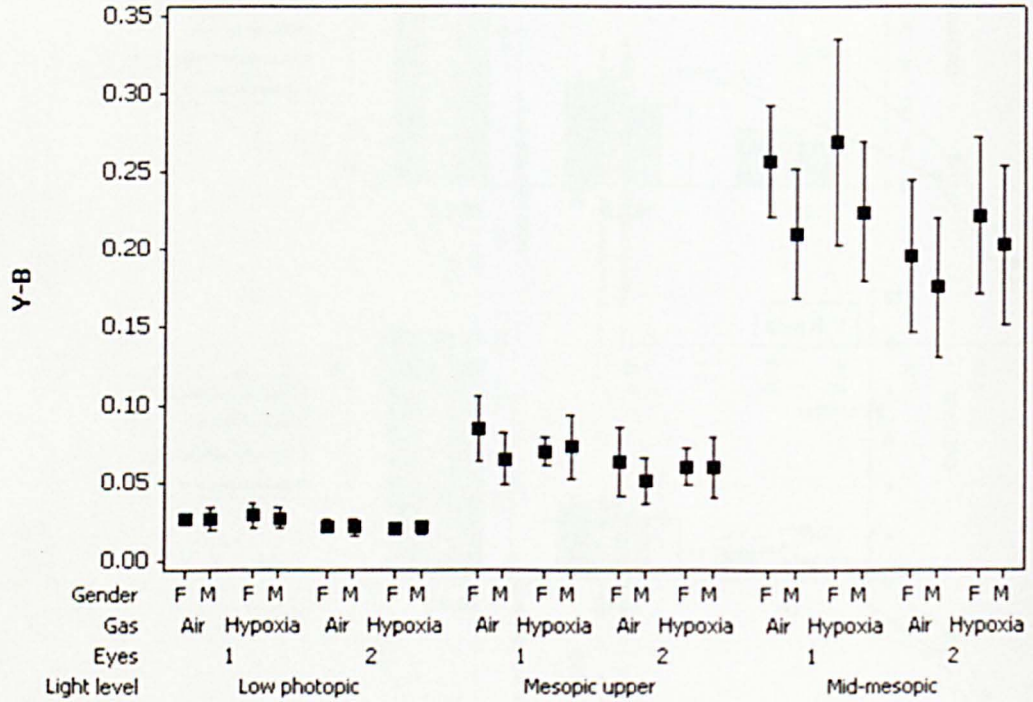


Figure 7.16 Tendency of mean Y-B axis length (CIE 1931) to be greater in females (95% CI)

Two trends were apparent when comparing group male and female thresholds for R-G and Y-B axes at each luminance, with the data disaggregated by gender, number of viewing eyes and breathing gas; R-G axes were consistently longer in males while Y-B axes were consistently longer in females, shown respectively in Figure 7.15 and Figure 7.16.

The data were examined in detail to identify idiosyncratic responses of individual subjects. One male was thereby identified as having consistently longer R-G axes and consistently shorter Y-B axes than the other subjects, while one female was found to have substantially longer Y-B axes than the other subjects, particularly at lower light levels. Thus, these two subjects introduce consistent orthogonal sources of bias that might explain the apparent effect of gender.

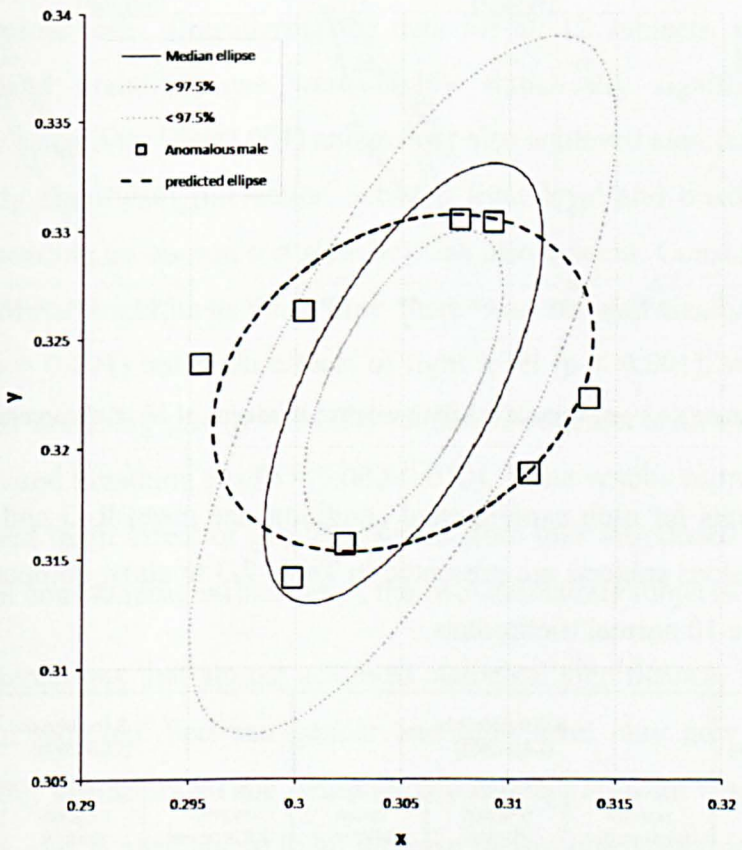


Figure 7.17 Anomalous male subject's R-G data (□) and predicted colour ellipse

The male subject's normoxic, photopic, binocular data were considered further and are represented in Figure 7.17, indicating anomalous R-G sensitivity with thresholds well beyond the 95% CI of the standard normal CAD observer. His mean R-G threshold of 1.79 CAD units is outside the range for normal trichromacy when using the CAD test and is consistent with the responses of subjects with minimal deuteranomaly (Barbur,

Rodriguez-Carmona and Harlow, 2006; Rodriguez-Carmona, Harlow, Walker *et al*, 2005).

The Y-B data of the female with an apparent tritan deficiency were amongst the worst in the group under all conditions but exhibited unambiguously the most severe Y-B loss at the lowest luminance tested, particularly to blue stimuli. She was the only female using hormonal contraception, known to influence colour vision (Menu, Ivan, Daumann *et al*, 2001). The binocular, normoxic (x, y) data of the deuteranomalous male (grey triangles) and the tritan female (white squares) are compared, at each light level, with the mean responses of the remaining 10 normal trichromats (black diamonds) in Figure 7.18, illustrating how their combined influences would be likely to introduce a main effect of gender through consistent and mutually orthogonal bias.

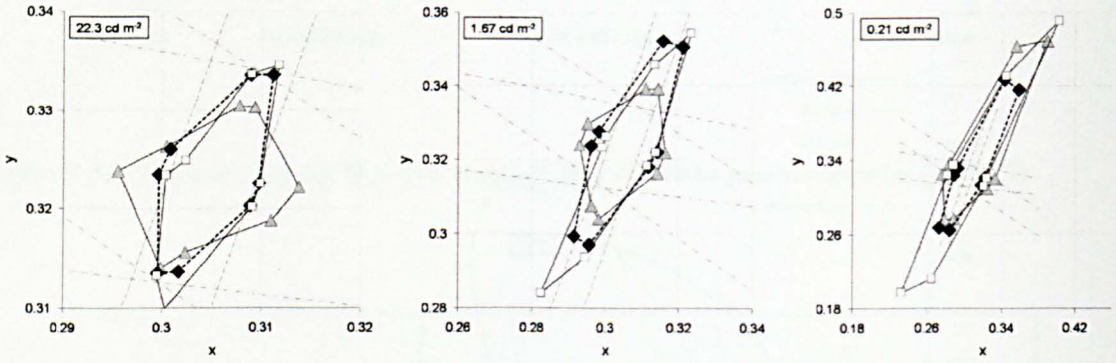


Figure 7.18 Anomalous male (▲) and female (□) data relative to means of 10 trichromats (♦)

Expressed in CAD units for each experimental condition, the mean R-G and Y-B axis data of the two anomalous subjects are presented in Table 7-3 to allow comparison with the mean results of the 10 normal trichromats.

Light level (cd m^{-2})	Number of viewing eyes	Breathing gas condition	R-G thresholds (CAD units)			Y-B thresholds (CAD units)		
			N = 10 normal trichromats Mean (SD)	Male minimal deutan	Female tritan deficiency	N = 10 normal trichromats Mean (SD)	Male minimal deutan	Female tritan deficiency
Direct viewing (22.3)	2	Air	1.14 (0.19)	1.79	1.01	0.99 (0.24)	0.77	1.12
		Hypoxia	1.06 (0.18)	1.42	0.95	0.95 (0.20)	0.87	1.02
	1	Air	1.36 (0.30)	1.78	0.90	1.24 (0.27)	0.79	1.30
		Hypoxia	1.37 (0.33)	1.80	1.39	1.25 (0.33)	0.98	1.66
ND 1.0 (1.67)	2	Air	1.99 (0.47)	2.67	1.64	2.64 (0.85)	1.64	3.06
		Hypoxia	2.00 (0.60)	2.94	1.56	2.68 (0.69)	2.09	3.37
	1	Air	2.29 (0.80)	2.42	2.03	3.37 (0.87)	2.19	4.40
		Hypoxia	2.52 (0.99)	2.82	1.95	3.29 (0.83)	2.20	3.49
ND 2.0 (0.21)	2	Air	3.78 (1.20)	6.03	4.68	7.74 (2.11)	9.42	12.82
		Hypoxia	4.32 (1.58)	7.67	4.42	9.21 (3.03)	9.99	11.92
	1	Air	5.07 (1.92)	6.83	4.99	10.45 (2.77)	10.17	10.98
		Hypoxia	6.15 (2.44)	8.64	8.26	10.55 (2.97)	11.41	15.77

Table 7-3 Detailed R-G and Y-B thresholds of 10 normal trichromats and two anomalous subjects

The results of both anomalous subjects were removed from the data set for Study 1. The mean (± SD) Y-B and R-G axis lengths of the remaining males and females compared using two-sample *t*-tests ($\alpha = 0.05$) and were not statistically significantly different between the remaining male and female subject groups ($p > 0.8$ on both occasions). The data of the remaining 10 subjects were examined again using balanced ANOVA and the effect of gender was no longer significant ($p = 0.073$), although this could be attributed to reduced subject numbers. Furthermore, the previous main effect of breathing gas now also failed to achieve statistical significance ($p = 0.064$).

To consider this further the data were analysed again using balanced multivariate ANOVA (MANOVA, $\alpha = 0.05$), on the basis that the colour axes behave as and may be treated as related dependent variables and that the effects of the independent variables may be expected to vary in absolute magnitude between the different R-G as compared to Y-B colour axes. Considering the data for all 12 subjects, light level, monocular viewing and breathing gas were highly statistically significant determinants of chromatic sensitivity ($p \leq 0.001$) and gender also achieved significance ($p = 0.016$). The statistically significant interaction between light level and breathing gas ($p = 0.001$-0.007 depending on chosen test statistic) was also evident. Considering the data for just the 10 normal trichromats, this time there was no statistically significant effect of gender ($p = 0.291$) but main effects of light level ($p < 0.001$), viewing condition ($p < 0.001$) and breathing gas ($p = 0.004$) remained, together with the interaction between light level and breathing gas ($p = 0.002$-0.010). These results support the contention that the apparent main effect of gender resulted from bias introduced by the consistent and orthogonal confounding influences of the two anomalous subjects.

Of the interactions that almost achieved statistical significance, those between gender and colour axis and between gender and light level may now be explained by the confounding influences of the two anomalous subjects. That between number of eyes and colour axis is attributable to an increase in absolute magnitude of each axis length when viewing monocularly as light level decreases.

7.4.2 Study 2

The summary data set for Study 2 and the results of initial balanced ANOVA are reproduced in Appendix 5. Light level, colour axis, number of viewing eyes and breathing gas were all highly statistically significant determinants of chromatic sensitivity ($p < 0.001$). A main effect of gender remained ($p = 0.001$), consistent with

continued orthogonal influences of the deuteranomalous male and the tritanomalous female. Significant interactions between light level and colour axis ($p < 0.001$) and between light level and number of viewing eyes ($p = 0.001$) were present as before. There was no significant interaction between light level and breathing gas ($p = 0.447$).

For each colour axis, mean (± SE) chromatic thresholds at each light level are shown for both viewing and both respiratory conditions in Figure 7.19. The graphs illustrate clearly all statistically significant main effects and interactions with the exception of the main effect of gender. The data are shown expressed in CAD units in Figure 7.20 while in Table 7-4 the data are segregated for comparison of the responses of the 10 normal trichromats with those of the minimally deuteranomalous male and the female with tritan deficiency.

Light level (cd m^{-2})	Number of viewing eyes	Respiratory condition	R-G thresholds (CAD units)			Y-B thresholds (CAD units)		
			N = 10 normal trichromats Mean (SD)	Male minimal deutan	Female tritan deficiency	N = 10 normal trichromats Mean (SD)	Male minimal deutan	Female tritan deficiency
ND 1.0 (1.67)	2	100% O_2	2.51 (2.13)	2.50	1.75	2.85 (0.90)	1.98	2.86
		Hypoxia	3.03 (1.85)	2.81	3.26	3.54 (1.39)	2.08	5.80
	1	100% O_2	2.17 (0.54)	2.45	1.62	3.30 (1.01)	2.10	3.62
		Hypoxia	3.93 (2.89)	2.97	5.37	4.60 (1.63)	3.02	6.21
ND 2.0 (0.21)	2	100% O_2	3.48 (1.04)	5.79	3.42	7.77 (2.03)	7.51	8.16
		Hypoxia	4.09 (1.30)	6.43	7.42	9.07 (2.81)	8.68	12.60
	1	100% O_2	4.96 (2.25)	5.71	4.34	10.06 (2.77)	8.09	10.20
		Hypoxia	5.58 (1.61)	8.10	10.33	11.35 (3.07)	14.36	10.45

Table 7-4 Chromatic thresholds of 10 normal trichromats and two anomalous subjects in Study 2

The results of Study 2 are broadly consistent with those of Study 1 with the obvious exception that chromatic sensitivity under hypoxic conditions when viewing through the ND 1.0 filter was generally poorer in Study 2, for both binocular and monocular testing, than under the matching hypoxic conditions in Study 1. These are compared in Figure 7.21. On the other hand, thresholds when breathing 100% O_2 in Study 2 were largely consistent with the corresponding thresholds breathing air in Study 1. Thus, hyperoxia did not enhance sensitivity by comparison to normoxia. On the other hand, there was no procedural explanation for the different group responses to the same level of hypoxia in the two studies.

As in Study 1, the data were subject to *post hoc* balanced MANOVA. Light level ($p < 0.001$), number of viewing eyes ($p = 0.011$) and breathing gas ($p = 0.002$) remained statistically significant determinants of Y-B and R-G chromatic sensitivity. No other main effects or interactions achieved significance and the main effect of gender was not statistically significant ($p = 0.1$).

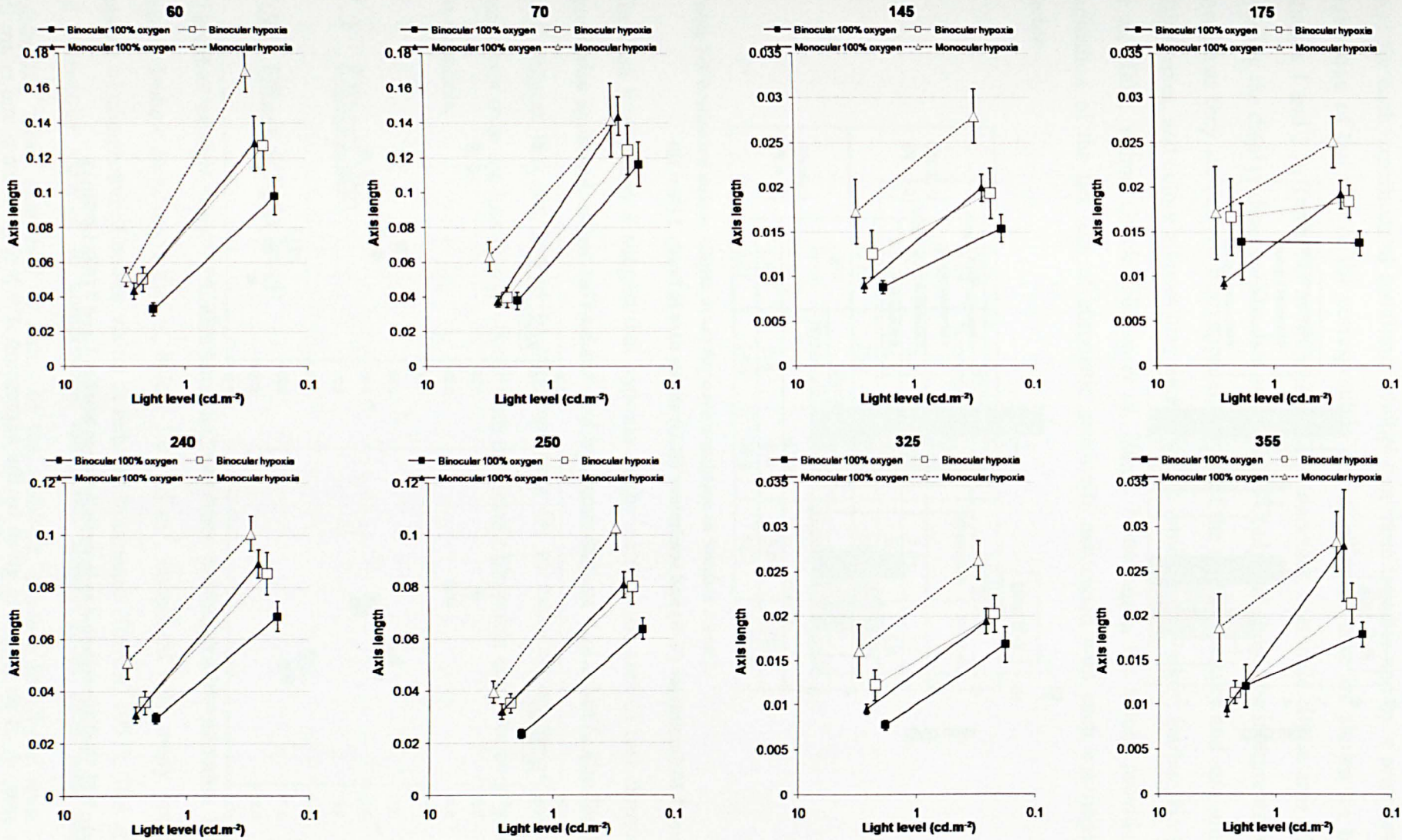


Figure 7.19 Group mean (± SE) chromatic thresholds for each colour axis in Study 2

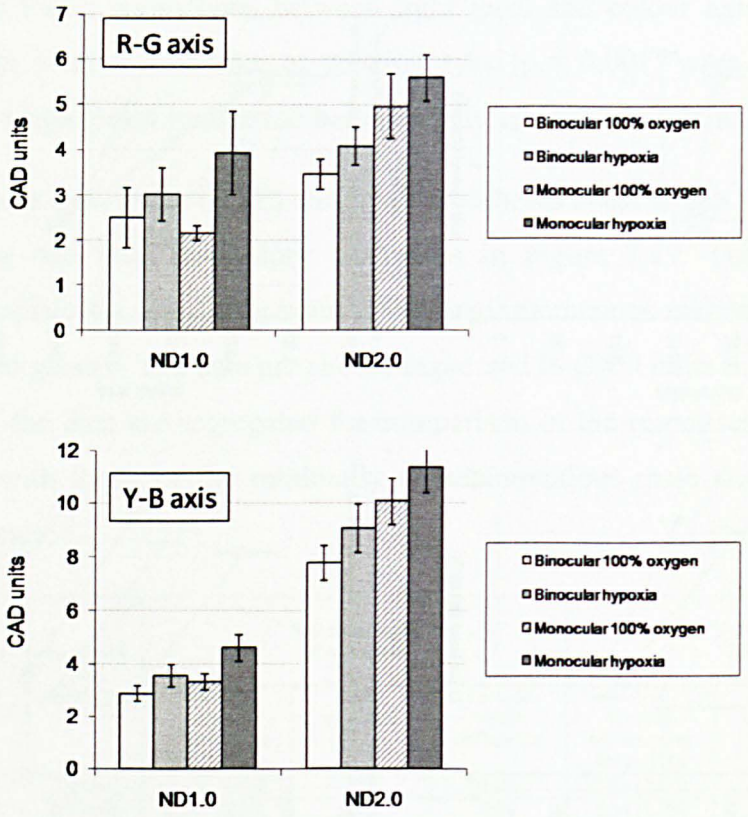


Figure 7.20 Semimajor (Y-B) and semiminor (R-G) ellipse axes in Study 2 (N = 10)

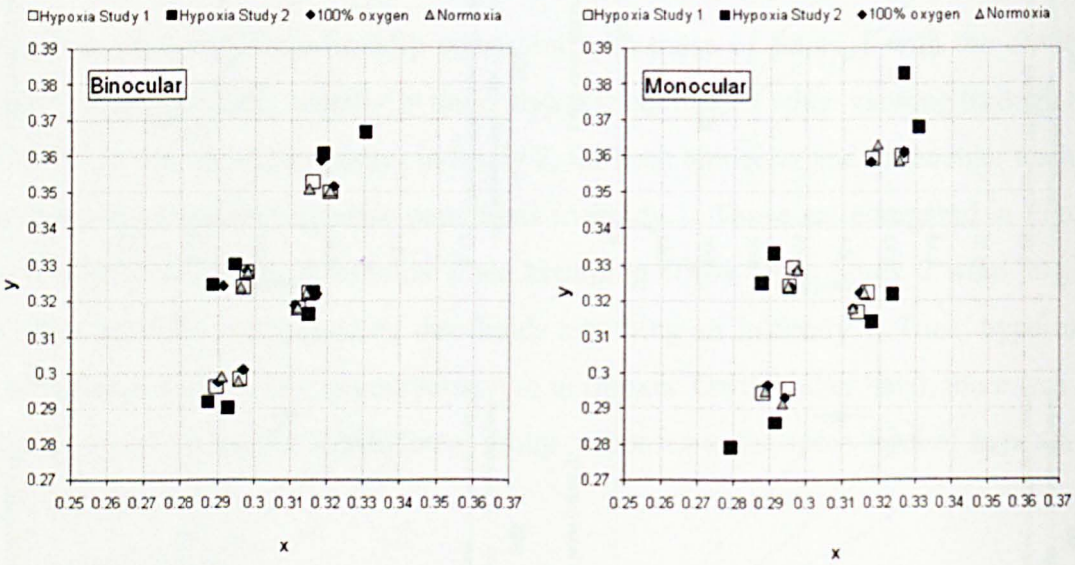


Figure 7.21 Variable response to hypoxia between Studies 1 and 2 (ND 1.0 filter)

The area, A, of an ellipse is given by the expression $A = \pi\, r_1\, r_2$ where r_1 and r_2 are the semi-major and semi-minor radii of the ellipse. For colour ellipses, the mean of each

four R-G and each four Y-B axis lengths approximate the semi-major and semi-minor axes for each experimental condition. Multiplying these together and by π provides an estimation of the area of the corresponding colour ellipse. These are shown for both Studies 1 and 2 in Table 7-5 as multiples of the binocular, normoxic ellipse area when viewing the display directly. The data are indicative only and should be interpreted with caution as they are derived from approximations of the true semi-major and semi-minor ellipse axes, and colour ellipses may tilt at reduced luminance (Walkey, Barbur, Harlow *et* al, 2001; Yebra, García, Nieves *et al*, 2001). Nonetheless, the values provide an indication of the net loss of chromatic sensitivity associated with each contributory factor.

	Light level (cd m^{-2})	Binocular		Monocular	
Study 1 (N = 12)		Normoxia	Hypoxia	Normoxia	Hypoxia
	22.3	1.0	0.9	1.4	1.5
	1.67	4.5	4.7	6.5	6.9
	0.21	28.7	37.4	46.7	61.8
Study 2 (N = 12)	Light level (cd m^{-2})	Binocular		Monocular	
		Hyperoxia	Hypoxia	Hyperoxia	Hypoxia
	1.67	5.8	9.4	5.9	15.6
	0.21	24.4	36.4	42.1	61.0

Table 7-5 Estimated colour ellipse areas for each condition in Studies 1 and 2

The data from Study 1 suggest that hypoxia might increase the area of lost chromatic sensitivity by about 5% at 1.67 cd m^{-2} and by about 30% at 0.21 cd m^{-2}. The Study 2 data suggest that the benefit of supplementary O_2 to maintain optimal chromatic sensitivity over the loss that can occur with this level of hypoxia could be even greater on occasion.

7.5 *Discussion*

7.5.1 Effect of light level

The effect of light level is fundamental and becomes profound with reduction below upper mesopic luminance, that is, below 1.67 cd m^{-2}, consistent with many previous reports of compromised colour vision at reduced luminance (Brown, 1951). The effect of monocular viewing to compromise chromatic sensitivity is consistent and is substantial when examined in terms of the resultant increase in ellipse area. The estimated ellipse areas at photopic luminance in Study 1 suggest that hypoxia has negligible effect on colour sensitivity at this light level. The effects of both monocular

viewing and hypoxia to compound the loss of chromatic sensitivity then appear to be progressive as light level falls.

Y-B thresholds appear to be compromised to a greater extent by falling light levels than do R-G thresholds and an asymmetry in Y-B thresholds is evident at the lowest light level tested, such that there is reduced sensitivity for S-cone decrements (corresponding to yellow thresholds) by comparison to the sensitivity for S-cone increments (blue thresholds). This is consistent with a previous report examining mesopic colour sensitivity using a version of the CAD test (Walkey, Barbur, Harlow *et al*, 2001). The ratios of Y-B to R-G axis lengths of the 10 normal trichromats are shown for each condition in Table 7-6. The results at photopic luminance are slightly less than unity because the group mean axis lengths for this subject sample are slightly longer than the standard normal CAD observer for the R-G axis and slightly shorter for the Y-B axis. The results show clearly that the Y-B axis elongates more than the R-G axis as light levels fall, that the ratio is reasonably consistent at each light level, and that the ratio is largely independent of the number of viewing eyes and respiratory status.

Study 1 (N = 10)	Light level (cd m^{-2})	Binocular		Monocular	
		Normoxia	Hypoxia	Normoxia	Hypoxia
	22.3	0.87	0.89	0.91	0.91
	1.67	1.33	1.34	1.47	1.31
	0.21	2.05	2.13	2.06	1.72
Study 2 (N = 10)	Light level (cd m^{-2})	Binocular		Monocular	
		Hyperoxia	Hypoxia	Hyperoxia	Hypoxia
	1.67	1.14	1.17	1.52	1.17
	0.21	2.23	2.22	2.03	2.03

Table 7-6 Group mean Y-B to R-G (CAD unit) axis length ratios for 10 normal trichromats

Since the Y-B to R-G ratios remain consistent at each light level, the effects of monocular viewing and hypoxia to compromise chromatic sensitivity along both Y-B and R-G axes indicate that both conditions compromise Y-B sensitivity to a greater extent, in terms of absolute distance from background chromaticity, than they do R-G sensitivity. This is to say that, for a given mesopic light level, both induce a relatively greater tritan deficiency than R-G deficiency, compounding the normal tendency to tritan loss as light level decreases. This effect of monocular viewing is the consequence of binocular summation, but a similar effect with hypoxia suggests a greater metabolic demand of the S-cone system that is further supported by the asymmetry for increments and decrements in S-cone signals at low light levels, shown in Figure 7.9. Thus, the results presented here support a possible role of hypoxia in the aetiology of disease-

induced tritan losses (Greenstein, Hood, Rich *et al*, 1989; Cho, Poulson, Ver Herve *et al*, 2000).

7.5.2 Effect of binocular summation

The effect of binocular summation to benefit chromatic sensitivity by comparison to monocular viewing may be gauged by examining the monocular to binocular ratios of mean R-G and Y-B axis lengths under each condition studied, shown in Table 7-7.

Study 1 (N = 10 normal trichromats)	Light level (cd m^{-2})	R-G		Y-B	
		Normoxia	Hypoxia	Normoxia	Hypoxia
	22.3	1.192	1.289	1.248	1.317
	1.67	1.149	1.259	1.278	1.227
	0.21	1.340	1.424	1.351	1.146
Study 2 (N = 10 normal trichromats)	Light level (cd m^{-2})	R-G		Y-B	
		Hyperoxia	Hypoxia	Hyperoxia	Hypoxia
	1.67	0.865	1.296	1.157	1.299
	0.21	1.426	1.366	1.295	1.251

Table 7-7 Binocular summation factors for R-G and Y-B axes in Studies 1 and 2

From these data, the mean R-G binocular summation factor is 1.26 SD ± 0.17 and the mean Y-B factor is similar at 1.26 ± 0.07. Because of the dynamic luminance masking employed by the CAD test, this effect of binocular summation relates specifically to detection of colour signals. This finding contradicts a recent report (Costa, Ventura, Perazzolo *et al*, 2006) but accords with a number of others (Verriest, Van Laetham, and Uvijls, 1982; Simmons and Kingdom, 1998; Jiménez, Medina, Jiménez del Barco *et al*, 2002; Forte, 2005). The values in Table 7-7 tend to fall well short of the $\sqrt{2}$ required to attribute the benefit of binocular viewing to neural summation and, instead, suggest that a form of probability summation is the more likely mechanism. The values do not exhibit any obvious dependence either on light level or on the prevailing respiratory conditions, further supporting the contention that the effects of light level, number of viewing eyes and hypoxia are summative.

7.5.3 Effect of gender

The minimally deuteranomalous male passed the colour vision screening tests for the study and was unaware that he had any form of colour deficiency. On questioning the medical examiner who conducted Ishihara screening, the subject hesitated over some of his responses and was given the opportunity to correct himself. Records from a previous (unpublished) study indicated that the same subject twice made two errors on Ishihara

testing some years earlier. During screening at that time he also made one mistake during testing with the Holmes-Wright Lantern, calling white for a green stimulus, and he had an error score of 24 on the FM100. In the current study his Rayleigh matches on the Nagel type I anomaloscope were Yellow 13.5, R-G 45; Yellow 13, R-G 46; and Yellow 13, R-G 48.5, giving means of Yellow 13.2 and R-G 46.5, comfortably within the range of group responses and consistent with normal trichromacy (Figure 7.1 and Figure 7.2). Unfortunately, his Nagel anomaloscope matching range data were not recorded and were just noted as 'normal'.

The photopic, binocular, normoxic R-G threshold of the minimally deuteranomalous male is 1.79 CAD units which places him just outside the acceptable boundary for normal trichromacy as assessed using the CAD test (Rodriguez-Carmona, Harlow, Walker *et al*, 2005; Barbur, Rodriguez-Carmona and Harlow, 2006). Such mild anomalous responses are characterised by the CAD test as deutan in nature, with even the mildest protanomalous losses exhibiting more substantial R-G deficiencies. At photopic and upper mesopic luminance the deuteranomalous male's R-G sensitivity is marginally worse than that of normal trichromats but his Y-B sensitivity is somewhat better. However, at the lowest light level his sensitivity to yellow deteriorates dramatically (Figure 7.18). Despite near-normal photopic and upper mesopic colour sensitivity, he manifests a specific loss of sensitivity to yellow at mid-mesopic luminance. Such responses call into question the suitability of conventional colour tests for determining the functional acceptability of colour vision at reduced luminance.

Equally, the tritanomalous female passed colour vision screening without difficulty but exhibits clear loss of Y-B sensitivity in comparison to her peers, most noticeably at reduced luminance and particularly to blue stimuli. Colour vision screening for this study did not include exclusion of tritan losses but may well not have detected the tritanomalous female as here loss of sensitivity only became substantial at reduced luminance. This subject was well-established on long-term oral hormonal contraception, which she used on a cyclical basis throughout these experiments. Long-term use of older hormonal contraceptives is known to promote tritanomalous defects in about a quarter of users and may exacerbate marginal underlying losses of chromatic sensitivity (Marré, Neubauer and Nemetz, 1974; Lakowski and Morton, 1978). Furthermore, changes in both Y-B and R-G sensitivity may occur in relation to stage of the ovarian cycle and use of hormonal contraception, albeit with considerable variability between subjects (Eisner, Burke and Toomey, 2004). In the current study, none of the other

females used hormonal contraception. The tritanomalous female was otherwise perfectly well, on no other medication, and no other obvious explanation exists for her loss of Y-B sensitivity. As with the deuteranomalous male, contemporary colour vision screening for military aircrew might not detect her tritan loss at reduced luminance and might fail to preserve occupational colour vision requirements under such conditions.

7.5.4 Effect of breathing gas

The effect of hypoxia to compromise chromatic sensitivity is unambiguous at mid-mesopic luminance and the data suggest a less dramatic effect at upper mesopic luminance. The effect is well established at a severity of hypoxia that is equivalent to breathing air at only 10,000 ft, and it is likely that milder levels of hypoxia will be associated with some lesser impairment of chromatic sensitivity, particularly at mid-mesopic luminance. However, chromatic sensitivity at photopic luminance is unaffected by hypoxia at 10,000 ft, contrary to past reports of threshold elevation (Brandl and Lachenmayr, 1994) and loss of colour discrimination (Karakucuk, Oner, Goktas *et al*, 2004).

Brandl and Lachenmayr (1994) used a hypobaric chamber to examine colour vision at GL (500 m above sea level) and at a purported simulated altitude of 10,000 ft. However, vision testing appears to have been conducted at 3500 m (11,480 ft), albeit with reference to preliminary testing at 2500 m (8200 ft) by way of familiarisation with the experimental procedures. Neither altitude can reasonably be said to represent 10,000 ft. The reported mean S_aO_2 of 83 ± 3% is also substantially lower than might reasonably be expected at 10,000 ft, while respiratory gas pressures are not reported. It is also not clear how many of the 48 subjects were tested at each altitude. Testing conducted using the Heidelberg Anomaloscope and Panel-D-15 tests were reported to be unaffected by hypoxia. A Humphrey Field Analyzer was used to test threshold sensitivity to white, green, red and blue stimuli presented at various distances from fixation along the four quadrant diagonals. The viewing conditions, visual adaptation state and response criteria for the test are not detailed, although it is concluded that hypoxia generated differences in light sensitivity in the photopic range, suggesting that this was primarily a test of differential brightness sensitivity to spot stimuli against a photopic background. In particular, it is unclear whether testing examined the threshold for stimulus detection or, for the chromatic ones, threshold for hue detection. Virtually identical linear plots correlating a reduction in log sensitivity (dB) against falling S_aO_2 are presented for the

four different stimuli. The rationale for the selection of data points in each graph is not given, but these vary in number between 9 and 13 for the four plots. Data points are scattered reasonably uniformly across a range of S_aO_2 varying between 80% and ~95% in each plot, begging questions as to how such a range could be produced by just one or two well-controlled hypobaric exposures and which values are believed to correspond to the test altitude(s). In sum, this study appears to assess mixed luminance/chromatic contrast thresholds to photopic stimuli at altitudes of up to nearly 11,500 ft and neither the visual nor respiratory test conditions have been well controlled. Conclusions in relation to chromatic sensitivity at altitude should probably not be based on this study.

Karakucuk, Oner, Goktas *et al* (2004) compared the performance of 16 young subjects (aged 14 to 17 y) on the FM100 test at altitudes of 1060 m (3480 ft) and 3000 m (9840 ft). However, tests at the lower altitude were conducted in an eye clinic one week before the tests at 3000 m were conducted in the open air, on a mountain, following a slow, 3 h ascent by bus and on foot. Furthermore, it is highly unlikely that testing was conducted under identical visual and respiratory conditions (particularly duration of exposure) for all subjects at 3000 m, and those tests are unlikely to have been performed under the same visual conditions as testing in the clinic one week before. Furthermore, the pre-test exercise may have confounded testing at altitude. The total errors made and errors made in each sector of the FM100 were summed across all 16 subjects and compared between altitudes using the McNemar Test. However, it is not clear how the paired data were categorised for this non-parametric, 2x2 table equivalent of the χ^2 test for unpaired data, while examining the relevance within subjects. In other words, it is unclear whether an increase in total number of errors is contributed by a majority or a minority of subjects and how relevant this increased error rate may be to the overall sample group or a wider population. Nonetheless, the authors report a statistically significant increase in the total number of errors and the number of errors in sectors 1 and 3, indicating impaired Y-B colour discrimination at the higher altitude under photopic viewing conditions.

Given the preservation of photopic Y-B chromatic sensitivity under the carefully controlled conditions in the current study, the robustness of the findings on the mountainside seems doubtful. For example, the number of errors in the sector 4 caps of the FM100 increased from 16 at 1060 m to 36 at 3000 m, that is, by 125%, yet was not statistically significant. On the other hand, sector 1 error rate increased from 31 to 54 (74%) and sector 3 error rate increased from 40 to 61 (52%), both achieving statistical significance at ($p = 0.007$ and $p = 0.013$ respectively). Furthermore, although the errors

made in sectors 2 and 4 were not considered significant, nonetheless they were included in the analysis of total error. A paired Wilcoxon test may have been more appropriate. Some of the same authors were involved in a very similar recent study which assessed FM100 scores of 16 subjects at altitudes of 1640 m (5380 ft) and 4200 m (13,780 ft). This time no statistically significant differences in error rates were detected when using the Wilcoxon signed rank test (Mazicioğlu, Karaküçük, Öner *et al*, 2006). Accordingly, the results of the earlier study should be treated with caution.

The data obtained breathing 100% O_2 in Study 2 are consistent with those breathing air in Study 1, suggesting that hyperoxia does not enhance chromatic sensitivity relative to normoxic performance under the conditions tested. Performance under hypoxia in Study 2 was clearly worse than that under identical hypoxia in Study 1, most notably when viewing at upper mesopic luminance through the ND 1.0 filter. Responses to hypoxia are notoriously variable both within and between subjects. It is quite possible that familiarity with the lengthy and challenging study procedure and an associated reduction in levels of psychological arousal may have increased susceptibility to the wider cognitive or attentional effects of hypoxia during later experiments. In this regard, there is always a balance to be struck between keeping hypoxic exposures sufficiently brief to avoid undue confounding effects of fatigue and changing glycaemic status, for example, and being able to conduct sufficient testing, following a period of respiratory adaptation, to avoid extended series of experiments.

7.5.5 Lessons for subsequent studies

The colour vision screening of subjects for future studies of chromatic sensitivity at reduced luminance under respiratory challenge should be conducted carefully to identify and, if appropriate, exclude mild or covert deficiencies. Consideration should be given to the likelihood of acquired (usually tritan) defects and subjects excluded, if necessary. This might require the exclusion of female subjects using hormonal contraception, for example. Furthermore, there is increasing evidence that males and females perceive colour differently, so it may be prudent to restrict future studies to either males or females, as appropriate (Bimler, Kirkland and Jameson, 2004).

The version of the CAD test employed in this study examined only 8 colour axes from background chromaticity. Future studies should consider employing a larger gamut of colour directions and investigating intermediate mesopic levels between 1.67 and 0.21 cd m^{-2}, as loss of chromatic sensitivity between these light levels is substantial.

Furthermore, at the lowest luminance tested, subjects occasionally approached the limits of the test. In particular, the deuteranomalous male repeatedly hit the limits of the test in the yellow direction while the tritan female hit the limits of the test in the blue direction.

In retrospect, the design of the experiments reported in this and the neighbouring chapters is more demanding for the subjects than might be desired. Although the respiratory exposure orders are balanced, it is possible that factors such as fatigue, inattention and changing glycaemic status might interact with exposure order to confound the results.

7.5.6 Summary and future work

The effect of hypoxia to compromise both Y-B and R-G chromatic sensitivity is progressive with reducing mesopic luminance and supports the hypothesis that progressive rod-driven retinal hypoxia makes foveal chromatic sensitivity vulnerable to exogenous hypoxia. On the other hand, supplementary O_2 maintains optimal chromatic sensitivity in the mesopic range. Contrary to previous reports, photopic colour vision does not appear to be affected by hypoxia at an equivalent altitude of 10,000 ft.

The results indicate a consistent effect of binocular summation to enhance chromatic sensitivity. This is independent of the luminance channel and appears to result from probability summation. Hypoxia compounds the loss of threshold chromatic sensitivity occasioned by viewing monocularly in a manner that appears additive and independent of binocular summation.

The CAD test is highly sensitive to colour vision deficiencies that may not be detected using conventional screening tests and which may manifest as significant losses of chromatic sensitivity in the mesopic range. The usual screening tests may be inadequate to confirm satisfactory colour vision for occupations requiring optimal chromatic sensitivity in the mesopic range.

The experiments reported here assessed foveal thresholds for Y-B and R-G chromatic sensitivity in a relatively young sample group. It is likely that colour vision away from the fovea may be more sensitive to hypoxic degradation as cone density decreases and rod density increases, as these factors are likely to exaggerate cone hypoxia with eccentricity. Furthermore, the chromatic thresholds of older subjects might exhibit greater losses of sensitivity under hypoxia (Knoblauch, Vital-Durand and Barbur, 2000).

It would be of interest to explore thresholds for colour discrimination against chromatic backgrounds under hypoxia to ascertain whether or not simultaneous colour contrast is likely to be degraded at altitude, and this has particular relevance to the use by aircrew of night vision devices that make use of green phosphors. Given that the effect of breathing 14.1% O_2 exhibits an unambiguous impairment of chromatic sensitivity, there is a requirement to consider the altitude at which such impairment might commence. Thus, further work is indicated to assess chromatic sensitivity under milder hypoxic conditions. The manner by which the chromatic sensitivity of normal trichromats is compromised at low light levels requires further study, as some individuals appear to fare far worse than others.

8 Visual Processing Speed

8.1 Abstract

Purpose. Hypoxia and decreasing light reduce the sensitivity of the peripheral visual field, while the extent of the functional visual field is related to peripheral stimulus contrast, task complexity and the presence of foveal stimuli and distractors. Various studies have suggested that hypoxia delays the speed at which visual information is processed and this may have contributed to past findings in relation to the functional extent of the visual field. This study aimed to confirm or refute an effect of hypoxia on the speed of visual information processing in relation to task complexity and decreasing stimulus contrast.

Methods. The UFOV® test was used to measure the stimulus duration necessary to achieve three increasingly complex visual tasks. UFOV® testing followed the tests of chromatic sensitivity reported in the previous chapter and therefore followed an identical method of visual and respiratory adaptation. The test display screen was viewed either directly or through ND 1.0 or 2.0 filters. Mild hypoxia was imposed breathing 14.1% O_2 and investigated relative to control exposures breathing air (normoxia) at each light level. Subsequently, hyperoxia, breathing 100% O_2, was assessed relative to hypoxia under the two mesopic conditions. The experimental design and the highly skewed nature of the UFOV® data precluded statistical analysis of the complete data sets using balanced ANOVA and a variety of parametric and non-parametric tests were employed.

Results. Hypoxia tends to impair central target discrimination to briefly presented, low contrast stimuli and may be offset by increasing presentation time and/or stimulus contrast. Hypoxia also tends to impair visual efficiency when a peripheral localisation task is added to the central discrimination task, particularly when viewing monocularly and at low stimulus contrast, and is clearly worse than when breathing 100% O_2. The tendency to impaired performance under hypoxia was promoted by increasing task complexity, when the peripheral localisation task was hidden among numerous distractors. Hyperoxia again provided a clear benefit in comparison to hypoxia.

Conclusions. The data support the contention that hypoxia compromises the detection and discrimination of supra-threshold visual stimuli presented briefly to the central visual field, particularly if the stimuli have lower contrast or the visual scene is cluttered. At the very least, supplementary O_2 appears to facilitate information extraction from cluttered visual displays by comparison with performance under hypoxia, particularly under low light, low contrast conditions. Hyperoxia may even enhance performance over that achieved breathing air when viewing complex, low contrast scenes.

8.2 *Introduction*

8.2.1 Background

If the functional or useful field of view (UFOV) is defined as the total area of visual field in which useful information can be acquired without eye and head movements, that is, within a single eye fixation, then the presence of a foveal stimulus is enough to compromise it (Leibowitz and Appelle, 1969). The detection of peripheral stimuli may then be compromised further by increasing the demand of the central visual task or by embedding the peripheral target amongst numerous distracting stimuli (Ball, Beard, Roenker *et al*, 1988). The sensitivity and extent of the peripheral visual field is also compromised progressively by increasingly severe hypoxia and by dimmer viewing conditions (Kobrick, 1970). However, response times to peripheral stimuli increase and become more variable with eccentricity in a manner that suggests an effect of hypoxia upon visual processing speed rather than on stimulus detection thresholds (Kobrick and Dusek, 1970). Surprisingly, given the findings of Leibowitz and Appelle (1969) this effect was mitigated by the addition of a central monitoring task (Kobrick, 1972), suggesting an attentional rather than visual effect. Nonetheless, a degree of hypoxic impairment of responses to peripheral stimuli remains and is well established by 30 minutes of exposure (Kobrick, 1974). Again surprisingly, the magnitude of the impairment of response time under hypoxia was less at mesopic light levels than in the previous photopic studies, which was attributed to the greater contrast of the mesopic stimuli relative to their dim background (Kobrick, 1975).

Kobrick's studies are highly relevant to aircrew visual performance generally and particularly to the acquisition of visual information from aircrew displays. However, it is unclear whether or not the effects observed in Kobrick's studies result from delayed visual processing, whether they occur under milder hypoxia, whether they may have been confounded by hypocapnia, or how decreasing luminance (and increasing rod QO_2) might affect the results if confounding factors are controlled out. Other studies have demonstrated effects of hypoxia on response times and suggest that protracted visual processing may contribute to the delays (Fowler and Nathoo, 1997). Furthermore, a possible relationship to light intensity has been implicated in the mesopic range (Fowler, Banner and Pogue, 1993).

The Useful Field Of View test (UFOV®, Visual Awareness Incorporated) has been developed as a means of assessing functional visual performance across the central visual field, specifically in relation to ageing and driving ability (Ball and Owsley, 1993). Ageing is associated with deterioration of UFOV® performance that begins in early adulthood and can be regarded as a reduction in the efficiency with which subjects extract information from a cluttered visual scene (Sekuler, Bennett and Mamelak, 2000). However, the young subject group in the current study would normally be expected to perform well on this test, providing a baseline against which to assess visual processing speed in relation to the extraction of information away from fixation at decreasing mesopic luminance and under respiratory challenge.

The UFOV® test employs high contrast, white stimuli against a dark background and measures the stimulus presentation time necessary to enable correct responses. Thus, the test does not estimate threshold sensitivity to stimulus detection and neither does it depend on response time. Rather, the test provides an estimate of the extent to which the speed or efficiency of visual processing is compromised in relation to a series of increasingly complex visual tasks. These comprise an initial, simple, central stimulus identification task that is subsequently combined with a contemporaneous, peripheral stimulus localisation task. The combined task is then made more difficult still by camouflaging the peripheral stimulus amongst a pattern of 'distractors'. The background is always dark, so stimulus contrast will decrease when viewed through filters of increasing OD. Accordingly, each reduction in stimulus contrast will also tend to make the task more difficult. Nonetheless, the stimuli are at all times supra-threshold in nature and it is the effect of the respiratory disturbances to affect performance at each light level that is of primary interest. For example, an effect of hypoxia to degrade performance that is of comparable magnitude at each light level will suggest a post-receptoral mechanism, while an effect that is progressive with decreasing luminance will implicate outer retinal hypoxia and hence the photoreceptors.

8.2.2 Hypotheses

Mild hypoxia might compromise the speed at which visual information is processed from dimmer and more complex scenes, requiring longer stimulus presentation times to maintain cognitive performance.

Supplementary O_2 might optimise visual processing efficiency.

8.2.3 Aims

The main aim of these experiments was to assess whether hypoxia requires supra-threshold visual stimuli to be presented to the central visual field for longer to maintain optimal visual performance, as this has implications for the use of aircrew display systems (section 8.5.3). In addressing this aim, the hypotheses have been considered (section 8.5.5).

8.2.4 Experimental design

The design of these experiments was outlined in section 6.2.4 and summarised again in section 7.2.4. The terms Study 1 and Study 2 retain the same meanings as in Chapters 6 and 7. The first three experimental series (Study 1) compared visual performance under conditions of normoxia and mild hypoxia when viewing the test display directly and through ND 1.0 and 2.0 filters. The final two series (Study 2) repeated the previous assessments viewing through ND 1.0 and 2.0 filters to compare performance under the same level of mild hypoxia with that achieved breathing 100% O_2. The experimental parameters are summarised in Table 6-1.

The timeline of each experimental exposure was illustrated at Figure 6.2. Vision testing using UFOV® followed that of chromatic sensitivity and occupied the period from about 50 to 60 minutes of exposure to each respiratory condition, varying slightly between subjects according to the time taken to complete the preceding tests. Initially, only binocular UFOV® tests were planned. However, following the first set of 'direct view' experiments in Study 1 it became apparent that sufficient time was available to repeat the tests monocularly and this was undertaken in subsequent experiments. Binocular UFOV® testing always preceded monocular testing to accord with the rationale espoused previously, that is, to preserve the validity of direct comparison between respiratory conditions of corresponding duration, although this is highly unlikely to have been a significant confounding factor on this occasion.

8.3 Materials and Methods

8.3.1 Subjects

Background subject details and screening requirements were detailed in Chapter 3, section 3.6.3. Specific subject details for the experiments in this chapter are as detailed

in section 6.3.1 and section 7.3.1. Subjects again acted as their own controls for all experiments and were masked to the exposure orders of the respiratory conditions, which were randomised and balanced between males and females. Subjects were trained on the vision test before conducting the experiment.

8.3.2 Equipment

Equipment details are given in section 6.3.2 with ND filter characteristics detailed in section 7.3.4.

8.3.3 Respiratory conditions

The respiratory conditions are detailed in section 6.3.3.

8.3.4 Vision testing

The version of the UFOV® test employed in the experiments reported here has three components that are undertaken consecutively. In order, the proprietary terms for these elements are Processing Speed, Divided Attention and Selective Attention, and they present progressively more challenging visual tasks.

The first task, Processing Speed, assesses focused visual attention. A high contrast white line drawing is presented briefly in a central fixation box and the subject makes a 2-AFC response to identify whether it was a 'car' or a very similar-looking 'truck'. In the Divided Attention test, the central task is the same but a contemporaneous peripheral stimulus is presented along one of 8 radials towards the periphery of the display, at approximately 15° of visual angle from the central stimulus. The subject has to make the same 2-AFC response to the central stimulus followed by an 8-AFC response to select the radial occupied by the peripheral stimulus. The peripheral target is only ever a 'car' and need not be identified, only localised. In the Selective Attention test the subject has to respond in the same 2-AFC/8-AFC fashion but this time the peripheral target is embedded among a field of 47 inverted triangular distractors, making the localisation task much more difficult.

The standard screening test was used in this study, running each of the three components in succession. For all tests, the dependent variable being measured is the shortest duration of stimulus presentation compatible with correct identification of the central target and correct localisation of the peripheral target. For the Processing Speed

test, the result is calculated as the minimum presentation time at which the central target can be correctly discriminated 75% of the time. For the Divided Attention and Selective Attention tasks, the score is derived from a best-fit line for the relationship between central target discrimination and peripheral target localisation, based on a 75% correct response rate for both components of these tasks. It is emphasized that these are not tests of response or reaction time. After the stimulus is presented the subject can take as long as he or she wishes in choosing the response to the 2-AFC and 8-AFC questions. It is the accuracy of the responses that is important. Thus, the test provides data relating to visual processing speed and the cognitive ability to extract visual information from imagery presented briefly to the central 30° or so of the visual field.

The test is computer-administered and computer-scored. Once the test has started the software automatically adjusts stimulus presentation time, shortening it after two correct responses and lengthening it after an incorrect response. Responses are tracked using this staircase technique until a stable estimate of correct response rate or the shortest possible presentation time (16.7 ms) is achieved. Thus the duration of the test depends on the consistency of subject responses.

In this study the UFOV® software (Version 5.6) was supported on a Dell Optiplex GX150 PC running Windows 98 to drive the recommended 17-inch ELO Touchsystems Entuitive TouchScreen display monitor (model ET 1725C-4SWE-3), known to be compatible with the UFOV® software. However, subjects responded to the test stimuli using a computer mouse rather than touching the display screen. The display was viewed along a matt black viewing tunnel, to exclude stray light and prevent reflections, from a distance of 60 cm. Only the display screen was visible to the subject when positioned for the test, resting a notch in the AR5 expiratory manifold against a chin rest. The whole display screen was visible to both eyes when viewing binocularly.

Normal performance values are given in section 8.4 when discussing the results achieved for each task. Very few scores fell outside the normal range but this is to be expected. The test was designed for subjects over the age of 55 y and those with a history of illness or relative disability that might impact on driving performance. The subjects in the current study were all young and healthy and would be expected to achieve near optimal stimulus presentation times under normal conditions.

8.3.5 Experimental procedure

The UFOV® tests followed those of contrast sensitivity and chromatic sensitivity, and the detailed procedures to that point are given in sections 6.3.5 and 7.3.5. Testing was conducted binocularly and then monocularly (dominant eye) in accordance with the same rationale for testing of spatial contrast thresholds outlined in section 6.2.4 except that testing under direct viewing in Study 1 was only conducted binocularly as it was considered initially that insufficient time would be available for monocular testing.

Having completed the monocular test of chromatic sensitivity, the Velcro patch over the subject's non-dominant eye was removed and the subject's chair rolled gently to the right to position for the UFOV® test. The binocular test commenced after approximately 50 minutes of respiratory exposure (Time 60 in Figure 6.2), to within a minute or two according to how long the subject had taken with the preceding tests. Upon completion, the subject relaxed, the results were saved, and the subject rested for a minute or two before the Velcro patch was re-applied to cover the non-dominant eye. When ready to continue, the monocular UFOV® test was performed. Upon completion the subject again relaxed, the results were saved, the breathing gas supply hose was disconnected from the AR5 inspiratory hose, monitoring was discontinued, and the subject doffed the AR5 and associated experimental equipment. Each UFOV® test occupied about 5 minutes, such that UFOV® testing occupied about 10 minutes in total and was completed by about 60 minutes of respiratory exposure (Time 70).

8.3.6 Physiological parameters

Physiological responses to these exposures are detailed in section 6.3.6.

8.3.7 Analysis

The original aim was to conduct only binocular experiments and to analyse the data in Studies 1 and 2 using balanced ANOVA following $\log_{10}$ transformation. Introducing the monocular studies unbalanced the Study 1 data and obliged binocular and monocular results to be considered separately if all three light levels were to be considered together. Additionally, subjects frequently achieved correct responses at optimal stimulus durations, so data sets were often impossible to normalise, preventing parametric analysis. Finally, one female subject was unable to complete UFOV® testing in Study 2, viewing through the ND 1.0 filter, owing to facial discomfort from

prolonged wear of the AR5 face mask. Thus, the ND 1.0 filter data set was also unbalanced, constraining subsequent analysis.

When the data could be normalised by $\log_{10}$ transformation, parametric tests for significance were performed, including ANOVA and paired *t* tests. When they could not, non-parametric tests were conducted, particularly Friedman analyses, to help infer the influences of the various imposed conditions.

8.4 Results

The summary data for the UFOV® tests in Studies 1 and 2 are at Appendix 6.

8.4.1 Processing Speed

In both Study 1 and Study 2, Processing Speed remained optimal when viewing directly or through the ND 1.0 filter, either binocularly and monocularly, regardless of breathing gas. All subjects achieved correct identification of the central target at the shortest possible stimulus duration of ~16.7 ms, with just two negligible exceptions when subjects achieved results of 20 ms. Interestingly, both of these occurred when subjects were hypoxic.

	Binocular		Monocular	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Number of subjects not achieving optimal stimulus duration of 16.7 ms (N=12)	2	3	3	8
Range (ms)	16.7 - 23.3	16.7 – 23.3	16.7 – 33.3	16.7 – 53.3
Mean (ms)	17.5	18.1	18.6	24.7
Median (ms)	16.7	16.7	16.7	23.3

Table 8-1 UFOV® Processing Speed under normoxia and hypoxia (ND 2.0 filter, Study 1)

Processing Speed was not so well preserved in Study 1 when viewing through the ND 2.0 filter (Table 8-1). Performance is clearly poorer at this lowest level of stimulus contrast. The data also suggest poorer performance when viewing monocularly and particularly when hypoxic. The normal value for Processing Speed is $\leq$ 30 ms and it is noteworthy that some individuals did not achieve this target when viewing monocularly. The ND 2.0 filter data were analysed using the Friedman test for differences between medians (α = 0.05). The four 'treatment' groups comprised the four possible combinations of breathing gas and number of viewing eyes, with the null hypothesis that there was no significant difference between the four groups. The results of the analysis are shown in Table 8-2. As there are many ties in the data sets, the uncorrected

test statistic is likely to be conservative and the adjusted value more reliable. While the adjusted p value could still be either liberal or conservative, the results suggest that Processing Speed for low contrast stimuli at fixation may be compromised by hypoxia when viewing monocularly.

Friedman Test: Processing Speed versus Condition blocked by Subject (ND 2.0 filter)			
Test statistic S = 4.85	Degrees of Freedom = 3		p = 0.183
Test statistic S = 7.86	Degrees of Freedom = 3		p = 0.049 (adjusted for ties)
Condition	N	Estimated median	Sum of Ranks
Binocular hypoxia	12	16.878	27.5
Binocular normoxia	12	16.462	26.5
Monocular hypoxia	12	21.457	38.5
Monocular normoxia	12	17.711	27.5
Grand median		18.127	

Table 8-2 Hypoxic impairment of monocular Processing Speed – Friedman analysis (Study 1)

A similar pattern of results was seen when viewing through the ND 2.0 filter in Study 2, but with only marginally poorer performance across the subject group when viewing monocularly under hypoxia by comparison with the other conditions (Table 8-3). Friedman analysis on these data was not statistically significant (adjusted p = 0.44).

	Binocular		Monocular	
	Hyperoxia	Hypoxia	Hyperoxia	Hypoxia
Number of subjects not achieving optimal stimulus duration of 16.7 ms (N=12)	1	3	2	4
Range (ms)	16.7 – 20.0	16.7 – 23.3	16.7 – 30.0	16.7 – 23.3
Mean (ms)	16.9	17.8	18.1	18.6
Median (ms)	16.7	16.7	16.7	16.7

Table 8-3 UFOV® Processing Speed under hyperoxia and hypoxia (ND 2.0 filter, Study 2)

Overall, the results suggest that there may be a marginal effect of combined monocular viewing and hypoxia to compromise Processing Speed for low contrast foveal stimuli presented to an otherwise empty visual field. Both monocular viewing and hypoxia were associated with greater numbers of suboptimal responses from some subjects, with poorest performance overall when both conditions were combined. The absolute magnitude of these effects is slight. Nonetheless, this was a simple, cued test that already suggests a tendency towards slower visual processing for dimmer stimuli.

8.4.2 Divided Attention

As with Processing Speed, performance on the Divided Attention test was also well preserved when viewing the display directly and through the ND 1.0 filter in both Study

1 and Study 2. Again, subjects typically achieved optimal performance with stimulus durations of 16.7 ms. There were 4 exceptions viewing through the ND 1.0 filter in Study 1, when subjects achieved 20, 20, 23.3 and 36.6 ms, all but the last when hypoxic. In Study 2, viewing through the ND 1.0 filter, all subjects achieved 16.7 ms when breathing 100% O_2 (N = 11), while failures to achieve this occurred under hypoxia, with presentation times of 20, 23.3 and 56.7 ms.

The results achieved viewing through the ND 2.0 filter in Study 1 again suggest impairment with reduced stimulus contrast, monocular viewing and hypoxia, with worst performance when these conditions are combined (Table 8-4).

	Binocular		Monocular	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Number of subjects not achieving optimal stimulus duration of 16.7 ms (N=12)	6	6	7	10
Range (ms)	16.7 - 36.7	16.7 – 60.0	16.7 - 33.3	16.7 - 53.3
Mean (ms)	20.8	23.6	21.7	27.5
Median (ms)	18.2	18.2	20	26.7

Table 8-4 UFOV® Divided Attention under normoxia and hypoxia (ND 2.0 filter, Study 1)

A Friedman analysis performed on these data again indicated poorest performance on the Divided Attention task when viewing monocularly under hypoxia, but did not achieve statistical significance (Table 8-5).

Friedman Test: Divided Attention versus Condition blocked by Subject (ND 2.0 filter)			
Test statistic S = 3.28	Degrees of Freedom = 3		p = 0.351
Test statistic S = 3.97	Degrees of Freedom = 3		p = 0.265 (adjusted for ties)
Condition	N	Estimated median	Sum of Ranks
Binocular hypoxia	12	18.959	28.0
Binocular normoxia	12	18.542	27.5
Monocular hypoxia	12	23.956	37.0
Monocular normoxia	12	19.376	27.5
Grand median		20.208	

Table 8-5 Performance on the Divided Attention task – Friedman analysis (Study 1)

In Study 2, breathing 100% O_2 appears to optimise performance by minimising mean stimulus duration and greatly reducing the variability in performance between individuals (Table 8-6). The difficulty of this test, compared to the Processing Speed task, reflects the added requirement to locate a peripheral target at an eccentricity of only 15°, of the same luminance and character as the central target, in an otherwise empty visual field. Despite the relative task simplicity, one subject failed to achieve a

'normal' score on this test, that is, she failed to achieve a presentation time of < 100 ms, when viewing monocularly under hypoxia.

	Binocular		Monocular	
	Hyperoxia	Hypoxia	Hyperoxia	Hypoxia
Number of subjects not achieving optimal stimulus duration of 16.7 ms (N=12)	5	8	7	9
Range (ms)	16.7 - 23.3	16.7 - 53.3	16.7 - 30.0	16.7 - 163.0
Mean (ms)	18.3	24.4	21.1	42.2
Median (ms)	16.7	20.0	21.7	23.3

Table 8-6 UFOV® Divided Attention under hyperoxia and hypoxia (ND 2.0 filter, Study 2)

These data were also analysed using the Friedman test and the results are shown in Table 8-7. The effect of condition almost achieves statistical significance to $\alpha = 0.05$ and the analysis suggests that this is as much due to hyperoxic optimisation of stimulus presentation time when viewing binocularly as to hypoxic impairment when viewing monocularly, with the median results of the other two conditions remaining close to the grand median. It seems probable that this analysis would achieve statistical significance if using a larger pool of subjects.

Friedman Test: Divided Attention versus Condition blocked by Subject (ND 2.0 filter)			
Test statistic S = 6.88	Degrees of Freedom = 3		p = 0.076
Test statistic S = 7.71	Degrees of Freedom = 3		p = 0.052 (adjusted for ties)
Condition	N	Estimated median	Sum of Ranks
Binocular hyperoxia	12	17.503	22.0
Binocular hypoxia	12	20.416	30.5
Monocular hyperoxia	12	19.584	29.0
Monocular hypoxia	12	25.828	38.5
Grand median		20.833	

Table 8-7 Hypoxic impairment and hyperoxic preservation of Divided Attention (Study 2)

8.4.3 Selective Attention

Study 1

The addition of distractor stimuli made the previous task much more difficult. Normal Selective Attention ability is defined generously as a score of < 350 ms and none of the young healthy subjects in the current studies achieved worse presentation times than this under any condition. However, in Study 1 subjects were generally unable to achieve optimal performance on the Selective Attention task.

The control data (normoxic, binocular, direct viewing) resulted in a mean (± SD) presentation time of 58.6 ± 24.3 ms and were consistent with a normal distribution (α =

0.1) using the AD test (AD value 0.167, p = 0.917). Accordingly, balanced ANOVA was conducted on the binocular data for all three viewing conditions and both respiratory conditions, identifying only a statistically significant main effect of light level (p = 0.01). Scatterplots of these data are shown in Figure 8.1, including the respective mean (square) ± SE and median (triangle) for each condition. Interestingly, performance when viewing through the ND 1.0 filter appears, if anything, slightly better than that achieved when viewing the display directly. Performance is clearly worst viewing through the ND 2.0 filter. The appearance of the ND 2.0 data suggests a possible effect of hypoxia, with only 2 subjects achieving presentation times of < 50 ms. Within-subject paired data for the two respiratory conditions were normalised by subtraction (AD value 0.45, p = 0.226) but the difference between them was not statistically significant on paired *t* test ($t = 0.93$, p = 0.37).

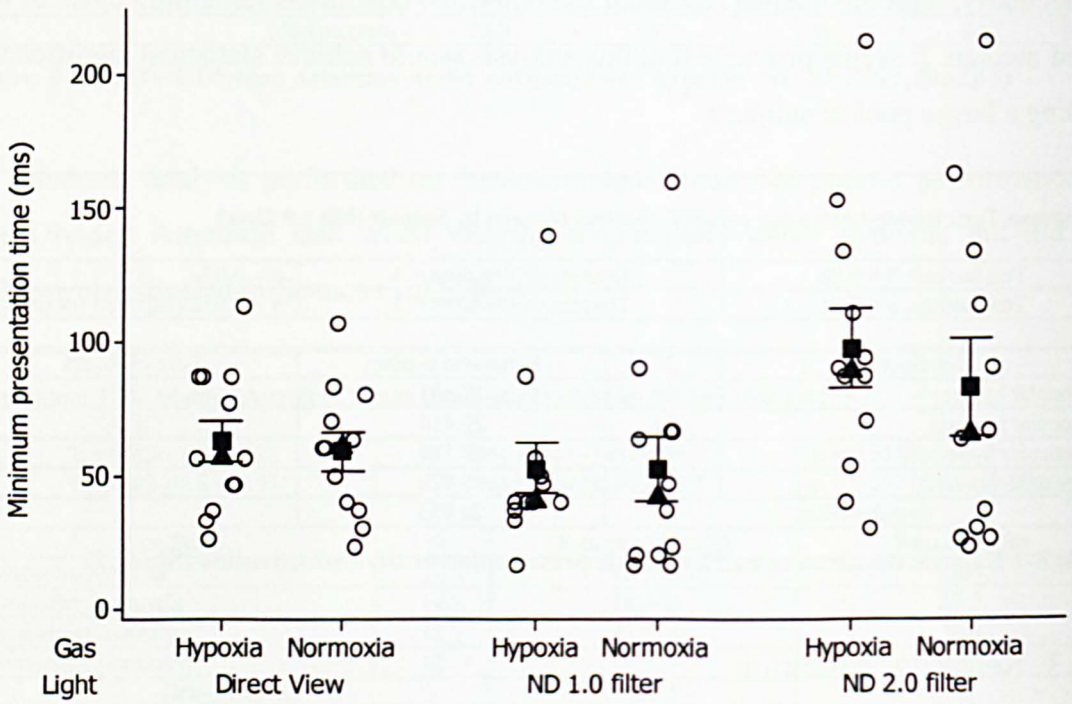


Figure 8.1 Mean (±SE) binocular Selective Attention in Study 1 (N = 12), with median (▲)

Considering the Study 1 monocular data, mean values are within 5 ms of their binocular counterparts, while the respective ranges and scatter are also very similar. There appears to be no meaningful effect of monocular, rather than binocular, viewing to compromise performance. However, monocular results appear compromised by both decreasing light level and hypoxia (Figure 8.2). The data were normalised by $\log_{10}$ transformation and analysed for main effects and interactions of light level and breathing gas using

balanced ANOVA (α = 0.05). A main effect of light level approached statistical significance (p = 0.057) but breathing gas was not significant (p = 0.548) and there was no significant interaction between light level and breathing gas (p = 0.64).

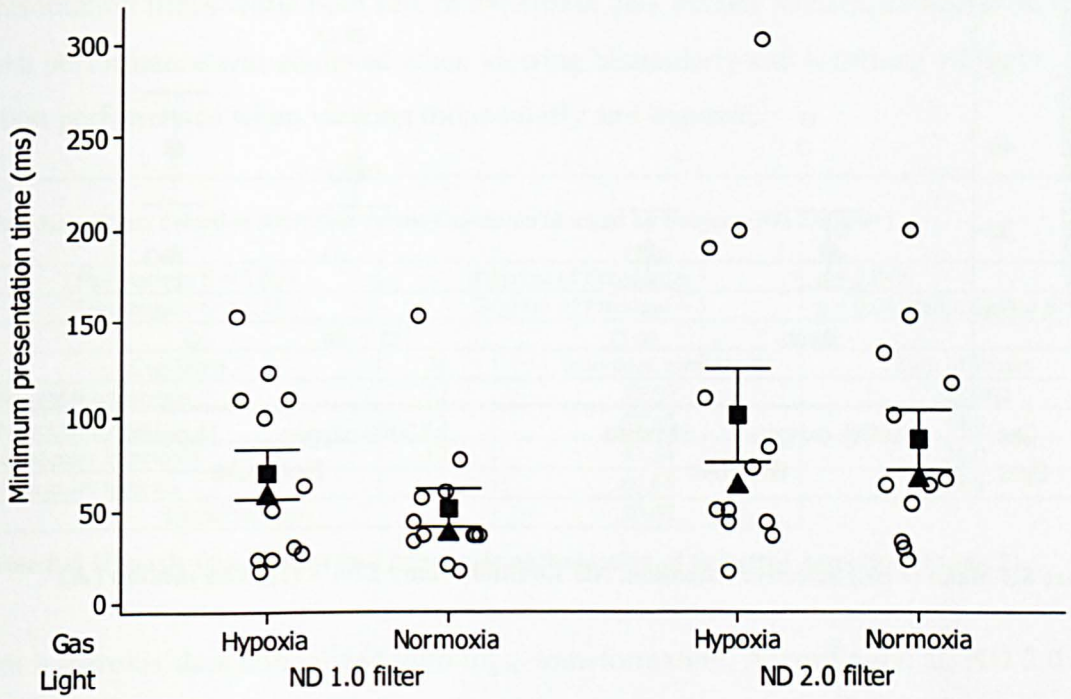


Figure 8.2 Mean (± SE) monocular Selective Attention in Study 1 (N = 12), with median (▲)

Study 2

Analysis of the Study 2 data was constrained by the absence of results for one subject viewing through the ND 1.0 filter, which unbalanced the data. Furthermore, overall performance on the Selective Attention task under hypoxia was better in Study 2 than under hypoxia in Study 1 for the corresponding viewing conditions, while performance under hyperoxia in Study 2 was better than that under normoxia in Study 1, such that many subjects achieved optimal performance when viewing through the ND 1.0 filter. As a result, analysis was also constrained by failure of $\log_{10}$ transformation to normalise the data at the lower light level.

The ND 1.0 filter data are shown in Figure 8.3 and suggest poorer performance when viewing monocularly. Subtracting the 22 available binocular values from the matching monocular values provided paired data that were compatible with a normal distribution (AD value 0.531, p = 0.155). A paired *t* test (α = 0.05) on these data confirmed a statistically significant difference between binocular and monocular viewing (t = 2.81, p = 0.011).

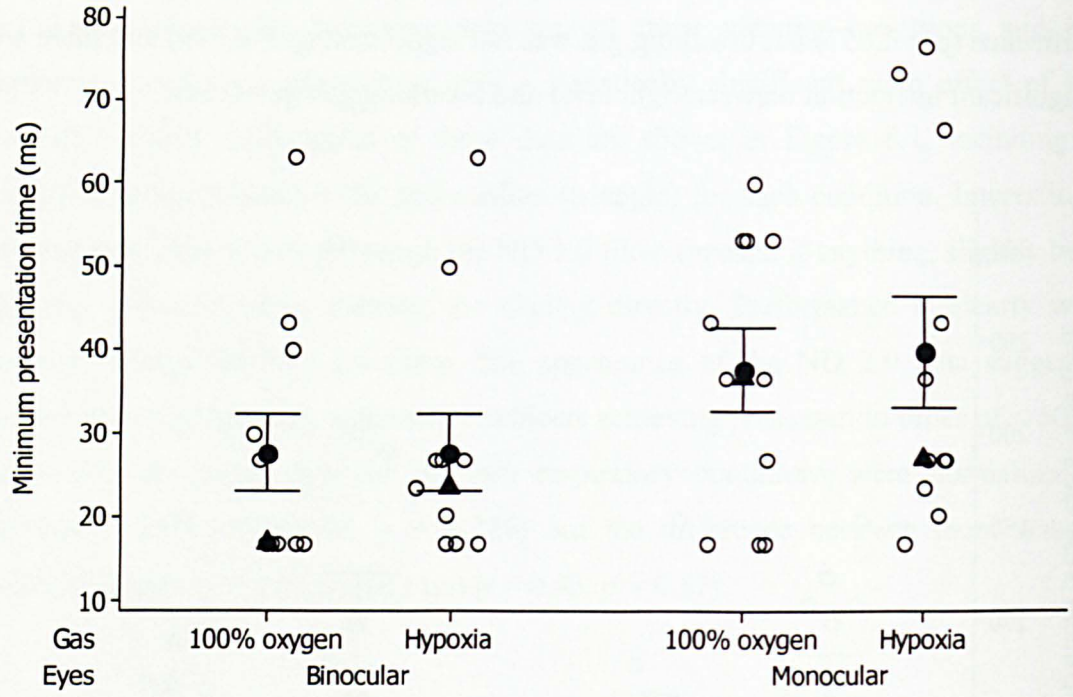


Figure 8.3 Mean (± SE) Selective Attention, ND 1.0 filter, Study 2 (N = 11), with median (▲)

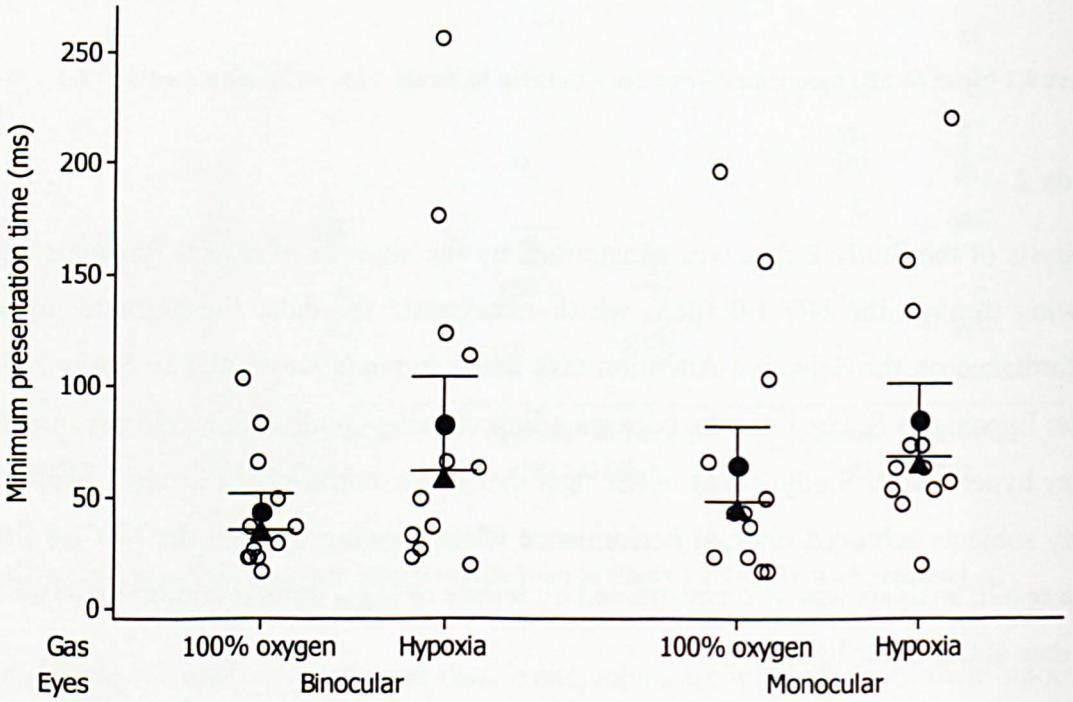


Figure 8.4 Mean (± SE) Selective Attention, ND 2.0 filter, Study 2 (N = 12), with median (▲)

The Selective Attention data when viewing through the ND 2.0 filter are shown in Figure 8.4 and suggest impairment under hypoxia (or a benefit of 100% O_2) for binocular and, perhaps, monocular viewing, while the effect of monocular viewing to

compromise performance is less evident than with the ND 1.0 filter results. The results of Friedman analysis on these data are shown in Table 8-8, indicating a statistically significant difference between the experimental conditions (α = 0.05, adjusted p = 0.041). With reference to the grand median, both sets of hypoxia data exhibit extended presentation times while both sets of hyperoxia data exhibit reduced presentation times. Best performance was achieved when viewing binocularly and breathing 100% O_2, with worst performance when viewing monocularly and hypoxic.

Friedman Test: Selective Attention versus Condition blocked by Subject (ND 2.0 filter)			
Test statistic S = 8.00	Degrees of Freedom = 3		p = 0.046
Test statistic S = 8.28	Degrees of Freedom = 3		p = 0.041 (adjusted for ties)
Condition	N	Estimated median	Sum of Ranks
Binocular hyperoxia	12	32.50	22.0
Binocular hypoxia	12	57.50	34.0
Monocular hyperoxia	12	43.34	26.0
Monocular hypoxia	12	66.66	38.0
Grand median		50.00	

Table 8-8 Hypoxic impairment and hyperoxic optimisation of Selective Attention (Study 2)

The hyperoxia data normalized with $\log_{10}$ transformation. Accordingly, all ND 2.0 data were $\log_{10}$ transformed and subject to balanced ANOVA (α = 0.05), confirming a main effect of breathing gas (F = 4.57, p = 0.038) but not of number of viewing eyes (F = 0.93, p = 0.34), and no significant interaction between them (F = 0.03, p = 0.872).

8.5 Discussion

8.5.1 Processing Speed

The Processing Speed central target discrimination task is easy and the level of stimulus contrast is such that it is readily detectable to the adapted eye when viewing through the ND 2.0 filter. Even so, the data suggest a consistent tendency towards marginal hypoxic impairment while the statistically significant hypoxic decrement when viewing monocularly at the lowest light level in Study 1 is remarkable. The magnitude of the effect is slight, increasing the median stimulus presentation time from 16.7 to only 23.3 ms, with only two subjects achieving presentation times greater than 30 ms. Nonetheless, it is enough to generate concern in relation to the acquisition of briefly presented visual information by mildly hypoxic aircrew when flying at night.

8.5.2 Divided Attention

Despite the addition of a peripheral target localisation element to the central stimulus discrimination task, the test remains easy if the visual field is otherwise empty. The high level of contrast enjoyed by the peripheral visual target makes it easy to localise, even when viewing through the ND 2.0 filter.

As with the Processing Speed task, marginal tendencies to increased presentation times were seen at the higher light levels, almost invariably when hypoxic. Although not statistically significant, the greatest hypoxic performance decrement was seen again when viewing monocularly at the lowest light level in Study 1. Additionally, the results in Table 8-6 and Table 8-7 support a benefit of hyperoxia on performance on this task at reduced stimulus contrast. The data suggest that hyperoxia optimises performance across the subject pool, decreasing maximum, mean and median presentation times relative to those under hypoxia. More subjects achieve optimal performance alongside better performance by those who do not. The effect is illustrated in Figure 8.5 in which subject performance is ranked in order for each condition.

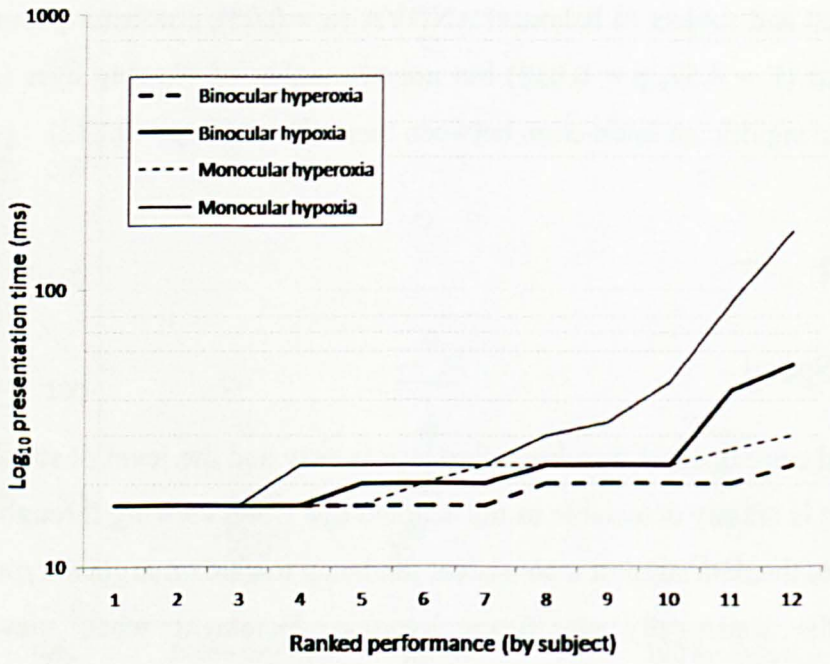


Figure 8.5 Effect of hyperoxia to optimise Divided Attention (ND 2.0 filter, N = 12)

The magnitude of the effects of respiratory condition are greater than for the Processing Speed task, and yet are still relatively slight for the majority of subjects under most conditions. Again, however, the results cause concern in relation to display viewing by

mildly hypoxic aircrew at night, since the peripheral target localisation task is well within the area likely to be bounded by a helmet-mounted display, for example. Adjustments of display brightness and contrast may need to be made carefully to avoid performance decrements under hypoxia, while supplementary O_2 is likely to maintain near-optimal performance on this type of task.

8.5.3 Selective Attention

The addition of numerous distractors makes the Selective Attention task considerably more difficult than the Divided Attention task, with subjects generally failing to achieve optimal performance in Study 1, even under the best viewing conditions. Some of the Study 1 data suggested poorer performance under hypoxia but none of the analyses achieved statistical significance. It is, arguably, possible that these trends would achieve statistical significance in a larger study. In Study 2, performance breathing 100% O_2 was clearly better than that achieved under otherwise equivalent normoxic conditions in Study 1. Although there was little difference between hyperoxia and hypoxia at the ND 1.0 filter level, the Friedman analysis and balanced ANOVA clearly support a benefit of 100% O_2 to decrease presentation times for the lowest contrast stimuli, illustrated using ranked data in Figure 8.6.

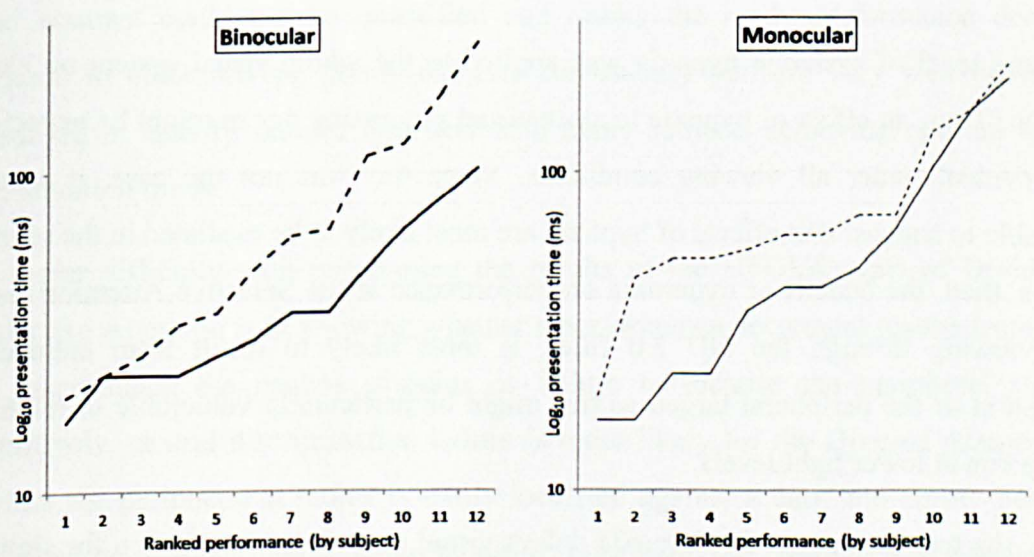


Figure 8.6 Ranked hyperoxic and hypoxic (dashed line) Selective Attention (ND 2.0 filter)

These findings suggest that supplementary O_2 may benefit the extraction of information from a cluttered visual field, such as a busy aircrew display, when presented at low luminance and low contrast. Furthermore, the benefit of 100% O_2 may be greater than that achieved by increasing slightly the luminance or contrast of the display. Binocular

performance when viewing through the ND 2.0 filter while breathing 100% O_2 in Study 2 was as good, if not better, across the subject group as a whole, than that achieved viewing through the ND 1.0 filter under normoxic conditions in Study 1 (Figure 8.7).

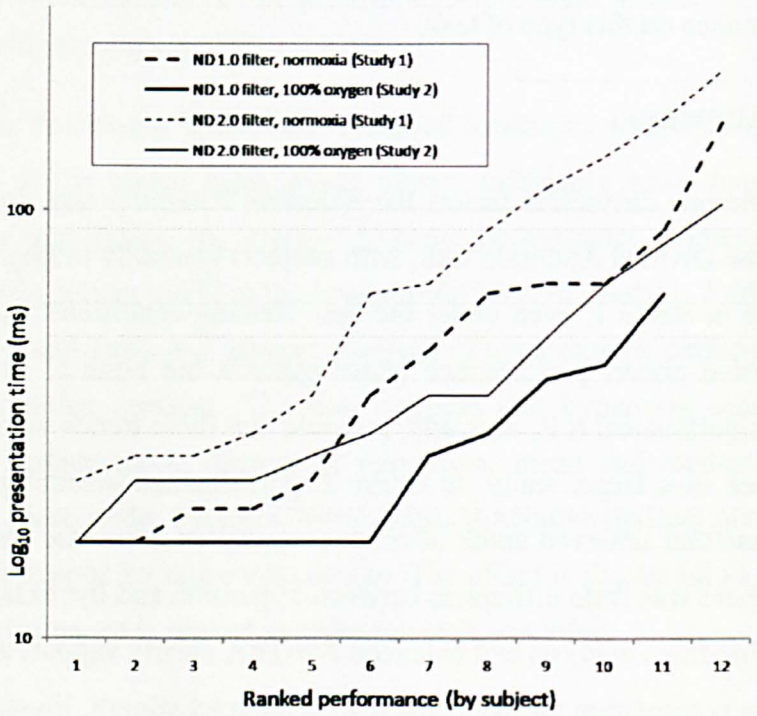


Figure 8.7 Comparison of binocular Selective Attention under normoxia and hyperoxia

The same level of systemic hypoxia was applied to the whole visual system on each occasion. Thus, an effect of hypoxia to slow visual processing *per se* might be expected to be evident under all viewing conditions. Since this was not the case, it seems reasonable to suggest that effects of hypoxia are most likely to be mediated in the retina. Perhaps, then, the benefit of hyperoxia on performance at the Selective Attention task, when viewing through the ND 2.0 filter, is most likely to result from enhanced localisation of the peripheral target, as this might be particularly vulnerable to relative rod hypoxia at lower light levels.

In sum, the results suggest that hypoxia delays visual processing speed even for simple foveal stimuli and that the effect becomes more pronounced for more complex visual tasks and dimmer viewing conditions (lower stimulus contrast). Viewing monocularly appears independently also to require longer stimulus presentation times. For the most complex visual tasks, supplementary O_2 may offer a performance benefit at lower mesopic light levels and stimulus contrast.

8.5.4 Lessons for subsequent studies

Subjects

In retrospect, it was ambitious to expect all subjects to complete all experiments in this series when wearing the AR5 for extended periods. In fact, given the inconvenience of wearing the AR5, data collection went remarkably well with only a single set of experimental data being lost as a result of a subject face-mask discomfort. Nonetheless, it was unfortunate that the UFOV® data set was incomplete and it would have been preferable to conduct the UFOV® experiments as a discrete study in its own right.

Experimental design

Data analysis in Study 1 was limited by the lack of monocular data at the highest light level. It would have been beneficial to time more carefully the duration of each vision test so that monocular UFOV® experiments could be included from the outset.

Vision testing

Contrary to expectation, given the extent to which the test has already been studied in relation to driving ability and accident risk, the UFOV® test is not well suited to investigation of the effect of hypoxia on visual processing speed. Stimulus luminance and contrast could not be quantified and unlike the ready performance decrement evident in older drivers, young subjects consistently perform very well on the test, resulting in heavily skewed data sets with many subjects achieving optimal stimulus presentation times.

A major difficulty with interpreting the results of the UFOV® tests of Divided and Selective Attention is in knowing whether a performance decrement results from failure to discriminate the central stimulus or failure to localise the peripheral stimulus. Intuitively, central discrimination failure is more likely for the Divided Attention task (when the peripheral stimulus is easily localised against a dark and empty peripheral field) while peripheral localisation failure is more likely for the Selective Attention task (when the central discrimination task should be no more difficult than in the Divided Attention task), but this is conjectural. Accordingly, it is difficult to infer with confidence the likely mechanism whereby oxygenation state might influence performance. Future studies of visual processing speed should consider the development of bespoke vision tests, giving due consideration to more robust forms of parametric data analysis. Even so, data sets are likely to be heavily skewed.

8.5.5 Summary and future work

Central target discrimination for low contrast stimuli is susceptible to impairment by hypoxia if presentation time is short, particularly when viewing monocularly, confirming the hypothesis that longer stimulus presentation times may be required to maintain cognitive performance. However, if this is due to hypoxic impairment of post-receptoral visual processing speed then it does not appear to be independent of stimulus contrast. The hypoxic degradation in Processing Speed discrimination at the fovea was only evident for low contrast targets, supporting the second hypothesis that an effect of hypoxia may become manifest when viewing dimmer stimuli.

The addition of the peripheral localisation task to the central discrimination task tended to compromise visual efficiency and a trend to poorer performance under hypoxia was apparent, particularly for low contrast stimuli viewed monocularly. Poorer performance under hypoxia is unambiguous when compared with breathing 100% O_2, clearly demonstrating an effect of respiratory condition on visual processing efficiency that is related to the level of stimulus contrast. The effect of hyperoxia supports the hypothesis that supplementary O_2 may benefit visual processing efficiency.

The further addition of numerous distractors to hamper the peripheral localisation element made the subjects' task much more difficult, and a tendency towards hypoxic impairment was apparent, supporting the hypothesis that visual target discrimination from increasingly cluttered visual scenes (increasing task complexity) may be more vulnerable to the effects of hypoxia. An unambiguous effect of respiratory condition was again seen when comparing responses under hypoxia with those breathing 100% O_2, supporting a benefit of hyperoxia to facilitate information extraction from cluttered visual displays under low light, low contrast conditions.

The main aim of these experiments was to assess whether hypoxia might compromise the detection and discrimination of briefly presented supra-threshold visual stimuli. The results suggest that it does, particularly if the stimuli are of lower contrast and the visual scene is complex or cluttered. Hyperoxia appears, at the very least, to mitigate the effect of hypoxia, if not to improve performance over that achieved breathing air when viewing complex, low contrast scenes.

It should not be assumed that effects of oxygenation state reported here are due to delayed visual processing rather than impaired sensitivity (threshold elevation). The level of hypoxia imposed here is unlikely to have compromised the inner retina, while

an outer retinal effect is more likely to manifest as reduced receptor sensitivity rather than inhibition of processing speed. However, it is perhaps possible that an effect on signal transmission from photoreceptor to bipolar cell, or perhaps on horizontal cell function, could result from rod-driven outer retinal hypoxia at low light levels (Feigl, Stewart and Brown, 2007). Further work is warranted to examine the nature of delayed visual processing under hypoxia and to derive the nature and retinal location of visual stimuli and tasks that are most likely to be compromised in this way.

The data suggest that the ability of the visual system to discriminate fine detail in dim light at low levels of stimulus contrast is compromised by mild hypoxia. The current study was unhelpful in quantifying such an effect meaningfully and further work to investigate this concern is reported in Chapter 10 which explores the effects of respiratory disturbance on low contrast acuity to ± 5° from fixation.

9 Temporal Contrast Sensitivity

9.1 Abstract

PURPOSE. It is well known that sensitivity to flicker is compromised by hypoxia but temporal contrast sensitivity at the fovea appears resistant. However, the effect of mild hypoxia has not been documented in relation either to temporal contrast thresholds beyond the fovea or to its effect on temporal contrast sensitivity in the mesopic range. This study investigated the effects of mild hypoxia, hyperoxia and hypocapnia on monocular thresholds to a temporally modulated contrast stimulus presented at photopic and mesopic light levels at various locations across the central 40° of visual field.

METHODS. Following a consistent process of visual adaptation, the dominant eye sensitivity of 12 subjects to temporal contrast gratings, having a *sf* of 0.25 cpd and temporal frequency of 25 Hz, was assessed using a standard FDT perimeter. Stimuli were viewed either directly (~100 cd m^{-2}) or via ND 1.0 (~10 cd m^{-2}) or 2.0 (~1 cd m^{-2}) filters. Mild hypoxia was imposed by breathing 14.1% O_2 and hyperoxia by breathing 100% O_2, with normoxic control exposures breathing air. Respired gas pressures were monitored continuously using mass spectrometry. Vision test data at each light level were analysed by eccentricity from fixation and visual field quadrant. The design allowed assessment of main effects and interactions of gender, breathing gas, exposure order sub-group and eccentricity or field quadrant using balanced ANOVA. In each experiment, a final voluntary hyperventilation condition enabled assessment of the effect of hypocapnia relative to the normoxic (normocapnic) data.

RESULTS. At 100 cd m^{-2}, mild hypoxia significantly decreased temporal contrast sensitivity beyond the fovea ($p < 0.001$). Male sensitivity exceeded that of females over the nasal hemifield, regardless of respiratory state, with no difference on the temporal side. Compared with breathing air, 100% O_2 enhanced female, but not male, sensitivity in all field quadrants, but all benefited from 100% O_2 relative to mild hypoxia. At ~10 cd m^{-2} female sensitivity exceeded that of males at all eccentricities but particularly over the temporal hemifield. Supplementary O_2 enhanced sensitivity across the visual field except at the fovea. At ~1 cd m^{-2} hyperoxia enhanced and hypoxia compromised sensitivity at all eccentricities and in all field quadrants. Hypocapnia enhanced sensitivity at the lower light levels.

CONCLUSIONS. The retinotopic hypoxic impairment of temporal contrast sensitivity beyond the fovea at 100 cd m^{-2} appears distinct in character from the influence of oxygenation state seen at 1 cd m^{-2}, supporting both regional and luminance-related influences on outer retinal O_2 demand. Gender differences in patterns of sensitivity changed with decreasing light level and are unexplained.

CITATION. The work at 100 cd m^{-2} has been subject to peer review and is published at **Connolly, D. M., and Hosking, S. L. (2008). Oxygenation and gender effects on photopic frequency-doubled contrast sensitivity. *Vision Research*, 48: 281-288.** The paper is reproduced at Appendix 9. An additional paper entitled 'Oxygenation state and temporal contrast sensitivity in the mesopic range' is currently under review.

9.2 *Introduction*

9.2.1 Background

Sensitivity to temporally modulated contrast stimuli represents the ability of the visual system to discriminate subtle fluctuations in spatial contrast over time and is particularly relevant to the sensation of motion in the visual periphery. Such visual characteristics have obvious relevance to piloting aircraft, for example, in relation to flight in low contrast conditions (poor weather), discrimination of display information (monochrome imagery), or detection of relative motion in the peripheral visual field (hovering or landing). However, few studies have examined the effect of respiratory disturbance on temporal contrast sensitivity at photopic luminance, none have examined thresholds to dynamic contrast stimuli away from the fovea, none document such aspects of visual performance with decreasing light level in the mesopic range, and none have explored how responses might vary in relation to retinal location. Yap *et al* (1995) studied temporal contrast sensitivity at the fovea and found no effect of hypobaric hypoxia at equivalent altitudes of 7000 and 12,000 ft. Enhanced sensitivity was reported by Benedek *et al* (2002) but is likely to have been confounded by hypocapnia secondary to hypoxia at an equivalent altitude of 18,000 ft. Accordingly, it is appropriate to explore again the effects of respiratory disturbance on temporal contrast sensitivity, at and beyond the fovea, at photopic and mesopic luminance.

As well as examining the effect of mild hypoxia in relation to normoxia, the potential benefit of supplementary O_2 was also explored. Additionally, given that flicker sensitivity is enhanced in relation to decreases in PCO_2, it was considered relevant to examine the effect of hypocapnia, generated by voluntary hyperventilation, in relation to normocapnia (that is, the normoxic condition), particularly at photopic luminance. A range of light levels were assessed to identify any changing patterns of response to respiratory disturbance with decreasing photopic to mesopic luminance.

The results from the work conducted to this point suggested that a significant effect of mild hypoxia would be unlikely at photopic luminance while hypocapnia would be unlikely to significantly enhance sensitivity to a temporally modulated stimulus in the mesopic range. Thus, the photopic experiments were intended to provide 'baseline' data against which to compare response patterns to altered PO_2 at reduced luminance while ensuring that the hypocapnia data were robust. On the other hand, the mesopic

experiments would need to be conducted carefully to ensure balanced analysis of the conditions involving altered oxygenation in relation to stable states of visual adaptation.

The results reported in the previous three chapters were obtained during prolonged, paired respiratory exposures with a short break between them. Approximately 40 minutes separated the end of the last vision test under the first respiratory condition before the first vision test began under the second respiratory condition, allowing plenty of opportunity for 'wash-out' of the first breathing gas condition and 'wash-in' of the second. In the current study four respiratory conditions were imposed in succession, demanding consideration of any possible delayed effects of preceding respiratory conditions on subsequent exposures, as this presents a potentially confounding influence that could have a physiological basis. Neuroglobin (Ngb) is a recently discovered mammalian globin that has been found in particularly high concentration in the retina, where it is located at the sites of greatest mitochondrial density and QO_2, including photoreceptor inner segments, OPL, IPL and GC (Schmidt, Giessl, Laufs *et al*, 2003; Bentmann, Schmidt, Reuss *et al*, 2005). The ligand binding properties of Ngb appear to be related to the redox state of the cell and suggest relatively slow changes in O_2 saturation in response to local fluctuations of PO_2 (Pesce, Bolognesi, Bocedi *et al*, 2002; Burmester and Henkeln, 2004). Possible roles of Ngb have been suggested to include local O_2 storage, regulation of mitochondrial QO_2 and protection from hypoxia (Sun, Jin, Mao *et al*, 2001; Hankeln, Ebner, Fuchs *et al*, 2005). The characteristics of Ngb suggest that imposed disturbances of retinal PO_2 could confound the results of vision testing under subsequent respiratory conditions.

9.2.2 Hypotheses

Mild hypoxia might compromise temporal contrast sensitivity in a manner that suggests retinotopic susceptibility, particularly at lower light levels.

Supplementary O_2 might optimise temporal contrast sensitivity at photopic and mesopic light levels by comparison to hypoxic performance.

Hypocapnia might enhance temporal contrast sensitivity, particularly at photopic luminance.

9.2.3 Aims

The primary aim of this piece of work was to document the effects of aviation-related respiratory disturbances on a measure of temporal contrast sensitivity, across the central visual field, at photopic and mesopic luminance. In so doing, the underlying hypotheses were investigated (sections 9.5.1 and 9.5.4).

It was intended subsequently to interpret the findings in relation to aircrew visual performance during flight and with respect to the use of aircrew display systems (section 11.1.3).

9.2.4 Experimental design

In order to minimise the overall number of individual experiments to be conducted, it was decided that each experiment would involve assessment of the four respiratory conditions at one light level. Light levels would be achieved by viewing the test display directly, at photopic luminance, or through ND filters to achieve borderline and upper mesopic levels, as illustrated in Figure 6.1. At each light level, testing would be conducted under normoxic, hypoxic, hyperoxic and hypocapnic respiratory conditions. However, the requirement to establish stable and procedurally reproducible states of visual adaptation to the lower light levels, at which the respiratory challenges could then be imposed in succession, each requiring 15 minutes of respiratory adaptation, would oblige experiments lasting over 100 minutes, wearing the respiratory equipment continuously. Experience with previous experiments suggested that this would be ambitious if using the AR5 to control visual and respiratory adaptation for the experiments at the lower light levels, as some subjects would find it difficult to tolerate the AR5 for this long without a break.

Consideration also had to be given to balancing the respiratory exposure orders. In this respect, the primary goal was to understand the influence of altered PO_2. Accordingly, the six possible orders of the normoxic, hypoxic and hyperoxic conditions were randomised and balanced across the subject pool. Thus, for each experiment, these three conditions were completed before attempting the final hypocapnic condition, the result of which could be compared to the normoxic (normocapnic) condition. This principle was upheld for the experiments at all light levels.

However, it was considered that many subjects would be unwilling to undertake the final hyperventilation condition at the end of such long experiments, while the main

purpose of investigating the effect of hypocapnia was to do so at photopic luminance, where prolonged initial visual adaptation and use of the AR5 was unnecessary. By wearing the more comfortable 'G' helmet and P/Q mask, and avoiding the initial retinal bleach and dark adaptation, the photopic experiments would be more likely to succeed in gaining the intended hypocapnia data, while still providing 'baseline' photopic information against which altered mesopic responses could be compared. It was accepted that not all subjects might be able to tolerate the AR5 for sufficiently long to generate the hypocapnia data under the mesopic conditions.

Thus, the study designs were slightly different for the direct view (photopic) and ND filter (borderline and upper mesopic) experiments and these are referred to, respectively, as Study A and Study B. Each subject undertook one photopic experiment in Study A and two low light (borderline and upper mesopic) experiments in Study B, each involving up to four respiratory conditions. All subjects completed the three respiratory conditions involving altered PO_2 at all light levels. Additionally, all completed the hypocapnia condition in Study A, but not in Study B.

9.3 Materials and Methods

9.3.1 Subjects

Numerous gender differences in ocular and visual function have been reported (Guttridge, 1993), including menstrual cycle-dependent changes in both spatial and temporal contrast sensitivity (Brabyn and McGuiness, 1979; Dunn and Ross, 1985; Johnson and Petersik, 1987), although this is not a consistent finding (Solberg and Brown, 2002). Gender differences in the visual performance of healthy subjects have also been reported using two different perimeter tests, (Cohn, DeAgostini, Aron-Rosa *et al*, 1994; Akar, Zulauf, Yucel *et al*, 2005). Accordingly, this study of temporal contrast sensitivity across the central visual field was balanced to allow comparison of responses by gender under the various respiratory challenges at each light level. The same 12 subjects (6M, 6F) completed both Study A and Study B. Their mean age was 30 y (range 23-37 y) and the age distributions of males and females were similar. The mean (± SD) age of the males was 31.8 ± 5.0 y while that of the females was 27.7 ± 4.1 y. There was no significant difference ($\alpha = 0.1$) between these distributions when assessed using a two-sample *t* test ($p = 0.148$).

Subject medical and ophthalmic screening requirements, exclusion and withdrawal criteria, training, payment, exposure limits, lifestyle requirements and compliance are as detailed in section 3.6.3. Specifically, subjects were all non-smokers who were asked to avoid alcohol for 24 h and caffeine on the day of their experiment. Females undertook a urine test to exclude pregnancy before beginning each experiment. All subjects had previous experience with breathing from pressure-demand regulators using aircrew masks, and, prior training in the technique of voluntary hyperventilation was considered unnecessary (Connolly and Hosking, 2007).

9.3.2 Equipment

For Study A, subjects were fitted with a P/Q mask supported from a 'G' helmet (Figure 3.6). This arrangement integrated well with the vision test apparatus. The mask was modified to provide an access port to the mask cavity, allowing continuous respiratory gas analysis using an Airspec MGA 2000 mass spectrometer. This was calibrated immediately before and after each experiment using various gas mixtures of known composition. As in previous experiments, breathing gases were supplied through dedicated, pressure-demand regulators with matching pressure/flow characteristics, so the gases were indistinguishable to the subjects who remained unaware of the presentation order throughout. The mass spectrometer trace was monitored continuously during each exposure to ensure that an adequate face-mask seal was maintained. Blood pressure, heart rate and S_aO_2 were monitored and recorded non-invasively using an Ohmeda Finapres 2300 blood pressure monitor and a Kontron 7840 pulse oximeter with finger probe. Analogue outputs were calibrated and recorded on an ADInstruments PC-based data recording system, together with the mass spectrometer data.

The experimental equipment was essentially the same in Study B, except that subjects wore a modified AR5 respirator (Figure 3.9) instead of the 'G' helmet and aircrew mask. This enabled them to undertake a process of retinal bleach (using the same light box as previously) and dark adaptation, followed by light adaptation to a controlled light level determined by the application of either ND 1.0 or 2.0 filters to the polycarbonate frame of the AR5. Contemporaneous respiratory adaptation could then be undertaken as detailed previously (section 3.3). Use of the AR5 again necessitated the use of blown air to prevent filter misting. The AR5 also integrated very well with the vision test apparatus.

9.3.3 Respiratory conditions

As previously, mild hypoxia was imposed breathing 14.1% O_2 (balance N_2) to simulate hypobaric hypoxia equivalent to breathing air at 10,000 ft, intending to generate a P_AO_2 of 55-60 mm Hg. The effect of supplementary O_2 was examined breathing 100% O_2 (hyperoxia). Normoxic, normocapnic control exposures were conducted breathing air at rest. Abbreviating the conditions of altered oxygenation as H for hypoxia, A for normoxia (air), and O for hyperoxia (O_2), the six possible exposure orders for these conditions are HAO, HOA, AOH, AHO, OHA and OAH. These were randomised and balanced such that one male and one female experienced each exposure order at each light level. Subjects unknowingly self-allocated to a particular exposure order according to their availability to undertake an experiment.

Moderate hypocapnia was induced by voluntary hyperventilation sufficient to lower $P_{ET}CO_2$ to 25 mm Hg, while breathing air from the appropriate regulator. This respiratory condition was always undertaken last, thereby avoiding any delayed and potentially confounding effects of the resulting acid-base disturbance (respiratory alkalosis) on the exposures involving altered PO_2. All respiratory conditions were imposed for precisely 15 minutes before undertaking the vision test.

9.3.4 Vision testing

Vision testing employed a standard Zeiss Humphrey® Visual Field Instrument incorporating Welch Allyn® Frequency Doubling Technology, commonly known simply as FDT or FDT perimetry. The perimeter is used in clinical practice as a supra-threshold screening test for glaucoma and to document the progress of any loss in sensitivity of the central visual field. Sinusoidal contrast stimuli are presented monocularly and comprise vertical gratings of low *sf* (0.25 cpd) with high temporal frequency counter-phase flicker (25 Hz). Thus, the gratings oscillate to produce a shimmering effect and an illusion that the *sf* of the gratings is twice the actual *sf*, hence the 'frequency doubling illusion'. The test was designed to isolate the magnocellular subset of retinal GC, believed to be most vulnerable to glaucoma as a result of their relatively small number and sparse distribution over the retina.

In this study, the perimeter was not used in its clinical screening mode. Instead the N-30 test protocol was used to generate threshold measurements of sensitivity to the temporal contrast stimulus, providing a test of functional dynamic contrast sensitivity across the

central visual field. The FDT N-30 programme was chosen as offering a brief, automated and easily repeated test using a dynamic contrast stimulus, while providing threshold information across the central visual field. The test was readily available, easy to administer and undertake consistently in conjunction with altered respiratory conditions, and had the potential to allow variations in threshold sensitivity to be interpreted retinotopically.

The test area encompasses a 40° x 40° square of central visual field, extending 20° of visual angle from its centre along the horizontal and vertical meridians. It comprises 17 discrete test areas, each with a maximum horizontal diameter of 10° of visual angle, arranged as shown in Figure 9.1, and a central fixation point is provided. The circular central test location encompasses fixation with a radius of 5° of visual angle. Beyond this lies an inner 'ring' of four irregularly-shaped test locations that extend to 10° from fixation along the horizontal and vertical meridians with their stimuli centred at about 9.6° along the 45° diagonal from fixation. The outer 'ring' of 12 square stimulus locations extends to 20° from fixation along the horizontal and vertical meridians such that the corner test areas have their stimuli centred at just over 21° from fixation. These three concentric test areas are referred to as 'centre', 'inner' and 'outer' respectively. The corresponding areas of retina differ in their anatomical structure, in their density and distributions of cone and rod photoreceptors, and by the nature of their vascular supply and, therefore, oxygenation.

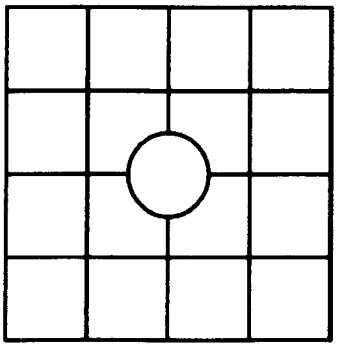


Figure 9.1 Spatial organisation of test areas for FDT perimetry

The automated test regime chooses test areas pseudorandomly and thresholds are assessed using a modified staircase technique. Subjects respond with a button press when a stimulus is seen. There being little, if any, difference between detection- and resolution-contrast thresholds for the frequency doubling illusion (McKendrick, Anderson, Johnson *et al*, 2003), in this study subjects remained unaware of the illusion and were asked simply to respond to unambiguous perception of a faint 'shimmering'

stimulus, so that the earliest threshold perception of the stimulus was determined for all participants under all conditions. Maximum stimulus duration is 720 ms with 400 ms at plateau contrast.

The stimuli are presented on a liquid crystal display and are focused at infinity. At the start of each test, the perimeter self-calibrates to a background field luminance of 100 cd m^{-2}, and this was confirmed at the start of the study by direct measurement using a Minolta CS1000 telespectroradiometer. In Study A, the testing was undertaken viewing the display directly. In Study B, experiments were undertaken viewing the display through ND 1.0 and 2.0 filters, approximating background fields of ~10 cd m^{-2} and ~1 cd m^{-2}, respectively.

All tests were conducted using the dominant eye, such that only two subjects used their left eyes; both were female. The design of the perimeter is such that the test area is obscured from view by the resting eye but this does not mask all stray light. While this may make little difference to relatively brief screening procedures in clinical practice, the N-30 threshold tests lasted 5-6 minutes. As a result, the first two experiments conducted at photopic luminance in Study A were found to be susceptible to binocular rivalry, apparent as pronounced dimming of the test field. Accordingly, these two experiments were abandoned and repeated. Subsequent experiments were completed uneventfully with the resting eye occluded gently by an eye patch and light cotton wool pledget (Study A) or by a light cotton wool dressing held in place by the filter frame (Study B).

The transmission characteristics of the ND 1.0 filters used over the right and left eyes were identical, but the ND 2.0 filter used over the left eye transmitted marginally less light, at all wavelengths, than that used before the right eye (Figure 7.6). This raised the possibility that thresholds for the two left-eye-dominant females might be compromised in comparison to their right-eye-dominant colleagues when viewing through the ND 2.0 filter. In practice, one of the left-eye-dominant females achieved normoxic thresholds that were close to the group means for each of the test locations. The other had poorer sensitivity that was, nonetheless, better than that achieved by two of the right-eye-dominant subjects. Since any slight differences in ND 2.0 filter transmission have, in any case, no bearing on the interpretation of differences attributable to the imposed respiratory conditions, they are not considered further.

The subjects were all screened using the FDT screening protocol to exclude pre-existing losses of visual field sensitivity when using the FDT test and all were normal for age. Subjects then undertook three 'training' FDT tests using the N-30 threshold protocol to counter any learning effect (Iester, Capris, Pandolfo *et al*, 2000; Heeg, Ponsioen and Jansonius, 2003). The 48 subsequent exposures accepted for analysis during Study A provided a cumulative total of 5 h of vision testing, between 12 subjects, and generated only 22 fixation errors (distributed equally between males and females), four false positive responses and a single false negative response, with no apparent relationship to respiratory condition. This is taken as indicating that subjects were well-rehearsed at conducting FDT perimetry and that attention was well maintained throughout. This will have mitigated any reduction in FDT sensitivity associated with immediate repeat testing (Artes, Nicolela, McCormick *et al*, 2003). Furthermore, this has not confounded interpretation of any effect of breathing gas due to the randomisation and balancing of exposure order. However, it remains possible that an effect of immediate repeat testing may have confounded interpretation of the effect of hypocapnia relative to the earlier normoxic normocapnic control test.

The N-30 protocol also assesses two additional nasal test areas but does so by changing the fixation point and altering the direction of regard, having completed the examination of the other 17 test areas. During the experiments it was noticeable that subjects appeared to relax for this final element of the test, in anticipation of test completion. Accordingly, the data from these nasal test areas is not included in the analysis.

9.3.5 Experimental procedure

The dim ambient illumination in the laboratory provided 1-2 lux at subject eye level in all experiments. Study A was conducted first and completed by all subjects before undertaking Study B. In all experiments, subjects adapted to the ambient light level for about 10 minutes while being briefed on the study and prepared for the first experiment.

In Study A, seated at rest, subjects donned the 'G' helmet and mask and were prepared for monitoring. The cotton wool pledget was lightly applied to occlude the eyelid of the non-dominant eye and held in place with non-allergenic tape. The hose supplying the first breathing gas was connected to the mask to start the experiment. As in previous experiments, each breathing gas condition was imposed for precisely 15 minutes, to achieve a respiratory steady state, before commencing the first vision test. Upon its completion, the breathing gas supply hose was disconnected and the subject rested for

2-3 minutes before repeating the process with the next breathing gas. When the randomised hypoxic, normoxic and hyperoxic conditions were complete, the subject was again supplied with air from the appropriate regulator and began hyperventilating until the $P_{ET}CO_2$ fell to 25 mm Hg. This was maintained for 15 minutes, with breath-by-breath monitoring of $P_{ET}CO_2$ throughout and verbal feedback from the experimenter to guide the subject's ventilatory effort. After 15 minutes, the subject commenced the final vision test, still hyperventilating. By this stage, typically, a steady ventilatory rhythm was well established such that minimal, if any, prompting was required to maintain a steady $P_{ET}CO_2$ during vision testing. Upon completion of the test, the subject relaxed and was monitored until $P_{ET}CO_2$ rose above 30 mm Hg.

In Study B, again seated at rest, subjects donned the AR5 respirator and were prepared for monitoring. The experiment began with 5 minutes of retinal bleach to both eyes, using the same light box as in earlier experiments (see section 3.5.1). This was followed by 15 minutes of dark adaptation using the procedure established previously. The filter frames, ND filters and cover were applied and secured a light cotton wool dressing over the non-dominant (non-test) eye. The air blower was turned on to provide the demist facility using ambient air. Five minutes into the process of dark adaptation, the hose supplying the first breathing gas was connected to the inspiratory hose of the AR5 and the subject began 15 minutes of respiratory adaptation. The last 5 minutes of respiratory adaptation coincided with 5 minutes of light (mesopic) adaptation of the test eye, initially viewing the dim ambient surroundings but including 2 minutes of adaptation to the FDT test luminance, viewed through the ND filter, immediately before commencing the vision test. Upon completion of the first vision test, the breathing gas supply hose was disconnected and the subject rested for 2-3 minutes before the supply hose was reconnected to commence 15 minutes adaptation to the next breathing gas. The last 2 minutes of this time were again spent adapting to the FDT test luminance before starting the test. This process was repeated again for the third breathing gas and vision test.

At this stage in Study B, some subjects chose to discontinue the experiment owing to various sensations of discomfort and fatigue associated with wearing the AR5 for a prolonged period (facial discomfort from the mask, feeling hot and sweaty, skin irritation, and fatigue). All such symptoms resolved spontaneously upon doffing the respirator. For those who were comfortable and willing, air was again supplied from the appropriate regulator and the subject began hyperventilating for 15 minutes as described

previously, before commencing the final vision test. Upon completion of this last test, the subject relaxed and was monitored until $P_{ET}CO_2$ rose above 30 mm Hg.

In Study B, subjects undertook the ND 1.0 filter experiment first and then followed up with the ND 2.0 study on a subsequent occasion.

9.3.6 Physiological parameters

The physiological responses to conditions of altered oxygenation imposed in Study A are shown in Table 9-1. They are consistent with those reported previously (section 6.3.6) when using the same methodology employed in Study B.

	P_IO_2 (mm Hg)	$P_{ET}CO_2$ (mm Hg)	S_aO_2 (%)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (bpm)
Control (normoxia, normocapnia)	158 ± 1	38 ± 3	97 ± 1	139 ± 20	90 ± 16	66 ± 10
Mild hypoxia (14.1% O_2)	107 ± 1	38 ± 2	90 ± 2	140 ± 14	89 ± 8	68 ± 10
Hyperoxia (100% O_2)	739 ± 6	33 ± 3	99 ± 1	139 ± 18	88 ± 16	61 ± 9
Hyperventilation (hypocapnia)	159 ± 1	23 ± 2	99 ± 1	145 ± 20	99 ± 19	63 ± 12

Table 9-1 Mean (± SD) cardio-respiratory responses in Study A

The imposed partial pressures of O_2 and CO_2 were generated as intended and were well controlled, resulting in the anticipated changes in S_aO_2. Not shown in Table 9-1, mild hypoxia induced a mean (± SD) $P_{ET}O_2$ of ~59 ± 4 mm Hg, reflecting the imposed P_AO_2, within the anticipated range (55-60 mmHg) and consistent with measurements made in the previous studies Table 6-3. As previously, hyperoxia was associated with a slight but consistent and reproducible fall in $P_{ET}CO_2$ by about 5 mm Hg that is explained by the Haldane Effect. When hyperventilating, all subjects maintained a steady state of hypocapnia, close to the target level of 25 mm Hg, before and during the vision test.

The same physiological parameters were monitored and recorded continuously during Study B. Responses throughout were generally consistent with those seen in Study A and are presented in Table 9-2. During Study B the group mean (± SD) $P_{ET}O_2$ during the hypoxia exposures tended to be slightly higher than during Study A, even though the group mean P_IO_2 and $P_{ET}CO_2$ were the same in each Study. However, the Study B measurements of $P_{ET}O_2$ are consistent with those measured in the hypobaric chamber at 10,000 ft (Table 4-2) and therefore accord with expectation. The differences in $P_{ET}O_2$

between Study A and Study B are slight and within acceptable day-to-day variability in responses to imposed respiratory disturbances. As evidence of this, group mean S_aO_2 was the same in both studies, indicating acceptably similar hypoxic challenges.

ND 1.0 (~10 cd m^{-2})	P_iO_2 (mm Hg)	$P_{ET}O_2$ (mm Hg)	$P_{ET}CO_2$ (mm Hg)	S_aO_2 (%)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (bpm)
Control (normoxia)	157 ± 3	111 ± 3	38 ± 2	97 ± 1	144 ± 27	89 ± 19	73 ± 8
Mild hypoxia (14.1% O_2)	106 ± 2	63 ± 3	38 ± 2	90 ± 1	141 ± 24	88 ± 14	77 ± 9
Hyperoxia (100% O_2)	731 ± 17	673 ± 21	33 ± 2	99 ± 1	143 ± 23	9 ± 17	68 ± 9

ND 2.0 (~1 cd m^{-2})	P_iO_2 (mm Hg)	$P_{ET}O_2$ (mm Hg)	$P_{ET}CO_2$ (mm Hg)	S_aO_2 (%)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (bpm)
Control (normoxia)	158 ± 3	114 ± 3	37 ± 3	97 ± 1	133 ± 21	82 ± 14	74 ± 8
Mild hypoxia (14.1% O_2)	108 ± 2	64 ± 3	38 ± 3	90 ± 1	139 ± 18	85 ± 12	78 ± 11
Hyperoxia (100% O_2)	735 ± 15	682 ± 15	33 ± 3	99 ± 1	142 ± 19	88 ± 13	70 ± 10

Table 9-2 Mean (± SD) cardio-respiratory responses in Study B

Blood pressures in both tables appear higher than normal due to an uncorrected hydrostatic pressure effect on measurements made using photoplethysmography; seating height varied between subjects. There was no effect of respiratory condition on blood pressure.

9.3.7 Analysis

The methodology of Study A was intentionally different from Study B and not all subjects undertook the hyperventilation conditions in Study B. Accordingly, the data at each discrete light level were analysed separately for effects of respiratory disturbance before considering trends by viewing condition. At each light level, the main goal was to assess differences due to altered oxygenation using a balanced analysis of the normoxia, hypoxia and hyperoxia data while excluding any confounding effects of gender or exposure order sub-group. Subsequent consideration was then given to any possible effect of hypocapnia relative to the normoxic (normocapnic) control condition.

FDT perimeter N-30 results are expressed as threshold measurements in dB, according to the manufacturer's proprietary formula (Kogure, Membrey, Fitzke *et al*, 2000). The raw data are presented in Appendix 8 for each of the 17 test area locations shown in

Figure 9.2. Dominant eyes were tested on all occasions and the data transposed, as necessary, to achieve common numbering of nasal and temporal field test sites.

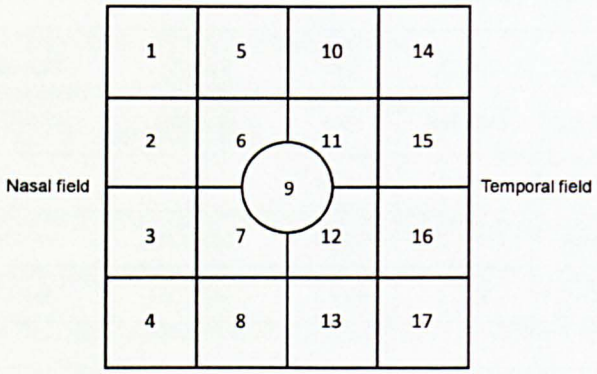


Figure 9.2 Test area numbering for dominant eye analysis

These data were then grouped appropriately to enable analysis of mean sensitivity firstly by retinal eccentricity, and secondly by visual field quadrant, as illustrated by diagrams A and B, respectively, in Figure 9.3. For the analysis by retinal eccentricity, each individual's means of the four 'inner' and 12 'outer' results were taken as the data points to feed, with the single 'centre' measurement, into group analysis. For the analysis by field quadrant, the 'centre' test location was ignored. For each respiratory condition, each subject's mean threshold of the four data points in each quadrant fed into the group analysis.

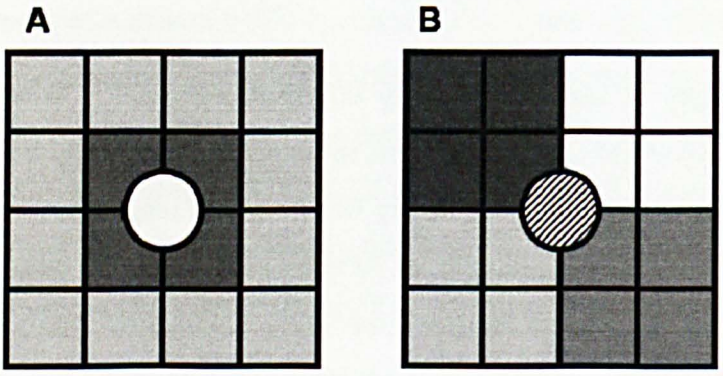


Figure 9.3 FDT analysis by retinal eccentricity (A) and visual field quadrant (B)

To exclude any possible effect of earlier respiratory disturbances confounding the results under subsequent conditions, the six possible PO_2 exposure orders were arranged into three groups according to whether hypoxia immediately preceded the 100% O_2 condition (termed 'H-O', incorporating the HOA and AHO data), 100% O_2 immediately

preceded hypoxia ('O-H', incorporating OHA and AOH data), or the hypoxia and 100% O_2 conditions were separated in time by the intervening air condition ('x-A-x', incorporating OAH and HAO data). Thus, the data from two male and two female subjects fed into each of these three exposure order sub-groups. Interactions between exposure order sub-group and breathing gas could then be excluded.

To validate subsequent statistical analysis, the normoxic retinal eccentricity and field quadrant data sets at each light level were examined using the Anderson-Darling test ($\alpha = 0.1$) and all were consistent with normal distributions. Normoxic retinal eccentricity and field quadrant data were also compared to exclude any underlying differences in sensitivity between males and females. For the eccentricity data, sensitivities were analysed using two-way ANOVA by gender and eccentricity ($\alpha = 0.05$), with no significant main effects of gender at 100 cd m^{-2} ($p = 0.732$), 10 cd m^{-2} ($p = 0.349$) or 1 cd m^{-2} ($p = 0.536$) and no interactions with eccentricity. Similarly, there were no statistically significant differences on two-sample *t* test ($\alpha = 0.05$), between grouped normoxic male and female field quadrant sensitivities at 100 cd m^{-2} ($p = 0.056$), 10 cd m^{-2} ($p = 0.278$) or 1 cd m^{-2} ($p = 0.606$), although the data at 100 cd m^{-2} approached statistical significance.

The retinal eccentricity data sets at each light level were analysed using balanced ANOVA to assess main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of breathing gas, gender, exposure order sub-group and retinal eccentricity. Field quadrant data sets at each light level were interrogated using balanced ANOVA for main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of breathing gas, gender, exposure order sub-group and field quadrant. Summary data sets and the results of initial balanced ANOVA at each light level are at Appendix 7. Mean field quadrant thresholds were also treated as four related dependent variables and analysed using balanced MANOVA to assess influences of the previous factors that might have varied systematically across the visual field. *Post hoc* tests included Tukey's Honestly Significant Difference for multiple comparisons ($\alpha = 0.05$) as well as other parametric (ANOVA and *t*) tests and non-parametric (Wilcoxon signed rank) tests as appropriate. In particular, having considered exposure order sub-group, global ANOVA were conducted to include consideration of light level as a main factor together with breathing gas, gender and either retinal eccentricity or field quadrant as appropriate. Also, the balanced ANOVA at each light level were repeated but excluding exposure order sub-group as a factor.

9.4 Results

9.4.1 Study A

Retinal Eccentricity at ~100 cd m^{-2}

Group mean threshold sensitivity data for the 'centre', 'inner' and 'outer' test areas, viewing the FDT display screen directly, are shown for each respiratory condition in Figure 9.4. Initial balanced ANOVA identified statistically significant main effects of retinal eccentricity ($p < 0.001$), breathing gas ($p = 0.004$) and exposure order sub-group ($p < 0.001$) and a statistically significant interaction between retinal eccentricity and exposure order sub-group ($p = 0.008$).

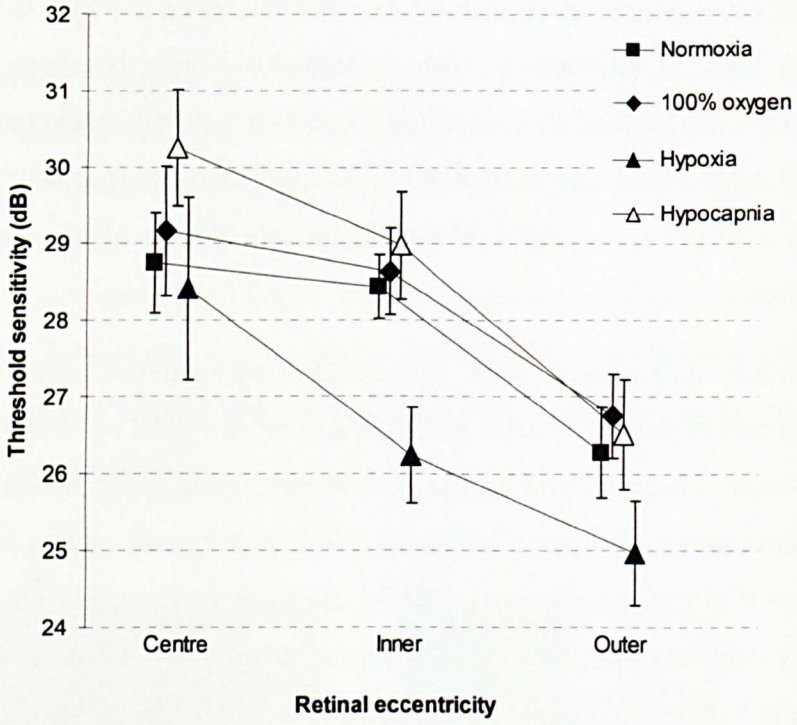


Figure 9.4 Mean (± SE) FDT threshold sensitivity by retinal eccentricity (~100 cd m^{-2})

The effect of retinal eccentricity is apparent in Figure 9.4 as impaired 'outer' sensitivity relative to 'centre' and 'inner' sensitivity. The graph also suggests that the main effect of breathing gas results from hypoxic impairment of threshold sensitivity for the 'inner' and possibly 'outer' test locations. These possible effects of hypoxia were investigated relative to normoxic performance using within-subject data paired by respiratory condition. The resulting data sets were normalised by subtraction, allowing assessment

using paired *t* tests ($\alpha = 0.05$). The effect of hypoxia was highly statistically significant for the 'inner' data ($p = 0.001$) and almost significant for the 'outer' data ($p = 0.051$).

The main effect of exposure order sub-group and its interaction with eccentricity are represented in Figure 9.5. In this, and all subsequent figure captions in this chapter, the term 'sensitivity' refers to threshold sensitivity to the FDT stimulus. Thresholds were consistently poorest in the sub-group that undertook hypoxia exposures immediately before the hyperoxia conditions (H-O), particularly for the 'centre' data. The effect is discussed further below in relation to the field quadrant data.

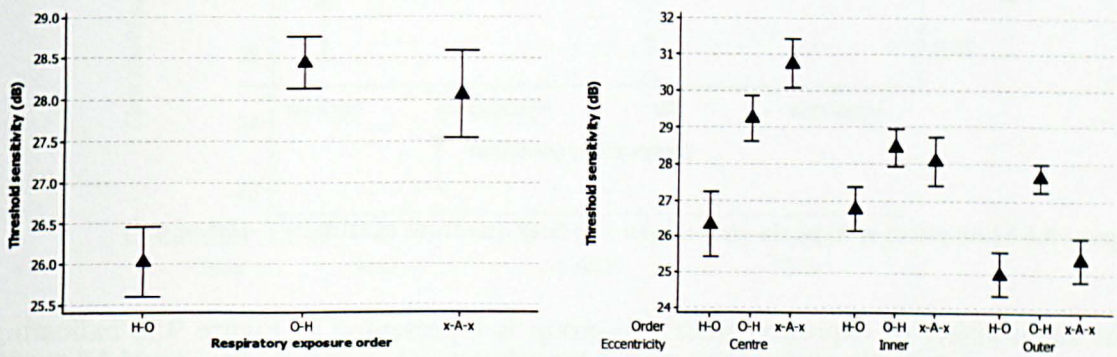


Figure 9.5 Main effect of exposure order sub-group and interaction with eccentricity (~100 cd m^{-2})

With regard to a possible effect of hypocapnia, from Figure 9.4, hypocapnia appears to do little except, perhaps, to enhance sensitivity slightly at the 'centre'. However, a paired *t* test comparing hypocapnic performance against the normoxic, normocapnic data was not statistically significant ($p = 0.207$).

Field Quadrant at ~100 cd m^{-2}

Mean (± SD) normoxic field quadrant threshold sensitivity was 26.8 ± 2.1 dB. Initial balanced ANOVA, including the hypocapnia data, identified a main effect only of breathing gas ($p < 0.001$), shown in Figure 9.6 to be a reduction in sensitivity by about 2 dB under mild hypoxia.

In order to conduct the primary analysis to assess the influence of PO_2, the hyperventilation data were excluded and the balanced ANOVA was repeated to include consideration of exposure order sub-group. The main effect of breathing gas remained highly statistically significant ($p < 0.001$) and a main effect of exposure order sub-group was also evident ($p < 0.001$). An interaction between gender and exposure order sub-

group approached statistical significance (p = 0.011). There was no main effect of field quadrant (p = 0.197) nor any interactions of field quadrant with the other factors.

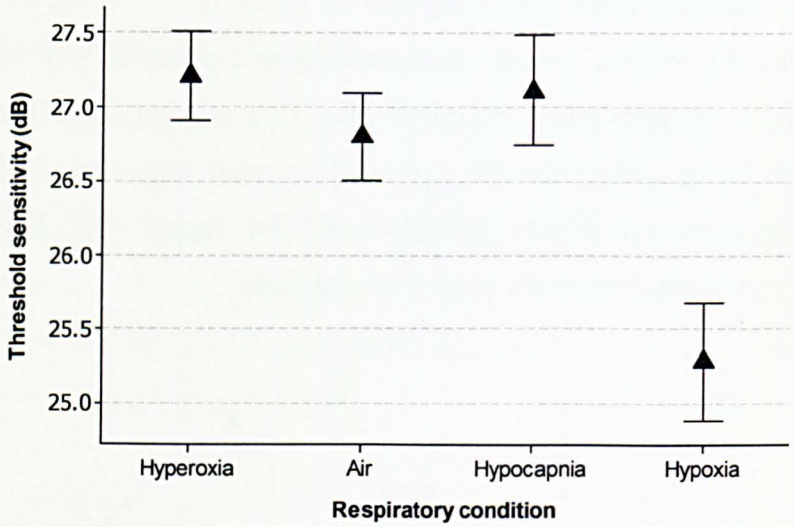


Figure 9.6 Main effect of hypoxia on mean (± SE) field quadrant sensitivity (~100 cd m^{-2})

The main effect of exposure order sub-group is represented in Figure 9.7, indicating greatest net sensitivity for the O-H sub-group and poorest net sensitivity for the H-O sub-group. Initially, this was interpreted as a net enhancement of sensitivity due to exposure to 100% O_2 prior to hypoxia exposure (O-H) and a decrement in sensitivity when hypoxia preceded hyperoxia, that is, as a physiological effect of exposure order.

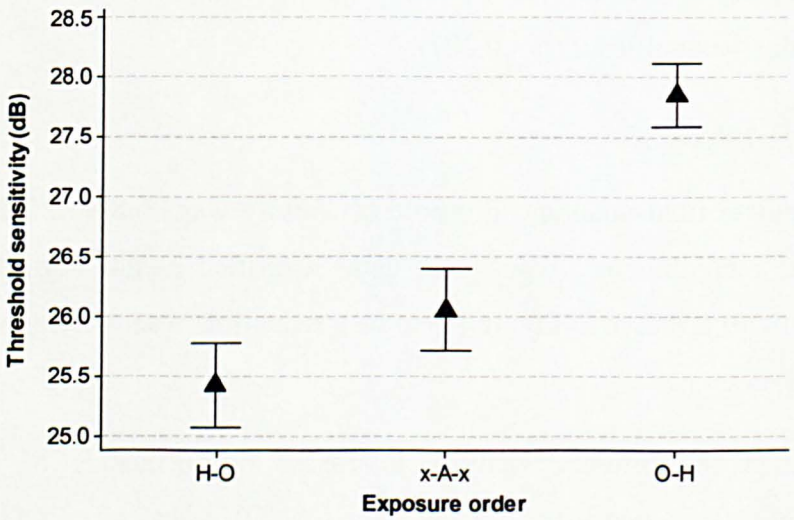


Figure 9.7 Exposure order sub-group and mean (± SE) field quadrant sensitivity (~100 cd m^{-2})

For each exposure order, the mean threshold sensitivity achieved with each breathing gas is shown in Figure 9.8, indicating a consistent benefit of supplementary O_2 over

hypoxia, regardless of exposure order sub-group, but also an apparent effect of exposure order to influence the overall sensitivities achieved with each breathing gas during each experiment. The data appear to suggest that prior hypoxia diminishes the benefit of subsequent O_2 and that prior 100% O_2 mitigates subsequent hypoxic impairment.

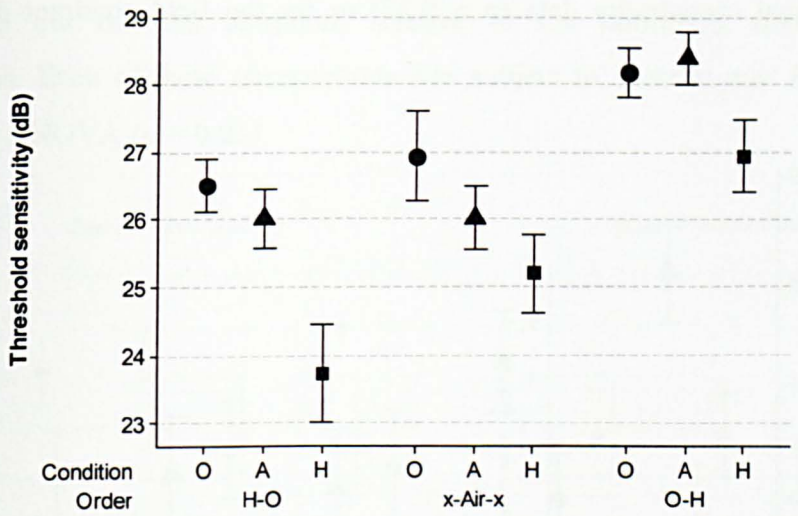


Figure 9.8 Mean (± SE) sensitivity by breathing gas and exposure order (~100 cd m^{-2})

This was interpreted as implying the existence of some form of 'retinal memory' for recent oxygenation state. However, later results from Study B demonstrated further highly statistically significant effects of exposure order sub-group that cannot have a physiological explanation based on delayed effects of respiratory disturbance. This prompted detailed review of the influence of exposure order sub-group in Study A. In fact, the apparent effect of exposure order in Study A is misleading. Only four of the 12 subjects contributed data to each sub-group and subjects were allocated to these randomly. Thus, subjects with differing underlying visual sensitivities to the FDT stimulus could be allocated to the different sub-groups and thereby bias the results under subsequent ANOVA. Subjects allocated to the O-H sub-group had a baseline (normoxic) mean (± SD) field quadrant sensitivity of 28.4 ± 1.6 dB, while the equivalent results for the subjects in the H-O and x-A-x subgroups were 26.0 ± 1.7 and 26.0 ± 1.9 respectively (Figure 9.8). While the variance was similar in all three sub-groups, the mean baseline sensitivity in the O-H sub-group was 2.4 dB greater than the others. Examining just the normoxic data using one-way ANOVA for exposure order sub-group, this difference was highly statistically significant ($p = 0.001$). This is attributable to allocation bias of subjects with greater underlying field quadrant sensitivity to the O-H sub-group in Study A. This explains both the main effect of

exposure order sub-group and its interaction with gender, as well as the effect of exposure order sub-group noted in relation to the retinal eccentricity data. Furthermore, genuine main effects of exposure order would be expected to generate statistically significant interactions with the breathing gas conditions. In fact, there were no interactions between breathing gas and exposure order sub-group on balanced ANOVA, either for the retinal eccentricity data ($p = 0.28$) or for the field quadrant data ($p = 0.491$).

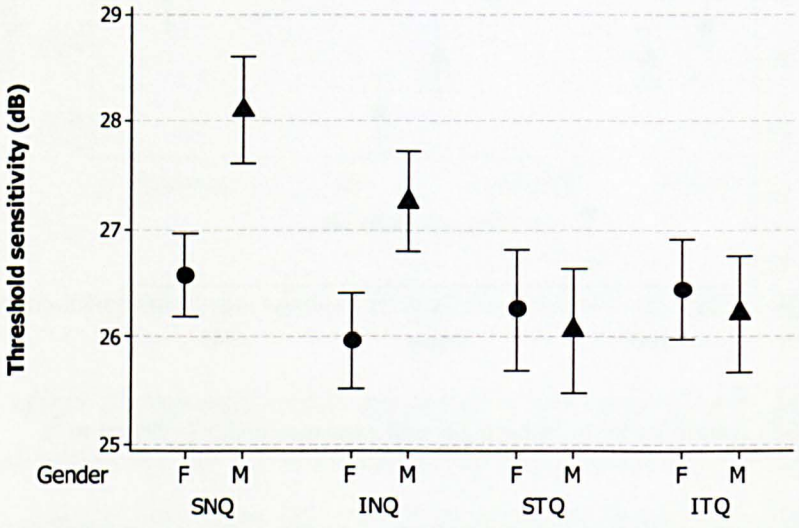


Figure 9.9 Male (▲) and female (●) sensitivity by field quadrant (~100 cd m^{-2})

Having excluded a main effect of field quadrant on balanced ANOVA, the four sets of quadrant data were examined as dependent variables using balanced MANOVA, for main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of breathing gas, gender and exposure order sub-group. Unexpectedly, only a main effect of gender achieved statistical significance ($p = 0.007$). This is represented in Figure 9.9, which shows mean (± SE) threshold sensitivity for males and females for the superior (SNQ) and inferior (INQ) nasal and superior (STQ) and inferior (ITQ) temporal field quadrants, indicating greater male sensitivity over the nasal hemifield. Accordingly, the data for all four field quadrants were examined in detail for trends in response by gender and are shown in their entirety in Figure 9.10.

Figure 9.10 shows mean (± SE) threshold FDT sensitivity for each field quadrant for males (black symbols) and females (white symbols) under each respiratory condition. Regardless of respiratory condition, female sensitivity is disadvantaged over the nasal hemifield relative to male sensitivity. Consistent with the main effect of breathing gas,

hypoxia tends to compromise the sensitivity of both males and females in all four quadrants. However, relative to normoxia, female sensitivity is consistently enhanced by 100% O_2 in all quadrants, while male sensitivity is not. However, both males and females benefit from 100% O_2, in all quadrants, compared to hypoxic sensitivity. Finally, hypocapnia appears to enhance male and female sensitivity over the nasal hemifield but not the temporal, relative to the normoxic, normocapnic control exposures. Each of these observations was subject to specific *post hoc* analysis using one-way ANOVA ($\alpha = 0.05$).

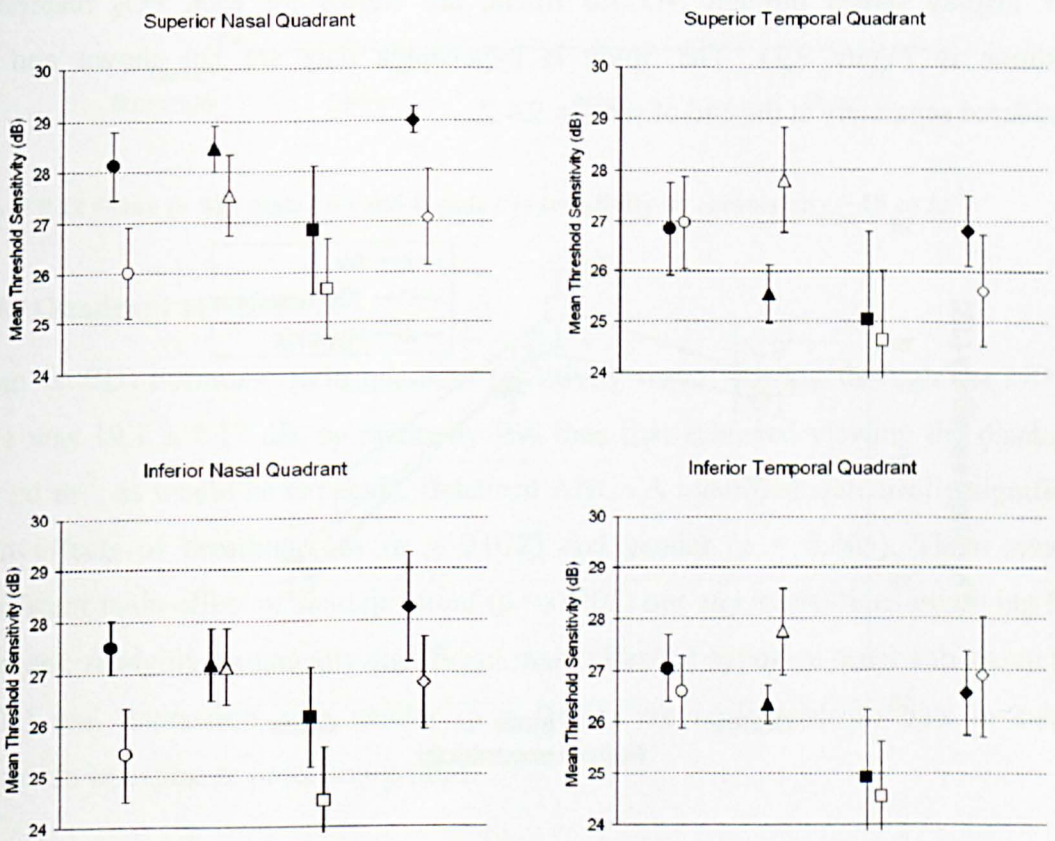


Figure 9.10 Mean (± SE) field quadrant sensitivity by gender and breathing gas (~100 cd m^{-2})

First, the effect of gender was examined in each of the four field quadrants individually, using the data from all four respiratory conditions, achieving statistical significance only in the SNQ ($p = 0.02$) and INQ ($p = 0.049$), and supporting greater male sensitivity over just the nasal hemifield. Secondly, the female hyperoxia data for all four quadrants were examined relative to normoxia, supporting a benefit of supplementary O_2 to enhance female sensitivity across the whole of the visual field ($p = 0.004$). The same analysis on the male data was not significant ($p = 0.487$). Thirdly, the hyperoxia data for all subjects

and field quadrants were compared to the hypoxia data, showing a highly statistically significant benefit of 100% O_2 ($p < 0.001$). Finally, the hypocapnia data for the nasal quadrants of both genders were examined relative to normocapnia (normoxia) and were not significantly different ($p = 0.119$).

9.4.2 Study B

Retinal Eccentricity at ~10 cd m^{-2}

Group mean threshold data for the 'centre', 'inner' and 'outer' test areas, viewing the FDT display screen through ND 1.0 filters, are shown for each PO_2 respiratory condition in Figure 9.11. The Study B hypocapnia data are not shown and are considered separately at the end of section 9.4.2.

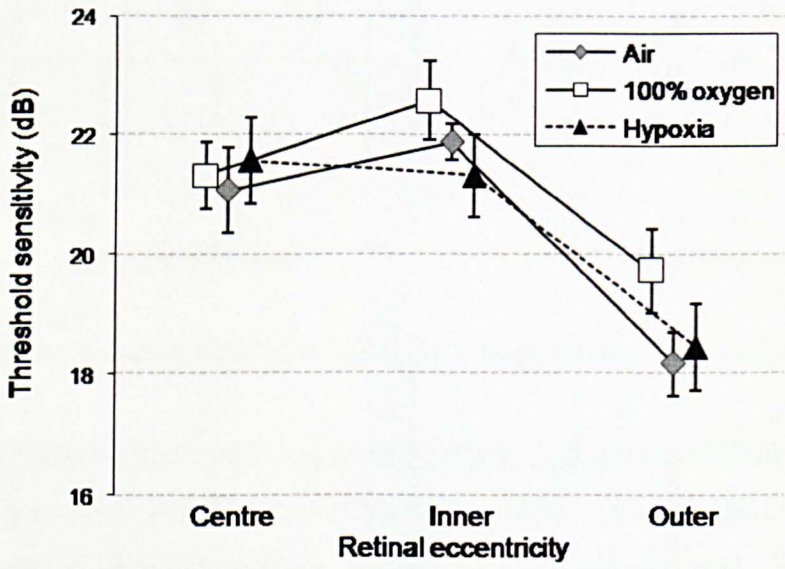


Figure 9.11 Mean (± SE) threshold sensitivity by retinal eccentricity (~10 cd m^{-2})

Balanced ANOVA confirmed a statistically significant effect of retinal eccentricity ($p < 0.001$) that is self-evident in Figure 9.11. 'Outer' sensitivity is clearly poorest, as it was at 100 cd m^{-2}. There was also a statistically significant main effect of gender ($p = 0.007$), illustrated for each eccentricity in Figure 9.12. Notwithstanding the absence of a gender difference when considering just the normoxic data, when examining the full data set female sensitivity was consistently greater than male sensitivity at all eccentricities. There was no significant effect of breathing gas (0.263), although the appearance of Figure 9.11 suggests slightly enhanced 'inner' and 'outer' sensitivity with hyperoxia.

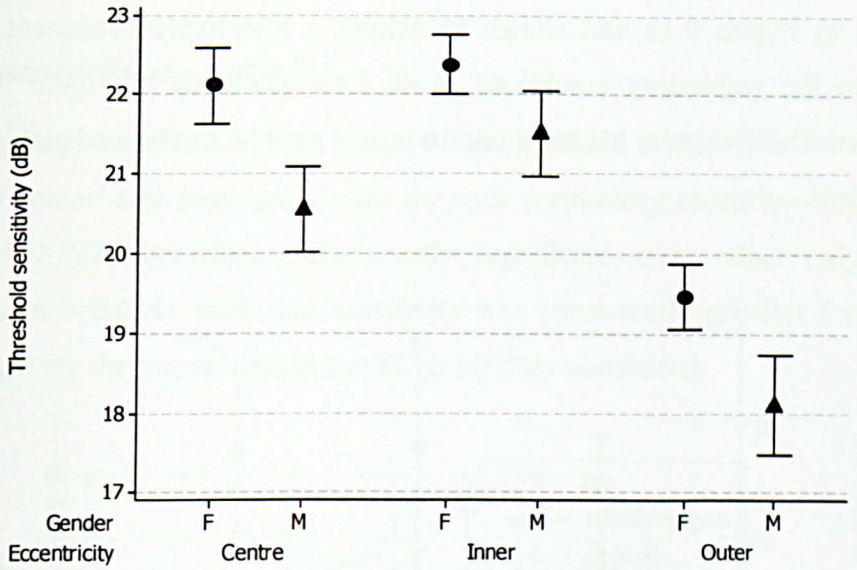


Figure 9.12 Mean (± SE) male (▲) and female (●) sensitivity by eccentricity (~10 cd m^{-2})

Field Quadrant at ~10 cd m^{-2}

Mean (± SD) normoxic field quadrant sensitivity when viewing through the ND 1.0 filter was 19.1 ± 2.17 dB, so markedly less than that achieved viewing the display at 100 cd m^{-2}, as would be expected. Balanced ANOVA identified statistically significant main effects of breathing gas ($p = 0.022$) and gender ($p = 0.005$). There was no significant main effect of field quadrant ($p = 0.902$) nor any interactions involving field quadrant. A highly statistically significant main effect of exposure order sub-group ($p < 0.001$) and interaction with gender ($p = 0.001$) are attributable to bias in subject allocation to exposure order sub-groups.

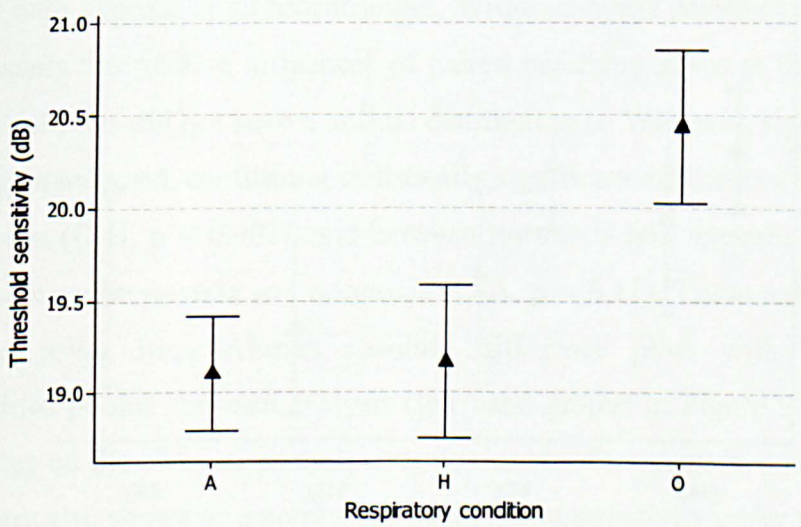


Figure 9.13 Effect of breathing gas on mean (± SE) field quadrant sensitivity (~10 cd m^{-2})

The main effect of breathing gas is an overall increase in sensitivity with hyperoxia, represented in Figure 9.13 and shown to exhibit a reasonably consistent pattern of responses to the respiratory conditions in all four quadrants in Figure 9.14. Mean threshold sensitivities under hypoxia and normoxia remain similar and stable across all field quadrants.

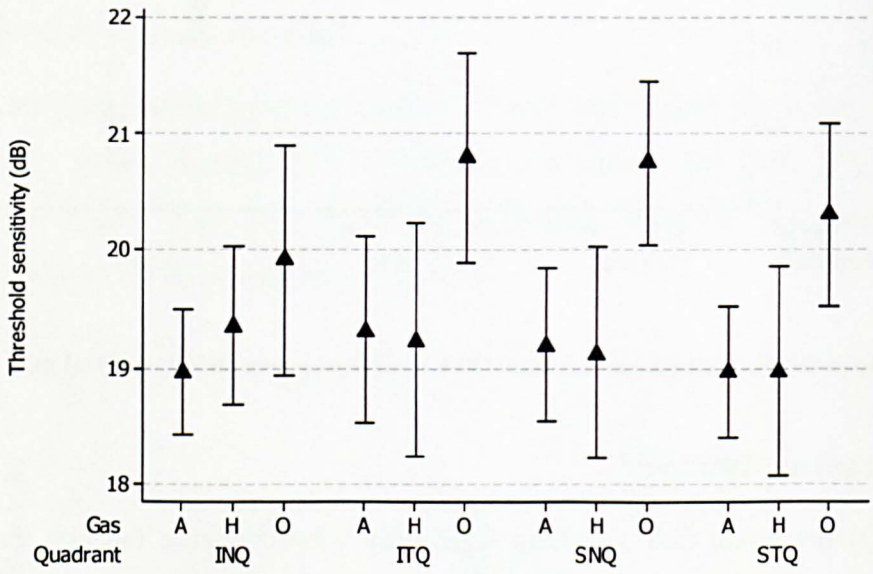


Figure 9.14 Benefit of hyperoxia on mean (± SE) sensitivity for each field quadrant (~10 cd m^{-2})

In contrast to the finding at 100 cd m^{-2} of greater male sensitivity over the nasal hemifield, the main effect of gender viewing through the ND 1.0 filter is attributable to greater female sensitivity, particularly across the temporal hemifield (Figure 9.15).

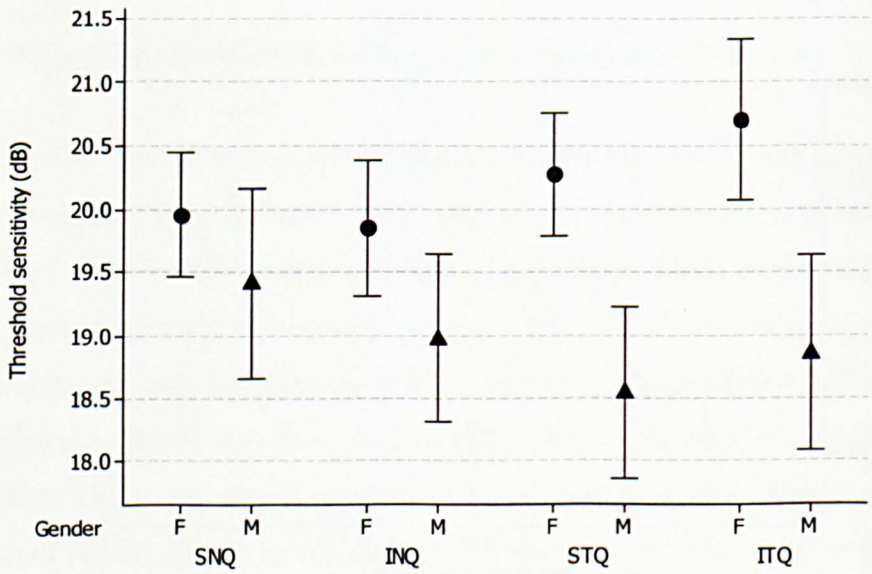


Figure 9.15 Male (▲) and female (●) sensitivity by field quadrant (~10 cd m^{-2})

There were no statistically significant findings from balanced MANOVA.

Retinal Eccentricity at ~1 cd m^{-2}

Viewing through the ND 2.0 filter, group mean threshold sensitivities for the 'centre', 'inner' and 'outer' test areas are shown for each respiratory condition in Figure 9.16. Balanced ANOVA identified a statistically significant main effect only of retinal eccentricity ($p = 0.034$), such that sensitivity was consistently greatest for the 'inner' data and least for the 'outer' data under all respiratory conditions.

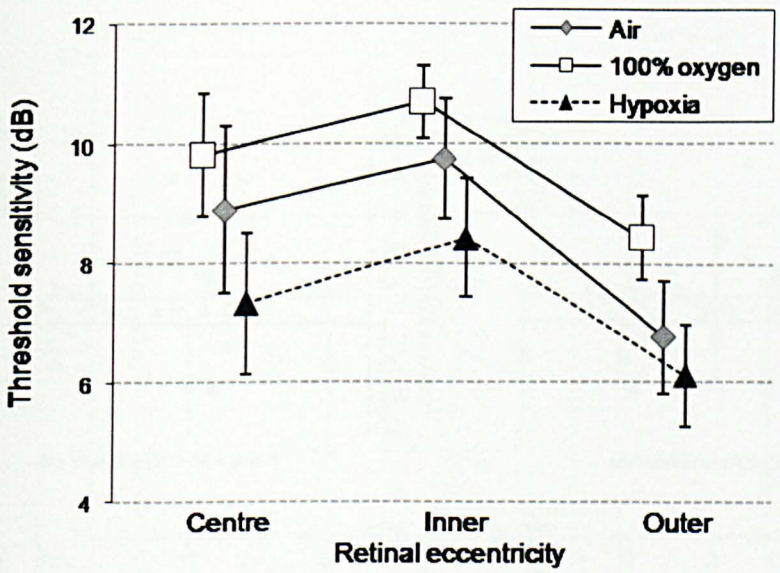


Figure 9.16 Mean (± SE) threshold sensitivity by retinal eccentricity (~1 cd m^{-2})

Consistent with the appearance in Figure 9.16, a main effect of breathing gas was close to achieving statistical significance ($p = 0.053$), sensitivity being greatest with 100% O_2 and least with hypoxia at all eccentricities. Within-subjects paired data were analysed to further assess the relative influences of paired breathing gases at this light level. The resulting data sets did not have a normal distribution so Wilcoxon signed rank tests ($\alpha = 0.05$) were conducted, confirming statistically significant differences between hyperoxia and hypoxia (O-H, $p < 0.001$), and between normoxia and hypoxia (A-H, $p = 0.016$), but not between hyperoxia and normoxia (O-A, $p = 0.11$). These paired conditions are illustrated using Bland-Altman absolute difference plots with the data for all eccentricities pooled for each analysis (left hand graphs in Figure 9.17). The range of sensitivities on the abscissa extends over one order of magnitude, so differences on the ordinate are also shown as a percentage of the mean sensitivity under the two conditions (Dewitte, Fierens, Stöckl and Thienpoint, 2002), weighting the differences in favour of

larger effects at low sensitivity and against smaller effects at high sensitivity (right hand graphs in Figure 9.17). Outlying values are contributed inconsistently by different subjects. Mean absolute differences are almost identical for the A-H (1.19) and O-A (1.18) data, which therefore contribute almost equally to the highly statistically significant O-H difference (2.37). Furthermore, expressing the differences as percentages of mean sensitivity gives very similar mean ± 95% limits of agreement for the A-H (20 ± 108) and O-A (19 ± 105) data. Both the A-H and the O-A differences appear meaningful, appearing to contribute similarly to the highly statistically significant O-H difference.

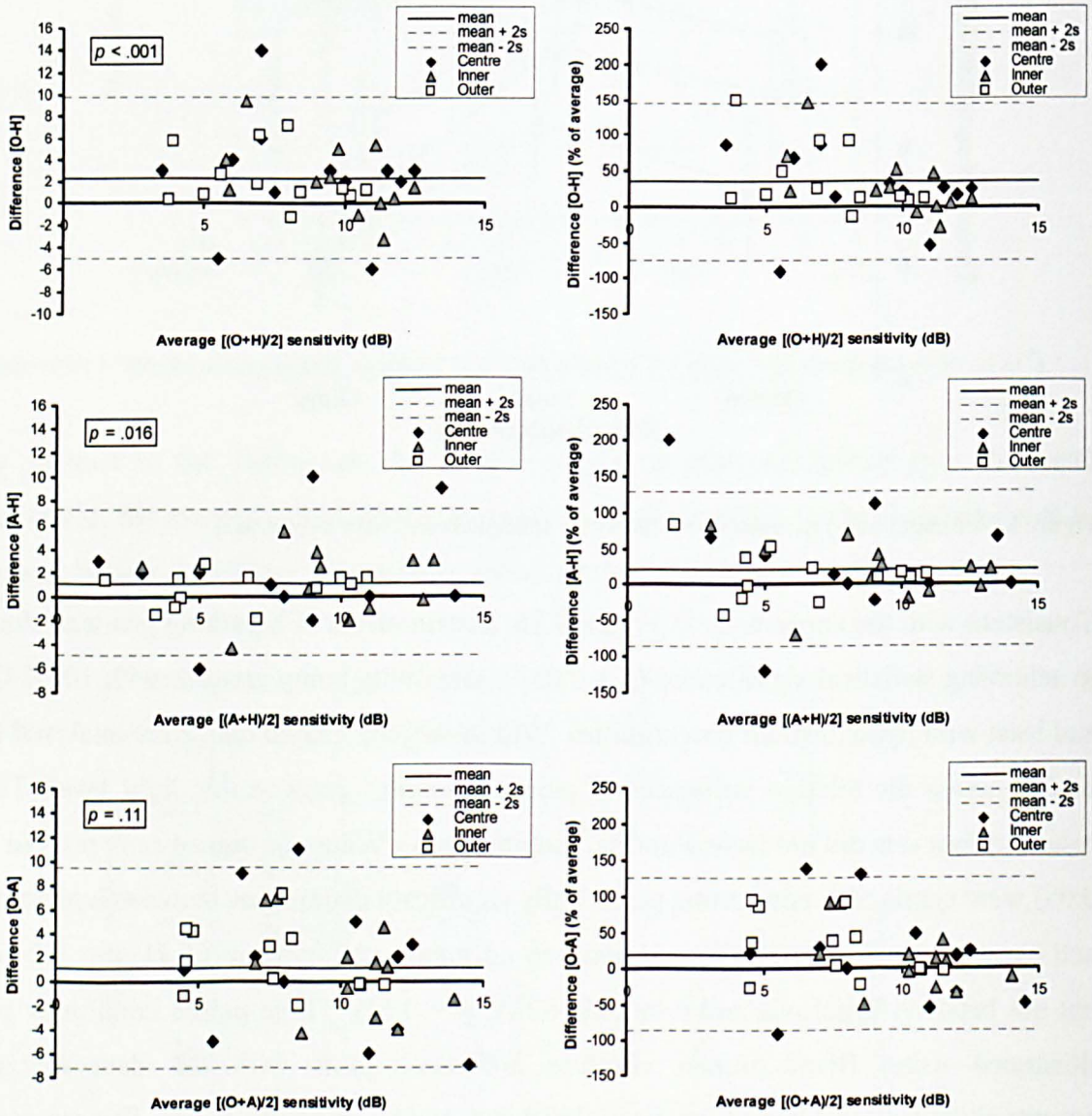


Figure 9.17 Bland-Altman difference plots of paired respiratory data (see text); Wilcoxon *p* values

Field Quadrant at ~1 cd m^{-2}

Mean (± SD) normoxic field quadrant sensitivity when viewing through the ND 2.0 filter was only 7.52 ± 3.44 dB. Thus, sensitivity to the FDT stimulus was grossly diminished at this light level. For these data balanced ANOVA revealed a main effect only of breathing gas ($p = 0.007$) with no effect of field quadrant (0.709). The effect is consistent across all field quadrants and is represented in Figure 9.18. In all field quadrants, hyperoxia enhances sensitivity while poorest sensitivity is seen under hypoxia.

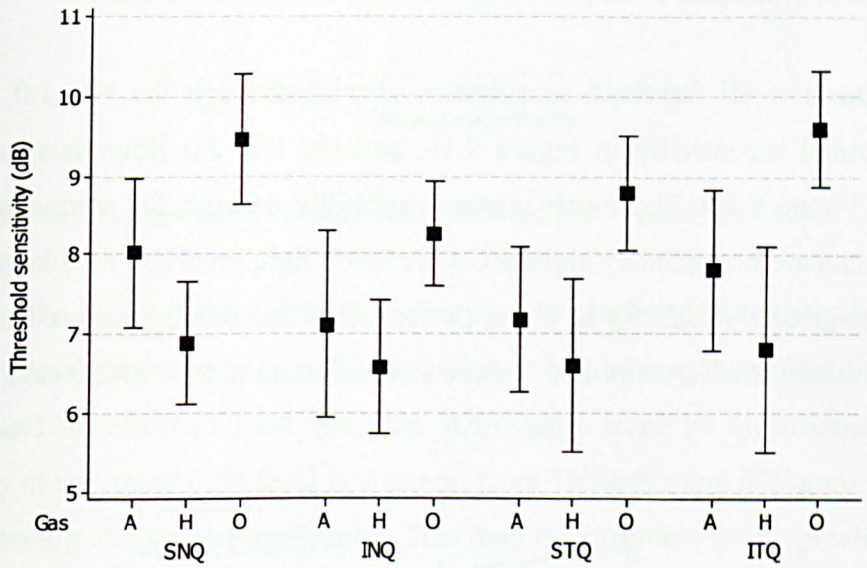


Figure 9.18 Effect of breathing gas (PO_2) on mean (± SE) field quadrant sensitivity (~1 cd m^{-2})

A statistically significant interaction between gender and respiratory exposure order sub-group was also evident on balanced ANOVA ($p = 0.002$) and is, again, attributable to allocation bias of subjects with differing underlying visual sensitivities to different sub-groups. There were no statistically significant effects from balanced MANOVA.

Hypocapnia

A total of 10 subjects (5M, 5F) completed the hypocapnia exposures when viewing through the ND 1.0 filter, but only 6 (5M, 1F) did so when viewing through the ND 2.0 filter. The drop out rate is attributable to general discomfort and fatigue associated with prolonged wear of the AR5 respirator, which is likely to have appeared subjectively to be more oppressive and claustrophobic at the lowest light level. The subjects completing the hypocapnia exposures achieved consistent $P_{ET}CO_2$ values close to the target level of 25 mm Hg. Group mean ± SD sensitivity during the hypocapnia

exposures are compared to those achieved during normoxia exposures in Table 9-3, showing a consistent increase in sensitivity with hypocapnia at each retinal eccentricity and in all field quadrants. The increase in sensitivity is greater at the higher light level, viewing through the ND 1.0 filter.

			Retinal eccentricity Mean ± SD (dB)			Field quadrant Mean ± SD (dB)			
Viewing condition	Number of subjects	Respiratory condition	Centre	Inner	Outer	SNQ	INQ	STQ	ITQ
ND 1.0 filter	10	Normoxia	21.5±2.1	21.8±1.0	18.3±1.4	19.1±1.7	19.3±1.8	18.9±1.4	19.5±2.8
		Hypocapnia	23.7±2.6	23.2±1.9	20.7±3.3	21.1±3.5	21.3±3.4	20.7±3.1	22.3±3.2
ND 2.0 filter	6	Normoxia	9.8±5.7	9.5±3.0	6.7±3.1	7.9±3.3	6.9±3.6	7.5±3.2	7.3±3.2
		Hypocapnia	11.5±3.7	10.8±2.6	7.5±2.0	9.4±3.6	7.6±1.4	8.7±2.1	7.7±2.5

Table 9-3 Effect of hypocapnia on FDT threshold sensitivity at reduced luminance

The group mean ± SE threshold sensitivities viewing through the ND 1.0 filter are shown by retinal eccentricity in Figure 9.19, and the ND 2.0 filter data are shown similarly in Figure 9.20. The much greater variability of both the normocapnia and hypocapnia data are immediately apparent at the lower light level and are likely to result both from the greater variability in visual performance that seems to occur at low light levels and from the smaller number of subjects contributing to the ND 2.0 data set.

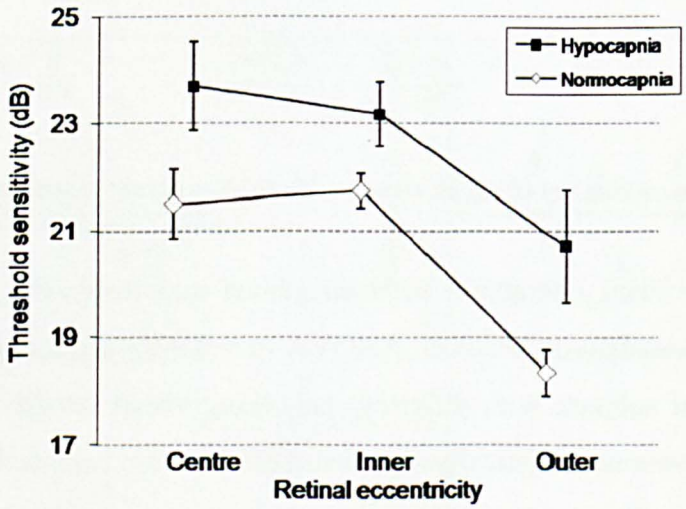


Figure 9.19 Effect of hypocapnia on mean (± SE) sensitivity at ~10 cd m^{-2} (N = 10)

At each light level, the available normocapnia and hypocapnia data were analysed for main effects and interactions of respiratory condition and either retinal eccentricity or field quadrant using two-way ANOVA ($\alpha = 0.05$). Main effects of both eccentricity ($p < 0.001$) and respiratory condition ($p = 0.001$) were evident for the ND 1.0 data (Figure 9.19). Two-way ANOVA applied to the ND 1.0 field quadrant data produced an equally

convincing effect of hypocapnia to enhance sensitivity ($p = 0.001$) across the visual field, with no effect of field quadrant ($p = 0.596$).

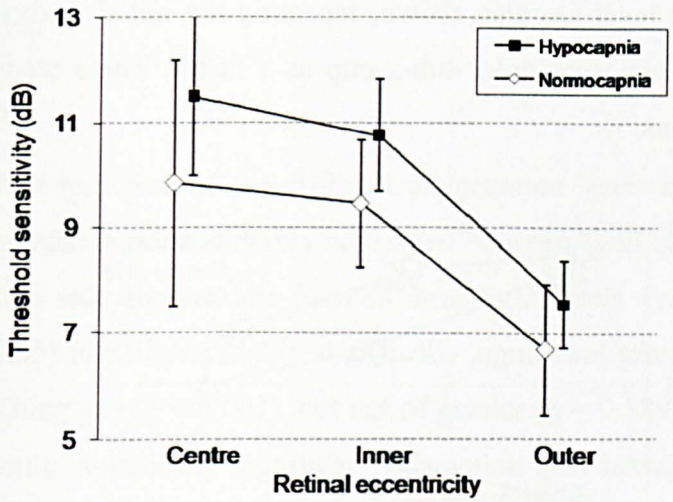


Figure 9.20 Effect of hypocapnia on mean (± SE) sensitivity at ~1 cd m^{-2} (N = 10)

Analysing the ND 2.0 filter data in the same way produced a statistically significant effect of retinal eccentricity ($p = 0.044$), but no effect of respiratory condition in either analysis and no effect of field quadrant. Although a trend of hypocapnia to enhance sensitivity at the lower light level is apparent from Table 9-3 and in Figure 9.20, it is far from achieving statistical significance. This may be attributed to the greater variability in response between subjects at this light level and an inadequate number of subjects completing this condition.

9.4.3 Extended *post hoc* analysis

Retinal eccentricity

Accepting that the Studies A and B were conducted using slightly different methodologies while nonetheless achieving comparable cardio-respiratory challenges, and having excluded any meaningful interactions between exposure order sub-group and breathing gas condition, a global ANOVA, encompassing all three retinal eccentricity data sets, was conducted to consider the net influence of PO_2 ($\alpha = 0.05$). Highly statistically significant effects of light level ($p < 0.001$), retinal eccentricity ($p < 0.001$) and breathing gas ($p < 0.001$) were seen, with no main effect of gender ($p = 0.264$) and no statistically significant interactions.

The changing nature of sensitivity to the FDT stimulus with respect to retinal eccentricity and oxygenation state is shown for all three light levels in Figure 9.21. The results of balanced ANOVA for main effects of retinal eccentricity, breathing gas and gender at each light level are also shown, repeating the initial analyses at each light level but excluding exposure order sub-group as a factor. There were no statistically significant interactions.

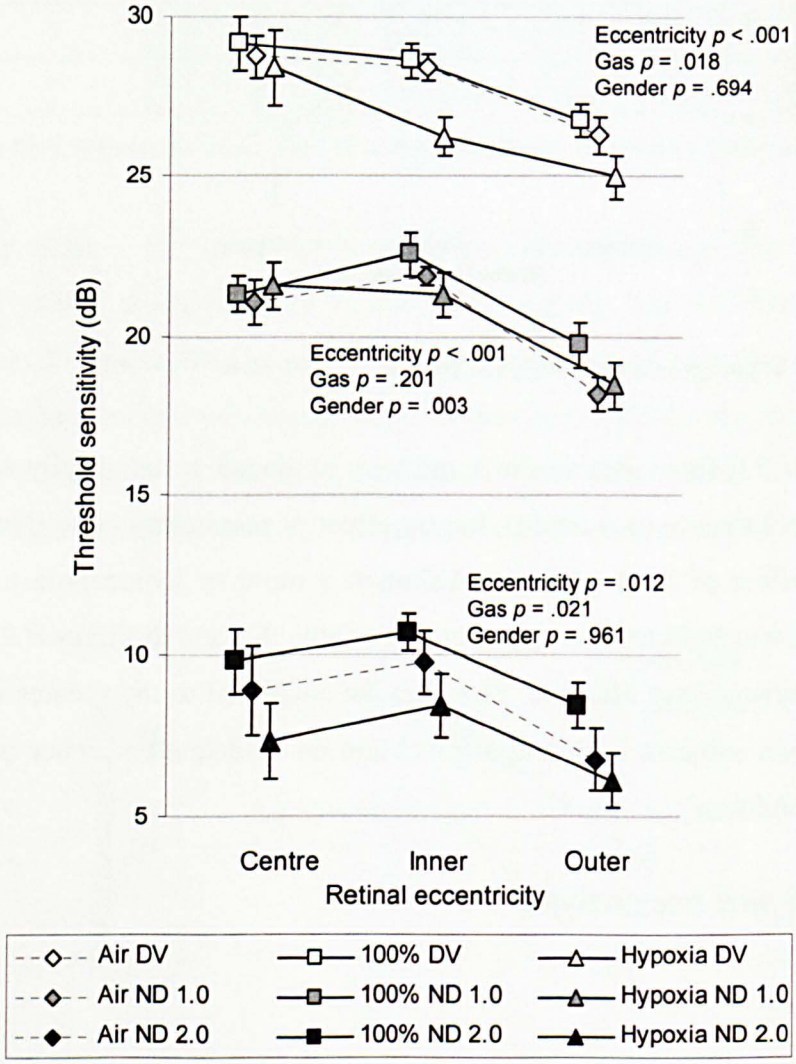


Figure 9.21 Mean (± SE) sensitivity by light level, retinal eccentricity and oxygenation state

Further *post hoc* analysis was conducted on the data at each light level using Tukey's Honestly Significant Difference to identify clearly significant differences between the breathing gas conditions ($\alpha = 0.05$). At 100 cd m^{-2} the hypoxia data were significantly worse than the hyperoxia data ($p = 0.019$) with a trend to be worse than the normoxia data ($p = 0.085$). Viewing through the ND 1.0 filter (~10 cd m^{-2}) there were no significant differences between the breathing gases. Viewing through the ND 2.0 filter

(~1 cd m^{-2}) sensitivity was significantly poorer under hypoxia than when breathing 100% O_2 ($p = 0.015$), with intermediate thresholds breathing air that were homogeneous with either the hypoxia or the hyperoxia subsets, consistent with the Bland-Altman assessment earlier.

Field quadrant

The field quadrant responses to the different oxygenation states at each light level are shown in Figure 9.22 to be remarkably consistent between field quadrants. As with the retinal eccentricity analysis, the data from all three light levels were subject to a global ANOVA ($\alpha = 0.05$) identifying highly statistically significant effects of light level ($p < 0.001$) and breathing gas ($p < 0.001$), but not of gender ($p = 0.189$) or field quadrant ($p = 0.312$). The only statistically significant interaction was between gender and light level ($p = 0.041$).

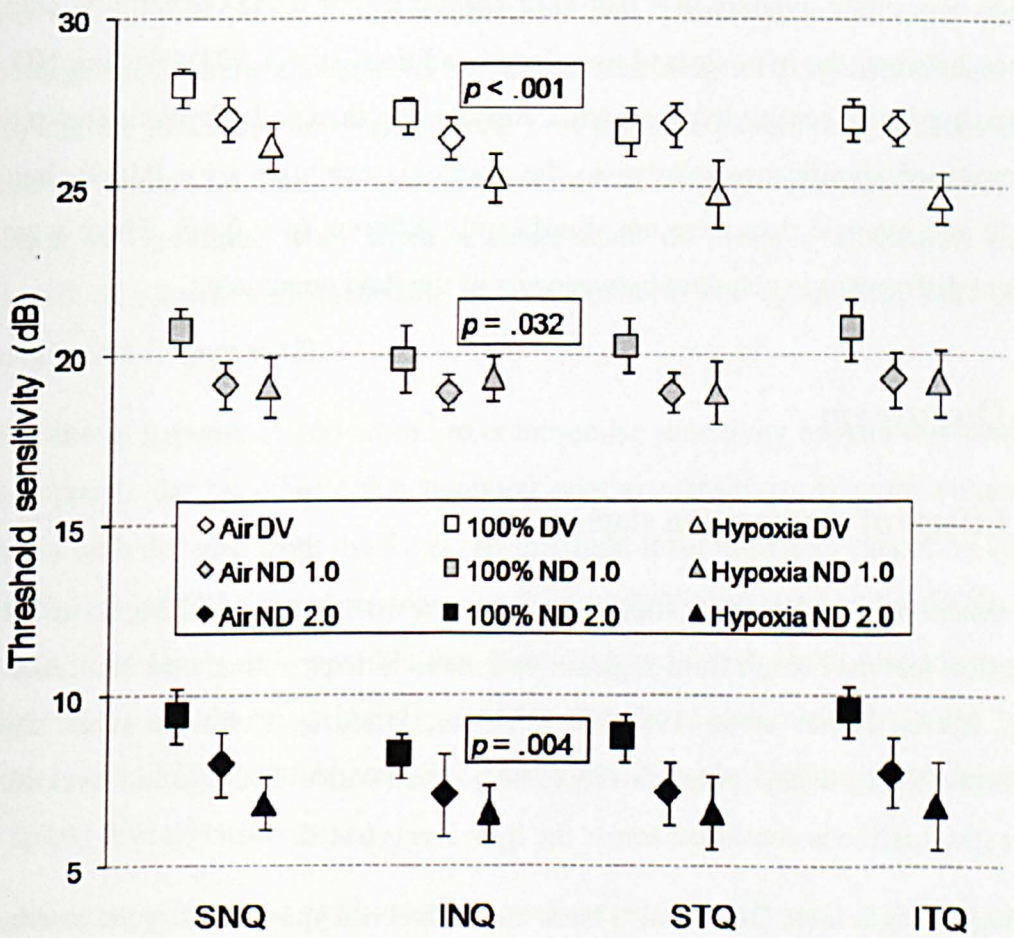


Figure 9.22 Effect of oxygenation state on mean field quadrant (± SE) FDT sensitivity

Turning to the analyses at each light level, repeat balanced ANOVA on the data measured at 100 cd m^{-2}, excluding consideration of exposure order sub-group, reaffirmed the highly statistically significant effect of hypoxia to compromise sensitivity beyond the fovea ($p < 0.001$), with no other statistically significant findings. Viewing through the ND 1.0 filter, repeat balanced ANOVA identified main effects of breathing gas ($p = 0.032$) and gender ($p = 0.008$). For the ND 2.0 filter data, balanced ANOVA identified a main effect only of breathing gas ($p = 0.004$). There were no statistically significant influences of field quadrant either when viewing directly ($p = 0.27$) or through the ND 1.0 ($p = 0.918$) or the ND 2.0 filter ($p = 0.672$), nor any interaction between field quadrant and breathing gas when viewing directly ($p = 0.951$) or through the ND 1.0 ($p = 0.997$) or ND 2.0 filter ($p = 0.999$). *Post hoc* analyses were again conducted using Tukey's Honestly Significant Difference to assess the relative influences of the breathing gases at each of the lower light levels ($\alpha = 0.05$). For the ND 1.0 condition, hyperoxic sensitivity just failed to achieve a statistically significant advantage over either hypoxic ($p = 0.067$) or normoxic ($p = 0.052$) sensitivity, with no difference between the hypoxia and normoxia conditions ($p = 0.993$). For the ND 2.0 condition, hyperoxic sensitivity was significantly greater than under hypoxia ($p = 0.003$) and approached significance relative to the normoxic condition ($p = 0.079$), but the normoxic and hypoxic data were not significantly different ($p = 0.46$). There were no significant differences in response between any of the field quadrants.

9.5 *Discussion*

9.5.1 Effects of oxygenation state

Visual sensitivity to the FDT stimulus appears resistant to any changes in retinal oxygenation that may result from regional variations of inner retinal vascular reactivity (Chung, Harris, Halter *et al*, 1999; Roff Hilton, Hosking, Cubbidge *et al*, 2003). Furthermore, the tendency for sensitivity to be greatest with 100% O_2 and least with the hypoxic gas mixture is consistent across the light levels tested.

At the highest light level (100 cd m^{-2}) there is an effect of hypoxia to degrade sensitivity beyond the fovea when examining the data by retinal eccentricity and this was supported by the field quadrant analysis. The findings support those of Yap, Garner, Legg *et al* (1995) that temporal contrast sensitivity is unaffected by mild hypoxia at the fovea. However, temporal contrast thresholds appear much more vulnerable away from

fixation. The geometric centres of the four test areas providing the 'inner' data lie just over 9° from the centre of the test display, suggesting significant impairment at the boundary of the macula, ~18° in diameter (Sharma and Ehinger, 2003), and beyond.

At the intermediate light level (~10 cd m^{-2}) there is no significant effect of hypoxia to compromise sensitivity but the field quadrant analysis suggests a slight benefit of supplementary O_2 to enhance sensitivity beyond the 'centre' test location (Figure 9.13 and Figure 9.14) relative to normoxic performance. This was very close to achieving *post hoc* statistical significance using Tukey's test ($p = 0.052$). Given that rod QO_2 increases as light levels fall, a benefit of supplementary O_2 to enhance sensitivity at this light level suggests possible facilitation of rod recruitment. A similar and consistent pattern of response was seen when examining the data by field quadrant (Figure 9.22).

At the lowest light level (~1 cd m^{-2}) hypoxia compromised and hyperoxia enhanced sensitivity across the central visual field (Figure 9.18). While the same pattern of altered sensitivity in relation to retinal eccentricity was preserved under each respiratory condition, that pattern appeared to be shifted according to oxygenation state (Figure 9.16) and the fovea was no longer spared. This response pattern suggests that threshold sensitivity to the FDT stimulus is O_2-dependent at this light level and, therefore, that rod function is O_2-limited even when a stable state of mesopic adaptation has been achieved. A similar and consistent response pattern was again seen with the field quadrant data (Figure 9.22).

The effect of hypoxia at 100 cd m^{-2} to compromise sensitivity beyond the 'centre' test area supports the hypothesis that temporal contrast sensitivity is more vulnerable to hypoxia with distance from the fovea. At this light level the effect cannot be attributed to increased rod QO_2 as rod responses will be virtually saturated and rod QO_2 will be close to basal levels. It is possible that increasing rod density with movement towards the periphery results in a high local QO_2 that is compromised by mild hypoxia. Certainly it is difficult to see how such mild hypoxia might exert an inner retinal effect that spares foveal sensitivity.

The changing patterns of response to hypoxia at ~10 cd m^{-2} and ~1 cd m^{-2} support a progressive increase in QO_2 at lower mesopic light levels as well as the contention that supplementary O_2 enhances temporal contrast sensitivity away from the fovea and in dim light. More specifically, breathing 100% O_2 benefits field quadrant sensitivity at all

light levels by comparison to hypoxia, implying that supplementary O_2 would benefit temporal contrast sensitivity at 10,000 ft, relative to breathing air at that altitude.

9.5.2 Gender

In contrast to the bias incurred when grouping subjects by exposure order, there were no underlying gender differences in visual sensitivity, under normoxic conditions, at any light level, although statistical significance was approached for the field quadrant data at 100 cd m^{-2} ($p = 0.056$). The difference in underlying nasal hemifield sensitivity between males and females then became apparent with repeat testing under the various respiratory conditions and was manifest on balanced MANOVA, with males having consistently greater sensitivity on that side of the visual field.

Right-handed, right-eye-dominant females have exhibited reduced sensitivity over the left hemifield using static Humphrey (24-2) perimetry, with no such difference apparent in male controls, no significant difference between the sexes for both eyes combined, or between the two eyes for either gender (Cohn, DeAgostini, Aron-Rosa *et al*, 1994). The finding was not related to the timing of the ovarian cycle. A recent study of randomly selected eyes found statistically significant, reduced, left hemifield sensitivity in right-handed females during the luteal phase of the ovarian cycle, but not during the follicular phase, using short wavelength automated perimetry (Akar, Zulauf, Yucel *et al*, 2005). In Study A, female sensitivity was consistently disadvantaged over the nasal hemifield by comparison with male sensitivity, adding FDT to the list of perimeter tests that have identified a gender-related difference in sensitivity across the vertical meridian, when accounting for ocular dominance. However, the gender difference in our subject group suggests greater male sensitivity over the nasal field, rather than a deficit in female sensitivity, at 100 cd m^{-2} (Figure 9.9).

Other (non-physiological) female sample groups have exhibited a tendency to reduced nasal field sensitivity during the luteal phase of the ovarian cycle (Apaydin, Akar, Akar *et al*, 2004; Yucel, Akar, Dora *et al*, 2005). In Study A, the females comprised three, presumed anovulatory, right-eye-dominant females using oral contraception, tested on days 7, 13 and 17; two left-eye-dominant, cycling females tested on days 14/26 and 15/33; and one right-eye-dominant, oligomenorrhoeic female tested on day 38/49. Thus, the data from this heterogeneous female sample exhibit no obvious trend in relation to stage of the ovarian cycle. Instead, the results suggest an underlying difference in hemifield sensitivity of the dominant eye between our male and female subjects at this

light level. This is already sufficient to refute the original hypothesis, that gender is unlikely to influence temporal contrast sensitivity under respiratory challenge.

A main effect of gender was also apparent at all eccentricities and in all field quadrants when testing at ~10 cd m^{-2}. However, this time female sensitivity was consistently greater than male sensitivity, and especially so over the temporal field quadrants. The male advantage over the nasal field at 100 cd m^{-2} turned into a disadvantage over the temporal field at the lower light level (Figure 9.15). In this experiment, the three females using oral contraception were tested on days 10, 14 and 15; the two cycling females were tested during the luteal phase, on days 5 and 12; and the oligomenorrhoeic female was tested on day 44 of the same cycle as previously. There was no significant difference between males and females at the lowest light level. This time the females using hormonal contraception were tested on days 8, 9 and 9; the cycling females were tested during the follicular phase on days 24 and 26; and the oligomenorrhoeic female was tested on day 15 of a cycle of unknown duration.

It is possible that testing at different stages of the ovarian cycle and the use of hormonal contraception by some of the female subjects may have confounded female sensitivity to the FDT stimulus at the different light levels (Wong and Tong, 1974; Ward, Stone and Sandman, 1978). Otherwise, there is no obvious explanation for the fluctuating gender differences in sensitivity over the nasal and temporal hemifields at the different light levels.

In Study A, hyperoxia was observed consistently to enhance female sensitivity by comparison to normoxia, while male sensitivity did not benefit. The relevance of this apparent gender difference in response to O_2 is also unclear but it may result, at least in part, from underlying differences in male and female susceptibility to visual disturbance across the vertical meridian. This is not necessarily a retinal phenomenon and may reflect a more fundamental gender difference in higher order visual processing (Kaufmann, Eibel, Gössl *et al*, 2001). Detailed assessment would require testing at set stages of the ovarian cycle and exclusion or consideration of the influences of hormonal contraception. At least at the lower light levels, both genders responded similarly to the various respiratory challenges, indicating consistent effects of breathing gas between males and females at lower light levels.

9.5.3 Hypocapnia

From Study A, the results obtained do not support the original hypothesis, that 'hypocapnia may enhance temporal contrast sensitivity, particularly at photopic luminance'. However, Study A was not designed specifically to examine this question and it is possible that the other prior respiratory disturbances may have confounded the hypocapnia results. On the other hand, the results at 10 cd m^{-2} do support an effect of hypocapnia to enhance temporal contrast sensitivity across the central visual field. A similar but smaller and less convincing trend was apparent at the lowest light level tested. However, the hypocapnia data from Study B should be interpreted with just as much caution as those from Study A on the basis that they might also have been confounded by the effects of other preceding respiratory disturbances and not all subjects completed the hypocapnia conditions. On balance it seems likely that a larger study would demonstrate that hypocapnia does enhance temporal contrast sensitivity.

9.5.4 Lessons for subsequent studies

Subjects

When considering the use of female subjects it is advisable to balance the numbers of males and females in order to at least identify the existence of potentially confounding gender influences on the results. However, while this may allow identification of whether or not an attribute may be vulnerable to gender-related influences, it is highly unlikely, in a small study, that the underlying mechanisms of such effects will be discernible. This would require consideration of the effects of hormonal contraception and testing at set times of the ovarian cycle.

Experimental design

Balancing the subjects by gender helped to eliminate any confounding influences that gender may have had to invalidate subsequent statistical analysis of the effect of breathing gas. However, checking for statistically significant gender differences in underlying normoxic sensitivity between the males and females was insufficient to prevent a gender difference from becoming manifest during repeated testing at 100 cd m^{-2} and again at ~10 cd m^{-2}. Possible confounding effects of gender must be considered when using both male and female subjects in studies of this nature.

Balancing the subjects by exposure order was important to avoid and account for any unknown and potentially confounding influences of successive respiratory conditions that could have masked main effects of breathing gas disturbances. The design was successful in this regard but promoted initial misinterpretation of the differences in response between exposure order sub-groups due to allocation bias of subjects with underlying differences in baseline FDT sensitivity to the various sub-groups. This became manifest as main effects and interactions of exposure order but these were entirely attributable to subject allocation bias and were not due to exposure order *per se*. Future studies should continue to balance respiratory exposure orders but care should be taken when analysing subsequent data sets that are not fully factorial. In order to attribute effects to successive respiratory conditions on balanced ANOVA, sub-groups would need to be larger or composed of individuals with matched visual sensitivities, in terms of both mean and variance. Alternatively, all subjects could undertake all exposure orders to produce a fully factorial data set. Another alternative would be to undertake normoxic exposures first, providing baseline data that would not be affected by prior respiratory disturbance, and then balance for the order of the other two conditions or conduct a crossover study. Subjects could also be allocated to ensure that means and variances are balanced appropriately using data from 'training' tests. On the other hand, the exposure orders may be balanced as used here providing no attempt is made to make inferences about possible effects of successive respiratory challenges without first establishing the baseline visual performance of the subjects in each sub-group.

In retrospect, it would have been preferable to omit the hypocapnia exposures, knowing that paired comparison with the control data would probably be questionable, as this would have allowed both Study A and Study B to have been conducted using exactly the same methodology and respiratory equipment. However, while the criticism that different methodologies were employed cannot be denied, it is considered unlikely to have made a meaningful difference to the outcomes in either Study A or Study B.

Vision testing

This study was conducted intentionally with a natural pupil to establish the impact of respiratory disturbance as it might relate to aircrew visual performance during flight, that is, net of any contribution from changing pupil size. The choice of FDT perimetry was made for sound reasons and it would have been difficult, if not impossible, to measure pupil size in conjunction with the experimental methods employed in the

current experiments. Furthermore, FDT perimetry is said to be relatively unaffected by changes in pupil size, providing the pupil is at least 2 mm in diameter (Johnson and Sample, 2003). However, other studies have shown that performance on FDT perimetry is affected by retinal illumination and, therefore, pupil diameter (Kogure, Membrey, Fitzke *et al*, 2000; Swanson, Dul and Fischer, 2005). Accordingly, it would have been helpful to document any influence of changing pupil size with respiratory disturbance and to have related this to changes in FDT stimulus sensitivity. Where possible, estimates of pupil size should be made in future experiments if changes in retinal illumination might influence visual sensitivity and performance.

The transmission characteristics of the ND 2.0 filters over the right and left eyes were slightly different (Figure 7.6), such that the two female subjects with dominant left eyes will have received very slightly less light than the right-eye-dominant subjects. The data of these two female subjects is consistent with their peers and neither produced outlying results. However, such potentially confounding influences should be avoided in future.

Respiratory exposures and respiratory and visual adaptation

The use of the AR5 is undesirable as it so obviously causes discomfort to the subjects over time, and, in any case, is generally distracting and unpleasant to wear. Less imposing methods of administering respiratory challenges are preferable and should, ideally, be no more stringent than the use of a helmet and mask as employed in Study A. The AR5 was useful in enabling total control of contemporaneous visual and respiratory adaptation but acceptable control of both is likely to be achievable, with care, using more subject-friendly procedures.

9.5.5 Summary and future work

The net effects of altered oxygenation state on FDT sensitivity at the different light levels are summarised in Table 9-4.

It is emphasised that these findings relate to a single and very specific temporal contrast stimulus, comprising a vertical grating of relatively low *sf* oscillating at relatively high temporal frequency. It is not at all certain that the same qualitative and quantitative findings would result if temporal contrast stimuli of different specification were employed. An exploration of the effects of altered oxygenation state on a range of such stimuli would be worthwhile.

More severe hypoxic challenges might be expected to result in greater losses of sensitivity, but when considering such challenges care would have to be taken to account for any confounding influence of concomitant hypocapnia. The more interesting question is what magnitude of hypoxia is necessary to compromise temporal contrast sensitivity at the different light levels? More specifically, what is the lowest equivalent altitude at which acute exposure might result in impaired temporal contrast sensitivity? This might most readily be addressed by repeating elements of the current experiment using milder hypoxic gas mixtures.

Light level (cd m^{-2})	Effects of altered oxygenation state	Benefit of supplementary O_2 over hypoxia?
100	Hypoxia compromises sensitivity at and beyond the macular periphery.	Moderate, ~2 dB (Figure 9.6, Figure 9.10, Figure 9.21)
~10	Supplementary O_2 appears to enhance sensitivity beyond the macula, perhaps by facilitating early rod recruitment.	Slight, ~1-1.5 dB (Figure 9.14, Figure 9.22)
~1	Hypoxia compromises and hyperoxia enhances sensitivity across the visual field.	Substantial, ~2-3 dB (Figure 9.16, Figure 9.18, Figure 9.22)

Table 9-4 Summary of effects of altered oxygenation state on sensitivity to FDT stimuli

The subjects in this study were all under 40 y of age. Ageing is associated with decreasing temporal contrast sensitivity, for example to low *sf* drifting gratings when older than 60 y (Owsley, Sekuler and Siemsen, 1983; Sloane, Owsley and Jackson, 1988). The nature of the influence of hypoxia on temporal contrast sensitivity with increasing age is worthy of attention as one might expect reduced sensitivity and greater susceptibility to hypoxia in the older eye.

The nature of the interactions between gender, ocular dominance, hemifield sensitivity, light level and oxygenation state are, unsurprisingly, poorly understood. That the male and female subjects in the current experiments responded differently at different light levels is undeniable. Explaining those differences is, at best, speculative. As a first step, a study focusing on the assessment of gender differences in hemifield sensitivity of the dominant and non-dominant eyes, accounting for stage of the ovarian cycle, would be worthwhile, at least in helping to define female subject sample criteria for subsequent studies. A range of perimetric assessment methods should be considered, but in the context of the current study, threshold assessments conducted using FDT Matrix perimetry could provide more data than standard FDT perimetry, with greater spatial resolution of differences in retinal sensitivity with eccentricity.

10 Contrast Acuity

10.1 Abstract

PURPOSE. Visual acuity is poor under low luminance, low contrast conditions that may be relevant in aviation but the effect of changes in oxygenation state on low contrast acuity in dim light is poorly documented. This study examined effects of hypoxia and hyperoxia on the contrast acuity thresholds of 12 healthy subjects (6M, 6F) at low photopic (12 cd m^{-2}), upper mesopic (1 cd m^{-2}) and mid-mesopic (0.1 cd m^{-2}) background field intensities.

METHODS. The Contrast Acuity Assessment (CAA) test was used to measure binocular contrast acuity thresholds (CAT) at fixation and eccentricities of ± 1.25°, ± 2.5° and ± 5° from fixation under conditions of mild hypoxia (breathing 14.1% O_2), hyperoxia (100% O_2) and normoxia (air). Contemporaneous respiratory and visual adaptation were followed by vision testing at the dimmest background field and then at progressively higher luminance. Respiratory exposure orders were randomised and balanced between the male and female subjects to exclude an interaction between exposure order and breathing gas condition. Pupil diameter was measured before and after each vision test using infrared imaging to enable consideration of the effect of pupil size on CAT. Pupil size and CAT data were analysed using repeated measures, balanced ANOVA.

RESULTS. Hypoxia promotes statistically significant elevation of CAT (decreased acuity) at all light levels and eccentricities. The effect of hypoxia at 12 and 1 cd m^{-2} is consistent for all retinal eccentricities examined, amounting to a mean 26-28% increase in contrast to maintain gap discrimination. At 0.1 cd m^{-2} supplementary O_2 enhances low contrast acuity over that achieved breathing air, while hypoxia compromises contrast acuity relative to normoxia. The hypoxic degradation in contrast acuity, relative to that achieved with hyperoxia, is greatest at fixation and amounts to a 50% increase in contrast (0.17 $\log_{10}$ unit) to maintain acuity. Mean pupil size decreases with hypoxia at all light levels, by about 0.4 mm, while pupil size increases slightly with hyperoxia at higher background field intensities. There is no direct correlation between the effect of oxygenation state on pupil size and its effect on CAT.

CONCLUSIONS. Contrast acuity is vulnerable to mild hypoxia at low photopic and upper mesopic light levels. Supplementary O_2 enhances low contrast acuity over that achieved breathing air at 0.1 cd m^{-2}. Effects of oxygenation state on the pupil do not explain the effects on CAT, such that low contrast acuity does not appear to be influenced meaningfully by slight changes in retinal illumination. At the light levels tested, the effect of hypoxia to promote a loss of acuity for fine detail is consistent and may warrant consideration of the use of supplementary oxygen in aviation.

CITATION. The work in this chapter is being prepared for peer review. This will include an assessment of the possible influences of macular pigment optical density and higher order aberrations on contrast acuity in relation to oxygenation state (Wooten and Hammond, 2002).

10.2 Introduction

10.2.1 Background

The experiments reported thus far have revisited the effects of respiratory disturbances on most of the visual performance attributes reviewed in section 1.6, with the obvious exception of VA. The studies by Rose (1949) and Leber, Roscoe and Southward (1986) suggested effects of relatively mild hypoxia on acuity-related measures, but the former was conducted in relation to recovery of visual sensitivity during dark adaptation, while the latter employed contrast gratings rather than testing for resolution of small visual targets. Otherwise, studies reporting an effect of hypoxia to degrade VA have either assessed acuity at very low (dark adapted) light levels or have imposed moderate to severe hypoxia equivalent to breathing air at equivalent altitudes well in excess of 10,000 ft.

The importance of considering VA in relation to background luminance and stimulus contrast was demonstrated by Johnson and Casson (1995), using Landolt C targets, with clear relevance to mesopic visual performance. Furthermore, an effect of hypoxia to compromise rod photoreceptor function at low light levels might be expected to impact adversely on low contrast acuity in the mesopic range. Under photopic conditions VA falls rapidly with increasing distance from fixation. At reduced light levels, an increasing rod contribution with eccentricity will benefit acuity but may be compromised by progressive effects of hypoxia as light level decreases. Thus, effects on net VA may be expected to vary in relation to testing at different retinal eccentricities and light levels, as well as between respiratory conditions. No studies to date appear to have assessed the effects of respiratory disturbance on VA to low contrast targets presented at functionally and occupationally meaningful background light levels. This final experimental chapter addresses this issue.

The Contrast Acuity Assessment (CAA) test was developed at the Department of Optometry and Visual Science of the Applied Vision Research Centre at City University, London, on behalf of the UK Civil Aviation Authority, to assess visual performance post-surgical correction of refractive error (Chisholm and Barbur, 2001). In addition, details of the test's development, validation and the limits of the 'standard normal observer' have been published (Chisholm, Evans, Harlow *et al*, 2003). The CAA test is perfect for assessing low contrast VA, at up to ± 5° of visual angle from fixation along the horizontal meridian, under a variety of ambient lighting conditions and

respiratory challenges. Furthermore, the initial CAA test parameters (target size, light levels and test eccentricities) were based on a detailed assessment of modern flight deck instrumentation design in commercial aviation. Of particular note, the test is sensitive to changes in contrast acuity thresholds that occur at low light levels and, hence, are relevant to night flying in the mesopic cockpit or flight deck (Barbur, Walker and Chisholm, 2005).

VA increases with pupil size as more light contributes to the retinal image until a pupil diameter of about 2.5 mm is achieved. It is then largely unaffected as pupil diameter increases to about 4 mm (Boff and Lincoln, 1988, p 226). At lower light levels, larger pupils will tend to be associated with increased optical aberrations which will compromise the quality of the retinal image. Accordingly, an assessment of low contrast acuity under respiratory disturbance must consider effects on pupil size and consequent changes in retinal illumination and optical aberrations. In this study, measurements of pupil diameter were made before and after each contrast acuity test.

10.2.2 Hypotheses

Mild hypoxia might compromise low contrast acuity progressively with increasing retinal eccentricity and decreasing light level.

Supplementary O_2 might enhance contrast acuity, particularly beyond the fovea and at dimmer light levels.

10.2.3 Aims

The primary aim of the work in this final section was to document any effects of hypoxia, and any benefits of supplementary O_2, on low contrast acuity at a range of photopic and mesopic background light levels, and at various retinal eccentricities up to $\pm$ 5° from fixation. In so doing, the underlying hypotheses were investigated (section 10.5.3).

As with previous work, it was intended subsequently to interpret the findings in relation to aircrew visual performance during flight and with respect to the use of current and future aircrew display systems (sections 10.5.1 and 11.1.3).

10.2.4 Experimental design

In keeping with previous experiments, contrast acuity was evaluated against low photopic, and upper and mid-mesopic background fields under hypoxic, hyperoxic and normoxic respiratory conditions. A display-based vision test was viewed directly and through ND filters to achieve upper and mid-mesopic levels (Figure 6.1). Subjects were again masked to the presentation order of the respiratory conditions. The process of concurrent visual and respiratory adaptation was modified to avoid the requirement to use the AR5 respirator. For each respiratory condition, successive vision tests were conducted at each of the background light levels, from dimmest to brightest.

In order to negate any possible effect of exposure order, a 15-20 minute recovery or 'washout' period was introduced between successive respiratory conditions such that vision tests began no sooner than 30-35 minutes following completion of a preceding respiratory condition. Thus, each subject undertook a total of nine contrast acuity assessments in one experimental session, comprising three vision tests during each of three respiratory exposures. The six possible orders of the normoxic, hypoxic and hyperoxic conditions were again randomised and balanced across the subject pool.

10.3 Materials and Methods

10.3.1 Subjects

In keeping with the previous experiments, the subject sample group was balanced by gender, with no expectation of a gender difference on contrast acuity testing. Twelve healthy subjects, comprising six males and six females, completed the study. Their mean (± SD) age at the time of the experiment was 28.3 ± 7.2 y (range 20.7 to 39.7 y) and there was no statistically significant difference between the ages of the males (28.3 ± 7.2 y, range 20.9 to 39.7 y) and the females (26.4 ± 6.9 y, range 20.7 to 37.5 y) on two-sample *t* test ($p = 0.394$).

Subject medical and ophthalmic screening requirements, exclusion and withdrawal criteria, training, payment, exposure limits, lifestyle requirements and compliance are as detailed in section 3.6.3. All subjects had healthy eyes and all achieved binocular Snellen acuity of 6/6 or better, with correction in four subjects. This was confirmed immediately prior to undertaking the experiment, viewing a backlit Snellen chart at a distance of 6 m in a dimly illuminated laboratory.

Female subjects undertook a urine test to exclude pregnancy before beginning each experiment. Three female subjects were using oral hormonal contraceptives at the time of the study. One of the other females was well-established on long-term isotretinoin (Roaccutane) therapy without side effects. Her data were not noticeably at odds with those of the other subjects.

There is a minimal learning component to performing the contrast acuity test, so each subject undertook representative vision test training before the experiment. Subjects were non-smokers who were asked to minimise caffeine and alcohol intake before an experiment. They were also asked to ensure a good night's rest before the experiment and one subject's study was rearranged to achieve this.

Contrast acuity measurements can be affected by higher order optical aberrations, particularly for larger pupil sizes, that is, under low light conditions (Barbur, Walker and Chisholm, 2005). In addition, individual variations in macular pigment density have been related to changes in visual performance measures including contrast acuity and colour discrimination (Kvansakul, Edgar, Barbur *et al*, 2004; Rodriguez-Carmona, Kvansakul, Harlow *et al*, 2006). In parallel to the assessment of the effects of respiratory disturbance on contrast acuity, each subject attended City University to undertake assessment of their macular pigment optical density, colour vision and higher-order (rms) wavefront aberrations, with the intention of correlating these with contrast acuity. These data are not reported here but may be published alongside the current work in due course.

10.3.2 Equipment

Subjects were fitted with a type P/Q aircrew O_2 mask, supported from a type 'G' cloth helmet, and an adequate and comfortable face-mask seal obtained. The first female subject to undertake the experiment required a special mask fit with short mask suspension chains and still struggled to maintain an acceptable face-mask seal. As a result, the quality of the imposed respiratory conditions was poor and her data could not be used. A replacement female subject was recruited who completed the experiment uneventfully.

As in previous experiments, the masks were modified to provide an access port to the mask cavity, enabling continuous respiratory gas analysis with an Innovision A/S Amis 2000 mass spectrometer. This was calibrated immediately before each imposed

respiratory condition, using a variety of gas mixtures of known composition, giving a measurement error of less than 1% for PO_2 and PCO_2 in the physiological range. The mass spectrometer traces were monitored in real time throughout every experiment to ensure that an adequate face-mask seal was maintained continuously.

As in previous experiments, the breathing gases for all exposures were supplied through dedicated Mk17F pressure-demand breathing gas regulators. The regulators supplied gas via a four-way selection tap to a common gas supply hose. The regulators had matching pressure/flow characteristics and all imposed minimal breathing resistance, so the breathing gases were indistinguishable to the subjects who remained unaware of the presentation order of the respiratory conditions.

Peripheral S_aO_2 was monitored using a Kontron 7840 pulse oximeter with a finger probe. Analogue outputs from the pulse oximeter and mass spectrometer were calibrated and recorded using an ADInstruments PC-based data recording and analysis system employing Powerlab/Chart software.

10.3.3 Respiratory conditions

Control exposures were conducted breathing air (A). Hypoxia (H) exposures were imposed by breathing 14.1% O_2 (balance N_2), to generate a P_AO_2 of 55-60 mm Hg, equivalent to breathing air at 10,000 ft and lowering peripheral S_aO_2 values to ~90%. Hyperoxia (O) was obtained by breathing 100% O_2.

The six possible exposure orders of these three respiratory conditions are AHO, AOH, OHA, OAH, HAO, and HOA. It was impractical for all subjects to undertake all exposure orders. Accordingly, they were balanced by gender such that one male and one female experienced each of the six possible exposure orders. In the previous study, respiratory exposures were imposed in quick succession. For this experiment, after each respiratory exposure the subject had a break that lasted no less than 15 minutes and no more than 20 minutes before starting the next respiratory condition. Each new condition was then imposed for 15 minutes before starting vision testing. Thus, an effect of prior respiratory exposure was considered exceptionally unlikely to persist sufficiently to influence testing under subsequent respiratory exposures. Accordingly, subjects were allocated randomly to the various exposure order sub-groups, recognising that interpretation of the effects of exposure order would rely on identification of statistically significant interactions between sub-group and breathing gas condition and that main

effects of sub-group would simply reflect allocation bias of subjects with varying contrast acuity.

The cardio-respiratory responses associated with these three respiratory conditions have been recorded in detail in earlier chapters and were not analysed in detail. In essence, the mild hypoxia condition is associated with a slight tachycardia while the $P_{ET}CO_2$ falls slightly when breathing 100% O_2, due to the Haldane Effect. Cardio-respiratory responses were monitored continuously throughout all experiments and were consistent with those reported previously. The respiratory challenges were imposed as intended and mask seals were well maintained by all 12 subjects completing the experiment.

10.3.4 Vision testing

The CAA test presents brief 'ring' stimuli that have a 'notch' or 'gap' in either the upper right, upper left, lower left or lower right quadrant. The size of the notch, one fifth of stimulus diameter, defines the acuity required to reliably determine the location of the notch as the differential brightness of the stimulus relative to its background, that is to say its contrast, is reduced. Thus, the stimulus is a 'Landolt C' rotated about its centre to one of four possible orientations. The subject's task is to identify the orientation of the 'C' by pressing one of four buttons on a response box, where each button corresponds to one possible location of the notch. Thus, the test employs a 4-AFC response paradigm well-suited to the determination of probabilistic psychophysical thresholds and for testing naive subjects (Jäkel and Wichmann, 2006). The Landolt ring is similar to many alphanumeric characters used in aircraft displays and is a simple visual stimulus for inexperienced observers to respond to.

The work conducted for the Civil Aviation Authority included a visual task analysis on a modern flight deck (Airbus A320) to determine relevant test parameters (Chisholm and Barbur, 2001; Barbur, Walker and Chisholm, 2005). Target size measurements indicated that the smallest alphanumeric characters of importance subtended 12 to 18 minutes of arc at the eye based on a mean viewing distance of 80 cm. Discrimination of these characters demands a minimum angle of resolution in the range 2.5 to 3.6 minutes of arc. Resolution of these relatively large characters is relatively easy for high contrast targets presented at photopic luminance. However, older aircraft instrument symbology and other objects of interest within the visual scene may be presented to the eye at lower light levels and much lower contrast. Furthermore, the spatial resolution of the retina falls rapidly with eccentricity, such that, even within the functional visual field

corresponding to the diameter of a typical aircraft display (± 5°), larger characters are required to enable resolution towards the periphery. This requirement is then exaggerated at lower light levels.

Normal data were established at two background light levels (Barbur, Walker and Chisholm, 2005). Photopic testing was conducted at 12 cd m^{-2}, a level above which increases in brightness have little benefit in enhancing contrast acuity. Mesopic testing was conducted at 0.05 cd m^{-2}, based on measured background light levels on the Airbus A320 flight deck at night. The CAA test presents stimuli at fixation and at ± 1.25°, ± 2.5°, and ± 5° from fixation along the horizontal meridian, seven test locations in all. The test determines the luminance contrast threshold at which the subject not only sees the Landolt ring but also discriminates correctly the orientation of its gap. To determine the appropriate size of stimulus to present at each location, initial experiments were conducted, first, to establish how spatial resolution changes with eccentricity at various fixed levels of contrast for each background luminance, and second, to establish the upper and lower contrast threshold limits for subjects with normal vision (Barbur, Walker and Chisholm, 2005). The results of these experiments allowed the target sizes at each retinal eccentricity to be adjusted such that the normal mean contrast acuity threshold (CAT), expressed as stimulus % contrast, is the same at all eccentricities. The variance of such limits in a normal sample group then defined the 'standard normal observer' for those tests.

The target sizes chosen for the test at photopic background luminance (12 cd m^{-2}) were those requiring a mean of 24% contrast for resolution at each eccentricity. Those chosen for the mesopic background (0.05 cd m^{-2}) required 48% contrast for resolution. The former gave target size thresholds that corresponded with the smallest angular subtense of the alphanumeric characters on flight deck displays. Higher contrast levels make VA less sensitive to retinal image degradation, while lower contrast levels demanded larger targets, were more difficult to perform reliably and promoted considerable variability between subjects. For the mesopic tests, lower contrast targets were extremely difficult to resolve and 48% contrast was chosen as the lowest level at which the test could be performed without undue difficulty. At each of these background field intensities, the correspondingly sized stimuli are located at transitional stages of respective curves indicating that changes in target contrast, resulting from retinal image degradation, are likely to have large effects on acuity. The size scaling experiments exhibited considerable variability between subjects, especially in the mesopic range, but there

were no significant differences between the nasal and corresponding temporal size thresholds to ± 5°. Subsequent testing of 100 normal observers gave mean (± SD) contrast acuity thresholds of 24 ± 8% at 12 cd m^{-2} and 48 ± 15% at 0.05 cd m^{-2}.

The current study intended to explore the changing nature of any effects of respiratory disturbance on visual performance at light levels decreasing from low photopic levels into the mesopic range. The obvious choice of background luminance for the photopic test, for the reasons outlined in the previous section, is 12 cd m^{-2}, employing target parameters established by the previous work. In order then to determine the influence of falling light level on any consequence of hypoxia, testing is most obviously performed under background light levels one and two orders of magnitude lower, achieved most readily by viewing an appropriately calibrated display through ND filters of corresponding OD. Using ND 1.0 and 2.0 filters, the CAA test and display monitor were calibrated to provide net background fields of 1.0 and 0.1 cd m^{-2}. The lowest of these light levels is sufficiently close to that employed by Barbur, Walker and Chisholm (2005) to allow use of the same target sizes with eccentricity, accepting that the resulting 'normal' data might not quite provide a steady contrast threshold across all retinal eccentricities.

The decision as to the target sizes to use for the 1 cd m^{-2} background field was more difficult as no underpinning size scaling work had been undertaken at this light level. It was decided to employ the same target sizes as for the photopic test at 12 cd m^{-2} on the basis that military aircrew might reasonably be required to discriminate alphanumeric characters of the same size at upper mesopic luminance and relatively low contrast, that is, functional vision involving high mesopic light adaptation is likely to require discrimination of 3 minutes of arc at fixation, just as it does at photopic luminance (corresponding to a Landolt ring subtending 15 minutes of arc). Thus, the chosen test at 1 cd m^{-2}, while unlikely to result in a flat CAT profile for all eccentricities, would be challenging, would give valuable data as to the loss of contrast acuity resulting from the transition from photopic to mesopic luminance, and should still be sensitive to any effects of altered oxygenation. For the lowest light level (0.1 cd m^{-2}), acuity would be expected to be substantially poorer and this light level is not frequently encountered in occupational tasks. At this light level a Landolt ring subtending 46 minutes of arc at the fovea presents a gap subtending 9 minutes of arc.

The Landolt C stimulus diameters employed at each retinal eccentricity are summarised for each background light level condition in Table 10-1. The requirement for a slightly

larger stimulus size at fixation than at ± 1.25°, when viewing at the lowest light level, results from the relative insensitivity of the fovea at reduced luminance due to the absence of rod photoreceptors.

ND filter optical density	Background light level (cd m^{-2})	Retinal eccentricity (degrees of visual angle from fixation)			
		0	± 1.25	± 2.5	± 5
None	12	15	17	21	32
1.0	1	15	17	21	32
2.0	0.1	46	44	48	61

Table 10-1 Landolt C stimulus diameter (in minutes of arc) at each retinal eccentricity

A fixation point was provided for 147 ms prior to stimulus presentation. The location of the fixation point is cued by four oblique guides that assist central fixation. Stimuli are presented for 187 ms, which is sufficiently brief to limit the influence of saccadic eye movements without compromising parvocellular processing speed. Saccade latency is generally greater than 200 ms. Baseline luminance and contrast parameters for each test are summarised in Table 10-2.

Background luminance (cd m^{-2})	Initial stimulus contrast (%)	Initial contrast increment (%)	Final contrast increment (%)	Fixation luminance (cd m^{-2})	Guide luminance (cd m^{-2})
12	56	12	1	6	3
1	80	16	1.6	0	0
0.1	100	20	2	0	0

Table 10-2 CAA test luminance and contrast parameters

A randomly interleaved staircase procedure with variable step sizes was used to assess contrast thresholds at each eccentricity. Two successive correct responses are required at each eccentricity before stimulus contrast is reduced for the next presentation at that location, reducing 'chance' correct responses to 1 in 16 when using a 4-AFC test procedure. A single incorrect response results in an increase in stimulus contrast the next time that eccentricity is chosen. A total of 12 reversals, above and below threshold, are estimated; the first six of these are ignored and the mean of the last six is calculated as the CAT.

The uniform background field was approximately equivalent to D65 'daylight' white at a colour temperature of 6500 K, with (x, y) chromaticity of (0.305, 0.323). An IBM-compatible PC ran the DOS-based CAA test program using a bespoke graphics card to drive a high resolution Sony Trinitron Multiscan G520 monitor. A luminance

calibration program was used in conjunction with a luminance photometer (LMT 1009) to calibrate automatically the luminance characteristics of the monitor, achieved by calibrating the luminance output against the applied voltage for each gun. The luminance calibration was similar to that shown in Figure 7.5. The monitor was allowed to warm up for at least an hour prior to calibration or testing in order to stabilise its luminance output. The spectral output of each display phosphor was measured using a telespectroradiometer (Minolta CS1000), providing the chromaticity coordinates of each phosphor. The phosphor calibration was very similar to that shown in Figure 7.4.

All testing was conducted binocularly and the test display was viewed along a matt black viewing tunnel from a distance of 150 cm. During the experiments, the laboratory was illuminated dimly by only three other displays, specifically those showing the mass spectrometer and pulse oximetry data that was being monitored and recorded; by the display used to set up and administer the vision tests; and by the display used for taking pupil measurements. The light from these displays was prevented from reaching the subject by a shroud from the viewing tunnel that encompassed the subject's upper torso when positioned for vision testing.

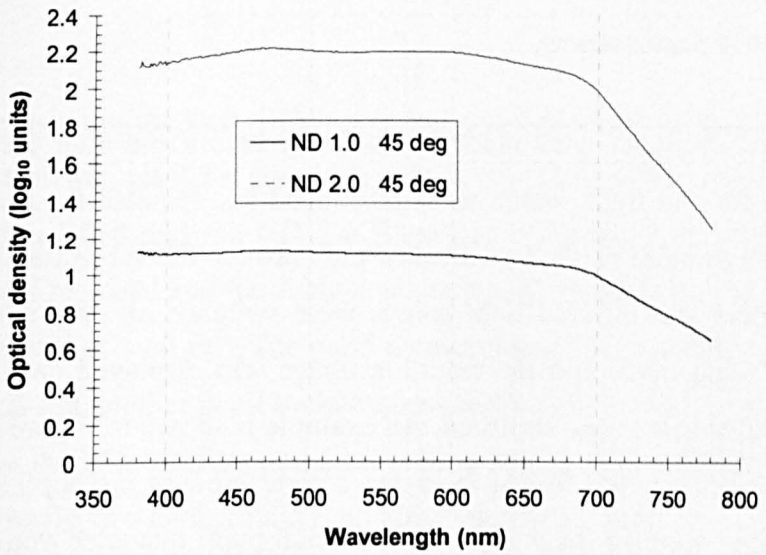


Figure 10.1 Neutral density filter calibration at 45° to the subjects' line of sight

The ND filters were soft gelatine squares that were held rigid in cardboard frames, giving a net visible filter size of 22 x 22 cm. The cardboard frames fitted into a fixed metal frame positioned between the subject and the display monitor. The metal frame was angled at 45° to minimise reflections from the filters, which were negligible. Subjects closed their eyes when the shroud was lifted to allow the filters to be changed

between vision tests. ND filter transmission was therefore calibrated at 45°, as this increases the thickness of filter through which light has to travel and decreases overall transmission. The net result is an increase in OD by about 10% over the light transmission expected if the filters were held perpendicular to the direction of incident light, as shown in Figure 10.1. The filter transmission data were incorporated into the CAA test software parameters such that the monitor display luminance was automatically adjusted to result in the desired net background luminance and stimulus contrast for each viewing condition.

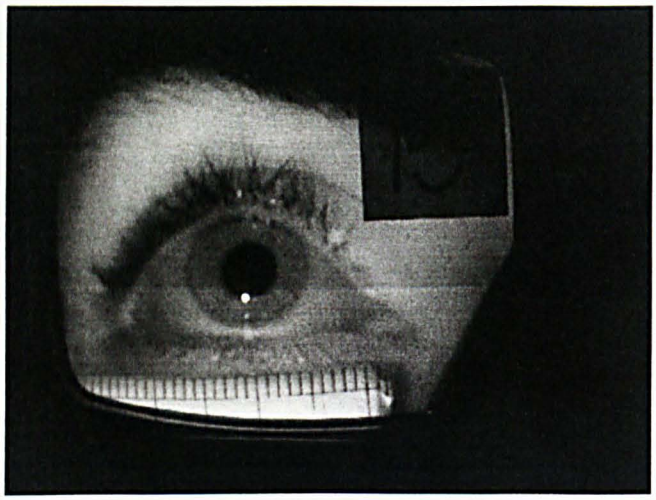


Figure 10.2 Measurement of pupil diameter

Measurements of pupil diameter were made immediately before and after each vision test. An infrared camera and light source were positioned just beneath the plane from the subject's eye to the bottom of the test display and between the subject and the ND filter frame. The camera and infrared light source were switched on only when pupil measurements were being made and the resulting image was displayed on a monitor from which still digital images were captured. An example is shown in Figure 10.2. On each occasion the subject was positioned to ensure a clear view of the pupil such that the margins of the pupil were in focus and the horizontal pupil diameter would not be affected by the camera viewing angle. The subject held a scale measured in mm against the lower eyelid such that the scale was also displayed and photographed. While not ideal, the digital images taken by this method enabled reasonable estimations to be made of pupil size, to within about ± 0.1 mm, using pixel widths aligned using the 'crop' facility of standard image editing software.

10.3.5 Experimental procedure

Subjects were seated at rest in front of the vision test and donned the cloth helmet and mask which were fitted to ensure a good face-mask seal. A halter was worn to support the common breathing gas supply hose. The mass spectrometer sampling line was connected securely to the mask port and the pulse oximeter was attached to a finger of the subject's choice. The laboratory was darkened and after a few minutes the experiment began by connecting the supply hose to the mask inspiratory hose to deliver the first breathing gas. At the same time the subject's eyes were closed and covered with a soft, opaque, material 'blind' to occlude all light. The subject then undertook 10 minutes of dark adaptation. Towards the end of this time, the blind was removed and the shroud from the viewing tunnel was drawn over the subject's head and upper body, such that the only light reaching the closed eyelids came from the test display. At 10 minutes he was instructed to open his eyes and adapt to the light from the display, viewed through the ND 2.0 filter to give a net background field intensity of 0.1 cd m^{-2}. After a further four minutes or so the subject positioned himself for a pupil measurement and then again for the first vision test which began after precisely 15 minutes of exposure to the respiratory condition. At the end of the vision test a further pupil measurement was made.

Upon completing the first vision test and pupil measurements, the results were saved and the test was reset for the next light level. The subject closed his eyes while the shroud was lifted and the ND 2.0 filter was substituted with the ND 1.0 filter. The shroud was replaced and the subject adapted to the new light level, at a net background field intensity of 1 cd m^{-2}. The pupil measurement was repeated, the second vision test performed and another pupil measurement made. Thereafter, a similar process followed to remove the ND 1.0 filter and allow vision testing and pupil sizing while viewing the screen directly, at a background field luminance of 12 cd m^{-2}.

Following the final pupil measurement, monitoring was discontinued and the instrumentation removed to allow the subject a 15-20 minute break. The whole process was then repeated for the next respiratory condition. After another 15-20 minute break, this process was then repeated a third time for the final respiratory condition. Thus, each respiratory exposure occupied about 45-50 minutes with each vision test and associated pair of pupil measurements occupying ~10 minutes. The whole experiment took ~3 h. Subjects experienced few symptoms and were unable to distinguish the respiratory

conditions, although mild fatigue or loss of concentration were sometimes associated with hypoxia.

10.3.6 Analysis

Pupillometry

For each vision test, the mean of the pre-test and post-test pupil diameters was calculated and fed into subsequent group analysis. The distribution of the normoxic data of the 12 subjects was then examined at each light level to confirm consistency with a normal distribution using the Anderson-Darling test ($\alpha = 0.1$). The entire data set was then interrogated using balanced analysis of variance (ANOVA) to assess main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of light level, gender, breathing gas and exposure order sub-group.

Contrast acuity

The photopic test parameters at 12 cd m^{-2} were validated against an older subject population than that engaged in the current study. A generally 'flat' contrast threshold could still be anticipated across the range of retinal eccentricities tested, but the current subject group should be expected to perform better than the 'standard normal observer' mean ± SD contrast reference values of 24 ± 8%. Under such circumstances, the data obtained from the current subject sample may be expected to be positively skewed away from a nominal 'high performance' mean threshold.

The upper mesopic study at 1 cd m^{-2} uses the same stimulus sizes at each retinal eccentricity as the photopic study at 12 cd m^{-2}, but has not been validated against a reference population. 'Normal' responses to these stimuli against this background light level are therefore unknown. It was considered unlikely that a 'flat' response would be generated, as the relative rod and cone contributions at each eccentricity would be likely to vary as a result of the reduced light level, while responses might or might not be positively skewed.

The mid-mesopic study at 0.1 cd m^{-2} again uses stimulus sizes that were validated against an older subject population than that engaged in the current study. The standard normal observer's mean ± SD contrast reference values of 48 ± 15% were also obtained against a slightly lower background field intensity of 0.05 cd m^{-2}. The slightly higher light level in the current study might be expected to decrease slightly the contrast thresholds obtained, while still generating a more or less 'flat' response. However, the

younger subject sample in the current study may be expected to require substantially less contrast than the reference population, lowering thresholds further. In addition, positively skewed data may be anticipated.

Most obviously, the data from all three test conditions could not be considered collectively since only two of the three used the same stimulus sizes. Thus, it was intended from the outset that each light level data set should be considered as a discrete analysis.

The normoxic responses at each light level were first analysed for a normal distribution using the Anderson-Darling test ($\alpha = 0.1$) on the data from all retinal test locations. Data sets were then normalised by logarithmic transformation as indicated. The design of the experiment was intended to enable balanced ANOVA to investigate main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of gender, breathing gas, retinal eccentricity and exposure order sub-group at each light level.

As with the data analysed in the previous chapter, for these analyses, the six possible respiratory exposure orders were arranged into three groups according to whether hypoxia immediately preceded the 100% O_2 condition (H-O, incorporating the HOA and AHO data), 100% O_2 immediately preceded the hypoxia condition (O-H, incorporating the OHA and AOH data), or the hypoxia and 100% O_2 conditions were separated in time by the intervening air (normoxic control) condition (x-A-x, incorporating the OAH and HAO data). Any statistically significant main effects would be considered in relation to normoxic variability between sub-groups before considering possible effects of respiratory exposure order. The latter were considered exceptionally unlikely given the duration of the timed rest periods imposed between successive respiratory conditions.

Finally, for normalised data sets, the contrast thresholds at the retinal locations under test were treated as seven related dependent variables and interrogated using balanced MANOVA to identify any influences of light level, gender, breathing gas and exposure order sub-group that might have varied systematically across the horizontal meridian.

Post hoc analysis was conducted using Tukey's Honestly Significant Difference test to assess the influences of breathing gas and retinal eccentricity on the data sets at each light level ($\alpha = 0.05$).

10.4 Results

10.4.1 Pupil size

The normoxic responses of the 12 subjects at each light level were assessed using the Anderson-Darling test ($\alpha = 0.1$) and all three sets of data were compatible with normal distributions (12 cd m-2, AD value 0.202, p = 0.842; 1 cd m-2, AD value 0.492, p = 0.175; 0.1 cd m-2, AD value 0.256, p = 0.659).

Respiratory condition (F_IO_2 %)		Light level (cd m^{-2})		
		12	1	0.1
Normoxia	(21%)	4.7 ± 0.5	5.8 ± 0.9	6.4 ± 0.8
Hypoxia	(14.1%)	4.4 ± 0.6	5.4 ± 0.9	6.2 ± 0.9
Hyperoxia	(100%)	5.0 ± 0.5	5.9 ± 0.8	6.4 ± 0.7

Table 10-3 Mean (± SD) pupil diameter for each light level and respiratory condition

The mean (± SD) results of pupillometry are shown for each light level and respiratory condition in Table 10-3, with mean ± SE results represented in Figure 10.3.

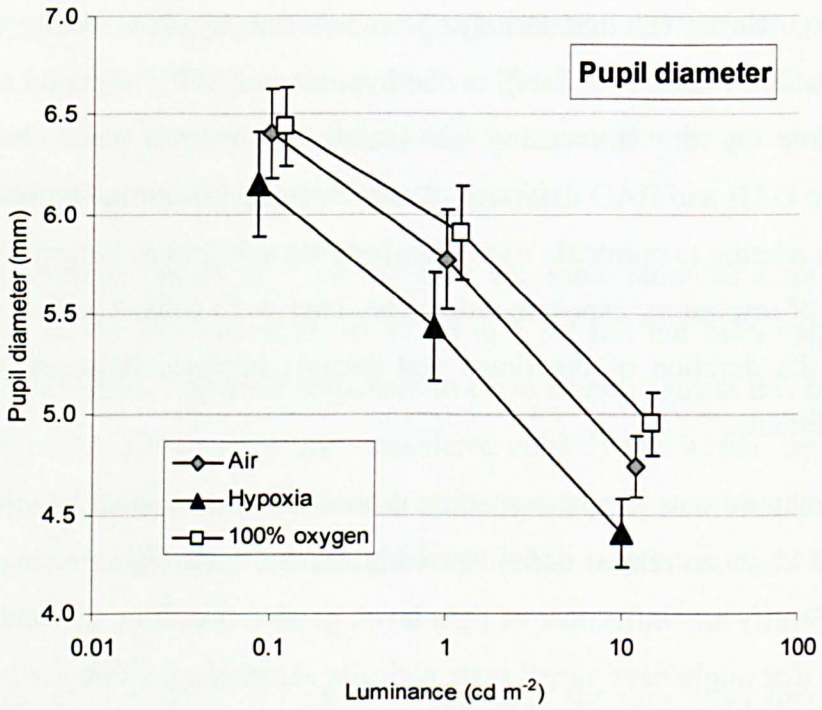


Figure 10.3 Mean (± SE) pupil diameter for each light level and respiratory condition

The anticipated effect of light level on pupil size is obvious. However, notwithstanding the normal wide variation in pupil size between individuals, the data also indicate a

clear trend for hypoxia to constrict the pupil across the range of light levels examined, with perhaps a less marked tendency for pupils to dilate when breathing 100% O_2.

The mean pupil diameters of all 12 subjects under all test conditions (N = 108 data points) were subject to balanced ANOVA to assess main effects (α = 0.05) and interactions (α = 0.01) of light level, gender, breathing gas and respiratory exposure order sub-group. The results are shown in Table 10-4 and confirm a statistically significant effect of respiratory condition (breathing gas) on pupil size (p = 0.042).

Factor	Type	Levels	Values
Light level	fixed	3	0.1, 1.0, 12.0 cd m^{-2}
Gender	fixed	2	F, M
Breathing gas	fixed	3	A, H, O
Exposure order sub-group	fixed	3	H-O, O-H, x-A-x

Source	DF	SS	MS	F	P
Light	2	48.7106	24.3553	45.25	**0.000**
Gender	1	0.0020	0.0020	0.00	0.951
Gas	2	3.6093	1.8047	3.35	**0.042**
Order	2	0.3911	0.1956	0.36	0.697
Light*Gender	2	0.7831	0.3916	0.73	0.488
Light*Gas	4	0.2225	0.0556	0.10	0.981
Light*Order	4	0.2437	0.0609	0.11	0.977
Gender*Gas	2	0.1414	0.0707	0.13	0.877
Gender*Order	2	20.8973	10.4487	19.41	**0.000**
Gas*Order	4	0.8157	0.2039	0.38	0.823
Light*Gender*Gas	4	0.0948	0.0237	0.04	0.996
Light*Gender*Order	4	1.5060	0.3765	0.70	0.596
Light*Gas*Order	8	1.1359	0.1420	0.26	0.975
Gender*Gas*Order	4	0.8519	0.2130	0.40	0.811
Light*Gender*Gas*Order	8	0.7807	0.0976	0.18	0.993
Error	54	29.0670	0.5383		
Total	107	109.2531			

S = 0.733674 R-Sq = 73.39% R-Sq(adj) = 47.28%

Table 10-4 Balanced ANOVA for pupil diameter

The highly statistically significant (p < 0.001) interaction between gender and exposure order sub-group was considered further. The basic form of the interaction is shown in Figure 10.4. For the x-A-x sub-group, mean female and male pupil diameters were similar at around 5.6 and 5.7 mm respectively. However, female mean pupil sizes were smaller (by ~0.5 mm) and male ones larger (~0.4 mm) for the subjects in the H-O sub-group. The converse was true for O-H sub-group, with mean female pupil sizes being larger (~0.4 mm) and male ones much smaller (~0.8 mm).

Disaggregating the data in Figure 10.4 by light level preserved the main effect of breathing gas for both males and females in all sub-groups while still demonstrating the interaction between gender and exposure order sub-group. Only two male or female subjects contribute to each of the data points in Figure 10.4 and variability between individuals contributes the bulk of the residual error observed when conducting the balanced ANOVA. Thus, it appears that the random allocation of subjects to each

exposure order sub-group has matched males having smallish pupils with females having largish pupils in the O-H sub-group, and vice versa for the H-O sub-group.

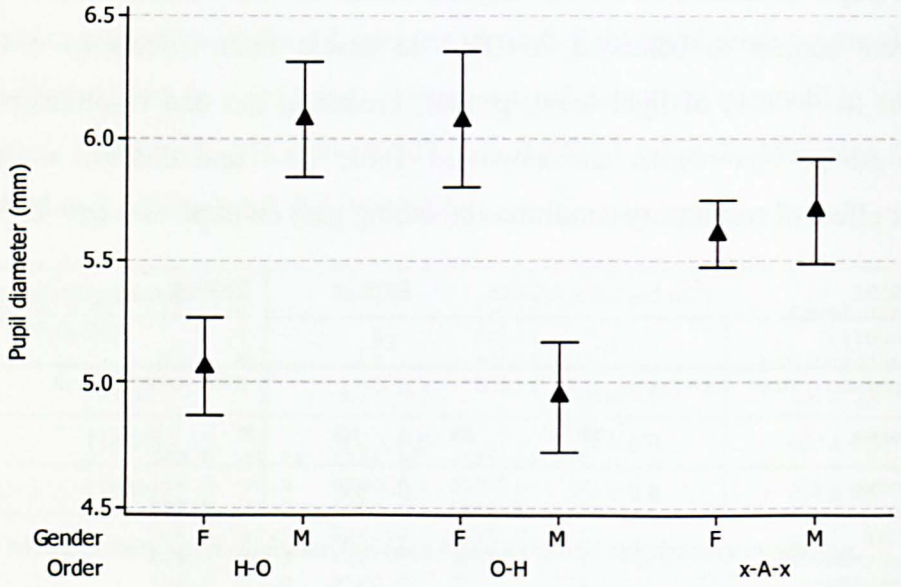


Figure 10.4 Interval plot of mean (± SE) pupil diameter by gender and exposure order sub-group

The range of pupil sizes obtained at each light level, regardless of breathing gas and exposure order, are consistent with the variability expected of 12 subjects viewing uniform fields corresponding to those used in this study (Spring and Stiles, 1948). Accordingly, it is assumed that the interaction between gender and exposure order is the result of allocation bias when randomising the subjects to exposure order sub-groups, and not a physiological effect of varying respiratory exposure orders.

10.4.2 Contrast acuity

At each light level the normoxic data from all retinal test locations were first examined for a normal distribution using the Anderson-Darling test ($\alpha = 0.1$). All three data sets were significantly positively skewed with none consistent with a normal distribution (12 cd m^{-2}, AD value 1.139, $p = 0.005$; 1 cd m^{-2}, AD value 1.028, $p = 0.01$; 0.1 cd m^{-2}, AD value 5.820, $p < 0.005$). Skewness values were, respectively, 0.80, 1.16 and 1.66. Log transformation normalised the data at 12 cd m^{-2} (AD value 0.261, $p = 0.699$) and 1 cd m^{-2} (AD value 0.135, $p = 0.135$), but not at 0.1 cd m^{-2} (AD value 2.774, $p < 0.005$). Skewness values for the transformed data were -0.15, -0.05 and 0.92. A histogram of the log transformed mid-mesopic data at 0.1 cd m^{-2} is shown in Figure 10.5 and a probability plot of the data relative to a fitted normal distribution is at Figure 10.6.

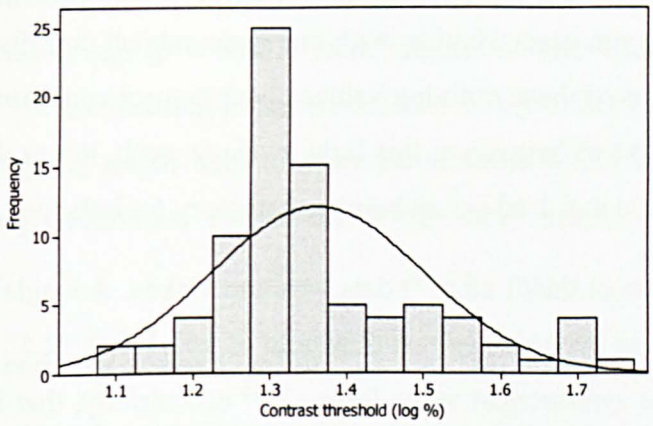


Figure 10.5 Histogram of $\log_{10}$ transformed data at 0.1 cd m^{-2} relative to 'normal'

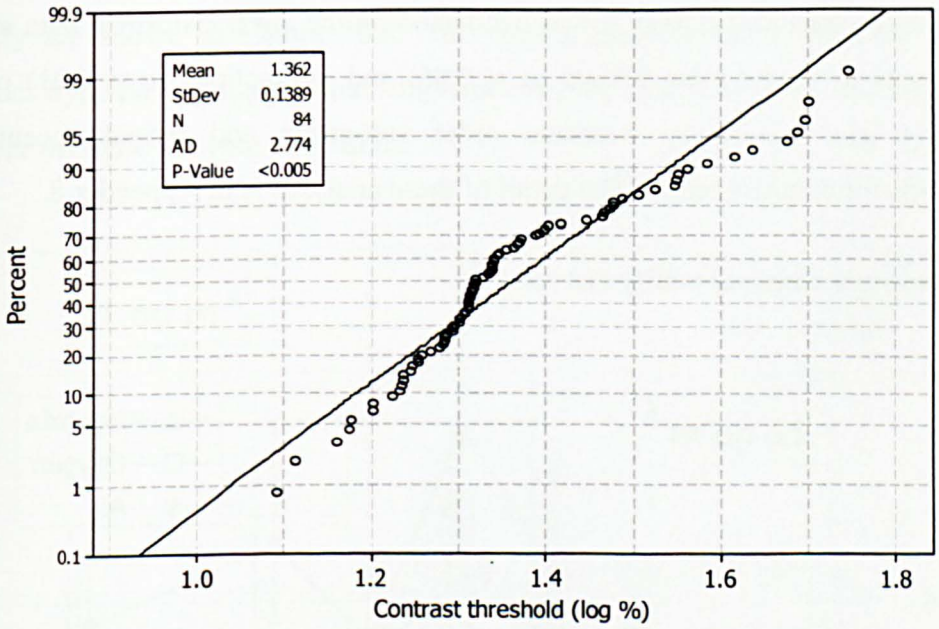


Figure 10.6 Probability plot of positively skewed, log-transformed data at 0.1 cd m^{-2}

While it is clear that the 0.1 cd m^{-2} data set remains skewed, log transformation has achieved a reasonably symmetrical distribution about a log contrast threshold of 1.3%, albeit with a few 'outliers' at about 1.7%. Severity of skewness was assessed by dividing by the standard error of the skew. The standard error of the skew is calculated as $\sqrt{(6/N)}$ where N is the number of subjects (Tabachnik and Fidell, 1996), giving a value of 0.92/0.707 = 1.19. The result is far less than twice the standard error of the skew (-1.96 to +1.96) and is therefore within an acceptable range for conducting ANOVA when considering the robustness of the method in relation to moderate departures from normality and homoscedasticity (Glass, Peckham and Sanders, 1972).

Seven of the 12 most positively skewed values were contributed by a single female subject's data at the seven eccentricities, with one male subject contributing three more of the outliers. The bias of these outlying values under control conditions would tend to militate against an effect of hypoxia at this light level. As such, it was decided to accept the log transformed data at 0.1 cd m^{-2} as being satisfactory for balanced ANOVA.

One other manipulation of the (1 cd m^{-2}) data was undertaken. A single grossly aberrant data point, indicating an hypoxic contrast threshold of 163.1% at +2.5° eccentricity was amended to reflect the symmetrical value from –2.5° eccentricity, that being 69.6% and in keeping with the order of magnitude of values obtained within and between subjects under comparable conditions. This change did not have any meaningful effect on the values for statistical significance obtained during subsequent analysis.

Accordingly, balanced ANOVA was conducted on the log transformed data at all three light levels to assess main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of gender, breathing gas, respiratory exposure order sub-group and retinal eccentricity on thresholds for contrast acuity. The detail of these analyses is in Appendix 8.

Low photopic contrast acuity (12 cd m^{-2})

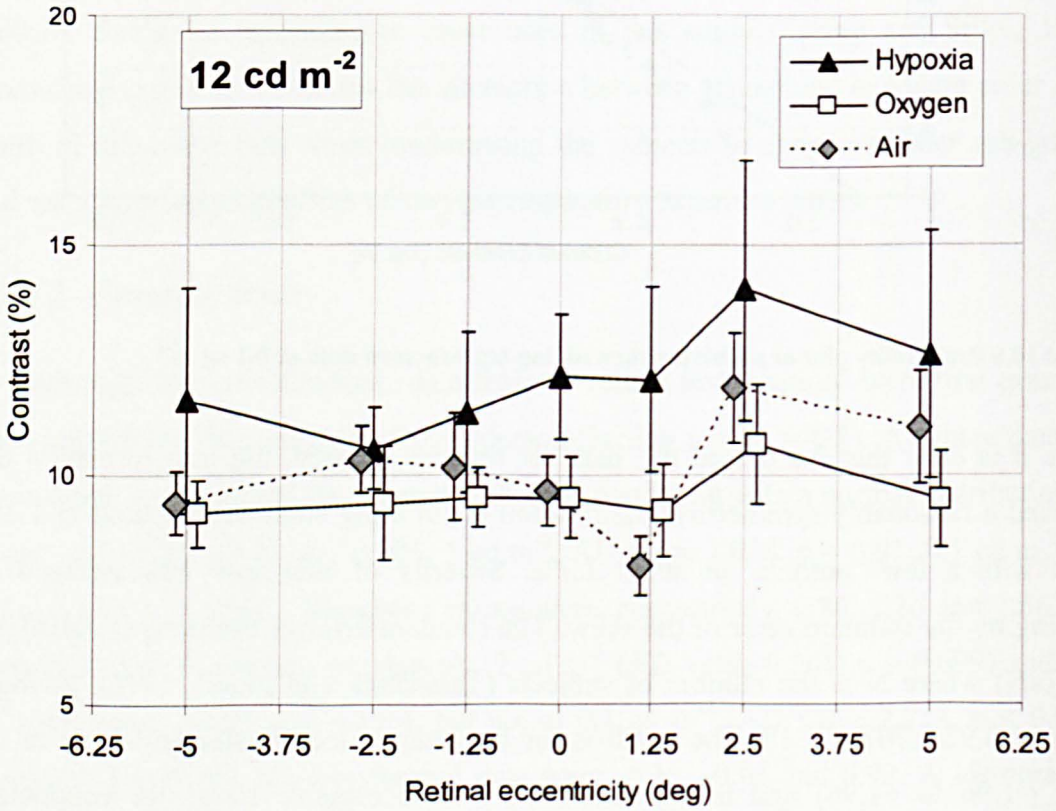


Figure 10.7 Mean (± SE) contrast acuity thresholds for a low photopic background (12 cd m^{-2})

Statistically significant main effects of breathing gas ($p = 0.016$) and respiratory exposure order sub-group ($p < 0.001$) were identified. The effect of breathing gas is shown in Figure 10.7, indicating impaired contrast acuity under hypoxia and suggesting that supplementary O_2 might help to optimise contrast acuity even under good (low photopic) viewing conditions. The effect of exposure order sub-group is considered separately below.

Upper mesopic contrast acuity (1 cd m^{-2})

At 1 cd m^{-2}, statistically significant main effects of breathing gas ($p = 0.016$) and exposure order sub-group ($p < 0.001$) were again identified alongside a main effect of retinal eccentricity ($p = 0.014$). The effect of exposure order sub-group is considered separately below. The effect of breathing gas and the variation attributable to retinal eccentricity are shown in Figure 10.8. The results again indicate impaired contrast acuity under hypoxia and suggest that supplementary O_2 should optimise contrast acuity under upper mesopic viewing conditions.

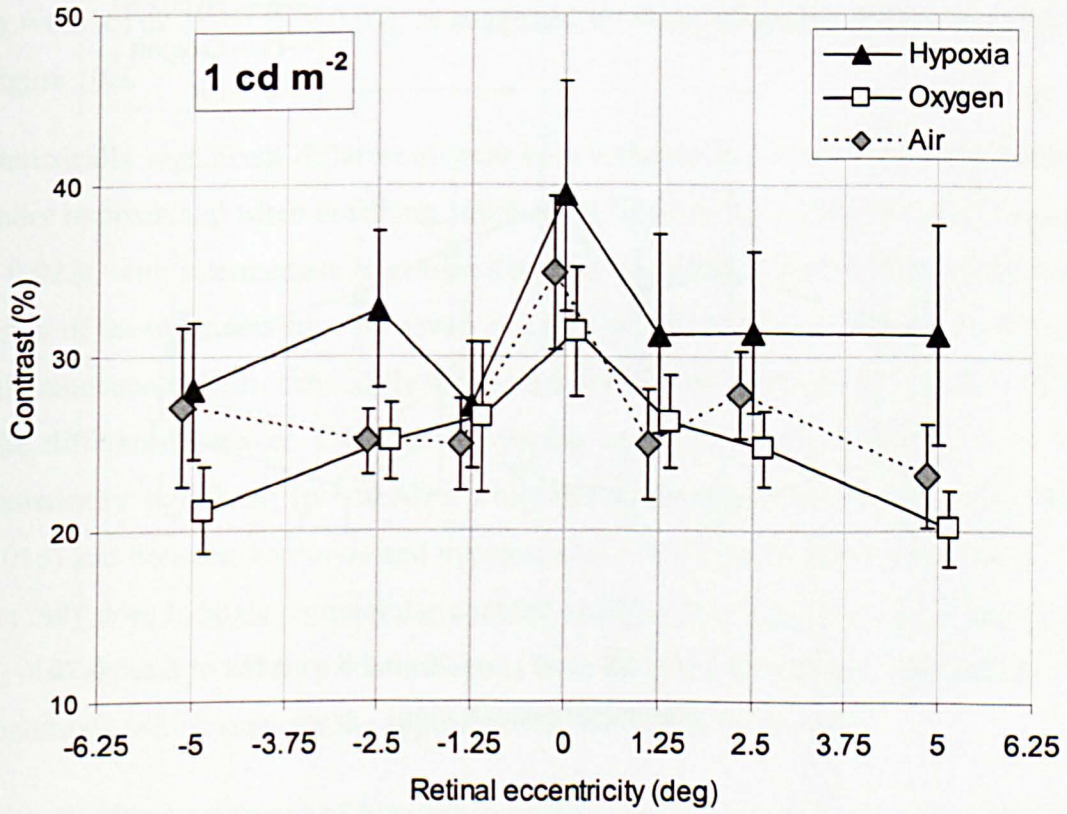


Figure 10.8 Mean (± SE) contrast acuity thresholds for an upper mesopic background (1 cd m^{-2})

The loss of sensitivity at the fovea, due to the absence of rods, is apparent as a peak (threshold elevation of contrast required) at 0° eccentricity, and explains the main effect

of retinal eccentricity seen on ANOVA. The graph also suggests that supplementary O_2 is particularly beneficial in optimising contrast acuity with distance from fixation, which is consonant with expectation given the increasing rod density with eccentricity.

Mid-mesopic contrast acuity (0.1 cd m^{-2})

Statistically significant main effects of breathing gas ($p < 0.001$) and respiratory exposure order sub-group ($p < 0.001$) were again identified. In addition, a statistically significant interaction between gender and exposure order sub-group was seen ($p < 0.001$). Main effects and interactions of exposure order sub-group are considered below. The effect of breathing gas is shown in Figure 10.9. Yet again, the results indicate impaired contrast acuity under hypoxia but now also suggest a clear benefit of supplementary O_2 over the contrast acuity obtained during the normoxic control exposures. The data indicate that supplementary O_2 enhances contrast acuity under mid-mesopic viewing conditions.

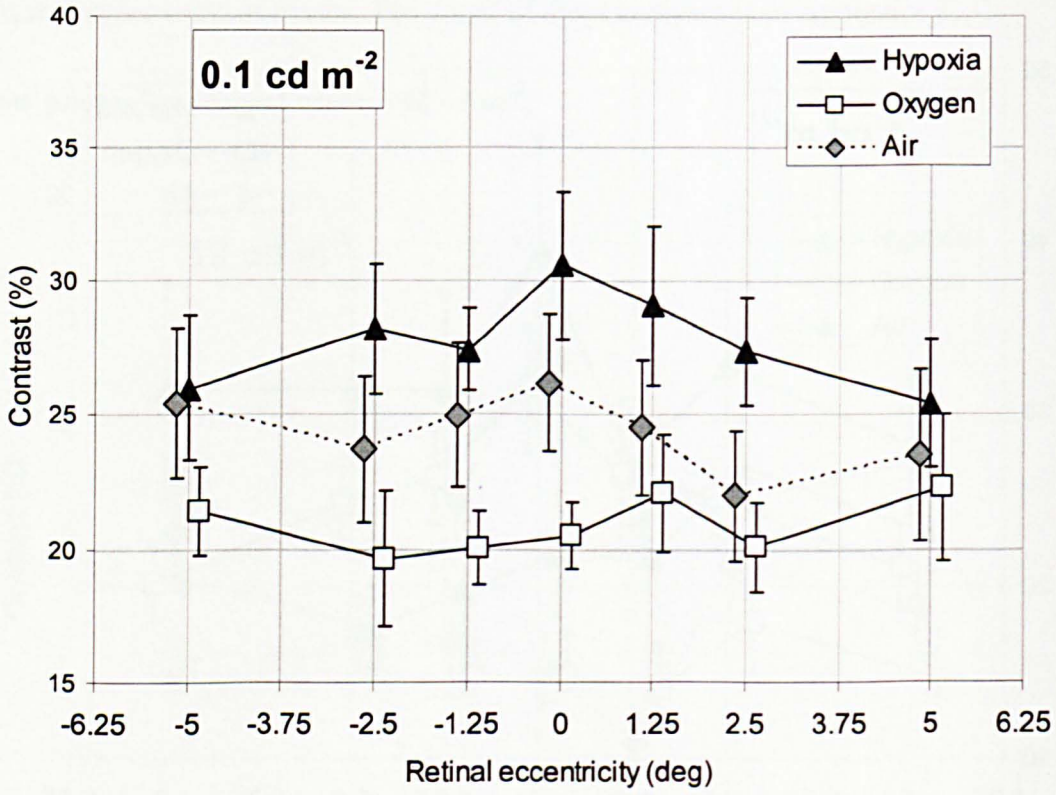


Figure 10.9 Mean (± SE) contrast acuity thresholds for a mid-mesopic background (0.1 cd m^{-2})

Effects of exposure order

A highly statistically significant ($p < 0.001$) main effect of respiratory exposure order sub-group was seen at every light level, such that contrast acuity was substantially

worse for the x-A-x sub-group than for either the H-O or O-H sub-groups. However, the main effects of breathing gas were preserved between sub-groups at each light level and there were no statistically significant interactions between sub-group and breathing gas, suggesting that exposure order *per se* had no influence on CAT under later respiratory conditions. The influence of exposure order sub-group undoubtedly results from allocation bias of subjects with differing underlying contrast acuities to different exposure order sub-groups. This also explains the highly statistically significant ($p < 0.001$) interaction between gender and exposure order at 0.1 cd m^{-2}. Similar trends were seen in the results at 12 and 1 cd m^{-2} but did not quite achieve statistical significance to $\alpha = 0.01$ ($p = 0.053$ and $p = 0.032$ respectively).

***Post hoc* analyses**

The data sets at each light level were analysed to assess the differences between breathing gases and retinal test eccentricities using Tukey's Honestly Significant Difference ($\alpha = 0.05$). There were no significant influences of retinal eccentricity except at 1 cd m^{-2} where contrast acuity at fixation was significantly greater than at either +5° ($p = 0.016$) or at –5° ($p = 0.05$), as suggested by the appearance of the results shown at Figure 10.8.

Statistically significant differences were seen between the contrast thresholds achieved under hypoxia and when breathing 100% O_2 at 12 cd m^{-2} ($p = 0.011$) and at 1 cd m^{-2} ($p = 0.023$), with intermediate normoxic data that could be considered homogeneous with either of the other data sets. However, at 0.1 cd m^{-2} all three breathing gas data sets were inhomogeneous with statistically significant differences between all possible pairings. The difference between CAT under hypoxia and those under 100% O_2 were highly statistically significant ($p < 0.001$), while those between normoxia and hypoxia ($p = 0.016$) and between normoxia and hyperoxia ($p = 0.012$) were also unambiguous. Thus, not only does hypoxia compromise contrast acuity at this light level, but supplementary O_2 also appears to enhance contrast acuity over that achievable under normal respiratory conditions, which supports the appearance of the data at Figure 10.9

Quantitative assessment of hypoxic impairment

If it is assumed that contrast acuity is optimal when breathing supplementary O_2, then the relative impairment under another respiratory condition may be calculated as the increase in contrast required as a percentage of the optimal hyperoxic threshold. This calculation is shown in Equation 18 where C_i is the hypoxic contrast impairment to be

expressed as a percentage of the optimal contrast threshold, C_h is the contrast threshold under hypoxia, and C_o is the contrast threshold breathing 100% O_2.

$$C_i = (C_h - C_o) / C_o \qquad \times 100\%$$

Equation 18 Calculation of contrast threshold impairment

The hypoxic impairment of contrast acuity to the smaller targets at 12 and 1 cd m^{-2} is shown in Figure 10.10. Across all eccentricities, the mean threshold elevation under hypoxia, as a percentage of optimal sensitivity, is similar between light levels, being about 26% at 12 cd m^{-2} and 28% at 1 cd m^{-2}.

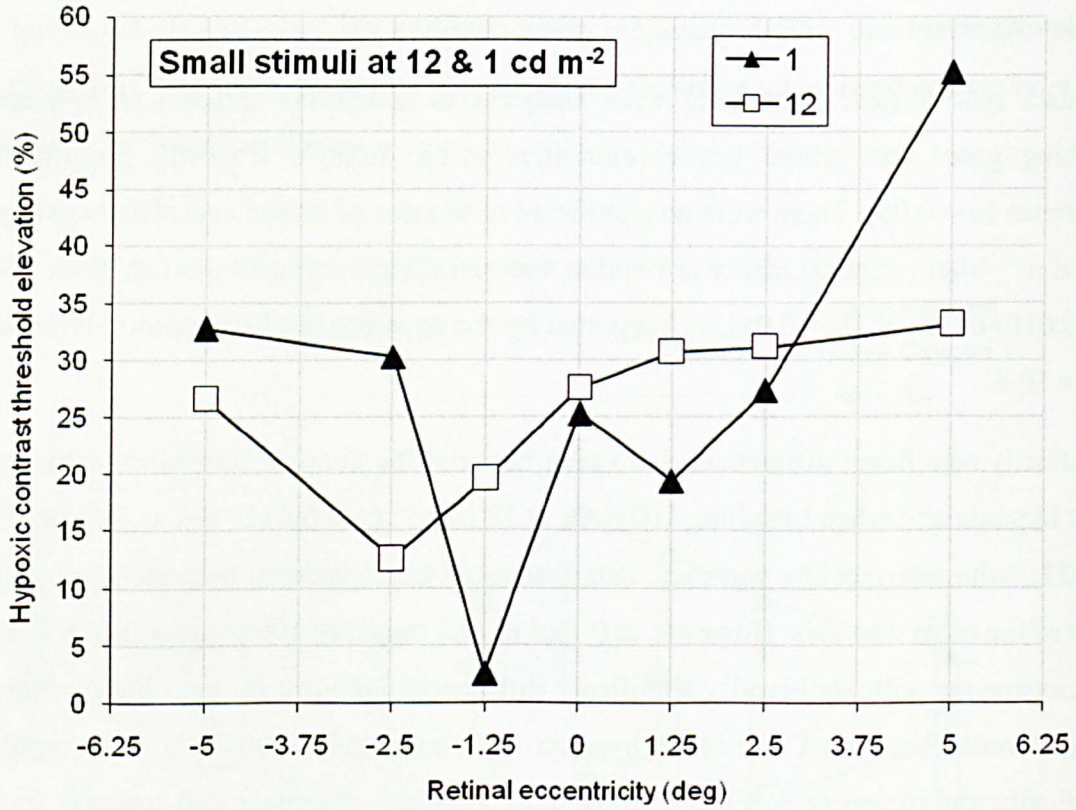


Figure 10.10 Hypoxic impairment relative to optimal (hyperoxic) contrast thresholds

The hypoxic impairment of contrast acuity to the larger stimuli at 0.1 cd m^{-2} is shown in Figure 10.11, amounting to a mean threshold elevation of 33%, so slightly greater than at the higher light levels. Figure 10.11 also shows the relative impairment of normoxic contrast thresholds at 0.1 cd m^{-2} compared to hyperoxia, by a mean of 17%.

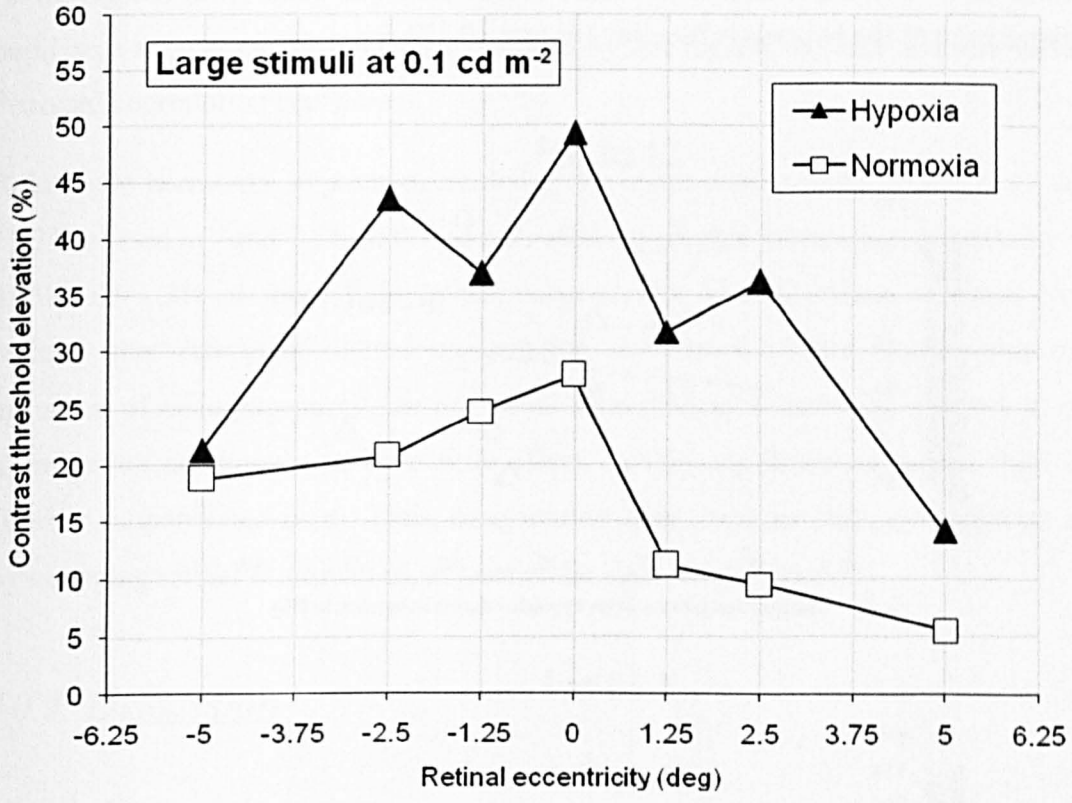


Figure 10.11 Threshold impairment relative to optimal (hyperoxic) contrast acuity at 0.1 cd m^{-2}

Notwithstanding the few aberrant data points, the general appearance of Figure 10.10 suggests that the effect of hypoxia to compromise thresholds at those light levels is probably reasonably uniform across the retinal eccentricities tested. On the other hand, the magnitude of the effect of hypoxia at 0.1 cd m^{-2} has a clear retinotopic distribution (Figure 10.11), such that foveal thresholds are elevated most and the effect of hypoxia becomes less pronounced with eccentricity. Relative to the optimal contrast acuity thresholds achieved with supplementary O_2, the effect of breathing air suggests a similar but less pronounced retinotopic pattern of impairment that is most pronounced at fixation. Accordingly, at this light level, breathing supplementary O_2 rather than air may be expected to be of greatest benefit to foveal contrast acuity.

10.4.3 Pupil diameter and contrast acuity

Effects of different oxygenation states to change pupil diameter may be anticipated to influence acuity either by altering retinal illumination or by modifying the influence of higher-order optical aberrations and, thereby, intraocular scatter. For each subject at each light level, the difference in pupil area under hyperoxia and hypoxia was plotted

against the corresponding difference in CAT at fixation, with both parameters expressed as a percentage of the hyperoxic baseline (Figure 10.12).

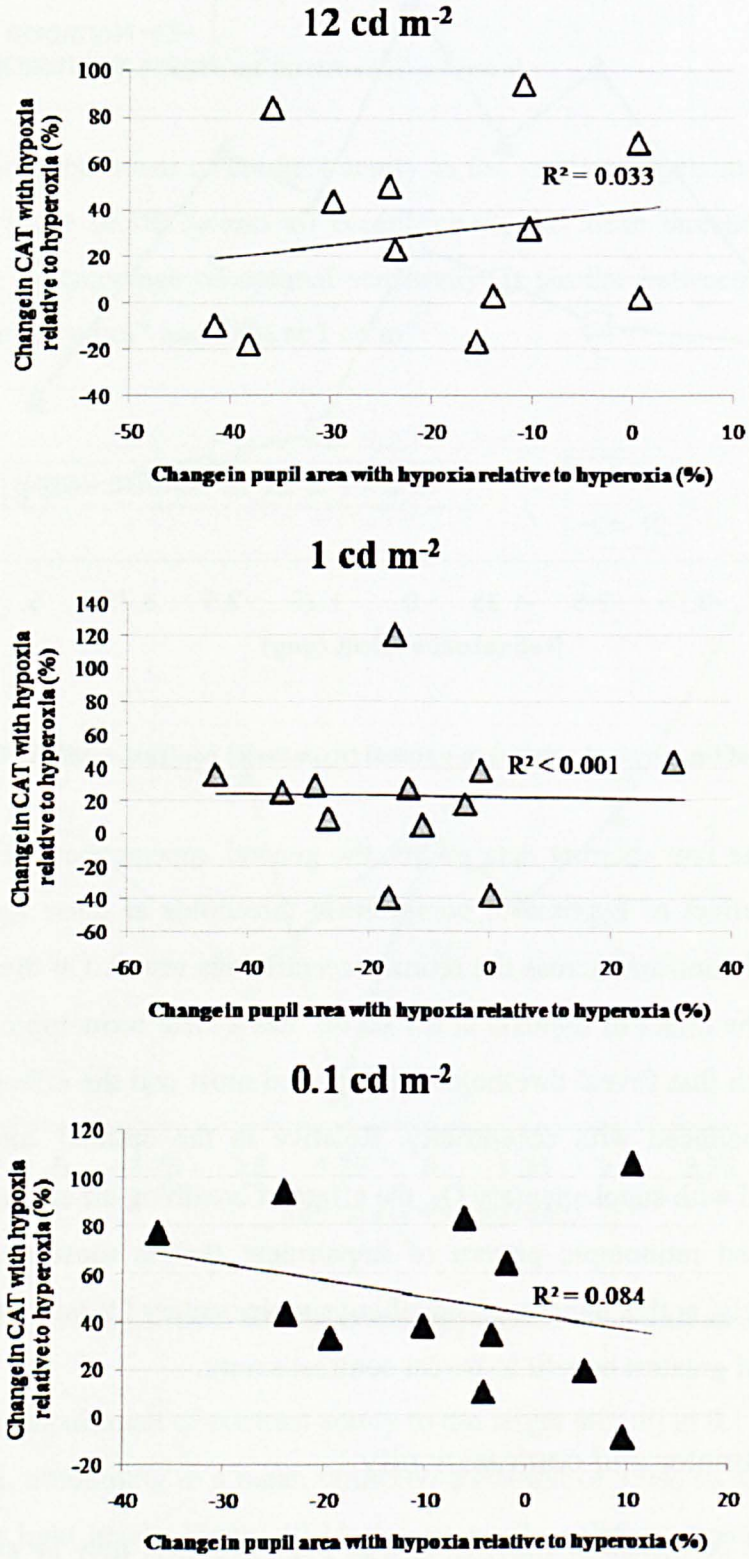


Figure 10.12 Correlation between changing pupil area and changing contrast acuity threshold under hypoxia relative to hyperoxia at each light level (N = 12)

At no light level is there any meaningful effect of, or association between, change in pupil area and change in CAT, as evidenced by flat regression lines and low values for Pearson's correlation coefficient.

Relative to normoxia, hypoxia decreased group mean pupil area by 13.6% at 12 cd m^{-2}, 11.3% at 1 cd m^{-2} and 7.7% at 0.1 cd m^{-2}, while hyperoxia increased pupil area by 9.4%, 4.5% and 1.3% at these light levels, respectively. The directions of these changes indicate that effects of altered oxygenation state on CAT are independent of any influence of changing pupil size on retinal illumination. Substantial changes in retinal illumination are known to have little effect on contrast acuity at higher light levels (Barbur, unpublished data). Thus, oxygenation state does not influence contrast acuity by changing retinal illumination, at least at the light levels tested.

10.5 Discussion

10.5.1 Oxygenation state

Midbrain pupilloconstrictor activity occurs spontaneously and originates from the Edinger-Westphal component of the oculomotor nucleus. This may be subject to central inhibition to produce pupillary dilatation, for example through stimulation of the hypothalamus (Sillito and Zbrozyna, 1970). If this central inhibition is itself inhibited, for example due to systemic and hence cerebral hypoxia, then relatively increased parasympathetic outflow will result in miosis (Figure 1.22). This has been suggested as the mechanism whereby terrestrial exposure to altitude results in pupillary constriction (Cymerman, Muza, Friedlander *et al*, 2005) and is supported by the more acute hypoxia imposed in the current study. The pupilloconstrictor effect of supranuclear hypoxic inhibition could be even stronger than is evident in the current study if concomitant hypoxic suppression of the afferent retinal signal is, at the same time, tending to dilate the pupil. Nonetheless, in the current study there appears to be little influence of alterations in pupil size to affect contrast acuity.

The hypoxic elevation of CAT is reasonably consistent across the various eccentricities at 12 and 0.1 cd m^{-2}. However, if same-sized stimuli were employed at all test locations, the effect of hypoxia at these light levels would be manifest as a non-monotonic loss of low contrast acuity with eccentricity, necessitating progressively more contrast to enable discrimination with distance from fixation. Thus, the effect of hypoxia is

retinotopic, supporting an ocular rather than central mechanism of action. The data at 1 cd m^{-2} indicate another trend, with worsening acuity at fixation as light level falls into the mesopic range, while rod recruitment benefits peripheral acuity. At this light level, cone-dominated acuity at fixation appears just as vulnerable to hypoxia as acuity further in the periphery. Perhaps surprisingly, at 0.1 cd m^{-2} acuity seems to benefit most from supplementary O_2 at fixation. This suggests either that cone oxygenation at fixation is O_2-limited in the mid-mesopic range or that it is spatially dependent on the increasing QO_2 of surrounding rods as light level falls. Given the relative sparsity of rods in the fovea, the former seems more likely.

Mild hypoxia equivalent to breathing air at 10,000 ft necessitates an increase in stimulus contrast by a mean of ~26% (~0.1 log unit) to maintain normal alphanumeric acuity across the central 10° of visual field at low photopic (12 cd m^{-2}) and upper mesopic (1 cd m^{-2}) luminance. At 0.1 cd m^{-2} a similar hypoxic impairment is accentuated by an effect of hyperoxia, of comparable magnitude, to enhance sensitivity over that achievable breathing air, suggesting that supplementary O_2 may benefit low contrast acuity at fixation by a mean of up to ~50% (0.17 log unit) at 10,000 ft. This benefit of supplementary O_2 at mid-mesopic luminance supports the hypothesis that local rod-driven tissue hypoxia renders the outer retina increasingly susceptible to further exogenous hypoxia as light levels fall. The results indicate hypoxic impairment at higher background field intensities than demonstrated previously (Kobrick, 1968; Kobrick and Appleton, 1971; Miller, 1958; McFarland and Halperin, 1940; Rose, 1949) and support a benefit of supplementary O_2 in the mesopic range, including at fixation.

The smallest alphanumeric characters of relevance on the flight deck subtend 12 to 18 minutes of arc at the eye, based on a mean viewing distance of 80 cm, and discrimination of these characters demands a minimum angle of resolution of 2.5 to 3.6 minutes of arc (Chisholm and Barbur, 2001; Chisholm, Evans, Harlow *et al*, 2003). This is relatively easy for high contrast targets viewed at photopic luminance but older symbology and other objects of importance in the visual scene may present at lower light levels and much lower contrast. Additionally, glare, shadows, rapidly fluctuating light levels and weather may make low contrast acuity tasks more difficult. Even within the functional visual field corresponding to the diameter of a typical aircraft display (± 5°), larger characters are required for resolution at the periphery. For a given adaptation state the functional range of visual sensitivity covers approximately 2 $\log_{10}$ units. The hypoxic decrement of 0.1 log units at fixation is small in absolute terms but nonetheless

represents a 5% loss of the most sensitive end of the dynamic range, for a given light level, that may be highly undesirable in aviation. Furthermore, the benefit of supplementary O_2 at an equivalent altitude of 10,000 ft approaches 9% of a '2 $\log_{10}$ unit' dynamic range at light levels that may be relevant during night flying (Chisholm, Evans, Harlow *et al*, 2003). These losses under hypoxia may be considered relatively slight but are nonetheless highly undesirable in the context of flight outside controlled airspace, at low level, and under conditions of low visibility, such as when viewing under dim cockpit lighting or searching the external scene in poor weather.

10.5.2 Lessons for subsequent studies

This experiment benefited considerably from those that preceded it. It would be helpful when recruiting subjects to exclude those who might have difficulty with fitting of experimental equipment. A more accurate method of measuring pupil size during vision testing would be desirable. Alternatively, dynamic pupillary responses should be assessed comprehensively under conditions of altered oxygenation at different light levels. Some minor modifications to the starting contrast levels and staircase step sizes during vision testing might help to enhance slightly the accuracy of within-subject data. Otherwise the experiment proceeded uneventfully and as intended.

10.5.3 Summary and future work

Mild hypoxia compromises low contrast acuity consistently for eccentricities up to ± 5° at background field intensities of 12 and 1 cd m^{-2}. At 0.1 cd m^{-2} supplementary O_2 enhances contrast acuity beyond that achieved under normoxic conditions. Relative to this 'optimised' baseline, hypoxia produces a retinotopic impairment of low contrast acuity that is worst at fixation (Figure 10.10). Thus the original hypotheses are only partly supported. The influence of oxygenation state on contrast acuity is progressive with decreasing light level but not with increasing retinal eccentricity, at least not out to ± 5°. The more obvious effect of hypoxia at 0.1 cd m^{-2} is relative to a benefit of supplementary O_2 at that light level that implies the presence of local retinal hypoxia under normal breathing conditions. Contrary to the second hypothesis, the benefit of supplementary O_2 over hypoxia at the lowest light level is greatest at fixation. The effects of altered oxygenation state on contrast acuity at the different light levels are summarised in Table 10-5.

Light level (cd m^{-2})	Effects of altered oxygenation state	Benefit of supplementary O_2 over hypoxia?
12	Across all eccentricities hypoxia elevates contrast acuity thresholds by a mean of 26%. There is no benefit of hyperoxia relative to normoxia.	Slight, 0.1 $\log_{10}$ unit (Figure 10.7)
1	Across all eccentricities hypoxia elevates contrast acuity thresholds by a mean of 28%. There is no benefit of hyperoxia relative to normoxia.	Slight, 0.1 $\log_{10}$ unit (Figure 10.8)
0.1	Hypoxia compromises and hyperoxia enhances contrast acuity across the central 10° of visual field, with a maximal differential effect at fixation amounting to 50% degradation under hypoxia relative to presumed optimal sensitivity with 100% O_2.	Moderate, 0.17 $\log_{10}$ unit (Figure 10.9)

Table 10-5 Summary of effects of altered oxygenation state on contrast acuity thresholds

It would be valuable to establish the level of hypoxia at which decrements in low contrast acuity first become evident at each light level and the rate at which acuity is compromised as hypoxia becomes progressively more severe. Furthermore, the modifying effect of stimulus and background colour warrants assessment to identify the circumstances under which detailed visual information might most readily be lost under hypoxia (Domenech, Seguí, Capilla *et al*, 1994). It is noteworthy that night vision devices present visual information at low photopic or upper mesopic light levels that may be vulnerable to the effects of mild hypoxia and this might warrant further investigation.

11 Discussion

11.1 The Aims of the Thesis

11.1.1 To identify visual effects of mild hypoxia in dim light

The primary aim of this thesis was to identify possible effects of mild acute hypoxia on aspects of visual performance at low light levels, thereby defining more clearly the limits and vulnerabilities of the human visual system at altitude. This aim is reviewed here in relation to the various experiments undertaken.

The dark adaptation study demonstrated that the rate of rod adaptation to dark depends fundamentally on outer retinal oxygenation. The rate of scotopic adaptation is delayed progressively by worsening hypoxia while supplementary O_2 hastens dark adaptation at GL, such that the time to cone rod inflection may vary by many minutes. By implication, the rod contribution to mesopic adaptation, sensitivity and visual performance will also depend on outer retinal oxygenation, at least during the process of adaptation to dim light levels. As well as the prevailing outer retinal PO_2, visual sensitivity will also be affected by the duration of exposure to a given low light level, the concomitant outer retinal PCO_2 (and therefore pH), and the considerable variability within and between individuals. Differences in scotopic threshold sensitivity between individuals and respiratory conditions will be most profound for the period immediately after cone rod inflection and may, briefly, amount to one $\log_{10}$ unit or so. Differences in mesopic sensitivity for a given time in dim light will be far less pronounced but are of more practical relevance to contemporary aviation at night. In practice, the rod contribution to visual performance will be compromised progressively with increasingly severe hypoxia from GL and to a greater extent at dimmer light levels.

The linear relationship between hypocapnia and flicker thresholds implies a close relationship between retinal PCO_2 (and therefore pH) and flicker sensitivity but indicates that the magnitude of the effect is slight and of little practical significance for low photopic/upper mesopic stimuli (3.8 cd m^{-2}) viewed against a dark background. Since sensitivity to flicker is greater for larger and brighter stimuli, the phenomenon may warrant further consideration in relation to viewing at photopic levels, since the

maximal effect of hypocapnia remains to be defined. Hypoxia is well known to compromise flicker sensitivity and was not examined specifically during the current work. In retrospect, it is likely that flicker sensitivity could be a sensitive indicator of the susceptibility of retinal function to the effects of hypoxia at different mesopic light levels and may warrant consideration in future studies.

The study of spatial contrast sensitivity at the fovea indicates that thresholds for detection of the chosen Gabor patch stimuli, at *sf* ranging from 0.5 to 16 cpd, were unaffected by changes in PO_2 across the range of background field intensities studied, that is, between about 0.3 and 28 cd m^{-2}.

On the other hand, chromatic sensitivity is compromised progressively by hypoxia as light levels fall in the mesopic range from about 1.7 to 0.2 cd m^{-2}. Hypoxia exaggerates the normal loss of chromatic sensitivity, over that occurring due to the reduction in light level alone, by ~5% at 1.7 cd m^{-2} and by ~30% at 0.2 cd m^{-2}. Photopic colour thresholds appear unaffected by hypoxia. Both R-G and Y-B thresholds are affected but hypoxia exaggerates the normal mesopic tritanopia by compromising Y-B thresholds more than R-G thresholds as light levels fall. The loss of Y-B sensitivity at the lowest light level is asymmetrical, with yellow thresholds compromised most. Hyperoxia maintains normal chromatic sensitivity but appears to offer no advantage relative to normoxia.

The study of visual processing speed using the UFOV® test suggested trends for hypoxia to compromise processing speed that are likely to be meaningful with regard to extraction of information during brief glances at cluttered displays under dim viewing conditions. However, hypoxia tends to require longer duration stimulus presentations to maintain discrimination, even for clearly suprathreshold stimuli presented against uncluttered backgrounds. The UFOV® test was a useful tool for undertaking an initial assessment of the effects of hypoxia on visual processing speed but a bespoke research test would be required to investigate these effects more definitively.

The examination of temporal contrast sensitivity using the FDT perimeter identified a range of responses to altered oxygenation at different light levels. Relative to normoxic performance, at 100 cd m^{-2} hypoxia compromised sensitivity beyond the fovea by up to ~2 dB, while at 10 cd m^{-2} hyperoxia enhanced sensitivity beyond the fovea by up to ~1.5 dB. At 1 cd m^{-2} hypoxia compromised and hyperoxia enhanced sensitivity across the central visual field, including at the fovea, with a benefit of supplementary O_2 relative to hypoxia amounting to over ~3 dB in some individuals. Of note, relative to

hypoxia beyond the fovea, supplementary O_2 offered a statistically significant benefit upon temporal contrast sensitivity at all light levels.

A similar pattern of benefit of hyperoxia over hypoxia was seen during the contrast acuity experiments. Hyperoxia enhanced contrast acuity relative to hypoxia at all light levels (12, 1 and 0.1 cd m^{-2}) with intermediate responses under normoxia. At 0.1 cd m^{-2} hyperoxia provided a statistically significant benefit over normoxia while hypoxia impaired contrast acuity relative to normoxia. At this light level the benefit of hyperoxia over hypoxia was greatest at fixation.

To summarise, significant new effects of hypoxia have been documented in relation to delayed dark adaptation at 10,000 ft and 15,000 ft, progressively impaired chromatic sensitivity with decreasing mesopic luminance, impaired temporal contrast sensitivity at photopic (100 cd m^{-2}) and mesopic (~1 cd m^{-2}) luminance, and impaired low contrast acuity at light levels of 12, 1 and 0.1 cd m^{-2}. Additionally, relative to normoxia, hyperoxia has been shown to facilitate dark adaptation and to enhance temporal contrast sensitivity at ~10 and ~1 cd m^{-2} and to benefit low contrast acuity at 0.1 cd m^{-2}, suggesting that these visual performance parameters are sub-optimal at these light levels under conditions of normal oxygenation.

11.1.2 To consider the underlying physiology of the visual system

The second aim of this thesis was to consider the findings for any insights they provide into the underlying physiology of the human visual system.

Quite unexpectedly, the dark adaptation experiments provided the most fundamental physiological conclusion of the entire programme of work. This is to say, human rod photoreceptors appear to be functionally hypoxic in the dark under normal respiratory conditions, that is, breathing 21% O_2 at sea level. Exposure to exogenous hypoxia then delays and worsens sensitivity but supplementary O_2 can hasten and enhance it. Additionally, rod sensitivity increases when outer retinal elimination of CO_2 is enhanced, although the mechanism for this is unclear and may be related to increasing tissue pH.

Hyperventilation clearly increases sensitivity to flicker. While the hypocapnia and associated tissue pH changes will affect the whole of the visual pathway, perhaps the prime suspect for the level at which the effect may be mediated is the GCL. However, given the clear increase in sensitivity of the outer retina under hyperventilation, it is

perhaps possible that GC output is being determined by more distal respiratory effects. The influence of outer retinal hypoxia on flicker sensitivity at reduced luminance would be of interest and the possible interaction between hypoxia and hypocapnia could be studied using mixed hypoxic/hypocapnic challenges.

The stimuli for the study of the spatial CSF at the fovea were predominantly parvocellular in nature, having relatively high spatial frequency, low temporal frequency, no contrast pedestal relative to background field intensity, and being presented at cone-dominant light levels to the area of retina with greatest cone density and fewest rods. Thus, parvocellular stimulus detection appears relatively resistant to mild hypoxia, at least for foveal stimuli presented against the background fields considered here. The temporal contrast stimuli considered further below were magnocellular in nature, having relatively low *sf* and high temporal frequency, which may perhaps explain their greater susceptibility to respiratory challenge.

The study of chromatic sensitivity demonstrated a number of features that have bearing on the underlying physiology. Firstly, the effect of hypoxia was progressive with reducing luminance and hence was related to the adaptation state of the outer retina, since an effect on the inner retina or elsewhere in the visual system would not be expected to exhibit this characteristic. Secondly, despite the stimuli being foveal and the fact that chromatic sensitivity is cone-mediated, modest descent into the mesopic range was sufficient to induce hypoxic impairment. This indicates either that cone O_2 requirements increase as light levels fall, in which case cones become susceptible to the mild hypoxia used here at below about 2 cd m^{-2}, or that rod QO_2 increases to such an extent that local tissue PO_2 falls even at the fovea, thereby compromising cone function. Given the high cone density and relative paucity of rods at the fovea, in relation to the distance from the choroid to the inner segments and likely PO_2 gradients, the former explanation seems most likely. Thirdly, the hypoxic impairment of Y-B sensitivity was greater than that of R-G sensitivity, and was worse for yellow (corresponding to S-cone decrements) than blue stimuli (S-cone increments) at the lowest light level. This suggests that S-cones may have been particularly sensitive to hypoxia and supports an increased metabolic requirement of S-cones relative to M- and L-cones (Greenstein, Hood, Ritch *et al*, 1989; Dean, Arden and Dornhurst, 1997; Cho, Poulsen, Ver Hoeve *et al*, 2000).

Considering the study of visual processing speed, the tendency of hypoxia to require longer stimulus presentation times to maintain discrimination is consistent with past

studies but it is impossible to draw any meaningful conclusions, in relation to specific sites of action of hypoxia, on the basis of the results obtained during the current work.

At 100 cd m^{-2} the effect of hypoxia to compromise temporal contrast sensitivity beyond the macular periphery was unexpected. Rod responses are close to saturation at this light level so their QO_2 will be close to basal levels, yet the lack of an effect of hypoxia at the fovea suggests a retinotopic susceptibility that implies an outer retinal effect. Also, the mild hypoxia imposed in these studies should be readily compensated by increased inner retinal Hb desaturation, inner retinal autoregulation and increased inner retinal blood flow, as necessary, to maintain the inner retinal PO_2. Furthermore, recent comparative studies in primates indicate that perifoveal photoreceptor QO_2 is greater than that at the fovea (Birol, Wang, Budzynski *et al*, 2007). The same study also indicates that primate QO_2 is more dependent on choroidal PO_2 in light than was previously suggested by work in cats. This all suggests that a smaller change in choroidal PO_2 than previously imagined might still impact on human photoreceptor metabolism under conditions of moderate illumination. It seems likely that the substantial reduction in choroidal PO_2 imposed in the current study, from normoxic levels of ~105-110 mm Hg down to about 55-60 mm Hg, that is, by almost 50%, was sufficient to compromise photoreceptor metabolism beyond the fovea, even under photopic viewing conditions.

There was no apparent effect on temporal contrast sensitivity of hypoxia at 10 cd m^{-2} but, instead, a benefit of hyperoxia over that achieved under control conditions. It is suggested that increasing rod recruitment and an increased rod contribution may have tended to benefit visual sensitivity at this light level, disguising any effect of hypoxia to compromise visual performance relative to normoxia. However, an increasing rod QO_2 will have tended to limit the benefit being occasioned, allowing supplementary O_2 to then enhance sensitivity beyond that expected under normoxic conditions. By 1 cd m^{-2} the rod contribution to visual perception can no longer disguise the consequences of increased outer retinal QO_2 such that exogenous hypoxic impairment and hyperoxic enhancement are both clearly evident relative to sensitivity under normoxic conditions. Thus this pattern of response is consistent with the notion that endogenous rod-driven outer retinal hypoxia might limit visual performance as light levels fall, and is consistent with the findings in relation to chromatic sensitivity.

Hypoxia was consistent in compromising low contrast acuity by between ~26 to 33% at 12, 1 and 0.1 cd m^{-2}, and the effect was broadly consistent across the various retinal

eccentricities tested. However, at the lowest light level hyperoxia exhibited a performance enhancement over that achieved under normoxic conditions, providing further evidence to support an effect of rod-driven retinal hypoxia to render the outer retina vulnerable to exogenous hypoxia as light levels fall in the mesopic range. The disparity between hypoxic and hyperoxic contrast acuity was greatest at fixation indicating that supplementary O_2 might significantly benefit acuity at fixation at relatively modest mesopic luminance.

It is noteworthy that the benefit of hyperoxia to enhance visual sensitivity over that achieved under normoxia was evident at a higher light level (~1 cd m^{-2}) when assessed using the temporal contrast sensitivity test than when using the contrast acuity test (0.1 cd m^{-2}), suggesting that the former is a more sensitive indicator to changes in outer retinal oxygenation. The FDT stimulus is intended to be magnocellular in nature and thereby sensitive to early glaucomatous ganglion cell injury. It is therefore perhaps unsurprising that it should also be sensitive to hypoxic impairment of magnocellular signalling and it may be anticipated that parvocellular contrast stimuli are less susceptible to hypoxic impairment (Pokorny and Smith, 1997; McAnany and Alexander, 2006). This would appear consistent with the absence of any effects of oxygenation state on mesopic spatial contrast thresholds at the fovea and the absence of any hyperoxic performance benefit on chromatic sensitivity at 0.21 cd m^{-2} relative to normoxic colour thresholds at that light level.

Oxygenation of the inner retina is effected by means of desaturation of Hb via a three-dimensional vascular network that is highly autoregulated to maintain a steady inner retinal PO_2. While more severe hypoxia may promote inner retinal dysfunction (for example, Kergoat, Hérard and Lemay, 2006), autoregulation should be able to compensate for the relatively slight desaturation of Hb occasioned by breathing 14.1% O_2 in the experiments reported in this thesis. On the other hand, the outer retina relies on a maintained choroidal PO_2 that was reduced by ~50% in the same experiments. In accordance with Fick's Law, this should also reduce O_2 delivery to the photoreceptor inner segments by ~50%. Even under conditions of good illumination (basal rod QO_2), this appears sufficient to induce retinotopic losses of visual (temporal contrast) sensitivity that broadly correspond with variations in photoreceptor density and net QO_2 (Birol, Wang, Budzynski *et al*, 2007).

In the dark, each human rod may require ~5,000,000 ATP molecules per second just to power the ion pumps (McIlwain, 1996, p71) and even mild hypoxia impairs them

(Linsenmeier and Steinberg, 1986). In light, the ion pumps work sub-maximally and the effect of hypoxia is no longer apparent. Under normoxic conditions, as light levels fall rod QO_2 will increase until the point at which photoreceptor function and visual sensitivity becomes O_2-limited, evidenced by a performance enhancement with supplementary O^2. Below this light level normoxic oxygenation is inadequate to maintain optimal performance. However, photoreceptor function may be compromised by mild exogenous hypoxia at much higher light levels below which visual decrements may be anticipated to be progressive either with decreasing light or worsening hypoxia or both.

11.1.3 To consider the implications for aircrew visual performance

General

These experiments have demonstrated relatively slight and subtle visual losses as a result of hypoxia equivalent to breathing air at an altitude of no more than 10,000 ft, possibly closer to 9000 ft when considering the data shown in Figure 1.12, Table 4-2 and Table 6-3. Civilian airliner cabin altitudes are generally less than this while military fast jet aircraft employ O_2 systems to prevent even these mild levels of hypoxia. However, the findings are relevant to flight in unpressurised aircraft that do not routinely use O_2 systems including helicopters, general aviation (light aircraft), older transport aircraft, gliders and other recreational vehicles. It is noteworthy that some of these may be flown in particularly challenging conditions including at low level over elevated terrain, at night, in conditions of poor visibility (weather), by instruments, and using a wide variety of binocular and monocular displays.

In the work presented here effects of hypoxia are always associated with visual performance decrements and, when present, these visual effects have easily achieved statistical significance despite testing only 12 subjects, suggesting a consistent and pervasive influence of hypoxia despite the wide variation in visual performance between subjects. The effects may therefore be regarded as unequivocal and appear to be well established at this level of hypoxia, suggesting that lesser deficits occur with milder levels of hypoxia.

It was anticipated that some visual losses might be found at low light levels, but hypoxia also compromised aspects of photopic vision, including temporal contrast sensitivity at 100 cd m^{-2} and low contrast acuity at 12 cd m^{-2}. The losses under dimmer

conditions nonetheless occurred at light levels at which excellent visual performance would normally be expected and which are relevant to flight at night in the mesopic cockpit or flight deck.

While each individual visual deficit under hypoxia might appear relatively minor, at upper mesopic luminance many of them will coexist and their cumulative influence may present a more meaningful degradation of overall visual task performance. Furthermore, the influence of hypoxia to delay rod adaptation indicates that under conditions of fluctuating ambient illumination the rod contribution to visual sensitivity will be degraded and delayed whenever light levels fall. While it is difficult to assess the level of general performance impairment and operational impact that will accompany this level of hypoxia, there is no doubt that fine acuity, sensitive chromatic discrimination and detection of subtle contrast fluctuations will be compromised. On the other hand, supplementary O_2 maintained normal visual performance relative to hypoxia and, at lower light levels, enhanced sensitivity and acuity relative to that associated with normoxia.

The implications of each experiment for the aviator will now be considered briefly in turn.

Dark adaptation

The temporal shifts of the early scotopic sensitivity curve during the dark adaptation experiments indicate that even mild hypoxia will compromise true night (scotopic) vision while hyperoxia will optimise light sensitivity. The influences of oxygenation state on early scotopic sensitivity and the rate of early scotopic adaptation are substantial, such that the delay times to achievement of sensitivity to a given scotopic luminance may vary by many minutes. Contemporary aviators will rely rarely on pure scotopic vision, but those who do should consider the benefit of hyperoxia to hasten adaptation to very low light levels. Accordingly, aircrew should consider using supplementary O_2 whenever they will be reliant upon viewing scotopic external scenes (illuminated by less than full moonlight perhaps) when flying at night. This will be most relevant to aircrew flying unpressurised aircraft at low level, especially over elevated terrain, without the benefit of night vision devices. However, flying in darkness increases the likelihood of accidents that are related to a scarcity of external visual cues, even in good weather (Wilson, 1999). The increased accident risk may exist during all phases of flight, including prior to take off (taxiway incidents) and during landing, when

runway incursions may go unnoticed. Thus supplementary O_2 could confer a visual benefit during ground movements at night. The risk may be greater when using unfamiliar or poorly illuminated airfields, especially those at significant elevation above sea level. For the majority of general aviation pilots flying light aircraft at night the use of supplementary O_2 will not be an option and the importance, under some circumstances, of allowing time for optimal dark adaptation will remain entirely relevant.

It should not be assumed that 100% O_2 is necessarily required to support optimal dark adaptation. A minimum local tissue PO_2 (that remains to be determined) would probably optimise rod function, and the F_IO_2 necessary to maintain the corresponding choroidal PO_2 would vary with altitude. This is likely to be well below 100% at sea level, although further studies would be necessary to confirm this.

By implication, supplementary O_2 is likely to facilitate the rod contribution to visual performance at mesopic luminance and to maintain visual sensitivity when light levels fluctuate in the mesopic range. The impairment with hypoxia and the benefit of hyperoxia are likely to be more pronounced at lower than higher mesopic levels. However, the results of the later experiments indicate that effects at the upper end of the mesopic range can also be significant, and it is possible that hyperoxia may offer a continuous advantage in a dynamic mesopic visual environment, particularly for those individuals who adapt more slowly to dim viewing conditions. The intriguing possibility exists that hyperoxia together with hypocapnia may have additive or compound effects to hasten dark adaptation and enhance visual sensitivity, particularly if they act through different mechanisms. Further study would be required to establish this.

At night, the cockpits and flight decks inhabited by professional (civilian and military) aircrew are typically illuminated at mesopic light levels and contain an increasing variety of sophisticated electro-optical displays that may be head-down, head-up, head or helmet-mounted, monocular or binocular, or, in the future, projected directly onto the retina. Monochrome displays demand optimal contrast discrimination while the use of colour is increasing (Menu, Ivan, Daumann *et al*, 2001). Viewing a dim external scene, aircrew must adjust cockpit lighting and display luminance to minimum levels that maintain visibility while avoiding glare (Rash and Manning, 2003). Accordingly, the studies of respiratory effects on mesopic vision are of greater relevance to the majority of professional aircrew who fly at night than the dark adaptation study.

Flicker sensitivity

The study of foveal flicker sensitivity supports a quantitative correlation between severity of hyperventilation and perception of flicker but also indicates that hypocapnia is unlikely to exert a meaningful effect at mesopic luminance. However, it is likely that the magnitude of the effect would be more pronounced in the photopic range (Granger and Ikeda, 1961). This retains contemporary relevance in aviation as a result of the profusion of CRT aircrew displays in recent years. Manufacturers have increased refresh rates to avoid flicker but about 50% of normal observers are able to detect flicker up to 72 Hz on a CRT with a mean luminance of 80 cd m^{-2} while 5% will still perceive flicker if the refresh rate is increased to 87 Hz (Bauer, Bonacker and Cavonius, 1983). Furthermore, performance on visual search and other tasks involving saccadic eye movements may be made worse if CRT refresh rates are increased insufficiently, possibly unless rates in excess of 115 Hz are achieved (Menozzi, Lang, Näpflin *et al*, 2001). While newer display technologies (such as liquid crystal displays) make the refresh rate issue redundant, existing aircrew displays will be used for years to come and new CRT displays continue to be developed. Other technologies, such as helmet mounted displays, are still specified with a refresh rate and may project imagery that is generated using CRTs. Thus, a more detailed investigation of the relationship between flicker perception and hyperventilation may be warranted using visual stimuli of more direct relevance to aircrew viewing CRT displays and imagery in flight.

Contrast sensitivity and acuity

Contrast sensitivity is reduced under low light conditions while visual acuity is degraded most under low light, low contrast conditions. In addition to the obvious relevance when viewing the external scene, a variety of contemporary displays employ monochrome (greyscale) imagery, so optimal contrast sensitivity and low contrast acuity will remain essential to ensure maximal operational effectiveness during missions involving such imagery.

The study of spatial contrast sensitivity provides reassurance that mild hypoxia will not compromise spatial contrast discrimination for foveal stimuli presented at low photopic and upper to mid-mesopic luminance. However, the FDT study suggests that temporal contrast sensitivity, specifically for stimuli that are likely to be detected through the magnocellular pathway, is much more susceptible to the effects of altered oxygenation, particularly beyond the fovea. The effects of hypoxia are likely to extend further into

the retinal periphery and risk compromising peripheral motion sensation. In addition, the low contrast acuity is relevant to discrimination of fine detail at photopic and mesopic luminance, within and beyond the cockpit, and also appears vulnerable at the fovea. Of concern, temporal contrast sensitivity and low contrast acuity were both compromised by hypoxia at photopic light levels, that is, under excellent viewing conditions. The impact of hypoxia was then of increasing relevance with descent into the mesopic range. At the lowest light levels, hyperoxia enhanced temporal contrast sensitivity and low contrast acuity beyond that achievable under normoxic conditions, clearly supporting a benefit of supplementary O_2 to provide visual performance enhancement at background fields of 0.1 to 1 cd m^{-2}.

The magnitudes of these effects were relatively slight but they nonetheless represent clear losses of fine sensitivity and acuity under viewing conditions and oxygenation states of direct relevance to contemporary flight. These losses might be considered acceptable under otherwise good flying conditions and in forms of aviation where they are unlikely to be safety critical. However, under more challenging conditions it may be considered appropriate to ensure optimal visual performance by breathing supplementary O_2. Examples might include flying at night, in poor visibility or poor weather, on military sorties where mission success might depend on optimal visual performance, when reliant on viewing displays at low luminance, viewing low contrast displays generating monochrome imagery, at provocative altitudes approaching or exceeding 10,000 ft, when flying at low level over elevated terrain, or under conditions of high luminance but low contrast (snow).

Chromatic sensitivity

Acceptable colour vision is essential to distinguish critical colours for air navigation, precision approach, warning and emergency purposes (Elliott, Moorhead and Evans, 2005; Menu, Ivan, Daumann, 2001). The increasing use of colour to code information is placing ever greater demands on aircrew colour vision, for example for the in-flight use of maps, manuals and contemporary electronic flight displays. This trend is likely to continue, as colour increases target conspicuity and enhances visual performance in coding or grouping and segmentation tasks, hastens information extraction and reduces errors (Barbur, Forsyth and Wooding, 1991; Macdonald and Cole, 1988; Post, Geiselman and Goodyear, 1999).

The effect of hypoxia progressively to compromise colour sensitivity with reducing mesopic luminance may impact on the discrimination of chromatic information by aircrew operating in unpressurised cabins approaching and over 10,000 ft. Besides the reduced conspicuity of coloured targets, colour deficiencies are associated with impaired visual performance as revealed by extended search times and higher error rates (Cole and Macdonald, 1988; Cole, Maddocks and Sharpe, 2004). Similar effects may be anticipated with the hypoxic impairment of colour vision at mesopic luminance. However, the consequences of reduced chromatic sensitivity go beyond impaired extraction of chromatic information. At mesopic levels the chromatic signal contributes to "effective" luminance contrast and reaction time (Walkey, Harlow, Barbur *et al*, 2005; Walkey, Harlow and Barbur, 2006), so hypoxic impairment of colour sensitivity might compromise wider aspects of aircrew visual performance.

Regardless of the effect of hypoxia, the occupational and aircrew requirements for colour vision at mesopic luminance are poorly defined. Further work would be necessary to define the "normal" mesopic chromatic sensitivity that is functionally acceptable at reduced luminance for those occupations in which colour vision deficiencies may be safety critical, including aviation.

Visual information processing

The net effect of hypoxia on visual discrimination is of more applied relevance to aircrew than theoretical considerations in relation to slight delays in visual information processing. However, the measurement of stimulus presentation times in relation to correct forced responses has direct relevance to visual task performance during flight, particularly with regard to display viewing. The study presented here indicates that hypoxia will compromise visual task performance in relation to the extraction of information from cluttered displays, particularly at low luminance. When turning down the brightness of a display to avoid glare, the contrast level may need to be increased to maintain visual discrimination performance and acquisition of visual information from brief saccades. To say the least, aircrew displays are complex, if not actually cluttered, and it may be appropriate to consider the use of supplementary O_2 when mission success relies heavily on timely decision making based on displayed visual information or imagery.

Summary

Many of the observed effects of hypoxia were highly statistically significant despite employing no more than 12 subjects in any particular study and often despite a wide variation in visual performance between subjects. While the magnitudes of the effects were generally slight, nonetheless they were unambiguous and well established at an equivalent altitude of only 10,000 ft. This suggests that milder losses of visual performance are likely at lower altitudes while more severe losses should be expected at higher altitudes. In general, aircrew are advised to use supplementary O_2 as a routine only for exposure to actual or cabin altitudes of greater than about 10,000 ft, and may be obliged to do so only at even greater altitudes of up to 14,500 ft, according to circumstance. Further work may be indicated to revise these limits in relation to visual performance requirements associated with particular forms of flight.

One final general point should be made. In these experiments the subjects were seated comfortably at rest in a quiet laboratory. They were trained in the visual task to be undertaken and, for the most part, had no distractions to prevent them doing their best on each test. Aircrew, on the other hand, are likely to have a high and stressful workload with numerous distractions while being exposed, for example, to concomitant thermal loads, vibration and disorienting manoeuvres, as well as mild hypoxia. Their visual scene will be changing constantly and could include switching between viewing at photopic luminance through night vision devices, viewing instruments and displays in a mesopic cockpit, and viewing a scotopic external environment. In addition, cognitive effects of hypoxia on reaction times, alertness, decision making, error rates and other aspects of visual task performance (Table 1-4) will not have been 'controlled out'. Subtle effects of hypoxia on individual attributes of visual perception will go entirely unrecognised yet may compound each other as well as risk compromising wider aspects of the aviation task.

11.2 Underpinning Hypothesis

Do the data support the hypothesis that progressive, endogenous, rod-driven, outer retinal hypoxia might render visual performance increasingly susceptible to exogenous hypoxia as the available light decreases through the mesopic range?

The dark adaptation data indicate clearly that rod function, in the dark, is limited by the availability of O_2, even under normoxic conditions. By implication, rod function is

likely to be compromised progressively by increasingly severe hypoxia and facilitated with supplementary O_2. Given that rod QO_2 increases as the available light decreases, and that the rod contribution to visual perception increases likewise, this dependence on O_2 may be anticipated to become more critical for normal visual performance during the transition from cone to rod vision, that is, in the mesopic range.

Three sets of results support this hypothesis. The progressive impairment of chromatic sensitivity indicates that the outer retina at the fovea becomes increasingly sensitive to mild exogenous hypoxia below ~2 cd m^{-2}. Since the exogenous hypoxic challenge is fixed, the endogenous, rod-driven element must be functionally meaningful at this light level. The 'centre' temporal contrast data support this at ~1 cd m^{-2}, with a benefit of hyperoxia over normoxic performance and therefore clearly O_2-dependent responses, since local tissue and functional hypoxia must exist under normoxic conditions if supplementary O_2 can manifest a performance benefit. The contrast acuity data manifest a similar O_2 dependency at 0.1 cd m^{-2}. These three findings provide compelling support for the existence of functionally-relevant, rod-driven outer retinal hypoxia in the mesopic range under normal respiratory conditions.

However, exogenous hypoxia impairs both temporal contrast sensitivity and low contrast acuity at higher light levels, relative to normoxia, the former at 100 cd m^{-2} (beyond the fovea) and the latter at 12 cd m^{-2} (from – 5° to + 5° from fixation). The patterns of response to altered oxygenation then change as light levels fall, and this is likely to reflect influences of changing rod and cone contributions to visual perception, changing photoreceptor QO_2, and changing rod and cone densities with eccentricity from fixation. It might be argued that some of the effects of hypoxia could lie in the inner retina or elsewhere in the visual system. However, the retinotopic nature of some of the data (for example, the effect of hypoxia on temporal contrast sensitivity at 100 cd m^{-2}) at least suggests clearly that the effect of altered oxygenation is within the retina. The changing nature of the effects of oxygenation state with decreasing light then supports a mechanism that is related to photoreceptor adaptation state and, hence, originates in the outer retina.

Linsenmeier and Braun (1992) indicate that photoreceptor QO_2 falls by around 50% when P_aO_2 is reduced to 50 mm Hg. On the other hand, inner retinal tissue PO_2 was well regulated under hypoxic conditions until the P_aO_2 fell below about 45 mm Hg in the dark, and was less affected in light. These observations correspond well to the human

exposures breathing 14.1% O_2 and further support an outer retinal mechanism of mild exogenous hypoxia to exaggerate innate rod-driven hypoxia in the mesopic range.

11.3 Lessons Learned

The lessons learned from the individual experiments are detailed in the relevant chapters and are not repeated here. However, when considering the sequence of experiments as a whole, a number of general lessons are identifiable.

The AR5 provided a means of ensuring absolute control of visual and respiratory adaptation states but only by imposing a degree of subject discomfort and potential distraction that is better avoided. Furthermore, it prevented measurement of pupil size during the experiments. With experience, and contrary to earlier expectation regarding voluntary eyelid closure to exclude light (see p 164), it was perfectly practicable to control both visual and respiratory adaptation using a more comfortable aircrew mask, a soft opaque blindfold, a shroud and careful instruction regarding eyelid closure, as employed during the contrast acuity experiments.

As the work progressed it became clear that a simpler regime of dark adaptation would allow stable mesopic adaptation states to be established without the need for prior retinal bleach or a full 15 minutes in the dark. This allowed a simpler experimental methodology for the contrast acuity experiments, reducing the duration of each experiment and the burden to the subjects.

In retrospect, the choice of vision tests could have been improved. Visual thresholds are typically probabilistic in nature and true forced-choice test techniques are well suited to their determination. Some of the tests used here (chromatic sensitivity, contrast acuity) employed 4-AFC paradigms that worked very well. The 'seen/not seen' response choice for the spatial contrast sensitivity test was not a true 2-AFC method but, fortunately, resulted in highly reproducible data that clearly indicate no meaningful effect of respiratory disturbance. Assessment of spatial contrast sensitivity away from fixation might better employ a 2-AFC or 4-AFC method. The use of commercially available clinical assessment tools (UFOV®, FDT) necessarily eliminated any possibility of tailoring the vision tests, each introducing methodological constraints and limiting analysis of the resulting data. For example, pupil measurement was impossible while using the FDT while highly skewed data sets constrained analysis of the UFOV® data. In future work, if time allowed, vision tests should be purposely designed to overcome

these issues. Any future study of flicker under respiratory challenge should employ a forced choice technique rather than the method of limits.

There is surprisingly little useful data available on the effect of changes in PO_2 and PCO_2 on pupil size. It was enormously beneficial for the contrast acuity experiment to be able to demonstrate that pupil size did not meaningfully contribute to the altered thresholds resulting from respiratory disturbance. In future studies care should be taken to ensure either that retinal illumination is controlled or that pupil size is measured, so that the data may be adjusted or regression analysis undertaken to exclude pupil size as a confounding factor, bearing in mind the influence of hypoxic miosis. Consideration might also be given to measuring respiratory effects on accommodative responses (Winn and Gilmartin, 1992) and to screening for axial length to exclude this as promoting variability in ocular blood flow amongst emmetropes (James, Trew, Clark *et al*, 1991; Mori, Konno, Hikichi *et al*, 2001).

The intention to control out an effect of exposure order by ensuring that these were balanced and randomised between the subjects was entirely appropriate, but it was not appreciated initially that this might introduce the possibility of a confounding effect of subject allocation bias to the different exposure order sub-groups. As a result, since the group data set is not fully factorial, a statistically significant effect of exposure order sub-group does not indicate a meaningful effect of exposure order, unless it is confirmed that baseline visual indices were normalised between sub-groups. In practice, once appreciated, it is obvious whether or not an initial subject allocation bias persists to produce a confounding effect of sub-group, and this approach remains valid when not wishing to expand the scope of an experiment to make all subjects complete all possible exposure orders. Doing so would have required a six-fold increase in the number of exposures for some of the studies and was clearly impractical. It will remain important to exclude an effect of exposure order in future work.

11.4 Summary and Future Work

When considering the effects of hypoxia on visual performance identified at different light levels it is convenient to add an altitude abscissa to Figure 1.4 and use the resulting graph to represent the effects of respiratory disturbance demonstrated during the course of the current work, as shown in Figure 11.1. Where benefits of hyperoxia have been demonstrated relative to normoxia, the supplementary O_2 is effectively enhancing visual

performance at sea level. Such benefits have been demonstrated across the mesopic range and are likely to result from enhanced rod function and sensitivity, and possibly increased rod QO_2. Hypoxia at 10,000 ft is likely to compromise both rod and cone function and appears to be progressive as light levels fall. Scotopic sensitivity is known to be compromised at altitudes between sea level and 10,000 ft, while various visual metrics have been shown to be affected by more severe hypoxia, equivalent to altitudes above 10,000 ft (see section 1.6). Most obviously from Figure 11.1, the nature of mesopic visual losses between sea level and 10,000 ft remain to be defined in any detail. As specific visual parameters are studied under various severities of hypoxia so an oxygenation threshold might be plotted on a graph similar to that at Figure 11.1, such that visual sensitivity at a given level of light adaptation might be unaffected above that threshold but compromised below it.

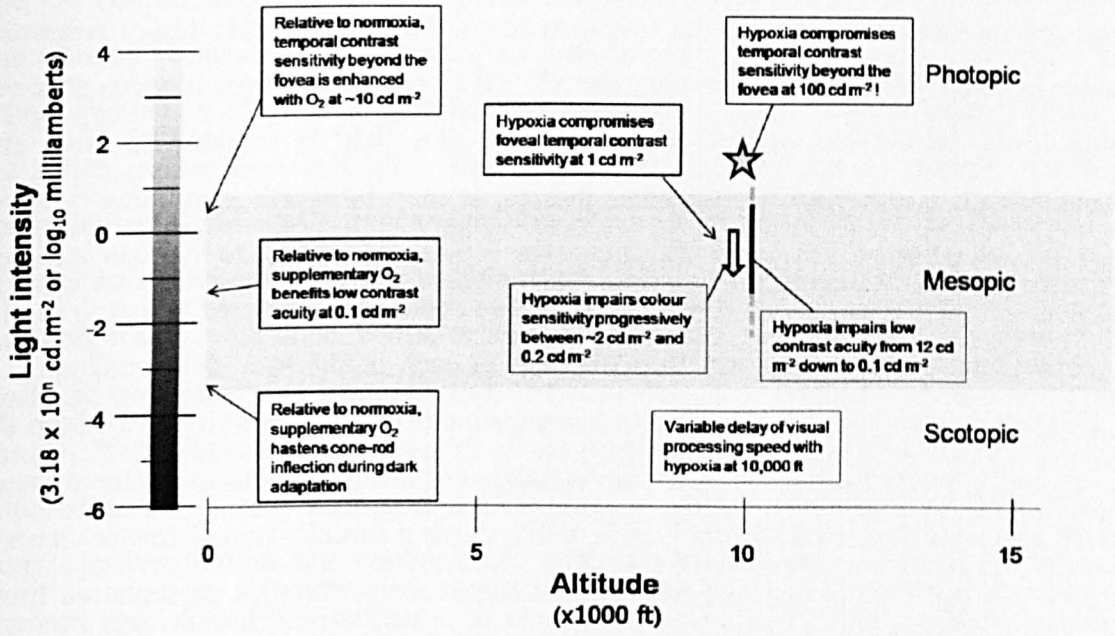


Figure 11.1 Novel effects of altered PO_2 on visual performance, related to equivalent altitude

Besides those shown on Figure 11.1, many other visual performance indices might warrant examination in relation to oxygenation. In particular, flicker thresholds are known to be sensitive to hypoxia and warrant consideration. Given the importance of flicker sensitivity in relation to movement detection and judgement of relative speed, it may be particularly worthwhile in the context of aviation to examine flicker thresholds further in the retinal periphery. From a more general perspective, besides considering achromatic flicker at different light levels, it would be interesting to consider the

possible increased metabolic requirements of the S-cone pathway using a blue-on-yellow heterochromatic flicker technique with different levels of hypoxia at different light levels.

This work has examined a single (magnocellular) temporal contrast stimulus, having a low spatial frequency and a high temporal frequency. It should not be assumed that other dynamic contrast stimuli would behave in the same way under respiratory disturbance and, ideally, a range of stimuli with higher spatial and lower temporal characteristics should be studied. Given that the initial work reported here has employed relatively large spatial sampling using the FDT perimeter, a simple way of extending this work to examine sensitivity with greater spatial resolution would be to repeat the study using the FDT Matrix perimeter's threshold protocol, accepting the limitations inherent in using a commercial tool.

The study of spatial contrast sensitivity could be extended to examining lower background field intensities at the fovea, to see if the possible early losses suspected under hypoxia when viewing through the ND 2.0 filter become more obvious at lower light levels. However, unaided vision in such dim light is unlikely to have any occupational or operational relevance. Instead, it may be worth examining whether spatial contrast sensitivity away from the fovea is more susceptible to hypoxia at higher light levels than at the fovea. A 4-AFC technique could easily be used to assess spatial contrast stimuli presented away from fixation in each of the four field quadrants. A similar approach could be used also to investigate chromatic sensitivity away from the fovea and to consider in more detail the extraction of information from cluttered visual fields against different background fields, while varying stimulus and distractor size and contrast. If possible, this would best be undertaken using stimulus presentation times that could allow both enhancement and degradation of stimulus discrimination, just as the choices of stimulus size and contrast allowed a contrast acuity performance enhancement with hyperoxia at the lowest light level.

While this work has demonstrated specific visual losses under hypoxia, it is not yet possible to quantify the overall net performance impairment in relation to functional activity or occupational or operational visual requirements, which, in any case, are poorly defined. For example, argument continues over the photopic occupational requirements for colour vision in aviation and the best screening procedures to use, while mesopic colour vision requirements are largely unknown and mesopic losses are not screened for. In order to more effectively interpret the consequences of hypoxia in

relation to, say, aviation, it would be helpful to develop a functional test of visual performance that could be applied to a variety of viewing conditions and was both representative of the flying task and, ideally, likely to discriminate between meaningful and inconsequential deficiencies. Normative data could then be compared with performance when hypoxic, or under other stressors, such as heat, cold and vibration.

11.5 Conclusion

Mild hypoxia compromises many aspects of visual performance including pronounced losses of chromatic sensitivity at ~0.2 cd m^{-2}, temporal contrast sensitivity at ~1 cd m^{-2}, and low contrast acuity at 0.1 cd m^{-2}. These indices appear more susceptible to hypoxia at lower mesopic light levels. Furthermore, relative to normoxia, hyperoxia not only hastens rod adaptation to dark but also appears to enhance temporal contrast sensitivity and low contrast acuity at the corresponding light levels. These findings support greater endogenous vulnerability to hypoxia as light levels fall in the mesopic range. The retinotopic nature of some of the effects indicates that the site of action of hypoxia is in the retina, rather than centrally. The mildness of the hypoxia is unlikely to have compromised significantly the oxygenation of the inner retina, given the effectiveness of inner retinal autoregulation to maintain local tissue PO_2. Studies often conclude that the inner retina is the more sensitive region to hypoxia, for example when examining electrophysiological responses (Janáky, Grósz, Tóth, *et al*, 2007; Kergoat, Hérard and Lemay, 2006). However, the severity of the hypoxia is usually more severe than that imposed in the current work and confounding influences of changes in CO_2 tension from hyperventilation are conveniently ignored. Instead, while the mild hypoxia imposed here should be transparent to the inner retina, the reduction of the choroidal 'pressure head' by almost 50% will greatly reduce the O_2 flux to the outer retina, in accordance with Fick's Law. As the light level falls and photoreceptor QO_2 increases, mild exogenous hypoxia may manifest visual performance impairment. The demonstration of a visual performance enhancement with supplementary O_2 indicates that outer retinal function can be limited by the local unavailability of O_2 even under normal respiratory conditions.

In healthy subjects, the practical physiological significance of such mild acute hypoxia is relevant only in aviation-related altitude exposure. Prolonged or gradual exposures typical of terrestrial altitude exposure, such as in mountaineering, provoke adaptive

responses and acclimatisation, with a rapid return to normal visual performance when assessed during controlled research exposures. In aviation, however, exposure is more abrupt, affording little opportunity for adaptation, and any consequent impairment of aircrew vision could have immediate detrimental effects and profound consequences for flight safety or mission success. It may be argued that the hypoxic impairment of visual sensitivity at low light levels is of such small absolute magnitude that it is biologically meaningless (Pierson, 1967). However, this fails to recognise that the usual dynamic range of visual sensitivity operates over only one or two orders of magnitude for a given state of light adaptation. At low light levels, a small absolute threshold elevation might nonetheless represent a meaningful loss of sensitivity when considered as a percentage of the likely dynamic range. Under circumstances in which a delay in reaction time by even a fraction of a second could be disastrous, such apparently slight losses in visual sensitivity may be entirely relevant. Further work is required to define more rigorously the potential consequences of the hypoxic losses described here and the possible requirement for supplementary O_2 to mitigate them during flight, particularly at night.

Many of the experiments reported here have served to highlight the profound reliance of the human retina on a maintained supply of O_2. Hypoxia has been implicated in the aetiology of various retinal pathologies that compromise vision, either as a result of systemic or ocular disease. Examples include diabetic retinopathy, glaucomatous damage, macular degeneration, vaso-occlusive disease, retinitis pigmentosa and various congenital disorders (Wangsa-Wirawan and Linsenmeier, 2003; Arjamaa and Nikinmaa, 2006). Furthermore, tissue oxygenation is intimately related to the regulation of retinal blood flow in health and disease (Pournaras, Rungger-Brändle, Riva *et al*, 2008). Additionally, there are a range of less well known clinical disorders that compromise mesopic vision (Petzold and Plant, 2006). As our understanding of the relevance of hypoxia to retinal function in health and disease progresses, it may perhaps become possible to screen for and monitor the progression of disordered retinal function and visual performance using finely tuned psychophysical tests of mesopic vision under mild hypoxic challenges.

References

Adler, F. H. (1945). Effect of anoxia on heterophoria and its analogy with convergent concomitant squint. *Archives of Ophthalmology*, 34: 227-232.

Adler, H. F., Burkhardt, W. L., Ivy, A. C., and Atkinson, A. J. (1950). Effect of various drugs on psychomotor performance at ground level and at simulated altitudes of 18000 feet in a low pressure chamber. *Aviation Medicine*, 21: 221-236.

Ahmed, J., Braun, R. D., Dunn, R. Jr., and Linsenmeier, R. A. (1993). Oxygen distribution in the macaque retina. *Investigative Ophthalmology and Visual Science*, 34(3): 516-521.

Akar, Y., Zulauf, M., Yucel, I., Akar, M. E., Erdem, U., and Trak, B. (2005). Menstrual-cycle dependent differences between right and left visual hemifields in perimetry. *Current Eye Research*, 30: 723-727.

Alder, V. A., and Constable, I. J. (1981). Effect of hypoxia on the maintained firing rate of retinal ganglion cells. *Investigative Ophthalmology and Visual Science*, 21: 450-456.

Alder, V. A., and Cringle, S. J. (1985). The effect of the retinal circulation on vitreal oxygen tension. *Current Eye Research*, 4: 121-129.

Alder, V. A., Cringle, S. J., and Constable, I. J. (1983). The retinal oxygen profile in cats. *Investigative Ophthalmology and Visual Science*, 24: 30-36.

Alder, V. A., Niemeyer, G., Cringle, S. J., and Brown, M. J. (1986). Vitreal oxygen tension gradients in the isolated perfused cat eye. *Current Eye Research*, 5: 249-256.

Alder, V. A., Yu, D., and Cringle, S. J. (1991). Vitreal oxygen tension measurements in the rat eye. *Experimental Eye Research*, 52: 293-299.

Alm, A., and Bill, A. (1970). Blood flow and oxygen extraction in the cat uvea at normal and high intraocular pressures. *Acta Physiologica Scandinavica*, 80: 19-28.

Alm, A., and Bill, A. (1972a). The oxygen supply to the retina, I. Effects of changes in intraocular and arterial blood pressures, and in arterial Po_2 and Pco_2 on the oxygen tension in the vitreous body of the cat. *Acta Physiologica Scandinavica*, 84: 261-305.

Alm, A., and Bill, A. (1972b). The oxygen supply to the retina, II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. *Acta Physiologica Scandinavica*, 84: 306-319.

Alpern, M., Faris, J., Eskildsen, P., and Garnett, P. (1954) Effect of hyperventilation on the human electroretinogram. *Science*, 121: 101-102.

Alpern, M., and Hendley, C. D. (1952). Visual functions as indices of physiological changes in the acid-base balance of the blood. *American Journal of Optometry*, 29: 301-314.

Anderson, B. (1968). Ocular effects of changes in oxygen and carbon dioxide tension. *Transactions of the American Ophthalmological Society*, 66: 423-474.

Anderson, B., and Saltzman, H. A. (1965). Hyperbaria, hybaroxia, and the retinal and cerebral vessels. *Headache*, 5(3): 73-77.

Anderson, B., Saltzman, H. A., and Frayser, R. (1967). Changes in arterial pCO_2 and retinal vessel size with oxygen breathing. *Investigative Ophthalmology and Visual Science*, 6: 416-419.

Anderson, A. J., and Vingrys, A. J. (2001). Multiple processes mediate flicker sensitivity. *Vision Research*, 41: 2449-2455.

Apaydin, K. C., Akar, Y., Akar, M. E., Zorlu, G. C., and Özer, H. O. (2004). Menstrual cycle dependent changes in blue-on-yellow visual field analysis of young diabetic women with severe non-proliferative diabetic retinopathy. Clinical and Experimental Ophthalmology, 32: 265-269.

Arden, G. B. (2001). The absence of diabetic retinopathy in patients with retinitis pigmentosa: implications for pathophysiology and possible treatment. *British Journal of Ophthalmology*, 85: 366-370.

Arden, G. B., Sidman, R. L., Arap, W., and Schlingemann, R. O. (2005). Spare the rod and spoil the eye. *British Journal of Ophthalmology*, 89: 764-769.

Arend, O., Harris, A., Martin, B. J., Holin, M., and Wolf, S. (1994). Retinal blood velocities during carbogen breathing using scanning laser ophthalmoscopy. *Acta Ophthalmologica*, 72: 332-336.

Arjamaa, O., and Nikinmaa, M. (2006). Oxygen-dependent diseases in the retina: Role of hypoxia-inducible factors. *Experimental Eye Research*, 83: 473-483.

Armstrong, R. A., Eperjesi, F., and Gilmartin, B. (2002). The application of analysis of variance (ANOVA) to different experimental designs in optometry. *Ophthalmic and Physiological Optics*, 22: 248-256.

Artes, P. H., Nicolela, M. T., McCormick, T. A., LeBlanc, R. P., and Chauhan, B. C. (2003). Effects of blur and repeated testing on sensitivity estimates with frequency doubling perimetry. *Investigative Ophthalmology and Visual Science*, 44: 646-652.

Baer, D. J. (1967). Hyperventilation effects on the critical flicker frequency of smokers and nonsmokers. *Journal of General Psychology*, 76: 201-206.

Bagshaw, M. (2006). The flight deck and cockpit. In Rainford, D. J., and Gradwell, D. P. (Eds). *Ernsting's Aviation Medicine* (4th ed). London: Hodder Arnold.

Ball, K. K., Beard, B. L., Roenker, D. L., Miller, R. L., and Griggs, D. S. (1988). Age and visual search: expanding the useful field of view. *Journal of the Optical Society of America A*, 5: 2210-2218.

Ball, K., and Owsley, C. (1993). The useful field of view test: a new technique for evaluating age-related declines in visual function. *Journal of the American Optometric Association*, 64: 71-79.

Barbur, J. L. (2004a). Learning from the pupil – studies of basic mechanisms and clinical applications. In Chalupa, L. M., and Werner, J. S. (Eds). *The Visual Neurosciences*. Cambridge, MA: MIT Press, Vol. 1, pp. 641-656.

Barbur, J. L. (2004b) 'Double-blindsight' revealed through the processing of color and luminance contrast defined motion signals. *Progress in Brain Research*, 144: 243-259.

Barbur, J. L., Forsyth, P. M., and Wooding, D. S. (1991). Colour, effective contrast and search performance. In: Schmid, R., and Zambarbieri, D. (Eds). *Oculomotor Control and Cognitive Processes*. North Holland: Elsevier Science Pub. B.V, pp 413-430.

Barbur, J. L., Harlow, A. J., and Plant, G. T. (1994). Insights into the different exploits of colour in the visual cortex. *Proceedings of the Royal Society B*, 258(1353): 327-334.

Barbur, J.L., Rodriguez-Carmona, M., and Harlow, A. J. Establishing the statistical limits of "normal" chromatic sensitivity. In: *Proceedings of the ISCC/CIE Expert Symposium 2006 "75 Years of the CIE Standard Colorimetric Observer"*; 16-17 May 2006, Ottawa, Ontario, Canada. CIE x030:2006.

Barbur, J. L., Walker, G., and Chisholm, C. (2005). Assessment of contrast acuity in the mesopic range. *Investigative Ophthalmology and Visual Science*, 46: E-abstract 3611.

Barnes, S. (1998). Modulation of vertebrate retinal function by pH. In Kaila, K., and Ransom, B. R. (Eds). *pH and Brain Function*. New York: Wiley-Liss, pp. 491-505.

Barr, R. E., and Silver, I. A. (1973). Effects of corneal environment on oxygen tension in the anterior chambers of rabbits. *Investigative Ophthalmology*, 12: 140-144.

Bartholomew, C. J., Jensen, W., Petros, T. V., Ferraro, F. R., Fire, K. M., Biberdorf, D., Fraley, E., Schalk, J., and Blumkin, D. (1999). The effect of moderate levels of simulated altitude on sustained cognitive performance. *International Journal of Aviation Psychology*, 9(4): 351-359.

Bauer, D., Bonacker, M., and Cavonius, C. R. (1983). Frame repetition rate for flicker-free viewing of bright VDU screens. *Displays*, 4: 31-33.

Bayer, A., Yumuşak, E., Şahin, Ö. F., and Uysal, Y. (2004). Intraocular pressure measured at ground level and 10,000 ft. *Aviation Space and Environmental Medicine*, 75: 543-545.

Baylor, D. A., Lamb, T. D., and Yau, K. W. (1979). Responses of retinal rods to single photons. *Journal of Physiology*, 288: 613-634.

Becker, H. F., Polo, O., McNamara, S. G., Berthon-Jones, M., and Sullivan, C. E. (1996). Effect of different levels of hyperoxia on breathing in healthy subjects. *Journal of Applied Physiology*, 81(4): 1683-1690.

Benedek, K., Kéri, S., Grósz, A., Tótka, Z., Tóth, E., and Benedek, G. (2002). Short-term hypobaric hypoxia enhances visual contrast sensitivity. *NeuroReport*, 13: 1063-1066.

Bentmann, A., Schmidt, M., Reuss, S., Wolfrum, U., Hankeln, T., and Burmester, T. (2005). Divergent distribution in vascular and avascular mammalian retinae links neuroglobin to cellular respiration. *Journal of Biological Chemistry*, 280: 20660-20665.

Berger, C., McFarland, R. A., Halperin, M. H., and Niven, J. I. (1943). The effects of anoxia on visual resolving power. *American Journal of Psychology*, 56: 385-407.

Bietti, G. (1953). Effects of experimentally decreased or increased oxygen supply in some ophthalmic diseases. *Archives of Ophthalmology*, 49: 491-513.

Billings, C. E. (1974). Evaluation of performance using the Gedye task. *Aerospace Medicine*, 45: 128-131.

Bimler, D. L., Kirkland, J., and Jameson, K. A. (2004). Quantifying variations in personal color spaces: are there sex differences in color vision? *Color Research and Application*, 29: 128-134.

Birch, J. (2003). *Diagnosis of Defective Colour Vision*, 2nd Ed. Edinburgh: Butterworth-Heinemann.

Birol, G., Wang, S., Budzynski, E., Wangsa-Wirawan, N. D., and Linsenmeier, R. A. (2007). Oxygen distribution and consumption in the macaque retina. *American Journal of Physiology – Heart and Circulatory Physiology*, 293: 1696-1704.

Birren, J. E., Fisher, M. B., Vollmer, E., and King, B. G. (1945). Effects of anoxia on performance at several simulated altitudes. *Journal of Experimental Psychology*, 35: 35-49.

Black, A. E., Prentice, A. M., and Coward, W.A. (1986). Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Human Nutrition: Clinical Nutrition*, 40C: 381-391.

Blake, R., Sloane, M., and Fox R. (1981). Further developments in binocular summation. *Perception and Psychophysics*, 30: 266-276.

Boehmer, R. D. (1987). Continuous, real-time, noninvasive monitor of blood pressure: Peñaz methodology applied to the finger. *Journal of Clinical Monitoring*, 3: 282-287.

Bohr, C., Hasselbalch, K. A., and Krogh, A. (1904) Ueber einen in biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung übt. *Scandinavian Archives of Physiology*, 16: 402-412.

Bonanno, J. A. (2001). Effects of contact lens-induced hypoxia on the physiology of the corneal endothelium. *Optometry and Vision Science*, 78: 783-790.

Borgdorff, P. (1975). Respiratory fluctuations in pupil size. *American Journal of Physiology*, 228: 1094-1102.

Bornstein, N. M., Gur, A. Y., Geyer, O., and Almog, Y. (2000). Vasomotor reactivity in the ophthalmic artery: different from or similar to intracerebral vessels? *European Journal of Ultrasound, 11*: 1-6.

Brabyn, L. B., and McGuiness, D. (1979). Gender differences in response to spatial frequency and stimulus orientation. *Perception and Psychophysics*, 26: 319-324.

Brandl, H., and Lachenmayr, B. (1994). Dependence of the sensitivity of the central visual field on hemoglobin-oxygen saturation. *Ophthalmologe*, 91: 151-155. (In German).

Braun, R. D., and Linsenmeier, R. A. (1995). Retinal oxygen tension and the electroretinogram during arterial occlusion in the cat. *Investigative Ophthalmology and Visual Science*, 36, 523-541.

Braun, R. D., Linsenmeier, R. A., and Goldstick, T. K. (1995). Oxygen consumption in the inner and outer retina of the cat. *Investigative Ophthalmology and Visual Science*, 36: 542-544.

Brennan, D. H., and Girvin, J. K. (1985). The flight acceptability of soft contact lenses: an environmental trial. *Aviation Space and Environmental Medicine*, 56: 43-48.

Brinchmann-Hansen, O., and Myhre, K. (1989). Blood pressure, intraocular pressure, and retinal vessels after high altitude mountain exposure. *Aviation Space and Environmental Medicine*, 60: 970-976.

Brinchmann-Hansen, O., and Myhre, K. (1990). Vascular response of retinal arteries and veins to acute hypoxia of 8000, 10000, 12500 and 15000 feet of simulated altitude. *Aviation Space and Environmental Medicine*, 61(2): 112-116.

Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S. N., Osborne, N. N., and Reichenbach, A. (2006). Müller cells in the healthy and diseased retina. *Progress in Retinal and Eye Research*, 25: 397-424.

Brown, W. R. (1951). The influence of luminance level on visual sensitivity to color differences. *Journal of the Optical Society of America*, 41: 684-688.

Brown, J. L., Hill, J. H., and Burke, R. E. (1957). The effect of hypoxia on the human electroretinogram. *American Journal of Ophthalmology*, 44: 57-67.

Buck, S., Knight, R., Fowler, G., and Hunt, B. (1998). Rod influence on hue-scaling functions. *Vision Research*,38: 3259-3263.

Burmester, T., and Hankeln, T. (2004). Neuroglobin: a respiratory protein of the nervous system. *News in Physiological Sciences*, 19: 110-113.

Cahoon, R. L. (1970). Vigilance performance under hypoxia. Journal of Applied Psychology, 54: 479-483.

Calcagnini, G., Censi, F., Lino, S and Cerutti, S. (2000). Spontaneous fluctuations of human pupil reflect central autonomic rhythms. *Methods of Information in Medicine*, 39: 142-145.

Calvert, P. D., Govardovskii, V. I., Arshavsky, V. Y., and Makino, C. L. (2002). Two temporal phases of light adaptation in retinal rods. *Journal of General Physiology*, 119: 129-145.

Cao, W., Govardovskii, V., Li, J. D., and Steinberg, R. H. (1996). Systemic hypoxia dehydrates the space surrounding photoreceptors in the cat retina. *Investigative Ophthalmology and Visual Science*, 37: 586-596.

Campbell, F. W., and Green, D. G. (1965a). Monocular vs. binocular visual acuity. *Nature*, 208: 191-192.

Campbell, F. W., and Green, D. G. (1965b). Optical and retinal factors affecting visual resolution. *Journal of Physiology*, 181: 576-593.

Carlisle, R., Lanphier, E. H., and Rahn, H. (1964). Hyperbaric oxygen and persistence of vision in retinal ischemia. *Journal of Applied Physiology*, 19: 914-918.

Carney, L. G. (1974). Studies on the basis of ocular changes during contact lens wear. PhD thesis. University of Melbourne.

Cheng, H. W., Ma, R. S., Ni, H. Y., and Wang, X. B. (1999). Effects of mild and moderate hypoxia on event-related potentials and the comparison of different stimulus paradigms. *Space Medicine and Medical Engineering (Beijing)*, 12: 23-27.

Chesler, M. (2003). Regulation and modulation of pH in the brain. *Physiological Reviews*, 83:1183-1221.

Chisholm, C. M., and Barbur, J. L. (2001). *The Contrast Acuity Assessment (CAA) Test.* Civil Aviation Authority Paper 2001/5, London, June 2001. As at 20 March 2008, available from http://www.caa.co.uk/docs/33/CAPAP200105.PDF

Chisholm, C. M., Evans, A. D. B., Harlow, J. A., and Barbur, J. L. (2003). New test to assess pilot's vision following refractive surgery. *Aviation Space and Environmental Medicine*, 74: 551-559.

Cho, N-C., Poulsen, G. L., Ver Hoeve, J. N., and Nork, M. (2000). Selective loss of S-cones in diabetic retinopathy. *Archives of Ophthalmology*, 118(10): 1393-1400.

Christensen, C. L., Gliner, J. A., Horvath, S. M., and Wagner, J. A. (1977). Effects of three kinds of hypoxias on vigilance performance. *Aviation Space and Environmental Medicine*, 48(6): 491-496.

Christiansen, J., Douglas, C. G., and Haldane, J. S. (1914). The absorption and dissociation of carbon dioxide by human blood. *Journal of Physiology (London)*, 48: 244-271.

Chung, H. S., Harris, A., Halter, P. J., Kagemann, L., Roff, E. J., Garzozi, H. J., Hosking, S. L., and Martin, B. J. (1999). Regional differences in retinal vascular reactivity. *Investigative Ophthalmology and Visual Science*, 40(10): 2448-2452.

Cioffi, G. A., Granstam, E., and Alm, A. (2003). Ocular circulation. In Kaufman, P. L., and Alm, A. (Eds). *Adler's Physiology of the Eye: Clinical Application* (10th ed). St Louis: Mosby, pp. 747-784.

Cohn, H., DeAgostini, M., Aron-Rosa, D., Laloum, L., and Boller, F. (1994). Sex differences in the left and right hemifields of normal subjects with computerised static perimetry. *British Journal of Ophthalmology*, 78: 837-841.

Cole, B. L., and Macdonald, W. A. (1988). Defective colour vision can impede information acquisition from redundantly colour-coded video displays. *Ophthalmic and Physiological Optics*, 8: 198-210.

Cole, B. L., Maddocks, J. D., and Sharpe, K. (2004). Visual search and the conspicuity of coloured targets for colour vision normal and colour vision deficient observers. *Clinical and Experimental Optometry*, 87: 294-304.

Collins, M., Davis, B., and Wood, J. (1995). Microfluctuations of steady-state accommodation and the cardiopulmonary system. *Vision Research*, 35: 2491-2502.

Connolly, D. M., and Hosking, S. L. (2006). Aviation-related respiratory gas disturbances affect dark adaptation: A reappraisal. *Vision Research*, 46: 1784-1793.

Connolly, D., and Hosking, S. (2007). Quantitative correlation of hyperventilation with flicker sensitivity. *Optometry and Vision Science*, 84: 529-534.

Connolly, D. M., Barbur, J. L., Hosking, S. L., and Moorhead, I. R. (2008). Mild hypoxia impairs chromatic sensitivity in the mesopic range. *Investigative Ophthalmology and Visual Science*, 49: 820-827.

Connolly, D. M., and Hosking, S. L. (2008). Oxygenation and gender effects on photopic frequency-doubled contrast sensitivity. *Vision Research*, 48: 281-288.

Connors, M. M. (1968). The effect of breathing 100% oxygen on foveal thresholds. *Human Factors*, 10(4): 377-384.

Cooke, J. P. (1970). Intraocular pressure and retinal responses of dogs at 45,000 ft and 80,000 ft. *Aerospace Medicine*, 41: 283-289.

Costa, M. F,, Ventura, D. F., Perazzolo, F., Murakoshi, M., and Silveira LC. (2006). Absence of binocular summation, eye dominance, and learning effects in color discrimination. *Visual Neuroscience*. 23: 461-469.

Cottrell, J. J., Lebovitz, B. L., Fennel, R. G., and Kohn, G. M. (1995). In-flight arterial saturation: continuous monitoring by pulse oximetry. *Aviation Space and Environmental Medicine*, 66: 126-130.

Craik, K. J. W. (1940). Origin of visual after-images. *Nature*, 154: 512.

Creel, D. J., Crandall, A. S., and Swartz, M. (1997). Hyperopic shift induced by high altitude after radial keratotomy. *Journal of Refractive Surgery*, 13: 398-400.

Cringle, S. J., Yu, D., Alder, V., and Su, E. (1999). Light and choroidal pO_2 modulation of intraretinal oxygen levels in an avascular retina. *Investigative Ophthalmology and Visual Science*, 40: 2307-2313.

Cringle, S. J., Yu, D., Yu, P., and Su, E. (2002). Intraretinal oxygen consumption in the rat *in vivo*. *Investigative Ophthalmology and Visual Science*, 43: 1922-1927.

Crow, T. J., and Kelman, G. R. (1973). Psychological effects of mild acute hypoxia. British Journal of Anaesthesia, 45: 335-337.

Curcio, C. A., Sloan, K. R., Kalina, R. E., and Hendrickson, A. E. (1990). Human photoreceptor topography. *Journal of Computational Neurology*, 292: 497-523.

Cusick, P. L., Benson, O. O., and Boothby, W. M. (1940). Effect of anoxia and of high concentrations of oxygen on the retinal vessels: Preliminary report. *Proceedings of the Mayo Clinic*, 15: 500-502.

Cymerman, A., Muza, S. R., Ditzler, D., Sharp, M., and Friedlander, A. (2003). Oculomotor and pupillary reflexes during acute exposure to hypobaric hypoxia. Technical Report USARIEM-T-03-4. United States Army Research Institute of Environmental Medicine, Natick, MA.

Cymerman, A., Muza, S. R., Friedlander, A. L., Fulco, C. S., and Rock, P. B. (2005). Saccadic velocity and pupillary reflexes during acclimatization to altitude (4300 m). *Aviation Space and Environmental Medicine*, 76: 627-634.

Cymerman, A., Rock, P. B., Muza, S. R., Lyons, T. P., Fulco, C. S., Mazzeo, R. S., Butterfield, G., and Moore, L. G. (2000). Intraocular pressure and acclimatization to 4300 M altitude. *Aviation Space and Environmental Medicine*, 71: 1045-1050.

Dacey, D. M. (1999). Primate retina: cell types, circuits and color opponency. *Progress in Retinal and Eye Research*, 18: 737-763.

Dautrabande, L., and Haldane, J. (1921). The effect of respiration of oxygen on breathing and circulation. *Journal of Physiology (London)*, 55: 296-299.

Dean, F. M., Arden, G. B., and Dornhurst, A. (1997). Partial reversal of protan and deutan colour defects with inhaled oxygen in insulin dependent diabetic subjects. *British Journal of Ophthalmology*, 81: 27-30.

De Groot, S. G., and Gebhard, J. W. (1952). Pupil size as determined by adapting luminance. *Journal of the Optical Society of America A*, 42: 492-495.

Demant, E., Nagahara, K., and Niemeyer, G. (1982). Effects of changes in systemic blood pressure on the electroretinogram of the cat: evidence for retinal autoregulation. *Investigative Ophthalmology and Visual Science*, 23: 683-687.

Denison, D M. (1981). High altitudes and hypoxia. In Edholm, O. G., and Weiner, J. S. (Eds). *The Principles and Practice of Human Physiology*. London: Academic Press, pp. 241-301.

Denison, D. M., Ledwith, F., and Poulton, E. C. (1966). Complex reaction times at simulated cabin altitudes of 5,000 and 8,000 ft. *Aerospace Medicine, 40*: 413-417.

Dewitte, K., Fierens, C., Stöckl, D., and Thienpoint, L. M. (2002). Application of the Bland-Altman plot for interpretation of method-comparison studies: a critical investigation of its practice. *Clinical Chemistry*, 48(5): 799-801.

Dollery, C. T., Bulpitt, C. J., and Kohner, E. M. (1969). Oxygen supply to the retina from the retinal and choroidal circulations at normal and increased arterial oxygen tensions. *Investigative Ophthalmology and Visual Science*, 8: 588-594.

Dollery, C. T., Hill, D. W., Mailer, C. M., and Ramalho, P. S. (1964). High oxygen pressure and the retinal blood vessels. *The Lancet*, 2: 291-292.

Domenech, B., Seguí, M. M., Capilla, P., and Illueca, C. (1994). Variation of the visual acuity – luminance function with background colour. *Ophthalmic Physiology*, 14:302-305.

Drasdo, N., Chiti, Z., Owens, D. R., and North, R. V. (2002). Effect of darkness on inner retinal hypoxia in diabetes. *The Lancet*, 359: 2251-2253.

Drummond, J. C., and Rebuck, A. S. (1981). Effect of hypoxia on the corneoretinal potential in man. *Ophthalmic Research*, 13: 213-223.

Du, J. Y., Li, X. Y., Zhuang, Y., Wu, X. Y., and Wang, T. (1999). Effects of acute mild and moderate hypoxia on human short memory. *Space Medicine and Medical Engineering (Beijing)*, 12: 270-273.

Dunn, C., and Ross, H. (1985). Gender, the menstrual cycle and visual contrast sensitivity. *Journal of Physiology*, 267: 19P (abstract in Proceedings of the Physiological Society).

Dyer, F. (1988). *Effects of Low and High Oxygen Tensions and Related Respiratory Conditions on Visual Performance: A Literature Review*. USAARL Report No. 88-7. Fort Rucker, AL: United States Army Aeromedical Research Laboratory.

Ebrey, T., and Koutalos, Y. (2001). Vertebrate photoreceptors. *Progress in Retinal and Eye Research*, 20: 49-94.

Efron, N., and Carney, L. G. (1979). Oxygen levels beneath the closed eyelid. *Investigative Ophthalmology and Visual Science*, 18: 93-95.

Elgin, S. S. (1964). Arteriovenous oxygen difference across the uveal tract of the dog eye. *Investigative Ophthalmology*, 3: 417-426.

Elliott, E. C., Moorhead, I. R., and Evans, A. D. (2005). A task analysis of minimum colour vision requirements for professional pilots [Abstract]. *Aviation Space and Environmental Medicine*, Abstract 328; 76(3-1): 282.

Eisenlohr, J. E., Langham, M. E., and Maumenee, A. E. (1962). Manometric studies of the pressure-volume relationship in living and enucleated eyes of individual human subjects. *British Journal of Ophthalmology*, 46: 536-548.

Eisner, A., Burke, S. N., and Toomey, M. D. (2004). Visual sensitivity across the menstrual cycle. *Visual Neuroscience*, 21: 513-531.

Eng, W. G., Rasco, J. L., and Marano, J. A. (1978). Low atmospheric pressure effects on wearing soft contact lenses. *Aviation Space and Environmental Medicine*, 49: 73-75.

Enroth-Cugell, C., Goldstick, T. K., and Linsenmeier, R. A. (1980). The contrast sensitivity of cat retinal ganglion cells at reduced oxygen tensions. *Journal of Physiology*, 304: 59-81.

Erkelens, C. J., and van Ee, R. (2002). Multi-coloured stereograms unveil two binocular colour mechanisms in human vision. *Vision Research*, 42: 1103-1112.

Ernest, J. T., and Krill, A. E. (1971). The effect of hypoxia on visual function. Psychophysical studies. *Investigative Ophthalmology*, 10: 323-328.

Ernsting, J. (1963). The ideal relationship between inspired oxygen concentration and cabin altitude. *Aerospace Medicine*, 34: 991-997.

Ernsting, J. (1965). Respiration and anoxia *and* The effects of anoxia on the central nervous system. In Gillies, J. A. (Ed), *A textbook of aviation physiology*. Oxford: Pergamon Press.

Ernsting, J. (1978). Prevention of hypoxia – acceptable compromises. *Aviation Space and Environmental Medicine*, 49: 495-502.

Ernsting, J., Ward, J., and Rutherford, O. M. (2006). Cardiovascular and respiratory physiology. In Rainford, D. J., and Gradwell, D. P. (Eds). *Ernsting's Aviation Medicine* (4th ed). London: Hodder Arnold.

Evans, J. N., and McFarland, R. A. (1938). The effects of oxygen deprivation on the central visual field. *American Journal of Ophthalmology*, 21: 968-980.

Fain, G. L., Matthews, H. R., and Cornwall, M. C. (1996). Dark adaptation in vertebrate photoreceptors. *Trends in Neuroscience*, 19: 502-507.

Fain, G. L., Matthews, H. R., Cornwall, M. C. and Koutalos, Y. (2001). Adaptation in vertebrate photoreceptors. *Physiological Reviews*, 81: 117-151.

Fallon, T. J., Maxwell, D., and Kohner, E. M. (1985). Retinal vascular autoregulation in conditions of hyperoxia and hypoxia using the blue field entoptic phenomenon. *Ophthalmology*, 92: 701-705.

Fatt, I., and Bieber, M. T. (1968). The steady-state distribution of oxygen and carbon dioxide in the in vivo cornea. I. The open eye in air and the closed eye. *Experimental Eye Research*, 7: 103.

Feigl, B., Stewart, I., and Brown, B. (2007). Experimental hypoxia in human eyes: implications for ischaemic disease. *Clinical neurophysiology*, 118: 887-895.

Feke, G. T., Tagawa, H., Deupree, D. M., Goger, D. G., Sebag, J., and Weiter, J. J. (1989). Blood flow in the normal human retina. *Investigative Ophthalmology and Visual Science*, 30: 58-65.

Feke, G. T., Zuckerman, R., Green, G. J., and Weiter, J. J. (1983). Response of human retinal blood flow to light and dark. *Investigative Ophthalmology and Visual Science*, 24: 136-141.

Fenn, W. O., Galambos, R., Otis, A. B., and Rahn, H. (1949). Corneo-retinal potential in anoxia and acapnia. *Journal of Applied Physiology*, 1: 710-716.

Ferree, C. E., and Rand, G. (1922). Perimetry: Variable factors influencing the breadth of the color fields. *American Journal of Ophthalmology*, 5: 886-895.

Figarola, R. R., and Billings, C. E. (1966). Effects of meprobamate and hypoxia on psychomotor performance. *Aerospace Medicine*, 37: 951-954.

Fiorica, V., Burr, M. J., and Moses, R. (1971). Effects of low-grade hypoxia on performance in a vigilance situation. *Aerospace Medicine*, 42: 1049-1055.

Firsov, M. L., Kolesnikov, A. V., Golobokova E. Y., and Govardovskii, V. I. (2005). Two realms of dark adaptation. *Vision Research*, 45: 147-151.

Fischer, F. P., and Jongbloed, J. (1935). Untersuchungen über die Dunkeladaptation bei herabgesetztem Sauerstoffdruck der Atmungsluft. *Archiv für Augenheilkunde*, 109, 432.

Fitch, C. L., Swedberg, S. H., and Livesey, J. C. (2000). Measurement and manipulation of the partial pressure of oxygen in the rat anterior chamber. *Current Eye Research*, 20: 121-126.

Fitzpatrick, D. T. (1994). Visual manifestations of neurologic decompression sickness. *Aviation Space and Environmental Medicine*, 65: 736-738.

Flower, R. A., Fryczkowski, A. W., and McLeod, D. S. (1995). Variability in choriocapillaris blood flow distribution. *Investigative Ophthalmology and Visual Science*, 36: 1247-1258.

Flower, R. W., and Patz, A. (1971). The effect of hyperbaric oxygenation on retinal ischaemia. *Investigative Ophthalmology and Visual Science*, 10: 605-616.

Flynn, W. J., Miller, R. E., Tredici, T. J., Block, M. G., Kirby, E. E., and Provines, W. F. (1987). Contact lens wear at altitude: subcontact lens bubble formation. *Aviation Space and Environmental Medicine*, 58: 1115-1118.

Forte, J. D. (2005). Binocular summation of color and luminance contrast gratings [Abstract]. *Journal of Vision*, 5(8):E-abstract 795a. doi:10.1167/5.8.795 (http://journalofvision.org/5/8/795/)

Fowler, K. T. (1969). The respiratory mass spectrometer. *Physics in Medicine and Biology*. 14: 185-199.

Fowler, B., and Kelso, B. (1992). The effects of hypoxia on components of the human event-related potential and relationship to reaction time. *Aviation Space and Environmental Medicine*. 63: 510-516.

Fowler, B., and Nathoo, A. (1997). Slowing due to acute hypoxia originates early in the visual system. *Aviation Space and Environmental Medicine*, 68: 886-889.

Fowler, B., and Prlic, H. (1995). A comparison of visual and auditory RT and P300 latency thresholds to acute hypoxia. *Aviation Space and Environmental Medicine*, 66: 645-650.

Fowler, B., Banner, J., and Pogue, J. (1993). The slowing of visual processing by hypoxia. *Ergonomics*, 36: 727-735.

Fowler, B., Elcombe, D. D., Kelso, B., and Porlier, G. (1987). The threshold for hypoxia effects on perceptual-motor performance. *Human Factors*, 29: 61-66.

Fowler, B., Paul, M., Porlier, G., Elcombe, D. D., and Taylor, M. (1985). A re-evaluation of the minimum altitude at which hypoxic performance decrements can be detected. *Ergonomics*, 28: 781-791.

Fowler, B., Taylor, M., and Porlier, G. (1987). The effects of hypoxia on reaction time and movement time components of a perceptual-motor task. *Ergonomics*, 30: 1475-1485.

Fowler, B., White, P. L., Wright, G. R., and Ackles, K. N. (1982). The effects of hypoxia on serial response time. *Ergonomics*, 25: 189-201.

Frayser, R., and Hickam, J. B. (1964). Retinal vascular response to breathing increased carbon dioxide and oxygen concentrations. *Investigative Ophthalmology and Visual Science*, 3: 427-431.

Frayser, R., Houston, C. S., Gray, G. W., and Bryan. A. C. (1971). The response of the retinal circulation to altitude. *Archives of Internal Medicine*, 127: 708-711.

Friedenwald, J. S. (1937). Contribution to the theory and practice of tonometry. *American Journal of Ophthalmology*, 20: 985-1024.

Friedman, E. (1970). Choroidal blood flow. Pressure-flow relationships. *Archives of Ophthalmology*, 83: 95-99.

Friedman, E., and Chandra, S. R. (1972). Choroidal blood flow. III. Effects of oxygen and carbon dioxide. *Archives of Ophthalmology*, 87: 70-71.

Friedmann, A. I. (1980). Outline of Visual Field Analyser. Mark II. *Documenta Ophthalmologica Proceedings Series*, 22: 65-67.

Frisby, J. P., Barrett, R. F., and Thornton, J. A. (1973). Effect of mild acute hypoxia on a decision-making task. *Aerospace Medicine*, 44: 523-526.

Frisén, L., and Lindblom, B. (1988). Binocular summation in humans: evidence for a hierarchic model. *Journal of Physiology*, 402: 773-782.

Fuchsjager-Mayrl, G., Malec, M., Amoako-Mensah, T., Kolodjaschna, J., and Schmetterer, L. (2003). Changes in choroidal blood flow during light/dark transitions are not altered by atropine or propranolol in healthy eyes. *Vision Research*, 43: 2185-2190.

Fuchsjager-Mayrl, G., Polska, E., Malec, M., and Schmetterer, L. (2001). Unilateral light-dark transitions affect choroidal blood flow in both eyes. *Vision Research*, 41(22): 2919-2924.

Fulk, G. W., and West, R. W. (1990). Effect of simulated altitude on the visual fields of glaucoma patients and the elderly. *Optometry & Vision Science*, 68(5): 344-350.

Funk, R. H. W. (1997). Blood supply of the retina. *Ophthalmic Research*, 29: 320-325.

Gallin-Cohen, P. F., Podos, S. M., and Yablonski, M. E. (1980). Oxygen lowers intraocular pressure. *Investigative Ophthalmology and Visual Science*, 19: 43-48.

Gavriysky, V. S. (1995). Influence of a twofold voluntary hyperventilation on visually evoked cortical potentials and human pupillogram. *Documenta Ophthalmologica*, 77: 213-224.

Gayeski, T. E. S., and Honig, C. R. (1988). Intracellular PO_2 in axis of individual fibers in working dog gracilis muscles. *American Journal of Physiology*, 254: H1179-1186.

Geiser, M. H., Riva, C. E., Dorner, G. T., Diermann, U., Luksch, A., and Schmetterer, L. (2000). Response of choroidal blood flow in the foveal region to hyperoxia and hyperoxia-hypercapnia. *Current Eye Research*, 21: 669-676.

Gellhorn, E. (1936a) The effect of O2-lack, variation in the CO2-content of the inspired air, and hyperpnea on visual intensity discrimination. *American Journal of Physiology*, 115: 679-684.

Gellhorn, E. (1936b). The effectiveness of carbon dioxide in combating the changes in visual intensity discrimination produced by oxygen deficiency. *American Journal of Physiology*, 117: 75-78.

Gellhorn, E., and Hailman, H. (1943). The effect of anoxia on sense organs. *Federation Proceedings*, 2: 122-126.

Gellhorn, E., and Hailman, H. (1944). The parallelism in changes of sensory function and electroencephalogram in anoxia and the effect of hypercapnia under these conditions. *Psychosomatic Medicine*, 6: 23-30.

Gellhorn, E., and Levin, J. (1945). The nature of pupillary dilatation in anoxia. *American Journal of Physiology*, 143: 282-289.

Gibson, T. M. (1979). Hyperventilation in aircrew: A review. *Aviation, Space and Environmental Medicine*, 50(7): 725-733.

Gibson, T. M. (1984). Hyperventilation in flight. *Aviation, Space and Environmental Medicine*, 55: 411-412.

Ginsburg, N. (1967). Ascending-descending differences in CFF measurement as a function of eccentricity and starting point. *Journal of Psychology*, 67: 161-167.

Glass, G. V., Peckham, P. D., and Sanders, J. R. (1972). Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research*, 43: 237-288.

Gouras, P., and Carr, R. E. (1965). Light-induced DC responses of monkey retina before and after central retinal artery interruption. *Investigative Ophthalmology and Visual Science*, 4: 310-317.

Gowdy, P. D., and Cicerone, C. M. (1998). The spatial arrangement of the L and M cones in the central fovea of the living human eye. *Vision Research*, 38: 2575-2589.

Gradwell, D. P. (1993). Human physiological responses to positive pressure breathing for high altitude protection. PhD Thesis. University of London.

Gradwell, D. P. (2006). Hypoxia and hyperventilation. In Rainford, D. J., and Gradwell, D. P. (Eds). *Ernsting's Aviation Medicine* (4th ed). London: Hodder Arnold.

Granger, G. W., and Ikeda, H. (1961). Effect of hyperventilation on foveal critical flicker frequency. *Vision Research*, 1: 287-300.

Gray, L. S., Gilmartin, B., and Winn, B. (2002). Accommodation microfluctuations and pupil size during sustained viewing of visual display terminals. *Ophthalmic and Physiological Optics*, 20: 5-10.

Gray, L. S., Winn, B., and Gilmartin, B. (1993). Effect of target luminance on microfluctuations of accommodation. *Ophthalmic and Physiological Optics*, 13: 258-265.

Green, R. G., and Morgan, D. R. (1985). The effects of mild hypoxia on a logical reasoning task. *Aviation Space and Environmental Medicine*, 56: 1004-1008.

Green, D. M., and Swets, J. A. (1966). *Signal Detection Theory and Psychophysics*. New York: Wiley.

Greenstein, V. C., Hood, D. C., Ritch, R., Steinberger, D., and Carr, R. E. (1989). S (blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. *Investigative Ophthalmology and Visual Science*, 30: 1732-1737.

Grehn, F. (1981). The sensitivity of the retinal nerve fibre layer to elevated intraocular pressure and graded hypoxia in the cat. *Vision Research*, 21: 1697-1701.

Grehn, F., Grüsser, O., and Stange, D. (1984). Effect of short-term intraocular pressure increase on cat retinal ganglion cell activity. *Behavioural Brain Research*, 14: 109-121.

Grunwald, J. E., Sinclair, S. H., and Riva, C. E. (1982). Autoregulation of the retinal circulation in response to decrease of intraocular pressure below normal. *Investigative Ophthalmology and Visual Science*, 23(1): 124-127.

Guttridge, N. M. (1993). Changes in ocular and visual variables during the menstrual cycle. *Ophthalmic and Physiological Optics*, 14: 38-48.

Hagins, W. A., Ross, P. D., Tate, R. I., and Yoshikami, S. (1989). Transduction heats of retinal rods. Tests of the role of cGMP by pyroelectric calorimetry. *Proceedings of the National Academy of Sciences of the USA*, 86: 1224-1228.

Halperin, M. H., McFarland, R. A., Niven, J. I., and Roughton, F. J. W. (1959). The time course of the effects of carbon monoxide on visual thresholds. *Journal of Physiology*, 146: 583-593.

Halstead, W. C. (1945). Chronic intermittent anoxia and the dynamic visual field. *Journal of Psychology*, 20: 49-56.

Hamdane, D., Kiger, L., Dewilde, S., Green, B. N., Pesce, A., Uzan, J., Burmester, T., Hankeln, T., Bolognesi, M., Moens, L., and Marden M. C. (2003). The redox state of the cell regulates the ligand binding affinity of human neuroglobin and cytoglobin. *Journal of Biological Chemistry*, 278: 51713-51721.

Hankeln, T., Ebner, B., Fuchs, C., Gerlach, F., Haberkamp M., Laufs, T. L., Roesner, A., Schmidt, M., Weich, B., Wystub, S., Saaler-Reinhardt, S., Reuss, S., Bolognesi, M., De Sanctis, D., Marden, M. C., Kiger, L., Moens, L., Dewilde, S., Nevo, E., Avivi, A., Weber, R. E., Fago, A., and Burmester, T. (2005). Neuroglobin and cytoglobin in search of their role in the vertebrate globin family. *Journal of Inorganic Biochemistry*, 99: 110-119.

Harris, A., Arend. O., Danis. R. P., Evans. D., Wolf. S., and Martin B. J. (1996). Hyperoxia improves contrast sensitivity in early diabetic retinopathy. *British Journal of Ophthalmology*, 80: 209-213.

Harris, A., Arend, O., Kopecky, K., Caldemeyer, K., Wolf, S., Sponsel, W., and Martin, B. (1994). Physiological perturbation of ocular and cerebral blood flow as measured by scanning laser ophthalmoscopy and color Doppler imaging. *Survey of Ophthalmology*, 38: Suppl, S81-S86.

Harris, A., Arend, O., Wolf, S., Cantor, L. B., and Martin, B. J. (1995). CO_2 dependence of retinal arterial and capillary blood velocity. *Acta Ophthalmologica Scandinavica*, 73: 421-424.

Harris, A., Kagemann, L., and Cioffi, G. A. (1998). Assessment of human ocular hemodynamics. *Survey of Ophthalmology, 42(6)*: 509-533.

Hecht, S., Hendley, C. D., Frank, S. R., and Haig, C. (1946). Anoxia and brightness discrimination. *Journal of General Physiology*, 29: 335-351.

Hecht, S., Shlaer, S., and Pirenne, M. H. (1942). Energy, quanta and vision. *Journal of General Physiology*, 25: 819-840.

Heeg, G. P., Ponsioen, T. L., and Jansonius, N. M. (2003). Learning effect, normal range, and test-retest variability of frequency doubling perimetry as a function of age, perimetric experience, and the presence or absence of glaucoma. *Ophthalmic and Physiological Optics*, 23: 535-540.

Helbig, H., Hinz, J. P., Kellner, U., and Foerster, M. H. (1993). Oxygen in the anterior chamber of the human eye. *German Journal of Ophthalmology*, 2: 161-164.

Hickam, J. B., and Frayser, R. (1966). Studies of the retinal circulation in man. *Circulation*, 33: 302-316.

Hickam, J. B., Frayser, R., and Ross, J. (1963). A study of retinal venous blood oxygen saturation in human subjects by photographic means. *Circulation*, 27: 375-385.

Hinshaw, H. C., Rushmer, W. M., and Boothby, W. M. (1943) The hyperventilation syndrome and its importance in aviation. *Journal of Aviation Medicine*, 14: 100-104.

Hirata, Y., and Nishiwaki, H. (2006). The choroidal circulation assessed by laser-targeted angiography. *Progress in Retinal and Eye Research*, 25: 129-147.

Hoang, Q. V., Linsenmeier, R. A., Chung, C. K., and Curcio, C. A. (2002). Photoreceptor inner segments in monkey and human retina: mitochondrial density, optics, and regional variation. *Visual Neuroscience*, 19: 395-407.

Holden, B. A., Sweeney, D. F., and Sanderson, G. (1984). The minimum precorneal oxygen tension to avoid corneal edema. *Investigative Ophthalmology and Visual Science*, 25: 476-480.

Holopigian, K., Blake, R., and Greenwald, M. J. (1986). Selective losses in binocular vision in anisometric amblyopes. *Vision Research*, 26: 621-630.

Home, R. (1978). Binocular summation: a study of contrast sensitivity, visual acuity and recognition. *Vision Research*, 18: 579-585.

Hongwei, C., Ruishan, M., and Li, L. (1997). The effects of sensory modalities on brain cognitive response under hypoxia. *Electroencephalography and Clinical Neurophysiology*, 103: 110.

Hoper, J., Funk, R., Zagorski, Z., and Rohen, J. W. (1989). Oxygen delivery to the anterior chamber of the eye – a novel function of the anterior iris surface. *Current Eye Research*, 8: 649-659.

Horng, C-T., Liu, C-C., Wu, Y-C., Chen, J-T., Chang, C-J., and Tsai, M-L. (2008). Visual fields during acute exposure to a simulated altitude of 7620 m. *Aviation Space and Environmental Medicine*, 79: 666-669.

Horsten, G. P. M., and Winkelman, J. E. (1957). Effect of temporary occlusion of the aorta on the electroretinogram. *Archives of Ophthalmology*, 57: 557-565.

Hosking, S. L., Evans, D. W., Embleton, S. J., Houde, B., Amos, J. F., and Bartlett, J. D. (2001). Hypercapnia invokes an acute loss of contrast sensitivity in untreated glaucoma patients. *British Journal of Ophthalmology*, 85: 1352-1356.

Huang, W-X., Yu, Q., and Cohen, M. I. (2000). Fast (3 Hz and 10 Hz) and slow (respiratory) rhythms in cervical sympathetic nerve and unit discharges of the cat. *Journal of Physiology*, 523: 459-477.

Hubel, D. H., and Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *Journal of Physiology*, 160: 106-154.

Hubel, D. H., and Wiesel, T. N. (1968). Receptive fields and functional architecture of monkey striate cortex. *Journal of Physiology*, 195: 215-243.

Iester, M., Capris, P., Pandolfo, A., Zingirian, M., and Traverso, C. E. (2000). Learning effect, short-term fluctuation, and long-term fluctuation in frequency doubling technique. *American Journal of Ophthalmology*, 130: 160-164.

Iscoe, S. I., and Fisher, J. A. (2005). Hyperoxia-induced hypocapnia. An underappreciated risk. *Chest*, 128: 430-433.

Iwasaki, M., and Inomata, H. (1986). Relation between superficial capillaries and foveal structures in the human retina. *Investigative Ophthalmology and Visual Science*, 27: 1698-1705.

Jackson, G. R., Owsley, C., and McGwin, G. Jr. (1999). Aging and dark adaptation. *Vision Research*, 39, 3975-3982.

Jäkel, F., and Wichmann, F. A. (2006). Spatial four-alternative forced-choice method is the preferred psychophysical method for naïve observers. *Journal of Vision*, 6: 1307-1322.

James, C. B., Trew, D. R., Clark, K., and Smith, S. E. (1991). Factors influencing the ocular pulse – axial length. *Graefe's Archive for Clinical and Experimental Ophthalmology*, 229: 341-344.

Janáky, M., Grósz, A., Tóth, E., Benedek, K., and Benedek, G. (2007).Hypobaric hypoxia reduces the amplitude of oscillatory potentials in the human ERG. *Documenta Ophthalmologica*, 114: 45-51.

Jensen, O., Hari, R., and Kaila, K. (2002). Visually evoked gamma responses in the human brain are enhanced during voluntary hyperventilation. *NeuroImage*,15: 575-586.

Jiménez, J. R., Medina, J. M., Jiménez del Barco, L., and Díaz, J. A. (2002). Binocular summation of chromatic changes as measured by visual reaction time. *Perceptual Psychophysics*, 64:140-147.

Jiménez, J. R., Valero, E., Anera, R. G., Martinez, J. A., and Salas, C. (2003). Chromatic changes in relation to binocular summation determined with contrast thresholds. *Color Research and Application*, 28: 366-370.

Johnson, C. A., and Casson, E. J. (1995). Effects of luminance, contrast, and blur on visual acuity. *Optometry and Vision Science*, 72(12): 864-869.

Johnson, C. A., and Sample, P. A. (2003) Perimetry and visual field testing. In P. L. Kaufman and A. Alm (Eds.), *Adler's Physiology of the Eye: Clinical Application* (10th ed.). St Louis: Mosby.

Johnson, N., and Petersik, J. T. (1987). Preliminary findings suggesting cyclic changes in visual contrast sensitivity during the menstrual cycle. *Perceptual and Motor Skills*, 64: 587-594.

Jorgensen, V. (1955). The effect of hyperventilation on flicker fusion frequencies with foveal images of different sizes. *Acta Ophthalmologica*, 33: 615-621.

Justo, M. S., Bermudez, M. A., Perez, R., and Gonzalez, F. (2004). Binocular interaction and performance of visual tasks. *Ophthalmic and Physiological Optics*, 24: 82-90.

Kang Derwent, J., and Linsenmeier, R. A. (2000). Effects of hypoxemia on the a- and b-waves of the electroretinogram in the cat retina. *Investigative Ophthalmology and Visual Science*, 41: 3634-3642.

Karakucuk, S., Oner, A. O., Goktas, S., Siki, E. and Kose, O. (2004). Color vision changes in young subjects acutely exposed to 3,000 m altitude. *Aviation Space and Environmental Medicine*, 75: 364-366.

Kardon, R. (2003). The pupil. In Kaufman, P. L., and Alm, A. (Eds). *Adler's physiology of the eye: clinical application*. (10th ed). St Louis: Mosby.

Kaufmann, C., Eibel, G-K., Gössl, C., Pütz, B., and Auer, D. P. (2001). Frequency dependence and gender effects in visual cortical regions involved in temporal frequency dependent pattern processing. *Human Brain Mapping*, 14: 28-38.

Kaufmann, H., Schotte, J. F., and Holtmann, H. W. (1971). Ocular tension during hyperventilation. *Ophthalmic Research*, 2: 261-265.

Kayama, Y. (1974). Evoked potentials of the central visual system during and after hypoxia in cats. *Electroencephalography and Clinical Neurophysiology*, 36: 619-628.

Kelman, G. R., and Crow, T. J. (1969) Impairment of mental performance at a simulated altitude of 8,000 feet. *Aerospace Medicine*, 40: 981-982

Kelman, G. R., Crow, T. J., and Bursill, A. E. (1969). Effect of mild hypoxia on mental performance assessed by a test of selective attention. *Aerospace Medicine*, 40: 301-303.

Kent, P. R. (1966). Oxygen breathing effects upon night vision thresholds. USNSMRC Report No 469. United States Naval Submarine Medical Research Center, Groton, CT.

Kergoat, H., and Faucher, C. (1999). Effects of oxygen and carbogen breathing on choroidal haemodynamics in humans. *Investigative Ophthalmology and Visual Science*, 40: 2906-2911.

Kergoat, H., Hérard, M-E., and Lemay, M. (2006). RGC sensitivity to mild systemic hypoxia. *Investigative Ophthalmology and Visual Science*, 47: 5423-5427.

Kergoat, H., Marinier, J., and Lovasik, J. V. (2005). Effects of transient mild systemic hypoxia on the pulsatile choroidal blood flow in healthy young human adults. *Current Eye Research*, 30: 465-470.

Kety, S. S., and Schmidt, C. F. (1946). The effects of active and passive hyperventilation on cerebral blood flow, cerebral oxygen consumption, cardiac output and blood pressure of normal young men. *Journal of Clinical Investigation*, 25: 107-119.

Kety, S. S., and Schmidt, C. F. (1948). The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men', *Journal of Clinical Investigation*, 27: 484-492.

Kiel, J. W. (1995). The effect of arterial pressure on the ocular pressure-volume relationship in the rabbit. *Experimental Eye Research*, 60: 267-278.

Kiel, J. W., and van Heuven, W. A. (1995). Ocular perfusion pressure and choroidal blood flow in the rabbit. *Investigative Ophthalmology and Visual Science*, 36(3), 579-585.

Kielar, R. A., Teraslinna, P., Kearney, J. T., and Barker, D. (1977). Effect of changes in PCO_2 on intraocular tension. *Investigative Ophthalmology and Visual Science*, 16: 534-537.

Kiss, B., Dallinger, S., Polak, K., Findl, O., Eichler, H., and Schmetterer, L. (2001). Ocular hemodynamics during isometric exercise. *Microvascular Research*, 61: 1-13.

Klemp, K., Lund-Andersen, H., Sander, B., and Larsen, M. (2007). The effect of acute hypoxia and hyperoxia on the slow multifocal electroretinogram in healthy subjects. *Investigative Ophthalmology and Visual Science*, 48: 3405-3412.

Klyce, S. D. (1981). Stromal lactate accumulation can account for corneal oedema osmotically following epithelial hypoxia in the rabbit. *Journal of Physiology*, 321: 49-64.

Knight, R., Buck, S. L., Fowler, G. A., and Nguyen, A. (1998). Rods affect S-cone discrimination on the Farnsworth-Munsell 100-hue test. *Vision Research*, 38: 3477-3481.

Knoblauch, K., Vital-Durand, F., and Barbur, J. L. (2000). Variation of chromatic sensitivity across the life span. *Vision Research*, 41: 23-36.

Kobrick, J. L. (1968). Effects of exposure to 12,800 feet and acetazolamide on visual performance. *Perceptual and Motor Skills*, 27: 939-944.

Kobrick, J. L. (1970). Effects of hypoxia and acetazolamide on color sensitivity zones in the visual field. *Journal of Applied Physiology*, 28: 741-747.

Kobrick, J. L. (1972). Effects of hypoxia on voluntary response time to peripheral stimuli during central target monitoring. *Ergonomics*, 15(2): 147-156.

Kobrick, J. L. (1974). Effects of hypoxia on peripheral visual response to rapid sustained stimulation. *Journal of Applied Physiology*, 37(1): 75-79.

Kobrick, J. L. (1975). Effects of hypoxia on peripheral visual response to dim stimuli. *Perceptual and Motor Skills*, 41: 467-474.

Kobrick, J. L. (1976). Effects of prior hypoxia exposure on visual target detection during later more severe hypoxia. *Perceptual and Motor Skills*, 42: 751-761.

Kobrick, J. L., and Appleton, B. (1971). Effects of extended hypoxia on visual performance and retinal vascular state. *Journal of Applied Physiology*, 31: 357-362.

Kobrick, J. L., Crohn, E., Shukitt, B., Houston, C. S., Sutton, J. R., and Cymerman, A. (1988). Operation Everest II: Lack of an effect of extreme altitude on visual contrast sensitivity. *Aviation Space and Environmental Medicine*, 59: 160-164.

Kobrick, J. L., and Dusek, E. R. (1970). Effects of hypoxia on voluntary response time to peripherally located visual stimuli. *Journal of Applied Physiology*, 29: 444-448.

Kobrick, J. L., Zwick, H., Witt, C. E., and Devine, J. A. (1984). Effects of extended hypoxia on night vision. *Aviation Space and Environmental Medicine*, 55:191-5.

Kogure, S., Membrey, W. L., Fitzke, F. W., and Tsukahara, S. (2000). Effect of decreased retinal illumination on frequency doubling technology. *Japanese Journal of Ophthalmology*, 44: 489-493.

Kuffler, S. W. (1953). Discharge patterns and functional organization of mammalian retina. *Journal of Neurophysiology*, 16: 37-68.

Kvansakul, J., Edgar, D. F., Barbur, J. L., Schalch, W., Barker, F. M., and Köpcke, W. (2004). Assessment of visual performance in relation to macular pigment density in human vision. *Investigative Ophthalmology and Visual Science*, 45: E-abstract 4340.

Kwan, M., and Fatt, I. (1971). A non-invasive method of continuous arterial oxygen tension estimation from measured palpebral conjunctival oxygen tension. *Anesthesiology*, 35: 309.

Kwan, M., Niinikoski, J., and Hunt, T. K. (1972). In vivo measurements of oxygen tension in the cornea, aqueous humor, and anterior lens of the open eye. *Investigative Ophthalmology*, 11(2): 108-114.

Lakowski, R., and Morton, A. (1978). Acquired colour losses and oral contraceptives. *Modern Problems in Ophthalmology*, 19: 314-318.

Lamb, T. D. (1990). Dark adaptation: a re-examination. In R. F. Hess, L. T. Sharpe, and K. Nordby (Eds). *Night Vision. Basic, Clinical and Applied Aspects* (1st ed.) Cambridge: Cambridge University Press, pp 177-222.

Lamb, T. D., and Pugh, E. N. Jr. (2004). Dark adaptation and the retinoid cycle of vision. *Progress in Retinal and Eye Research*, 23, 307-380.

Landers, M. B. (1978). Retinal oxygenation via the choroidal circulation. *Transactions of the American Ophthalmological Society*, 76: 528-556.

Landis, C. (1954). Determinants of the critical flicker-fusion threshold. *Physiological Reviews*, 34: 259-286.

Langham, M. E., Farrell, R. A., O'Brien, V., Silver, D. M., and Schilder, P. (1989). Blood flow in the human eye. *Acta Ophthalmologica Supplementa*, 191; S9-S13.

Larke, J. R., Parrish, S. T., and Wigham, C. G. (1981). Apparent human corneal oxygen uptake rate. *American Journal of Optometric and Physiological Optics*, 58(10): 803-805.

Leber, L. L., Roscoe, S. N., and Southward, G. M. (1986). Mild hypoxia and visual performance with night vision goggles. *Aviation Space and Environmental Medicine*, 57(4): 318-324.

Ledwith, F. (1970). The effects of hypoxia on choice reaction time and movement time. *Ergonomics*, 13: 465-482.

Legge, G. E. (1984a). Binocular contrast summation – 1. Detection and discrimination. *Vision Research*, 24: 373-383.

Legge, G. E. (1984b). Binocular contrast summation – 2. Quadratic summation. *Vision Research*, 24: 385-394.

Leibowitz, H. W., and Appelle, S. (1969). The effect of a central task on luminance thresholds for peripherally presented stimuli. *Human Factors*, 11: 387-392.

Lewis, D. H., and Duane, T. D. (1956). Electroretinogram in man during blackout. *Journal of Applied Physiology*, 9: 105-110.

Li, X. Y., Wu, X. Y., Fu, C., Shen, X. F., Yang, C. B., and Wu, Y. H. (2000). Effects of acute exposure to mild or moderate hypoxia on human psychomotor performance and visual reaction time. *Space Medicine and Medical Engineering (Beijing)*, 13: 235-239.

Lilienthal, J. L., and Fugitt, C. H. (1946). The effect of low concentrations of carboxyhemoglobin on the "altitude tolerance" of man'. *American Journal of Physiology*, 145: 359-364.

Linsenmeier, R. A. (1986). Effects of light and darkness on oxygen distribution and consumption in the cat retina. *Journal of General Physiology*, 88: 521-542.

Linsenmeier, R. A. (1990). Electrophysiological consequences of retinal hypoxia. *Graefe's Archives of Clinical and Experimental Ophthalmology*, 228: 143-150.

Linsenmeier, R. A., and Braun, R. D. (1992). Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *Journal of General Physiology*, 99: 177-197.

Linsenmeier, R. A., Mines, A. H., and Steinberg, R. H. (1983). Effects of hypoxia and hypercapnia on the light peak and electroretinogram of the cat. *Investigative Ophthalmology and Visual Science*, 24: 37-46.

Linsenmeier, R. A., and Padnick-Silver, L. (2000). Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. *Investigative Ophthalmology and Visual Science*, 41: 3117-3123.

Linsenmeier, R. A., and Steinberg, R. H. (1986). Mechanisms of hypoxic effects on the cat DC electroretinogram. *Investigative Ophthalmology and Visual Science*, 27: 1385-1394.

Linsenmeier, R. A., and Yancey, C, M. (1989). Effects of hyperoxia on the oxygen distribution in the intact cat retina. *Investigative Ophthalmology and Visual Science*, 30(4), 612-618.

Livingston, P. C. (1944). Visual problems of aerial warfare: II. Day: Studies in photopic vision. *The Lancet*, 2: 67-73.

Lowenstein, O., Feinberg, R., and Loewenfeld, I. E. (1963). Pupillary movements during acute and chronic fatigue: a new test for the objective evaluation of tiredness. *Investigative Ophthalmology*, 2: 138-157.

Longo, A., Geiser, M., and Riva, C. E. (2000). Subfoveal choroidal blood flow in response to light-dark exposure. *Investigative Ophthalmology and Visual Science*, 41: 2678-2683.

Lythgoe, R. (1931). Dark-adaptation and the peripheral colour sensations of normal subjects. *British Journal of Ophthalmology*, 15: 193-210.

Maag, C. H. (1957). Characteristics of mental impairment in hypoxia. *American Journal of Psychology*, 73: 243-247.

MacAdam, D. L. (1942). Visual sensitivities to colour differences in daylight. *Journal of the Optical Society of America*, 32: 247-274.

Macdonald, W. A., and Cole, B. L. (1988). Evaluating the role of colour in a flight information cockpit display. *Ergonomics*, 31: 13-37.

Macri, F. J. (1964). The intraocular and vascular pressures of the cat eye. *Experimental Eye Research*, 3: 266-282.

Mader, T. H., and White, L. J. (1995). Refractive changes at extreme altitude after radial keratotomy. *American Journal of Ophthalmology*, 119: 733-737.

Mandell, R. B., and Farrell, R. (1980). Corneal swelling at low atmospheric oxygen pressures. *Investigative Ophthalmology and Visual Science*, 19: 697-702.

Margrain, T. H. (1997). Recovery of spatial vision following intense light adaptation. PhD Thesis. City University, London.

Marmor, M. F., Donovan, W. J., and Gaba, D. M. (1985). Effects of hypoxia and hyperoxia on the human standing potential. *Documenta Ophthalmologica*, 60: 347-352.

Marré, M., Neubauer, O., and Nemetz, U. (1974). Colour vision and the "pill". *Modern Problems in Ophthalmology*, 13: 345-348.

Mastropasqua, L., Ciancaglini, M., Di Tano, G., Carpineto, P., Lobefalo, L., Loffredo, B., Bosco, D., Columbaro, M., and Falcieri, E. (1998). Ultrastuctural changes in rat cornea after prolonged hypobaric hypoxia. *Journal of Submicroscopic Cytology and Pathology*, 30: 285-293.

Mattiello, M., Maneiro, M., and Gastelú, R. (2001). Relation between retinal hypoxia and alteration of the chromatic vision in anemias and glaucomas. *Color Research and Application*, 26: S277-S280.

Mazicioğlu, M., Karaküçük, S., Öner, A., Ulusal, H., and Mirza, G. E. (2006). Effects of high altitude on colour vision of climbers during an expedition to Mt Ağri. *Erciyes Medical Journal*, 28: 134-137.

McAnany, J. J., and Alexander, K. R. (2006). Contrast sensitivity for letter optotypes vs. gratings under conditions biased toward parvocellular and magnocellular pathways. *Vision Research*, 46: 1574-1584.

McBee, J. K., Palczewski, K., Baehr, W., and Pepperberg, D. R. (2001). Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Progress in Retinal and Eye Research*, 20, 469-529.

McCarthy, D., Corban, R., Legg, S., and Faris, J. (1995). Effects of mild hypoxia on perceptual-motor performance: a signal-detection approach. *Ergonomics*, 38(10): 1979-1992.

McDonald, R., and Adler, F. H. (1939). Effect of anoxemia on the dark adaptation of the normal and of the vitamin A-deficient subject. *Archives of Ophthalmology*, 22: 980-988.

McFarland, R. A. (1932). The psychological effects of oxygen deprivation (anoxaemia) on human behaviour. *Archives of Psychology*, 145: 2-135.

McFarland, R. A. (1937). Psycho-physiological studies at high altitude in the Andes. II Sensory and motor responses during acclimatization. *Journal of Comparative and Physiological Psychology*, 23: 227-258.

McFarland, R. A. (1971). Human factors in relation to the development of pressurized cabins. *Aerospace Medicine*, 12: 1303-1318.

McFarland, R. A., and Edwards, H. T. (1937). The effects of prolonged exposures to altitudes of 8000 to 12000 feet during trans-Pacific flights. *Journal of Aviation Medicine*, 8(3): 156-177.

McFarland, R. A., and Evans, J. N. (1939). Alterations in dark adaptation under reduced oxygen tensions. *American Journal of Physiology*, 127: 37-50.

McFarland, R. A., Evans, J. N., and Halperin, M. H. (1941). Ophthalmic aspects of acute oxygen deficiency. *Archives of Ophthalmology*, 26: 886-913.

McFarland, R. A., and Forbes, W. H. (1940). The effects of variations in the concentration of oxygen and of glucose on dark adaptation. *Journal of General Physiology*, 24: 69-98.

McFarland, R. A., and Halperin, M. H. (1940). The relation between foveal visual acuity and illumination under reduced oxygen tensions. *Journal of General Physiology*, 23: 613-630.

McFarland, R. A., Halperin. M. H., and Niven, J. I. (1944). Visual thresholds as an index of physiological imbalance during anoxia. *American Journal of Physiology*, 142: 328-349.

McFarland, R. A., Holway, A. H., and Hurvich, A. H. (1942). *Studies of Visual Fatigue*. Graduate School of Business Administration Report. Boston: Harvard University.

McFarland, R. A., Knehr, C. A., and Berens, C. (1937a). The effects of oxygen deprivation on eye movements in reading. *Journal of Experimental Psychology*, 21: 1-25.

McFarland, R. A., Knehr, C. A., and Berens, C. (1937b). The effects of anoxaemia on ocular movements while reading. *American Journal of Ophthalmology*, 20: 1204-1219.

McKendrick, A. M., Sampson, G. P., Walland, M. J., and Badcock, D. R. (2007). Contrast sensitivity changes due to glaucoma and normal aging: low-spatial-frequency losses in both magnocellular and parvocellular pathways. *Investigative Ophthalmology and Visual Science*, 48: 215-2122.

McKendrick, A. M., Anderson, A. J., Johnson, C. A., and Fortune, B. (2003). Appearance of the frequency doubling stimulus in normal subjects and patients with glaucoma. *Investigative Ophthalmology and Visual Science*, 44: 1111-1116.

McLaren, J. W., Dinslage, S., Dillon, J. P., Roberts, J. E., and Brubaker, R. F. (1998). Measuring oxygen tension in the anterior chamber of rabbits. *Investigative Ophthalmology and Visual Science*, 39: 1899-1909.

McMann, M. A., Parmley, V. C., Brady, S. M., White, L. J., Raymond, W. R., Mazzoli, R. A., Hansen, E. A., and Mader, T. H. (2002). Analysis of anterior and posterior corneal curvature changes using Orbscan technology in radial keratotomy eyes exposed to hypoxia. *Journal of Cataract and Refractive Surgery*, 28: 289-294.

McNulty, R., Wang, H., Mathias, R. T., Ortwerth, B. J., Truscott, R. J. W., and Bassnett, S. (2004). Regulation of tissue oxygen levels in the mammalian lens. *Journal of Physiology*, 559: 883-898.

McRipley, M. A., Ahmed, J., Chen, E. P-C., and Linsenmeier, R. A. (1997). Effects of adaptation level and hypoglycaemia on function of the cat retina during hypoxemia. *Visual Neuroscience*, 14: 339-350.

Medina, J. M. (2006). Binocular interaction in random chromatic changes at isoluminance. *Journal of the Optical Society of America A*, 23: 239-246.

Medina, J. M., and Mullen, K. T. (2007). Colour-luminance interactions in binocular summation. Vision Research, 47: 1120-1128.

Meek, K. M., Dennis, S., and Khan, S. (2003). Changes in the refractive index of the stroma and its extrafibrillar matrix when the cornea swells. *Biophysical Journal*, 85: 2205-2212.

Meese, T. S., and Williams, C. B. (2000). Probability summation for multiple patches of luminance modulation. *Vision Research*, 40: 2101-2113.

Mellergård, P., and Siesjö, B. K. (1998). Cerebral energy metabolism and pH. In Kaila, K., and Ransom, B. R. (Eds). *pH and Brain Function*, (1st ed). New York: Wiley-Liss, pp. 67-91.

Menozzi, M., Lang, F., Näpflin, U., Zeller, C., and Krueger, H. (2001). CRT versus LCD: effects of refresh rate, display technology, and background luminance in visual performance. *Displays*, 22: 79-85.

Menu, J-P., Ivan, D., Daumann, F-J., Diamantopoulos, I., Firth, J. L., Heikens, M-F., LeBail, B., Leger, A., Walraven, J., Alferdinck, J., and Yates, J. T. *Operational colour vision in the modern aviation environment.* Working Group 24 of the Human Factors and Medicine Panel of the North Atlantic Treaty Organization Research and Technology Organization. RTO Technical Report 16. Brussels, Belgium: NATO; 2001.

Mercier, A., and Duguet, J. (1950). Physiopathology of the flyer's eye. Translated from the French 'Physiopathologie oculaire de l'aviateur'. Randolph Field, TX: U. S. Air Force School of Aviation Medicine.

Miller, E. F. (1958). Effect of breathing 100 per cent oxygen upon visual field and visual acuity. *Journal of Aviation Medicine*, 29: 598-602.

Millodot, M., and O'Leary, D. J. (1980). Effect of oxygen deprivation on corneal sensitivity. *Acta Ophthalmologica*, 58: 434-439.

Mills, M. D. (2000). An assessment of intraocular pressure rise in patients with gas-filled eyes during simulated air flight. *Ophthalmology*, 108: 40-44.

Morad, Y., Lemberg, H., Yofe, N., and Dagan, Y. (2000). Pupillography as an objective indicator of fatigue. *Current Eye Research*, 21: 535-542.

Morgan, A. J. (2003). *Analysis of the intraocular pressure pulse.* PhD Thesis. Aston University.

Mori, F., Konno, S., Hikichi, T., Yamaguchi, Y., Ishiko, S., and Yoshida, A. (2001). Factors affecting pulsatile ocular blood flow in normal subjects. *British Journal of Ophthalmology*, 85: 529-530.

Nagy, A. L., and Doyal, J. A. (1993). Red-green colour discrimination as a function of stimulus field size in peripheral vision. *Journal of the Optical Society of America*, 10: 1147-1156.

Neely, J. C. (1951). The effect of high altitude on ocular muscle balance. *Transactions of the Ophthalmological Society of the United Kingdom*, 71: 663-678.

Nelson, W. H. (1967). The effects of reduced partial pressures of oxygen (hypoxia) on the size of the visual field in humans. *Dissertation Abstracts (Psychology)*, 28(5-B): 2163-2164.

Newman, N. J., Wolfe, J. M., Stewart, M. I., and Lessell, S. (1991). Binocular visual function in patients with a history of monocular optic neuritis. *Clinical Vision Sciences*, 6: 95-107.

Newton, N. L., Clark, W. B., Culver, J. F., and Moore, J. T. (1963). Intraocular pressure, aqueous outflow and glaucoma at altitude. *American Journal of Ophthalmology*, 56: 223-229.

Ng, J. D., White, L. J., Parmley, V. C., Hubickey, W., Carter, J., and Mader, T. H. (1996). Effects of simulated high altitude on patients who have had radial keratotomy. *Ophthalmology*, 103: 452-457.

Nguyen, T., Soni, P. S., Brizendine, E., and Bonanno, J. A. (2003). Variability in hypoxia-induced corneal swelling is associated with variability in corneal metabolism and endothelial function. *Eye and Contact Lens*, 29: 117-125.

Nicholls, J. V. V. (1950). The relationship of heterophoria to depth perception in aviation: Part II. *American Journal of Ophthalmology*, 33: 1775-1787.

Nichols, C. W., and Lambertsen, C. J. (1969). Effects of high oxygen pressures on the eye. *New England Journal of Medicine*, 281: 25-30.

Niemeyer, G., Nagahara, K., and Demant, E. (1982). Effects of changes in arterial pO_2 and pCO_2 on the electroretinogram in the cat. *Investigative Ophthalmology and Visual Science*, 23: 678-683.

Nio, Y. K., Jansonius, N. M., Lamers, P., Mager, A., Zeinstra, J., and Kooijman, A. C. (2005). Influence of the rate of contrast change on the quality of contrast sensitivity assessment: a comparison of three psychophysical methods. *Ophthalmic and Physiological Optics*, 25: 18-26.

Noell, W. (1951). Site of asphyxial block in mammalian retinae. *Journal of Applied Physiology*, 3: 489-500.

Noell, W., and Chinn, H. I. (1950). Failure of the visual pathway during anoxia. *American Journal of Physiology*, 161: 573-590.

O'Hanlon, J. F., and Horvath, S. M. (1973). Neuroendocrine, cardiorespiratory and performance reactions of hypoxic men during a monitoring task. *Aerospace Medicine*, 44: 129-134.

Ohlbaum, M. K. (1969). The effects of hypoxia on certain aspects of visual performance. *American Journal of Optometry*, 46: 235-249.

Ohtsuka, K., Asakura, K., Kawasaki, H., and Sawa, M. (1988). Respiratory fluctuations of the human pupil. *Experimental Brain Research*, 71: 215-217.

Okamoto, N., Nishimura, Y., Goami, K., and Harino, S. (1998). Effect of hyperbaric oxygen on ophthalmic artery blood velocity in patients with diabetic neuropathy. *Japanese Journal of Ophthalmology, 42*: 406-410.

O'Leary, D. J., Wilson, G., and Henson, D. B. (1981). The effect of anoxia on the human corneal epithelium. *American Journal of Optometry and Physiological Optics*, 58: 472-476.

Østerberg, G. (1935). Topography of the layer of rods and cones in the human retina. *Acta Ophthalmologica*, 6: 1-103.

Ostroy, S. E., Gaitatzes, C. G., and Friedmann, A. L. (1993). Hypoxia inhibits rhodopsin regeneration in the excised mouse eye. *Investigative Ophthalmology and Visual Science*, 34: 447-452.

Otis, A. B., Rahn, H., Epstein, M. A., and Fenn, W. O. (1946). Performance as related to composition of alveolar air. *American Journal of Physiology*, 146: 207-223.

Owsley, C., Sekuler, R., and Siemsen, D. (1983). Contrast sensitivity throughout adulthood. *Vision Research*, 23: 689-699.

Pakola, S. J., and Grunwald, J. E. (1993). Effects of oxygen and carbon dioxide on human retinal circulation. *Investigative Ophthalmology and Visual Science*, 34(10): 2866-2870.

Pandey Vimal, R. L., Pokorny, J., Smith, V. C., and Shevell, S. K. (1989). Foveal cone thresholds. *Vision Research*, 29: 61-78.

Pardhan, S., and Whitaker, D. (2003). Binocular summation to gratings in the peripheral field in older subjects is spatial frequency dependent. *Current Eye Research*, 26: 297-302.

Papst, N., Demant, E., and Niemeyer, G. (1982). Changes in pO_2 induce retinal autoregulation in vitro. *Graefe's Archives of Clinical and Experimental Ophthalmology*, 219: 6-10.

Parver, L. M., Auker, C. R., and Carpenter, D. O. (1983). Choroidal blood flow. III. Reflexive control in human eyes. *Archives of Ophthalmology*, 101(10): 1604-1606.

Paul, M. A., and Fraser, W. D. (1994). Performance during mild acute hypoxia. *Aviation Space and Environmental Medicine*, 65: 891-899.

Paulson, H. M., and Ryan, A. P. (1981). The F-M 100 Hue Test for assessing the effect of oxygen on colour vision. United States Naval Submarine Medical Research Center, Report No USNMRL-956, Groton, CT.

Payne, J. P., Bushman, J. A., and Hill, D. W. (Eds). (1979). *The Medical and Biological Application of Mass Spectrometry*. London: Academic Press.

Peñaz, J. (1973). Photoelectric measurement of blood pressure, volume and flow in the finger. *Digest of the 10th International Conference on Medical and Biological Engineering*. Dresden, 104.

Pepe, I. M. (2001). Recent advances in our understanding of rhodopsin and phototransduction. *Progress in Retinal and Eye Research*, 20: 733-759.

Perkins, G. A., Ellisman, M. H., and Fox, D. A. (2003). Three-dimensional analysis of mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. *Molecular Vision*, 9: 60-73.

Pesce, A., Bolognesi, M., Bocedi, A., Ascenzi, P., Dewilde, S., Moens, L., Hankeln, T., and Burmester, T. (2002). Neuroglobin and cytoglobin. Fresh blood for the vertebrate globin family. *EMBO reports*, 3: 1146-1151.

Pestilli, F., and Carrasco, M. (2005). Attention enhances contrast sensitivity at cued and impairs it at uncued locations. *Vision Research*, 45: 1867-1875.

Petzold, A., and Plant, G. T. (2006). Clinical disorders affecting mesopic vision. *Ophthalmic and Physiological Optics*, 26: 326-341.

Picaud. S. (2003). Retinal biochemistry. In Kaufman, P. L., and Alm, A. (Eds). *Adler's physiology of the eye: clinical application.* (10th ed). St Louis: Mosby, pp 382-408.

Pierson, W. R. (1967). Night vision and mild hypoxia. *Aerospace Medicine*, 38(10): 993-994.

Pinson, E. A. (1940). Intra-ocular pressure at high altitudes. *Journal of Aviation Medicine*, 11: 108-111.

Pirenne, M. H. (1943). Binocular and uniocular thresholds of vision. *Nature*, 152: 698-699.

Pirenne, M. H. (1948). *Vision and the Eye.* London: Pilot Press Ltd.

Pokorny, J. (1968). The effect of target area on grating acuity. *Vision Research*, 8: 543-554.

Pokorny, J., and Smith, V. C. (1997). Psychophysical signatures associated with magnocellular and parvocellular pathway contrast gain. *Journal of the Optical Society of America A*, 14: 2477-2486.

Polse, K. A., and Decker, M. (1979). Oxygen tension under a contact lens. *Investigative Ophthalmology and Visual Science*, 18: 188-193.

Polse, K. A., and Mandell, R. B. (1970). Critical oxygen tension at the corneal surface. *Archives of Ophthalmology*, 84: 505-508.

Polyak, S. L. *The Retina.* Chicago: University Chicago Press; 1941.

Post, D. L., Geiselman, E. E., and Goodyear, C. D. (1999). Benefits of color coding weapons symbology for an airborne helmet-mounted display. *Human Factors*, 41: 515-523.

Pournaras, C. J., Rungger-Brändle, E., Riva, C. E., Hardarson, S. H., and Stefansson, E. (2008). Regulation of retinal blood flow in health and disease. *Progress in Retinal and Eye Research*, 27: 284-330.

Powell, T. J., Carey, T. M., Brent, H. P., and Taylor, W. G. R. (1957). Episodes of unconsciousness in pilots during flight in 1956. *Aerospace Medicine*, 28: 374-386.

Pretorius, H. A. (1970). Effect of oxygen on night vision. *Aerospace Medicine*, 41: 560-562.

Provis, J. M. (2001). Development of the primate retinal vasculature. *Progress in Retinal and Eye Research*, 20: 799-821.

Provis, J. M., Penfold, P. L., Cornish, E. E., Sandercoe, T. M., and Madigan, M. C. (2005). Anatomy and development of the macula: specialisation and the vulnerability to macular degeneration. *Clinical and Experimental Optometry*, 88: 269-281.

Pugh, E. N. and Cobbs, W. H. (1986). Visual transduction in vertebrate rods and cones: a tale of two transmitters, calcium and cyclic GMP. *Vision Research*, 26: 1613-1643.

Pugh, E. N., and Lamb, T. D. (1990). Cyclic GMP and calcium: the internal messengers of excitation and adaptation in vertebrate photoreceptors. *Vision Research*, 30: 1923-1948.

Rabin, J. (1995). Two eyes are better than one: binocular enhancement in the contrast domain. *Ophthalmic and Physiological Optics*, 15: 45-48.

Rash, C. E., and Manning, S. D. (2003). On the flight deck, lighting must satisfy a variety of needs. *Human Factors and Aviation Medicine*, 50(5): 1-12.

Ren, X., Fatemian, M., and Robbins, P. A. (2000). Changes in respiratory control in humans induced by 8 h of hyperoxia. *Journal of Applied Physiology*, 89: 655-662.

Richalet, J-P., Duval-Arnould, G., Darnaud, B., Keromes, A., and Rutgers, V. (1988). Modification of colour vision in the green/red axis in acute and chronic hypoxia explored with a portable anomaloscope. *Aviation Space and Environmental Medicine*, 59: 620-623.

Richalet, J-P., Rutgers, V., Bouchet, P., Rymer, J-C., Kéromès, A., Duval-Arnould, G., and Rathat, C. (1989). Diurnal variations of acute mountain sickness, colour vision, and plasma cortisol and ACTH at high altitude. *Aviation Space and Environmental Medicine*, 60: 105-111.

Rieke, F., and Baylor, D. A. (1998). Origin of reproducibility in the responses of retinal rods to single photons. *Biophysical Journal*, 75: 1836-1857.

Riva, C. E., Grunwald, J. E., and Petrig, B. L. (1986). Autoregulation of human retinal blood flow. An investigation with laser Doppler velocimetry. *Investigative Ophthalmology and Visual Science*, 27: 1706-1712.

Riva, C. E., Grunwald, J. E., and Sinclair, S. H. (1983). Laser Doppler velocimetry study of the effect of pure oxygen breathing on retinal blood flow. *Investigative Ophthalmology and Visual Science*, 24: 47-51.

Riva, C. E., Grunwald, J. E., Sinclair, S. H., and Petrig, B. L. (1985). Blood velocity and volumetric flow rate in human retinal vessels. *Investigative Ophthalmology and Visual Science*, 26: 1124-1132.

Riva, C. E., Pournaras, C. J., and Tsacopoulos, M. (1986). Regulation of local oxygen tension and blood flow in the inner retina during hyperoxia. *Journal of Applied Physiology*, 61(2): 592-598.

Riva, C. E., Sinclair, S. H., and Grunwald, J. E. (1981). Autoregulation of retinal circulation in response to decrease of perfusion pressure. *Investigative Ophthalmology and Visual Science*, 21(1 Pt 1): 34-38.

Riva, C. E., Titze, P., Hero, M., and Petrig, B.L. (1997). Effect of acute decreases of perfusion pressure on choroidal blood flow in humans. *Investigative Ophthalmology and Visual Science*, 38(9): 1752-1760.

Rodriguez-Carmona, M. L., Harlow, A. J., Walker, G., and Barbur, J. L. (2005). The variability of normal trichromatic vision and the establishment of the "normal" range. In: *Proceedings of the 10th Congress of the International Colour Association*. Granada: pp 979-982.

Rodriguez-Carmona, M., Kvansakul, J., Harlow, J. A., Köpcke, W., Schalch, W., and Barbur, J. L. (2006). The effects of supplementation with lutein and/or zeaxanthin on human macular pigment density and colour vision. *Ophthalmic and Physiological Optics*, 26: 137-147.

Roff, E. J., Harris, A., Chung, H. S., Hosking, S. L., Morrison, A. M., Halter, P. J., and Kagemann, L. (1999). Comprehensive assessment of retinal, choroidal and retrobulbar haemodynamics during blood gas perturbation. *Graefe's Archives of Clinical and Experimental Ophthalmology*, 237: 984-990.

Roff, E. J., Harris, A., Morrison, A. M., Halter, P. J., Chung, H. S., Hosking, S. L., and Kagemann, L. (1998). Ocular blood flow changes measured by colour Doppler imaging and pulsatile ocular blood flow during blood gas perturbation. *Ophthalmic and Physiological Optics*, 18(4): 384-385.

Roff Hilton, E. J., Hosking, S. L., Cubbidge, R. P., and Morgan, A. J. (2003). Regional variability in visual field sensitivity during hypercapnia. *American Journal of Ophthalmology*, 136: 272-276.

Rokseth, R., and Lorentzen, F. V. (1954). Combined effect of alcohol and hypoxia on flicker fusion frequency. *Journal of Applied Physiology*, 6: 559-565.

Rose, H. W. (1949). Study of acclimatisation during a two-week exposure to moderate altitude (10000 ft): effect of altitude adaptation on night vision and ocular muscle balance. United States Air Force School of Aviation Medicine, USAF SAM Report No AD-491 745/6, Randolph Air Force Base.

Rose, H. W. (1950a). Visual acuity and refraction. Ch IX-A in *German Aviation Medicine in World War II*, Department of the Air Force, US Government Printing Office, Washington DC, Vol II.

Rose, H. W. (1950b). Night vision. Ch IX-F in *German Aviation Medicine World War II*, Department of the Air Force, US Government Printing Office, Washington DC, Vol II.

Rosenthal, C. M. (1939). Changes in angioscotomas associated with inhalation of oxygen. *Archives of Ophthalmology*, 22: 385-392.

Saltzman, H. A., Anderson, B., Hart, L., Duffy, E., and Sieker, H. O. (1964). The retinal vascular and functional response to hyperbaric oxygenation in normal subjects and in patients with retinal vascular disease. In Ledingham (Ed) *Hyperbaric oxygenation: Proceedings of the Second International Congress Glasgow*. Edinburgh: E & S Livingstone Ltd.

Samuel, J. R., and Beaugié, A. (1974). Effect of carbon dioxide on the intraocular pressure in man during general anaesthesia. *British Journal of Ophthalmology*, 58: 62-67.

Santucci, G., Menu, J. P., and Valot, C. (1982). Visual acuity in color contrast on cathode ray tubes: role of luminance, hue, and saturation contrasts. *Aviation Space and Environmental Medicine*, 53(5): 478-484.

Schaefer, K. E., and Carey, C. (1953). Effect of increased CO_2 concentrations on flicker fusion frequency and alpha blocking. *Federation Proceedings*, 12: 125-126.

Schlaepfer, E., Bartsch, P., and Fisch, H. U. (1992). Paradoxical effects of mild hypoxia and moderate altitude on human visual perception. *Clinical Science (London)*, 83: 633-636.

Schellart, N. A. M., Pollen, M., and Van der Kley, A. (1997). Effect of dysoxia and moderate air-hyperbarism on red-green color sensitivity. *Undersea and Hyperbaric Medicine*, 24: 7-13.

Schmetterer, L., Findl, O., Strenn, K., Graselli, U., Kastner, J., Eichler, H-G., and Wolzt M. (1997). Role of NO in the O_2 and CO_2 responsiveness of cerebral and ocular circulations in humans. *American Journal of Physiology*, 273(6) Pt 2: R2005-2012.

Schmetterer, L., Lexer, F., Findl, O., Graselli, U., Eichler, H., and Wolzt, M. (1996). The effect of inhalation of different mixtures of O_2 and CO_2 on ocular fundus pulsations. *Experimental Eye Research*, 63: 351-355.

Schmetterer, L., Wolzt, M., Lexer, F., Alschinger, C., Gouya, G., Zanaschka, G., Fassolt, A., Eichler, A. G., and Fercher, A. F. (1995). The effect of hyperoxia and hypercapnia on fundus pulsations in the macular and optic disc region in healthy young men. *Experimental Eye Research*, 61: 685-690.

Schmidt, I. (1950). Color vision. Ch IX-D in *German Aviation Medicine in World War II*, Department of the Air Force, US Government Printing Office, Washington DC, Vol II.

Schmidt, M., Giessl, A., Laufs, T., Hankeln, T., Wolfrum, U., and Burmester, T. (2003). How does the eye breathe? *Journal of Biological Chemistry*, 278: 1932-1935.

Scow, J., Krasno, L. R., and Ivy, A. C. (1950). The immediate and accumulative effect on psychomotor performance of exposure to hypoxia, high altitude and hyperventilation. *Journal of Aviation Medicine*, 21: 79-81.

Seendy, J-L., Lovasik, J. V., and Kergoat, H. (2005). Systemic hyperoxia and retinal vasomotor responses. *Investigative Ophthalmology and Visual Science*, 46: 1714-1720.

Seitz, C.P. (1940). Effects of anoxia on visual function; a study of critical flicker frequency. *Archives of Psychology*, 257: 5-38.

Sekuler, A. B., Bennett, P. J., and Mamelak, M. (2000). Effects of aging on the useful field of view. *Experimental Aging Research*, 26: 103-120.

Sen Gupta, J., Mathew, L., and Gopinath, P. M. (1979). Effect of physical training at moderate altitude (1850m) on hypoxic tolerance. *Aviation Space and Environmental Medicine*, 50: 714-716.

Seshadri, J., Christensen, J and Lakshminarayanan, V. (2005). Evaluation of the new web-based "Colour Assessment and Diagnosis" test. *Optometry and Vision Science*, 82: 882-885.

Severinghaus, J. W., Naifeh, K. H., and Koh, S. O. (1988). Errors in 14 pulse oximeters during profound hypoxia. *Journal of Clinical Monitoring*, 5: 72-81.

Sharma, R. K., and Ehinger, B. E. J. (2003). Development and structure of the retina. In P. L. Kaufman and A. Alm (Eds.), Adler's Physiology of the Eye: Clinical Application (10th ed.). St Louis: Mosby.

Sheard, C. (1946). Effects of increased intrapulmonary pressure on dark adaptation. *Journal of Aviation Medicine*, 17: 525-532.

Shui, Y-B., Fu, J-J., Garcia, C., Dattilo, L. K., Rajagopal, R., McMillan, S., Mak, G., Holekamp, N. M., Lewis, A., and Beebe, D. C. (2006). Oxygen distribution in the rabbit eye and oxygen consumption by the lens. *Investigative Ophthalmology and Visual Science*, 47: 1571-1580.

Sillito, A. M., and Zbrozyna, A. W. (1970). The activity characteristics of the preganglionic pupilloconstrictor neurones. *J Physiol*, 211: 767-779.

Simmons, D. R., and Kingdom, F. A. A. (1998). On the binocular summation of chromatic contrast. *Vision Research*, 38:1063-1071.

Simonsen, E., and Brožek, J. (1952). Flicker fusion frequency: background and applications. *Physiological Reviews*, 32: 349-378.

Simonson, E., and Winchell, P. (1951). Effect of high carbon dioxide and of low oxygen concentration on fusion frequency of flicker. *Journal of Applied Physiology*, 3: 637-641.

Simpson, W. A., and Manahilov, V. (2005). Two eyes: twice as good as one? [Abstract]. *Journal of Vision*, 5:

Sloane, M. E., Owsley, C., and Jackson, C. A. (1988). Aging and luminance-adaptation effects on spatial contrast sensitivity. *Journal of the Optical Society of America A*, 5: 2181-2190.

Smith, E. L., and Harwerth, R. S. (1979). Suprathreshold binocular interactions: the effects of prolonged monocular occlusion. *American Journal of Optometry and Physiological Optics*, 56: 681-688.

Smith, W. L. (1965). Recovery rates with descent from hypoxia-induced peripheral visual field loss. In: *Collected Papers Presented at the twenty-second Meeting of the AGARD Aerospace Medical Panel*, Advisory Group for Aeronautical Research and Development Conference Proceedings No 2, Sep 1965.

Smith, V. C., Ernest, J. T., and Pokorny, J. (1976). Effect of hypoxia on FM 100-Hue Test performance. *Modern Problems of Ophthalmology*, 17: 248-256.

Smith, V. C., and Pokorny, J. (1975). Spectral sensitivity of the foveal cone photopigments between 400 nm and 500 nm. *Vision Research*. 15: 161-171.

Smith, G. M., Seitz, C. P., and Clark, K. B. (1946). Variations in the angioscotoma in response to prolonged mild anoxia. *Aviation Medicine*, 17: 590-595.

Solberg, J. L., and Brown, J. M. (2002). No sex differences in contrast sensitivity and reaction time to spatial frequency. *Perceptual and Motor Skills*, 94: 1053-1055.

Somjen, G. G., and Tombaugh, G. C. (1998). pH modulation of neuronal excitability and central nervous system functions. In: Kaila, K., and Ransom, B.R. (Eds). *pH and Brain Function*. New York: Wiley-Liss, pp.373-393.

Somner, J. E. A., Morris, D. S., Scott, K. M., MacCormick, I. J. C., Aspinall, P., and Dhillon, B. (2007). What happens to intraocular pressure at high altitude? *Investigative Ophthalmology and Visual Science*, 48: 1622-1626.

Sparing, R., Dafotakis, M., Buelte, D., Meister, I. G., and Noth, J. (2007). Excitability of human motor and visual cortex before, during, and after hyperventilation. *Journal of Applied Physiology*, 102: 406-411.

Sponsel, W. E., Harrison, J., Elliott, W. R., Trigo, Y., Kavanagh, J., and Harris, A. (1997). Dorzolamide hydrochloride and visual function in normal eyes. *American Journal of Ophthalmology*, 123: 759-766.

Spring, K. H., and Stiles, W. S. (1948). Variation of pupil size with change in the angle at which the light stimulus strikes the retina. *British Journal of Ophthalmology*, 32: 340.

Stabell, U., and Stabell, B. (1977). Wavelength discrimination of peripheral cones and its change with rod intrusion. *Vision Research*, 17: 423-426.

Stabell, B., and Stabell, U. (1996). Peripheral colour vision: effects of rod intrusion at different eccentricities. *Vision Research*, 36: 3407-3414.

Stabell, B., and Stabell, U. (1998). Chromatic rod-cone interaction during dark adaptation. *Journal of the Optical Society of America A*, 15: 2809-2815.

Stefánsson, E., Foulks, G. N., and Hamilton, R. C. (1987). The effect of corneal contact lenses on the oxygen tension in the anterior chamber of the rabbit eye. *Investigative Ophthalmology and Visual Science*, 28: 1716-1719.

Stefánsson, E., Wolbarsht, M. L., and Landers, M. B. (1983). The corneal contact lens and aqueous humor hypoxia in cats. *Investigative Ophthalmology and Visual Science*, 24: 1052-1054.

Stefánsson, E., Wolbarsht, M. L., and Landers, M. B. (1983). In vivo O_2 consumption in rhesus monkeys in light and dark. *Experimental Eye Research*, 37: 251-256.

Steinberg, R. H. (1987). Monitoring communications between photoreceptors and pigment epithelial cells: effects of "mild" systemic hypoxia. *Investigative Ophthalmology and Visual Science*, 28: 1888-1904.

Stivalet, P., Leifflen, D., Poquin, D., Savourey, G., Launay, J., Barraud, P., Raphe, C., and Bittel, J. (2000). Positive expiratory pressure as a method for preventing the impairment of attentional processes by hypoxia. *Ergonomics*, 43(4): 474-485.

Strauss, O. (2005) The retinal pigment epithelium in visual function. *Physiological Reviews*, **85**: 845-881.

Strenn, K., Menapace, R., Rainer, G., Findl, O., Wolzt, M., and Schmetterer, L. (1997). Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in PO_2. *British Journal of Ophthalmology*, 81: 360-364.

Sun, Y., Jin, K., Mao, X. O., Zhu, Y., and Greenberg, D. A. (2001). Neuroglobin is up-regulated by and protects neurons from hypoxic-ischemic injury. *Proceedings of the National Academy of Sciences of the USA*, 98: 15306-15311.

Swanson, W. H., Dul, M. W., and Fischer, S. E. (2005). Quantifying effects of retinal illuminance on frequency doubling perimetry. *Investigative Ophthalmology and Visual Science*, 46: 235-240.

Tabachnik, B. G., and Fidell, L. S. (1996). *Using multivariate statistics* (3rd ed). New York: Harper Collins.

Ten Doesschate, G. (1955). Heterophoria. In *Collected Papers in Aviation Medicine*, London, Butterworth. Pp 186-194.

Thorn, F., and Boynton, R. M. (1974). Human binocular summation at absolute threshold. *Vision Research*, 14: 445-458.

Throck Watson, J. (1976). *Introduction to mass spectrometry: biomedical, environmental and forensic applications*. New York: Raven Press.

Tinjust, D., Kergoat, H., and Lovasik, J. V. (2002). Neuroretinal function during mild systemic hypoxia. *Aviation Space and Environmental Medicine*, 73: 1189-1194.

Tipton, D. A. (1984). A review of vision physiology. *Aviation Space and Environmental Medicine*, 55(2): 145-149.

Tredici, T. J. (1985). Ophthalmology in aerospace medicine. In DeHart, R. L. (Ed), *Fundamentals of aerospace medicine*, Ch.15. Philadelphia: Lea and Febiger.

Trezona, P. (1970). Rod participation in the 'blue' mechanism and its effect on colour matching. *Vision Research*, 10: 317-332.

Trokel, S. (1965). Effect of respiratory gases upon choroidal haemodynamics. *Archives of Ophthalmology*, 73: 838-842.

Tsacopoulos, M., Baker, R., Johnson, M., Strauss, J., and David, N. J. (1973). The effect of arterial pCO_2 on inner-retinal oxygen availability in monkeys. *Investigative Ophthalmology*, 12: 449-455.

Tsacopoulos, M., and David, N. J. (1973). The effect of arterial pCO_2 on relative retinal blood flow in monkeys. *Investigative Ophthalmology*, 12(5): 335-347.

Tune, G. S. (1964). Psychological effects of hypoxia: review of certain literature from the period 1950-1963. *Perceptual and Motor Skills*, 19: 551-562.

Turner, P. (1965). The modification of critical flicker fusion frequency by an adapting stimulus of flickering light. *Vision Research*, 5: 463-469.

Vaernes, R. J., Owe, J. O., and Myking, O. (1984). Central nervous reactions to a 6.5 hour altitude exposure at 3048 meters. *Aviation Space and Environmental Medicine*, 55: 921-926.

Verriest, G., Van Laetham, J., and Uvijls, A. (1982). A new assessment of the normal ranges of the Farnsworth-Munsell 100-hue test scores. *American Journal of Ophthalmology*, 93: 635-642.

Vingrys, A. J., and Garner, L. F. (1987). The effect of a moderate level of hypoxia on human color vision. *Documenta Ophthalmologica*, 66: 171-185.

Vollmer, E., King, B. G., Birren, J. E., and Fisher, M. B. (1945). Effects of carbon monoxide on three types of performance at simulated altitudes of 10000 ft and 15000 ft. *Journal of Experimental Psychology*, 36: 244-251.

Wald, G., Harper, P. V., Goodman, H. C., and Krieger, H. P. (1942). Respiratory effects upon the visual threshold. *Journal of General Physiology*, 25: 891-902.

Walkey, H. C., Barbur, J. L., Harlow, J. A., and Makous, W. (2001). Measurements of chromatic sensitivity in the mesopic range. *Color Research and Application*, 26(S1): S36-S42.

Walkey, H. C., Barbur, J. L., Harlow, J. A., Hurden, A., Moorhead, I. R., and Taylor, J. A. F. (2005). Effective contrast of colored stimuli in the mesopic range: a metric for perceived contrast based on achromatic luminance contrast. *Journal of the Optical Society of America A*, 22(1): 17-28.

Walkey, H. C., Harlow, J.A., and Barbur, J. L. (2006). Characterising mesopic spectral sensitivity from reaction times. *Vision Research*, 46: 4232-4243.

Wangsa-Wirawan, N. D., and Linsenmeier, R. A. (2003). Retinal oxygen. Fundamental and clinical aspects. *Archives of Ophthalmology*, 121: 547-557.

Ward, M. M., Stone, S. C., and Sandman, C. A. (1978). Visual perception in women during the menstrual cycle. *Physiology and behaviour*, 20: 239-243.

Wässle, H., and Boycott, B. B. (1991). Functional architecture of the mammalian retina. *Physiological Reviews*, 71: 447-480.

Weissman, B. A. (1986). Critical corneal oxygen values: a summary. *Journal of the American Optometric Association*, 57(8): 595-597.

Welsh, K., Bennett, Q., and Kislin, B. (1975). Effects of hyperbaric air pressure on keratometry. *American Journal of Optometry and Physiological Optics*, 52: 192.

White, W. J. (1960). Variations in absolute visual thresholds during acceleration stress. Wright Air Development Division Technical Report 60-34, United States Air Force Air Research and Development Command, Wright-Patterson Air Force Base, Ohio.

White, L. J., and Mader, T. H. (1993). Refractive changes with increasing altitude after refractive keratotomy. *American Journal of Ophthalmology*, 115: 821-823.

Whiteside, T. C. D. (1957). The problems of vision in flight at high altitude. Advisory Group for Aeronautical Research and Development, AGARDograph 13. London: Butterworths Scientific Publications.

Wildsoet, C., Wood, J., Maag, H., and Sabdia, S. (1998). The effect of different forms of monocular occlusion on measures of central visual function. *Ophthalmic and Physiological Optics*, 18: 263-268.

Wilmer, W. H., and Berens, C. (1918). Medical Studies in Aviation: V. The Effect of Altitude on Ocular Functions. *Journal of the American Medical Association*, 71: 1382-1400. Reprinted (1989) *Aviation, Space and Environmental Medicine*, 10: 1018-1023.

Wilson, D. R. (1999). Darkness increases risks of flight. *Human Factors and Aviation Medicine*, 46(6): 1-8.

Wilson, T. M., Le May, M., Holloway, K. B., Strang, R., and McKenzie, E. (1974). Experimental and clinical study of factors influencing choroidal blood flow. *Transactions of the Ophthalmic Society of the United Kingdom*, 94: 378-381.

Winkle, R. K., Mader, T. H., Parmley, V. C., White, L. J., and Polse, K. A. (1998). The etiology of refractive changes at high altitude after radial keratotomy. *Ophthalmology*, 105: 282-286.

Winn, B., and Gilmartin, B. (1992). Current perspective on microfluctuations of accommodation. *Ophthalmic and Physiological Optics*, 12: 252-256.

Winn, B., Pugh, J. R., Gilmartin, B., and Owens, H. (1990). Arterial pulse modulates steady-state ocular accommodation. *Current Eye Research*, 9: 971-975.

Winn, B., Whitaker, D., Elliott, D. B., and Phillips, N. J. (1994) Factors affecting light-adapted pupil size in normal human subjects. *Investigative Ophthalmology and Visual Science*, 35: 1132-1137.

Wolbarsht, M. L., Stefansson, E., and Landers, M. B. (1987). Retinal oxygenation from the choroid in hyperoxia. *Experimental Biology*, 47: 49-52.

Wolf, E., and Nadroski, A. S. (1971). Extent of the visual field. Changes with age and oxygen tension. *Archives of Ophthalmology*, 86(6): 637-642.

Wong, S., and Tong, J. E. (1974). Menstrual cycle and contraceptive hormonal effects on temporal discrimination. *Perceptual and Motor Skills*, 39: 103-108.

Wooten, B. R., and Hammond, B. R. (2002). Macular pigment: influences on visual acuity and visibility. *Progress in Retinal and Eye Research*, 21(2):225-240.

Wu, X., Li, X., Han, L., Wang, T., and Wei, Y. (1998). Effects of acute moderate hypoxia on human performance of arithmetic. *Space Medicine and Medical Engineering (Beijing)*, 11: 391-395.

Wukitsch, M.W., Petterson, M. T., Tobler, D. R., and Pologe, J. A. (1988). Pulse oximetry: analysis of theory, technology, and practice, *Journal of Clinical Monitoring*, 4: 290-301.

Yamamoto, F., Borgula, G. A. and Steinberg, R. H. (1992). Effects of light and darkness on pH outside rod photoreceptors in the cat retina. *Experimental Eye Research*, 54, 689-697.

Yamamoto, F., and Steinberg, R. H. (1992). Effects of systemic hypoxia on pH outside rod photoreceptors in the cat retina. *Experimental Eye Research*, 54, 699-709.

Yancey, C. M., and Linsenmeier, R. A. (1988). The electroretinogram and choroidal PO_2 in the cat during elevated intraocular pressure. *Investigative Ophthalmology and Visual Science*, 29: 700-707.

Yancey, C. M., and Linsenmeier, R. A. (1989). Oxygen distribution and consumption in the cat retina at increased intraocular pressure. *Investigative Ophthalmology and Visual Science*, 30: 600-611.

Yang, Y. C., Hulbert, M. F., Batterbury, M., and Clearkin, L. G. (1997). Pulsatile ocular blood flow measurements in healthy eyes: reproducibility and reference values. *Journal of Glaucoma*, 6: 175-179.

Yap, M. K. H., Garner, L. F., Legg, S., and Faris, J. (1995). Effects of exposure to simulated altitudes on visual fields, contrast sensitivity, and dazzle recovery. *Aviation Space and Environmental Medicine*, 66: 243-246.

Yebra, A., García, J. A., Nieves, J. L., and Romero, J. (2001). Chromatic discrimination in relation to luminance level. *Color Research and Application*, 26: 123-131.

Young, D., Jewkes, C., Spittal, M., Blogg, C., Weissman, J., and Gradwell, D. Respose time of pulse oximeters assessed using acute decompression. *Anesthesia and Analgesia*, 74(2): 189-195.

Yu, D., and Cringle, S. J. (2001). Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Progress in Retinal and Eye Research*, 20: 175-208.

Yu, D., and Cringle, S. J. (2002). Outer retinal anoxia during dark adaptation is not a general property of mammalian retinas. *Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology*, 132(1): 47-52.

Yu, D., and Cringle, S. J. (2005). Retinal degeneration and local oxygen metabolism. *Experimental Eye Research*, 80: 745-751.

Yu, D., Cringle, S. J., and Alder, V. A. (1990). The response of rat vitreal oxygen tension to stepwise increases in inspired percentage oxygen. *Investigative Ophthalmology and Visual Science*, 31: 2493-2499.

Yu, D., Cringle, S. J., Alder, V. A., and Su, E-N. (1999). Intraretinal oxygen distribution in the rat with graded systemic hyperoxia and hypercapnia. *Investigative Ophthalmology and Visual Science*, 40: 2082-2087.

Yu, D., Cringle, S. J., and Su, E-N. (2005). Intraretinal oxygen distribution in the monkey retina and the response to systemic hyperoxia. *Investigative Ophthalmology and Visual Science*, 46: 4728-4733.

Yucel, I., Akar, M. E., Dora, B., Akar, Y., Taskin, O., and Özer, H. O. (2005). Effect of the menstrual cycle on standard achromatic and blue-on-yellow visual field analysis of women with migraine. *Canadian Journal of Ophthalmology*, 40: 51-57.

Zhang, H. R. (1994). Scanning electron-microscopic study of corrosion casts on retinal and choroidal angioarchitecture in man and animals. *Progress in Retinal and Eye Research*, 13: 243-270.

Züst, F. (1940). Das Verhalten der Pupille bei Abnehme des Luftdrucks. *Luftfahrtmedizin*, 4: 209-224.

Bibliography

Boff, K. R., and Lincoln, J. E. (1988). *Engineering data compendium: human perception and performance.* Armstrong Aeromedical Research Laboratory, Wright-Patterson Air Force Base, OH.

Boynton, R. M. (1979). *Human colour vision.* New York: Holt, Rinehart and Winston.

Bruce, V., Green, P. R., and Georgeson, M. A. (2003). *Visual perception: physiology, psychology and ecology.* Hove and New York: Psychology Press.

Carpenter, R. H. S., and Robson, J. G. (1998). *Vision Research.* Oxford: University Press.

Davson, H. (1972). *The Physiology of the Eye* (3rd ed). Edinburgh: Churchill Livingstone.

Forrester, J. V., Dick, A. D., McMenamin, P. G., and Lee, W. R. (2002). *The eye; basic sciences in practice.* (2nd ed). Edinburgh: Harcourt.

Hess, L. T., Sharpe, L. T., and Nordby, K. (Eds). (1990). *Night Vision. Basic, Clinical and Applied Aspects.* (1st ed). Cambridge: University Press.

Hogan, M. J., Alvarado, J. A., and Weddell, J. E. (1971). *Histology of the Human Eye.* Philadelphia: Saunders.

Hubel, D. H. (1995). *Eye, brain and vision.* New York: Scientific American Library.

Kaila, K., and Ransom, B. R. (Eds). (1998). *pH and Brain Function.* New York: Wiley-Liss

Kaufman, P. L., and Alm, A. (2003). *Adler's physiology of the eye: clinical application.* (10th ed). St Louis: Mosby.

Lumb, A. B. (2000). *Nunn's applied respiratory physiology* (5th ed). Oxford: Butterworth-Heinemann.

McIlwain, J. T. (1996). *An Introduction to the Biology of Vision.* Cambridge: University Press.

Norton, T. T., Corliss, D. A., and Bailey, J. E. (2002). *The psychophysical measurement of visual function.* Amsterdam: Butterworth-Heinemann.

Rainford, D. J., and Gradwell, D. P. (2006). *Ernsting's Aviation Medicine* (4th ed). London: Hodder Arnold.

Rodieck, R. W. (1998). *The first steps in seeing.* Sunderland: Sinauer.

Schwartz, S. H. (2004). *Visual perception. A clinical orientation.* (3rd ed). New York: McGraw-Hill.

Van Liere, E. J., and Stickney, J. C. (1963). *Hypoxia.* Chicago: University of Chicago Press.

Ward, M. P., Milledge, J. S., and West, J. B. (2000). *High altitude medicine and physiology.* London: Arnold.

West, J. B. (1988). *High life. A history of high-altitude physiology and medicine.* Oxford: University Press.

Wyszecki, G., and Stiles, W. S. (1982). *Color science: concepts and methods, quantitative data and formulae.* (2nd ed). New York: Wiley.

Zeki, S. (1993). *A vision of the brain.* Oxford: Blackwell.

Appendix 1 Relevant Gas Laws

Henry's Law

Henry's Law states that the mass of gas absorbed by a mass of liquid is directly proportional to the partial pressure of the gas above the liquid at a given temperature. Thus, at equilibrium, the partial pressure in the liquid phase will match that of the gas phase. This assumes that there is no chemical interaction between the gas and the liquid. The absolute amount of gas in physical solution also depends on the solubility of the gas. Thus, if the partial pressure of a gas in a liquid is reduced, for example by reducing the pressure of the gas mixture above the liquid, then the amount of dissolved gas will be reduced in proportion.

Graham's and Fick's Laws

Gaseous diffusion is the process by which molecules move from regions of higher to lower concentration in a liquid or gas mixture. Graham's Law states that the rate of diffusion of a gas is proportional to the solubility of the gas in the liquid and inversely proportional to the square root of the molecular weight of the gas, giving the diffusion constant. The net flux of a gas across a fluid membrane is given by Fick's Law which states that the rate of gas transfer ($\dot{V}_{gas}$) is proportional to the difference between the pressures of the gas at the two points of interest ($P_1 - P_2$), proportional to the membrane area and inversely proportional to membrane thickness. Combining these Laws, and assuming that the molecular weight and solubility of O_2, diffusion area and distance from the choroid to the photoreceptors will remain unchanged, a simple expression for the rate of O_2 transfer from the choroid to the photoreceptor inner segments becomes:

$$\dot{V}O_2 \propto P_{Choroid} - P_{Inner\ segment}$$

Equation 19 Simple approximation for the relative O_2 flux from choroid to photoreceptors

Dalton's Law

Dalton's Law of partial pressures states that the pressure exerted by a mixture of gases is equal to the sum of the pressures that each constituent gas would exert if it alone filled the space occupied by the mixture, that is, its partial pressure. If P_t is the total

pressure of the gas mixture and P_1, P_2, P_3...P_n are the partial pressures of each constituent gas, then Dalton's law may be expressed mathematically as:

$$P_t = P_1 + P_2 + P_3 \ldots + P_n$$

Equation 20 Expression for Dalton's Law of partial pressures

As a result, the partial pressure, P_x, of a gas in a mixture may be determined from its fractional concentration in the mixture, F_x, and the total pressure of the mixture, P_t, in accordance with the relationship:

$$P_x = F_x \times P_t$$

Equation 21 Expression for the partial pressure of a gas in a mixture

Appendix 2 Medical Screening Questionnaire

Name ..... Date of birth ...../...../.....

Age ..... Sex: Male / Female

1. Have you ever been told that you have high blood pressure? YES / NO

If YES give details.....

.....

2. Have you had any history of heart trouble? YES / NO

If YES give details.....

.....

3. Have you a family history of heart disease/stroke? YES / NO

If YES give details.....

.....

4. Have you ever been told by a doctor that you have asthma? YES / NO

5. Do you suffer from a wheezy chest? YES / NO

6. Do you ever have pains in your heart and chest? YES / NO

7. Do you ever feel faint or have spells of dizziness? YES / NO

8. Has a doctor told you that you have a bone or joint problem which could be made worse by exercise?

YES / NO

9. Have you been in hospital at all in the last 2 years? YES / NO

If YES give reason and outcome.....

.....

10. Have you had any operations or major illness in the last 6 months? YES / NO

If YES give details.....

.....

11. Are you undergoing treatment or having any regular medication at the moment? YES / NO

If YES give details.....

.....

12. Are you taking any pills or any other medicines, including inhalers, for any of the following:

heart trouble, angina?..... YES / NO

chest pains or blood pressure?..... YES / NO

asthma or other chest diseases?..... YES / NO

or for anything else?..... YES / NO

If YES give full details (from bottle labels)

.....

.....

13. Do you have any physical disabilities of any kind? YES / NO

If YES give details.....

.....

14. Have you ever suffered any complications from previous trials or treatment? YES / NO

If YES give details.....

.....

15. Do you suffer from any allergies? YES / NO

If YES give details.....

.....

16. Have you ever had an adverse reaction to a medicine or a food? YES / NO

If YES give details.....

.....

17. Specifically, for trial SP 0659, do you have a history of any of the following:

- Decompression illness? YES / NO
- Ear or sinus barotrauma? YES / NO
- Recent dental surgery? YES / NO
- History of any significant chest disease such as emphysema, pneumothorax chest surgery, pneumonia, bronchitis, infection or any other lung disease? YES / NO
- History of glucose intolerance or diabetes? YES / NO
- History of any ear, nose or throat surgery in the last 6 months? YES / NO
- History of any ear, sinus or nasal conditions? YES / NO
- History of inability to perform Valsalva (make your ears pop)? YES / NO
- Recreational exposure to increased pressure (eg SCUBA diving)? YES / NO
- Recreational exposure to reduced pressure (eg flying, parachuting)? YES / NO

If YES to any then please give details.....

.....

.....

18. Specifically, for trial SP 0659, do you smoke (answer YES if any within last month)? YES / NO

If YES then please give details.....

19. Specifically, for trial SP 0659, with respect to your eyes and your vision, do you have a history of any of the following:

- Any past or present eye disease, abnormality or need for eye surgery? YES / NO
- Any past or recent history of strabismus (squint) or double vision? YES / NO
- Any past or current requirement for spectacles or contact lenses? YES / NO
- Any history of significant eye injury, especially penetrating injury? YES / NO
- Any need for medication (including eye drops) for an eye condition? YES / NO
- Any family history of significant eye disease? YES / NO
- Any impairment of vision in either eye (including abnormal colour vision)? YES / NO
- Any other condition that affects your eyes or vision (eg hay fever)? YES / NO

If YES to any then please give details.....

.....

.....

20. Specifically, for trial SP 0659, do you have or have you ever had any anxiety over exposure to darkness or confined spaces? YES / NO

21. Specifically, for trial SP 0659, have you any history of a psychiatric disorder, anxiety or a psychological problem that you consider may be aggravated by being in the dark and in a confined space? YES / NO

22. Specifically, for trial SP 0659, do you have any history of fits, faints, blackouts or any unexplained loss of consciousness, severe headaches, migraine or any other neurological condition, including epilepsy, especially any such conditions that may be triggered by exposure to flashing or flickering lights? YES / NO

23. Specifically, for trial SP 0659 and deliberate exposure to hypoxia, are any of your ancestors of Afro-Caribbean or Mediterranean origin or are you aware of any hereditary susceptibility to the effects of hypoxia on the blood, such as sickle cell anaemia or thalassaemia? YES / NO

Appendix 3 Lifestyle Questionnaire

INITIALS_____**DATE/TIME** _____**CONDITION** _____

GENERAL HEALTH

Do you feel well, comfortable, relaxed, rested and fully alert? _____

Are you currently taking any medication (including hormonal contraception)?_____

SLEEP Did you sleep normally last night? _____How many hours?_____

NUTRITION Do you currently take a full normal balanced diet? _____

Please outline what you have had to eat and drink over the last 24 hrs (since 6pm yesterday):

Yesterday evening:_____

Breakfast:_____

Lunch:_____

Snacks/Drinks:_____

BEVERAGES Do you feel thirsty?

Please outline how many **caffeine**-containing drinks you have had today? _____

(including tea, coffee, hot chocolate, coca cola etc) _____

Please outline how much **alcohol** you have taken in the last 24 hours?_____

_____

ENVIRONMENTAL STRESSORS

Please outline any exercise or undue physical exertion you have undertaken in the last 24 hrs_____

_____

Please outline any hypobaric (flying, parachuting) or hyperbaric exposures (diving) in the last 48 hrs_____

_____

Please outline any human studies you have participated in as subject or experimenter requiring exposure to unusual environments, drug administration or non-air breathing gas use during the last 48 hrs (eg inside observer/safety person)

_____

FEMALE SUBJECTS ONLY

Please indicate the **first** day of your last menstrual period (LMP)? _____

Was your LMP normal for you? ____YES / NO *____ [K= /]

Do you believe there is any possibility that you may be pregnant? _____

Please indicate the result of a pregnancy test taken today?__POSITIVE / NEGATIVE *__

*delete as appropriate

I have been informed about the nature of this specific experiment and consent to participating.

Subject Name _____Signature_____

Principal Investigator_____Signature_____

Appendix 4 Contrast Sensitivity Data and ANOVA

Study 1

Summary Data Set

Measuring luminance contrast ratio (no units)					Spatial frequency (cpd)						
Subject	**Light level**	**Gender**	**Eyes**	**F_1O_2 (%)**	**0.5**	**1**	**2.1**	**4.1**	**5.5**	**8.3**	**16.5**
A	Direct viewing	Male	2	21	44.72	91.55	112	128	112	88	53.62
B					20.29	72.14	101	99.81	129	68.49	19.01
C					46.72	79.14	79.14	76.85	96.04	65.4	17.99
D					35.39	54.02	84.71	66.49	66.49	57.63	24.43
E					39.74	67.49	84.71	74.85	84.71	60.59	22.94
F					30.71	57.31	109	96.52	140	162	38.93
G		Female			16.24	40.28	52.99	46.55	52.99	22.3	3.22
H					21.1	86.3	88	84.23	89.36	46.55	9.07
I					49.86	71.94	83.01	83.01	83.01	49.86	15.33
J					54.13	78.3	113	113	120	71.94	109
K					49.86	85.34	96.45	96.74	96.74	63.2	15.82
L					47.58	89.36	211	88	136	52.99	22.41
A	Direct viewing	Male	1	21	23.5	53.58	112	101	88	59.63	27.27
B					9.86	43.42	81	59.63	59.63	35.3	7.46
C					30.6	61.35	110	76.85	88	27.27	17.12
D					25.21	39.74	75.87	50.99	59.35	26.27	14.42
E					26.68	46.01	65.99	48.7	65.99	43.17	30.2
F					42.63	39.74	74.85	65.99	76.34	42.66	19.22
G		Female			12.18	21.24	35.3	27.27	21.24	8.46	1.13
H					23.33	76.85	68.49	113	74.63	52.02	9.55
I					15.3	33.53	63.53	56.13	49.49	33.97	13.73
J					15.22	64.69	167	82.73	56.13	39.71	11.95
K					29.67	51.36	96.04	109	83.6	66.49	13.95
L					24.42	50.99	76.85	62.59	68.49	31.51	21.8
A	Direct viewing	Male	2	14.1	35.58	90.65	112	112	101	112	35.3
B					31.97	59.63	136	99.81	112	76.85	16.24
C					40.79	92.94	88	89.36	90.18	49.34	16.49
D					49.04	75.74	124	84.23	97.99	60.22	19.57
E					48.7	67.01	86.66	74.85	84.23	46.01	23.63
F					86.3	59.35	84.71	96.74	97.99	46.01	17.52
G		Female			23.74	57.63	68.49	67.99	59.63	32.05	6.38
H					24.42	76.85	112	138	110	55.93	15.8
I					38.51	64.99	97.77	83.01	64.99	38.51	12.02
J					33.05	64.99	157	83.6	187	113	33.05
K					43.22	96.74	258	182	162	109	51.09
L					71.24	151	74.1	108	121	59.63	21.54
A	Direct viewing	Male	1	14.1	21.89	48.7	123	129	76.85	61.35	24.66
B					19.1	50.99	76.85	76.85	76.85	39.74	18.42
C					30.6	142	88.66	67.99	91.66	29.5	10.85
D					17.8	88.9	87.89	57.63	73.25	40.6	18.94
E					19.7	50.99	57.63	54.97	63.93	44.13	26.27
F					24.12	102	74.85	65.99	120	97.99	22.12
G		Female			12.6	23.74	38.51	30.6	21.38	14.71	2.23
H					24.32	51.36	118	59.63	104	46.25	8.88
I					19.7	29.8	49.49	49.49	44.51	22.35	9.81
J					25.52	38.24	137	64.99	56.13	49.33	23.41
K					35.68	74.85	140	124	96.74	67.01	19.49
L					21.24	65.77	117	94.89	57.63	40.18	10.5

Subject	Light level	Gender	Eyes	F_iO_2 (%)	0.5	1	2.1	4.1	5.5	8.3	16.5
A	ND 1.0	Male	2	21	27.07	130	141	112	76.85	65.24	16.24
B					16.24	72.14	88.66	67.99	76.85	47.58	4.03
C					23.74	59.35	125	122	35.03	18.6	4.82
D					18.6	67.38	59.63	59.63	44.86	32.5	5.78
E					44.72	67.49	89.99	67.99	59.63	21.1	12.32
F					40.53	86	81	46.72	35.58	39.51	15.48
G		Female			23.74	35.58	76.85	48.7	35.3	13.95	2.41
H					24.42	67.91	103	60.22	69.82	22.97	4.04
I					30.2	53.36	59.63	46.72	44.49	21.1	10.89
J					29.59	59.63	76.85	89.85	76.85	70.7	8.58
K					18.89	58.68	76.85	65.24	67.99	56.3	14.16
M					30.6	76.85	129	76.85	68.49	32.5	11.84
A	ND 1.0	Male	1	21	19.97	50.54	126	68.49	64.95	27.07	11.62
B					10.85	35.58	59.63	54.02	87.59	25.97	2.66
C					21.1	57.38	86.66	35.58	49.74	17.01	3.64
D					12.6	32.05	49.82	40.53	27.27	19.02	4.89
E					32.05	39.51	57.63	48.7	44.72	14.48	7.74
F					20.97	33.24	65.3	42.02	23.14	11.78	33.95
G		Female			12.18	24.71	25.86	16.24	9.56	3.53	1
H					27.79	44.6	114	67.91	87.14	18.89	6.76
I					22.79	30.6	44.72	35.58	30.6	18.89	6.82
J					33.95	44.96	55.78	43.95	35.3	36.09	9.31
K					21.24	52.99	90.18	46.66	53.95	30.2	6.44
M					32.41	113	113	58.6	68.49	23.74	6.47
A	ND 1.0	Male	2	14.1	42.22	83.96	112	84.23	166	53.36	71.99
B					20.97	46.01	76.85	59.63	88	35.77	3.38
C					28.64	52.99	72.14	44.72	26.19	14.55	3.8
D					19.35	39.74	75.74	32.37	33.89	22.85	8.27
E					40.28	61.35	67.99	61.35	39.74	27.07	12.67
F					30.6	75.36	72.04	74.85	52.99	23.74	12.6
G		Female			21.24	35.58	59.63	40.02	30.6	13.63	2.37
H					32.02	74.52	88	53.36	102	17.48	5.88
I					30.6	61.35	74.73	40.56	43.95	23.74	6.34
J					29.73	51.36	112	79.14	61.35	46.92	15.17
K					36.32	65.87	99.81	76.85	80.51	72.29	12.89
M					29.5	73.31	167	101	81.42	41.57	16.49
A	ND 1.0	Male	1	14.1	21	48.93	62.05	59.63	68.59	30.6	12.6
B					10.85	46.72	76.85	46.01	39.74	24.91	3.62
C					18.98	35.3	50.99	35.3	23.74	14.48	3.11
D					31.48	24.42	27.07	25.97	40.02	19.37	9.43
E					45.7	27.27	52.99	39.74	30.6	16.24	6.66
F					34.69	55.57	54.97	40.18	27.07	17.88	3.62
G		Female			14.37	23.74	27.07	21.38	16.24	6.66	1.27
H					22.07	47.31	68.49	43.37	56.54	12.54	4.34
I					12.6	27.27	46.01	40.28	38.19	18.74	3.41
J					19.49	27.27	51.43	48.66	35.58	29.89	6.82
K					23.98	46.72	67.99	52.99	59.63	30.6	12.64
M					23.74	58.68	127	54.52	46.55	18.89	5.76
A	ND 2.0	Male	2	21	26.87	64.95	59.63	50.87	42.8	19.74	4.76
B					5.72	27.27	61.22	33.24	30.86	15.28	1.29
C					16.24	44.96	39.56	11.28	7.46	3.54	1.13
D					11.89	45.05	35.58	37.74	32.06	6.17	2.98
E					21.7	35.03	40.28	27.27	28.72	11.28	3.06
F					12.6	28.7	34.86	18.6	21.38	9.02	1.45
G		Female			8.88	21.1	27.07	16.24	11.2	5.49	1
H					24.71	54.9	53.36	158	31.23	27.55	65.69
I					12.6	23.74	27.27	19.06	15.82	7.4	2.52
J					18.98	35.77	46.01	40.28	32.92	20.65	2.02
K					22.41	61.22	50.24	27.23	33.39	14.68	2.77
M					21.48	74.6	81.69	39.74	27.07	15.25	3.11

Subject	Light level	Gender	Eyes	F_IO_2 (%)	0.5	1	2.1	4.1	5.5	8.3	16.5
A	ND 2.0	Male	1	21	15.23	21.1	39.74	21.24	29.88	12.84	3.06
B					7.47	29.5	30.6	23.74	34	8.22	1.29
C					18.98	30.6	40.88	13.73	11.46	3.87	1.52
D					9.6	16.24	21.38	14.48	13.15	5.31	2.24
E					22.71	23.74	30.6	46.25	16.24	8.64	1.93
F					15.49	28.86	23.74	16.43	16.56	5.97	1
G		Female			7.52	12.6	14.37	10.21	5.76	1.93	1
H					40.71	27.23	66.61	27.04	9.45	3.64	1
I					9.87	19.83	21.24	16.24	12.89	3.43	1.66
J					15.68	32.52	30.6	17.08	15.05	4.9	1.46
K					22.07	55.19	44.72	12.39	7.52	4.56	1.11
M					20.12	46.45	43.95	31.51	24	12.18	2.66
A	ND 2.0	Male	2	14.1	23.57	48.7	46.66	49.05	51.81	14.23	5.94
B					7.4	28.72	60.31	30.6	37.54	15.71	1.3
C					21.52	27.07	44.96	11.28	14.4	3.96	1.11
D					17.35	25.99	47.1	27.07	21.1	8.3	2.84
E					15.23	70.07	35.3	42.02	33.42	10.85	2.94
F					17.08	41.57	80.44	21.75	22.41	6.44	1.76
G		Female			8.51	16.24	27.07	14.05	10.85	3.34	1
H					18.04	21.24	30.6	34.12	18.12	13.08	6.87
I					14.37	21.1	27.07	18.6	19.24	8.51	2.93
J					18.6	27.27	103	28.86	33.8	13.3	2.25
K					19.48	43.95	86.66	44	35.58	19.77	4.57
M					24.35	44.72	71.94	47.16	35.58	17.8	3.39
A	ND 2.0	Male	1	14.1	12.9	16.55	32.05	27.07	12.6	13.73	2.39
B					7.59	18.89	32.25	21.38	14.78	9.85	1.22
C					17.12	21.1	25.97	7.81	7.13	2.43	1
D					9.64	14.83	21.24	17.25	8.4	7.33	2.4
E					14.53	20.94	54.25	21.54	19.23	7.63	1.3
F					7.52	14.37	21	19.67	12.28	4.27	1.5
G		Female			4.52	9.4	8.51	4.15	2.95	1.41	1
H					10.5	17.12	41.43	28.59	11.38	3.54	2.77
I					8.88	14.37	21	18.92	14.29	3.6	1.51
J					11.2	19.06	45.05	18.6	26.19	16.47	1.29
K					16.24	31.97	36.49	11.35	5.53	1.89	1
M					15.49	31.97	46.72	23.74	24.2	11.2	3.54

Balanced ANOVA (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$)

Factor	Type	Levels	Values
Light level	fixed	2	Low photopic, Upper mesopic, Mid-mesopic
Gender	fixed	2	Female, Male
Eyes	fixed	2	Monocular, Binocular
Gas	fixed	2	14.1% oxygen, 21% oxygen
sf	fixed	7	0.5, 1.0, 2.1, 4.1, 5.5, 8.3, 16.5 cpd

Source	DF	SS	MS	F	P
Light level	2	294168.1	147084.1	346.15	**0.000**
Gender	1	1.6	1.6	0.00	0.950
Eyes	1	63677.3	63677.3	149.86	**0.000**
Gas	1	0.0	0.0	0.00	0.998
sf	6	395451.7	65908.6	155.11	**0.000**
Light level*Gender	2	385.8	192.9	0.45	0.635
Light level*Eyes	2	5023.9	2512.0	5.91	**0.003**
Light level*Gas	2	4146.9	2073.5	4.88	**0.008**
Light level*sf	12	59515.0	4959.6	11.67	**0.000**
Gender*Eyes	1	469.9	469.9	1.11	0.293
Gender*Gas	1	165.6	165.6	0.39	0.533
Gender*sf	6	2585.5	430.9	1.01	0.415
Eyes*Gas	1	213.9	213.9	0.50	0.478
Eyes*sf	6	8310.9	1385.1	3.26	**0.004**
Gas*sf	6	669.4	111.6	0.26	0.954
Light level*Gender*Eyes	2	1395.6	697.8	1.64	0.194

Light level*Gender*Gas	2	812.4	406.2	0.96	0.385
Light level*Gender*sf	12	2454.4	204.5	0.48	0.926
Light level*Eyes*Gas	2	163.2	81.6	0.19	0.825
Light level*Eyes*sf	12	3031.4	252.6	0.59	0.848
Light level*Gas*sf	12	3967.7	330.6	0.78	0.674
Gender*Eyes*Gas	1	886.2	886.2	2.09	0.149
Gender*Eyes*sf	6	562.9	93.8	0.22	0.970
Gender*Gas*sf	6	2044.2	340.7	0.80	0.569
Eyes*Gas*sf	6	1737.9	289.6	0.68	0.665
Light level*Gender*Eyes*Gas	2	1854.2	927.1	2.18	0.113
Light level*Gender*Eyes*sf	12	3372.5	281.0	0.66	0.789
Light level*Gender*Gas*sf	12	1798.1	149.8	0.35	0.979
Light level*Eyes*Gas*sf	12	1680.5	140.0	0.33	0.984
Gender*Eyes*Gas*sf	6	1363.2	227.2	0.53	0.782
Light level*Gender*Eyes*Gas*sf	12	3606.1	300.5	0.71	0.745
Error	840	356925.8	424.9		
Total	1007	1222441.7			

S = 20.6134 R-Sq = 70.80% R-Sq(adj) = 65.00%

Study 2

Summary Data Set

Measuring luminance contrast ratio (no units)					Spatial frequency (cpd)						
Subject	**Light level**	**Gender**	**Eyes**	**F_IO_2 (%)**	**0.5**	**1**	**2.1**	**4.1**	**5.5**	**8.3**	**16.5**
A	ND 1.0	Male	2	14.1	47.1	84.71	140	97.05	80.51	45.26	32.27
B					13.92	50.99	111	72.04	67.99	30.6	2.35
C					23.74	54.1	59.63	49.66	35.03	23.5	4.97
D					18.6	67.49	101	50.99	35.58	14.23	4.1
E					37.54	119	165	67.99	76.85	46.92	16.49
F					15.45	38.19	27.27	35.58	80.14	24.91	6.22
G		Female			14.48	39.51	52.99	32.16	27.07	11.52	1.66
H					14.05	34.99	46.66	87.92	39.8	35.48	13.62
I					21.24	39.74	59.63	59.63	60.13	18.89	14.6
L					26.47	54.02	164	66.03	28.64	16.48	13.37
K					52.98	38.49	76.85	49.49	67.99	66.28	8.46
M					42.02	88	148	76.85	76.85	38.19	12.02
A	ND 1.0	Male	1	14.1	29.49	38.49	75.87	56.19	57.26	26.27	4.46
B					8.79	44.26	151	44.72	41.63	22.07	1.38
C					14.37	35.3	72.14	51.36	40.87	17.33	3.92
D					25.79	47.89	46.72	63.39	27.27	9.87	4.11
E					36.59	66.88	59.63	49.24	75.05	26.36	6.47
F					13.65	28.68	40.87	35.03	25.23	11.57	5.2
G		Female			9.45	21.1	35.58	21.1	18.6	7.4	1.52
H					11.28	56.51	55.9	17.01	28.7	10.31	8.42
I					18.04	20.3	44.26	34.86	27.07	18.6	5.64
L					22.68	32.25	46.72	42.06	21.24	19.02	1.93
K					25.26	27.27	51.85	76.98	59.35	20.65	5.37
M					21.1	53.36	90.85	81	59.63	30.6	10.85
A	ND 1.0	Male	2	100	35.3	61.35	116	76.85	79.14	35.3	9.45
B					14.72	67.49	123	59.63	68.49	40.21	2.78
C					27.07	44.72	72.14	51.36	52.43	16.24	5.22
D					23.74	50.99	68.49	63.45	47.87	18.89	3.99
E					52.07	73.31	117	67.99	59.63	30.6	8.88
F					67.31	69.75	67.99	112	103	27.07	16.75
G		Female			12.97	35.3	48.7	31.51	21.1	10.35	1.44
H					34.85	56.96	99.24	275	33.44	9.56	1.23
I					27.27	35.03	59.63	39.56	40.28	23.98	8.88
L					30.6	51.36	68.41	42.89	37.39	18.98	4.04
K					35.03	52.99	88	68.49	88.66	81.63	18.89
M					40.28	67.99	145	101	76.85	35.03	9.45

Subject	Light level	Gender	Eyes	F_1O_2 (%)	0.5	1	2.1	4.1	5.5	8.3	16.5
A	ND 1.0	Male	1	100	27.27	61.56	132	40.02	53.36	33.41	5.72
B					7.46	35.03	60.74	48.92	39.56	18.6	1.33
C					21.38	35.03	67.99	40.02	27.27	14.37	3.93
D					17.12	36.87	52.99	39.74	32.02	22.09	2.41
E					44.49	52.99	81	108	74.14	48.66	5.02
F					18.99	53.36	44.84	35.03	40.53	25.97	6.82
G		Female			9.45	18.74	23.74	14.48	10.78	2.97	1
H					283	52.99	60.37	68.36	54.02	22.02	1.81
I					14.37	27.27	40.02	30.6	29.5	11.2	5.72
L					18.74	30.6	46.55	40.87	31.51	14.48	2.92
K					24.42	46.72	109	59.63	60.74	23.74	9.86
M					27.07	59.63	67.99	76.85	60.37	30.6	11.28
A	ND 2.0	Male	2	14.1	21.35	60.74	286	63.7	31.97	13.88	5.34
B					5.02	27.27	49.4	40.18	32.5	12.19	1.22
C					14.48	37.03	27.07	12.18	8.46	5.72	1.37
D					12.6	28.72	40.88	16.24	13.15	5.08	1.81
E					19.02	41.36	39.74	30.6	21.1	17.25	3.93
F					11.2	27.27	45.3	27.2	17.4	6.25	4.41
G		Female			13.79	23.74	27.07	17.74	11.2	6.06	1
H					24.78	49.06	172	19.62	24.94	162	13.55
I					23.87	27.07	30.2	21.38	14.16	14.18	3.96
J					25.99	23.74	216	31.66	18.74	15.49	2.69
K					16.97	38.22	44.72	35.18	17.12	12.37	1.29
M					18.74	52.99	72.04	61.95	58.25	15.25	3.43
A	ND 2.0	Male	1	14.1	16.81	27.07	75.26	40.87	35.35	10.4	1.99
B					4.46	33.41	47.87	17.12	23.5	8.4	1
C					12.6	14.37	30.6	22.82	5.76	4.51	1.65
D					7.4	32.71	20.45	12.6	9.45	3.93	1.46
E					14.89	19.06	32.05	30.01	18.6	8.46	2.49
F					11.32	23.5	22.6	15.93	11.85	4.99	1
G		Female			8.51	16.14	15.82	7.46	6.47	2.45	1
H					23.63	29.71	39.89	22.97	21.87	11.63	1.82
I					13.88	16.24	21.1	23.36	9.87	11.74	1.58
J					23.46	25.97	35.83	21.24	23.5	11.28	2.29
K					11.2	19.1	23.63	16.24	12.6	4.74	1.07
M					16.73	35.3	59.35	35.3	26.27	13.82	3.06
A	ND 2.0	Male	2	100	95.16	69.7	313	106	86.32	20.4	3.59
B					5.76	49.34	50.99	62.94	37	14.91	1.94
C					21.33	34.86	49.4	15.59	10.76	4.46	2.29
D					20.23	36.49	35.58	19.29	14.5	11.48	2.05
E					39.27	46.55	61.17	44.72	32.52	14.37	4.59
F					16.24	35.3	48.6	24.77	18.74	11.28	3.47
G		Female			15.73	23.74	27.27	12.6	9.45	3.76	1
H					28.57	46.01	52.99	50.78	37.67	13.39	4.47
I					16.99	22.94	35.3	21.1	22.65	8.4	3.02
J					32.37	32.37	40.02	35.9	40.02	27.46	1.37
K					35.94	35.03	52.99	37.67	30.6	21.1	3.62
M					23.74	74.35	90.18	94.34	35.58	14.48	5.22
A	ND 2.0	Male	1	100	33.28	40.88	67.91	152	18.6	6.84	5.25
A					33.28	40.88	67.91	152.0	18.60	6.84	5.25
B					5.24	39.51	35.58	21.1	23.38	16.31	2.88
C					14.48	34.86	35.03	14.37	11.44	2.66	2.38
D					7.46	21.7	23.74	41.46	17.19	6.56	1
E					17.36	38.23	37.39	38.91	16.97	9.45	3.28
F					12.97	21.24	32.05	13.64	10.04	4.34	4.47
G		Female			8.88	16.24	13.15	8.46	6.57	2.03	1
H					15.79	23.74	45.7	21.1	23.62	6.84	21.79
I					12.54	20.97	30.6	21.52	11.2	5.72	1.83
J					15.8	38.4	40.74	43.42	21.1	11.28	2.29
K					16.24	23.74	54.02	20.3	13.23	4.63	1.29
M					17.01	35.03	51.36	35.03	23.5	9.87	4.46

Balanced ANOVA (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$)

Factor	Type	Levels	Values
Light level	fixed	2	Upper mesopic, Mid-mesopic
Gender	fixed	2	Female, Male
Eyes	fixed	2	Monocular, Binocular
Gas	fixed	2	14.1% oxygen, 100% oxygen
sf	fixed	7	0.5, 1.0, 2.1, 4.1, 5.5, 8.3, 16.5 cpd

Source	DF	SS	MS	F	P
Light level	1	48933.7	48933.7	62.57	**0.000**
Gender	1	1160.8	1160.8	1.48	0.224
Eyes	1	31208.2	31208.2	39.91	**0.000**
Gas	1	1636.0	1636.0	2.09	0.149
sf	6	245306.9	40884.5	52.28	**0.000**
Light level*Gender	1	216.2	216.2	0.28	0.599
Light level*Eyes	1	6.9	6.9	0.01	0.925
Light level*Gas	1	39.2	39.2	0.05	0.823
Light level*sf	6	10997.8	1833.0	2.34	0.030
Gender*Eyes	1	1.5	1.5	0.00	0.965
Gender*Gas	1	280.5	280.5	0.36	0.550
Gender*sf	6	4807.2	801.2	1.02	0.408
Eyes*Gas	1	265.5	265.5	0.34	0.560
Eyes*sf	6	16672.4	2778.7	3.55	**0.002**
Gas*sf	6	5667.5	944.6	1.21	0.300
Light level*Gender*Eyes	1	72.5	72.5	0.09	0.761
Light level*Gender*Gas	1	2173.5	2173.5	2.78	0.096
Light level*Gender*sf	6	2481.6	413.6	0.53	0.787
Light level*Eyes*Gas	1	23.5	23.5	0.03	0.863
Light level*Eyes*sf	6	3588.0	598.0	0.76	0.598
Light level*Gas*sf	6	945.9	157.6	0.20	0.976
Gender*Eyes*Gas	1	754.5	754.5	0.96	0.326
Gender*Eyes*sf	6	2590.4	431.7	0.55	0.768
Gender*Gas*sf	6	1046.0	174.3	0.22	0.969
Eyes*Gas*sf	6	1931.9	322.0	0.41	0.871
Light level*Gender*Eyes*Gas	1	162.9	162.9	0.21	0.648
Light level*Gender*Eyes*sf	6	1454.5	242.4	0.31	0.932
Light level*Gender*Gas*sf	6	1169.6	194.9	0.25	0.960
Light level*Eyes*Gas*sf	6	2149.2	358.2	0.46	0.839
Gender*Eyes*Gas*sf	6	3045.0	507.5	0.65	0.691
Light level*Gender*Eyes*Gas*sf	6	2202.6	367.1	0.47	0.831
Error	560	437942.5	782.0		
Total	671	830934.4			

S = 27.9650 R-Sq = 47.30% R-Sq(adj) = 36.85%

Appendix 5 Chromatic Sensitivity Data and ANOVA

Study 1

Summary Data Set – measuring axis length in CIE 1931 (x, y) colour space

Subject exposure order: * = hypoxia precedes normoxia

Subject & Order	Light level	Gender	Eyes	F_IO_2 (%)	Colour direction (°)							
					60	70	145	175	240	250	325	355
A	Direct viewing	Male	2	21	0.00848	0.00793	0.00566	0.00951	0.01032	0.00784	0.00724	0.00879
B					0.00951	0.01160	0.00609	0.00469	0.00845	0.00599	0.00327	0.00343
C *					0.01709	0.01491	0.00343	0.00533	0.01364	0.01303	0.00331	0.00406
D					0.00998	0.01006	0.00609	0.00467	0.01263	0.01260	0.00628	0.00706
E *					0.01436	0.01806	0.00456	0.00520	0.00766	0.01303	0.00484	0.00681
F *					0.00907	0.00887	0.00414	0.00729	0.01029	0.00871	0.00541	0.00398
G *		Female			0.01350	0.01021	0.00541	0.00520	0.01292	0.00976	0.00475	0.00467
H					0.01047	0.00885	0.00609	0.00603	0.00951	0.00701	0.00533	0.00439
I *					0.01199	0.00887	0.00381	0.00361	0.00937	0.00819	0.00309	0.00434
J *					0.01340	0.01136	0.00327	0.00469	0.01113	0.01386	0.00482	0.00482
K					0.01760	0.01081	0.00609	0.00502	0.01586	0.01206	0.00467	0.00361
L					0.00871	0.01039	0.00609	0.00696	0.01080	0.01062	0.00533	0.00587
A	Direct viewing	Male	1	21	0.01133	0.00994	0.01099	0.00691	0.00769	0.00848	0.00429	0.00873
B					0.01081	0.01212	0.00381	0.00434	0.01206	0.00533	0.00315	0.00384
C *					0.02082	0.01427	0.00647	0.00533	0.02309	0.01887	0.00533	0.00563
D					0.00971	0.01735	0.00767	0.00729	0.01322	0.01610	0.00486	0.00789
E *					0.01639	0.01366	0.00439	0.00702	0.01519	0.01311	0.00662	0.00807
F *					0.01337	0.01203	0.00681	0.00887	0.01281	0.01343	0.00814	0.00733
G *		Female			0.01706	0.01767	0.00734	0.00518	0.01020	0.01643	0.00589	0.00912
H					0.00922	0.01436	0.00432	0.00327	0.00954	0.00922	0.00480	0.00530
I *					0.01611	0.01306	0.00659	0.00524	0.01296	0.01029	0.00533	0.00566
J *					0.01274	0.01796	0.00327	0.00324	0.01701	0.01060	0.00578	0.00331
K					0.01272	0.01365	0.00483	0.00650	0.01606	0.01284	0.00480	0.00467
L					0.01655	0.01197	0.00673	0.00361	0.01528	0.01371	0.00781	0.00748
A	Direct viewing	Male	2	14.1	0.00991	0.01012	0.00693	0.00735	0.00960	0.00903	0.00518	0.00533
B					0.01084	0.00966	0.00381	0.00467	0.00855	0.00658	0.00327	0.00449
C *					0.01551	0.00997	0.00327	0.00361	0.01332	0.01156	0.00429	0.00467
D					0.01340	0.01076	0.00330	0.00620	0.01392	0.01376	0.00628	0.00486
E *					0.01552	0.01198	0.00651	0.00696	0.01181	0.01085	0.00613	0.00446
F *					0.00964	0.00729	0.00399	0.00520	0.00908	0.00641	0.00486	0.00706
G *		Female			0.00935	0.00887	0.00414	0.00434	0.01114	0.01038	0.00533	0.00807
H					0.00843	0.01131	0.00432	0.00467	0.00807	0.00626	0.00533	0.00449
I *					0.00718	0.01244	0.00323	0.00327	0.00880	0.00685	0.00327	0.00449
J *					0.01091	0.01146	0.00489	0.00412	0.01253	0.01079	0.00418	0.00331
K					0.01491	0.01248	0.00327	0.00520	0.01361	0.01103	0.00467	0.00365
L					0.01091	0.01281	0.00609	0.00467	0.01136	0.00937	0.00486	0.00327
A	Direct viewing	Male	1	14.1	0.01291	0.01215	0.00767	0.00785	0.00954	0.00903	0.00722	0.00864
B					0.01398	0.01012	0.00327	0.00449	0.01331	0.00691	0.00323	0.00343
C *					0.02354	0.01338	0.00427	0.00482	0.02423	0.01580	0.00533	0.00490
D					0.00799	0.01179	0.00567	0.00729	0.01621	0.01821	0.00361	0.00697
E *					0.01867	0.01586	0.00482	0.00729	0.01173	0.01331	0.00647	0.00814
F *					0.01158	0.01206	0.00767	0.00902	0.01139	0.01204	0.00695	0.00897
G *		Female			0.01235	0.01637	0.00734	0.00696	0.01785	0.00781	0.00486	0.00697
H					0.01356	0.01136	0.00568	0.00533	0.01142	0.00700	0.00689	0.00631
I *					0.01091	0.01017	0.00533	0.00696	0.01036	0.01006	0.00480	0.00587
J *					0.01827	0.01719	0.00725	0.00696	0.01785	0.02066	0.00429	0.00686
K					0.01774	0.02285	0.00327	0.00813	0.02139	0.01470	0.00486	0.00819
L					0.01248	0.01576	0.00562	0.00971	0.01592	0.01533	0.00913	0.00833

Subject & Order	Light level	Gender	Eyes	F_IO_2 (%)	60	70	145	175	240	250	325	355
A *	ND 1.0	Male	2	21	0.01867	0.01714	0.01191	0.01208	0.01752	0.02000	0.01113	0.01126
B					0.02416	0.02036	0.00642	0.00818	0.02135	0.01166	0.00533	0.00739
C *					0.03867	0.02776	0.00614	0.00869	0.03154	0.03001	0.00814	0.00607
D					0.03060	0.03662	0.00875	0.00938	0.02566	0.03869	0.01144	0.01096
E					0.03859	0.03034	0.01079	0.01079	0.03036	0.02852	0.00847	0.00889
F *					0.01745	0.02041	0.00999	0.01327	0.02098	0.02246	0.01258	0.01424
G		Female			0.02489	0.01624	0.01049	0.00813	0.02796	0.02819	0.00769	0.00551
H					0.02230	0.02236	0.00767	0.00665	0.02355	0.02097	0.00755	0.00664
I *					0.03397	0.03034	0.00551	0.00754	0.02121	0.02345	0.00327	0.00897
J					0.03628	0.02443	0.00609	0.00595	0.04477	0.03098	0.00781	0.00873
K *					0.05243	0.06882	0.01018	0.00995	0.03910	0.04342	0.01058	0.00854
L *					0.03665	0.03869	0.00767	0.00575	0.02934	0.02698	0.00805	0.01213
A *	ND 1.0	Male	1	21	0.03207	0.02829	0.00767	0.00988	0.01697	0.02047	0.01184	0.01270
B					0.02597	0.02792	0.00659	0.00729	0.02744	0.01630	0.00549	0.00490
C *					0.05778	0.04374	0.00818	0.00821	0.04074	0.03669	0.00921	0.00808
D					0.03209	0.03837	0.00851	0.01045	0.03139	0.04288	0.00831	0.01106
E					0.04873	0.03035	0.01140	0.00887	0.03393	0.03794	0.00904	0.00998
F *					0.02979	0.03463	0.01686	0.01938	0.02685	0.02451	0.01711	0.02086
G		Female			0.02981	0.03483	0.00901	0.00887	0.03502	0.02416	0.00898	0.00963
H					0.03922	0.03489	0.00734	0.00684	0.02886	0.03165	0.00804	0.00998
I *					0.05794	0.06868	0.00767	0.00995	0.04228	0.05060	0.00681	0.01011
J					0.05583	0.06659	0.00833	0.00854	0.03031	0.04363	0.00814	0.01029
K *					0.04340	0.04416	0.00985	0.00995	0.03259	0.02467	0.00913	0.00971
L *					0.03941	0.05567	0.00996	0.01286	0.04732	0.05148	0.01062	0.01496
A *	ND 1.0	Male	2	14.1	0.02187	0.02978	0.01064	0.01485	0.02470	0.01669	0.01030	0.01529
B					0.02095	0.02104	0.00516	0.00475	0.02652	0.01858	0.00503	0.00601
C *					0.04492	0.02254	0.00947	0.00713	0.03792	0.02987	0.00805	0.00864
D					0.02964	0.03252	0.01033	0.00847	0.03638	0.03405	0.01108	0.01208
E					0.06066	0.06281	0.01041	0.00793	0.03557	0.04115	0.00745	0.00912
F *					0.01917	0.01701	0.01363	0.00984	0.02548	0.02330	0.01610	0.01549
G		Female			0.03397	0.03743	0.01324	0.01026	0.02705	0.02847	0.01015	0.00948
H					0.01951	0.02783	0.00331	0.00668	0.02386	0.02169	0.00781	0.00743
I *					0.02529	0.02921	0.00667	0.00327	0.02880	0.01352	0.00518	0.00789
J					0.03996	0.03830	0.00693	0.00469	0.03889	0.03313	0.00814	0.00742
K *					0.03237	0.03420	0.00767	0.00971	0.03405	0.02875	0.00898	0.01011
L *					0.03154	0.03678	0.00784	0.01012	0.03447	0.02672	0.00736	0.00879
A *	ND 1.0	Male	1	14.1	0.02918	0.02640	0.01240	0.00771	0.02257	0.02014	0.01564	0.01336
B					0.04359	0.02225	0.00560	0.00691	0.02872	0.01640	0.00698	0.00699
C *					0.07845	0.04476	0.00818	0.01056	0.04556	0.02751	0.00739	0.00897
D					0.04250	0.04392	0.00985	0.00842	0.03663	0.05293	0.00566	0.01674
E					0.05676	0.04147	0.01299	0.00986	0.04035	0.02983	0.01445	0.01121
F *					0.03698	0.04116	0.02081	0.01624	0.02795	0.02097	0.02005	0.02897
G		Female			0.02812	0.03801	0.01199	0.01120	0.02271	0.03353	0.00984	0.00897
H					0.03531	0.02449	0.00915	0.00971	0.02792	0.02722	0.00898	0.00983
I *					0.03498	0.04965	0.00842	0.00938	0.03330	0.02279	0.00847	0.00795
J					0.04475	0.03343	0.00647	0.00745	0.04571	0.03197	0.01099	0.00897
K *					0.04597	0.04248	0.00700	0.00729	0.03085	0.03241	0.00847	0.00897
L *					0.03027	0.05217	0.01559	0.01184	0.03933	0.03712	0.01479	0.01293
A	ND 2.0	Male	2	21	0.16877	0.14957	0.02517	0.02871	0.06320	0.04876	0.02325	0.02786
B					0.10503	0.05184	0.01168	0.01079	0.04929	0.03953	0.00714	0.00981
C *					0.10403	0.08343	0.01576	0.01635	0.07328	0.06133	0.01404	0.01046
D					0.13509	0.10306	0.01194	0.01298	0.06087	0.06838	0.02348	0.01876
E *					0.10965	0.21903	0.01906	0.02821	0.03858	0.09076	0.02113	0.01838
F *					0.07239	0.09414	0.02689	0.02775	0.06301	0.03895	0.02807	0.02762
G		Female			0.06858	0.09258	0.01519	0.01127	0.06786	0.06614	0.01622	0.01449
H					0.17309	0.06232	0.01396	0.01256	0.07208	0.06854	0.01113	0.01864
I *					0.09789	0.06842	0.01513	0.01532	0.06348	0.07162	0.01064	0.00907
J					0.19446	0.11517	0.02098	0.02363	0.14468	0.11725	0.01880	0.01831
K *					0.11600	0.17090	0.01643	0.01694	0.07993	0.02966	0.01513	0.02245
M *					0.07549	0.14361	0.00918	0.01763	0.07424	0.06598	0.01442	0.02211

Subject & Order	Light level	Gender	Eyes	F_IO_2 (%)	60	70	145	175	240	250	325	355
A	ND 2.0	Male	1	21	0.12105	0.19491	0.03871	0.02318	0.06791	0.07977	0.02963	0.02933
B					0.14922	0.06907	0.01235	0.01724	0.06870	0.06032	0.01453	0.01247
C *					0.20030	0.04493	0.01270	0.01419	0.06773	0.07313	0.02187	0.02130
D					0.16573	0.11366	0.01935	0.01934	0.06990	0.10204	0.01632	0.01580
E *					0.20844	0.15254	0.02473	0.02548	0.06866	0.10220	0.02589	0.01334
F *					0.06520	0.09448	0.06072	0.04079	0.04514	0.09016	0.03684	0.03677
G		Female			0.11375	0.16244	0.02580	0.02242	0.06906	0.10949	0.02463	0.01731
H					0.10291	0.13986	0.01792	0.01385	0.07288	0.10408	0.01930	0.02229
I *					0.20986	0.06905	0.01800	0.02271	0.10187	0.12852	0.01696	0.02021
J					0.07995	0.21903	0.02625	0.01419	0.07260	0.11816	0.01902	0.02737
K *					0.21039	0.21903	0.01694	0.02485	0.06789	0.11766	0.01633	0.02629
M *					0.18134	0.14726	0.02433	0.01993	0.10537	0.11732	0.02083	0.02971
A	ND 2.0	Male	2	14.1	0.07807	0.21689	0.03845	0.03135	0.07566	0.07512	0.02930	0.03427
B					0.11065	0.06606	0.01084	0.01138	0.07061	0.03508	0.01182	0.00864
C *					0.20997	0.04098	0.01066	0.01326	0.12632	0.06408	0.01113	0.01841
D					0.13485	0.21819	0.02396	0.02018	0.06639	0.09762	0.02386	0.03002
E *					0.13840	0.12476	0.02348	0.01887	0.09407	0.10724	0.02009	0.02079
F *					0.09020	0.06722	0.02352	0.01990	0.06826	0.06209	0.03118	0.02358
G		Female			0.06354	0.09610	0.01815	0.00721	0.09713	0.08842	0.01737	0.02162
H					0.10613	0.07091	0.01579	0.00907	0.06951	0.04951	0.01553	0.01734
I *					0.11354	0.19581	0.00801	0.01904	0.09406	0.08091	0.01689	0.01575
J					0.19527	0.10591	0.01472	0.01882	0.12583	0.10477	0.02131	0.02206
K *					0.08393	0.19776	0.02711	0.03300	0.13178	0.06361	0.02886	0.04074
M *					0.16768	0.16729	0.01867	0.01237	0.07135	0.08437	0.01828	0.01724
A	ND 2.0	Male	1	14.1	0.15108	0.21903	0.05291	0.03139	0.07076	0.06811	0.03015	0.03682
B					0.11970	0.07477	0.01791	0.01169	0.09391	0.07690	0.01345	0.01697
C *					0.06952	0.10360	0.01644	0.01792	0.09674	0.09566	0.02396	0.01948
D					0.04124	0.21903	0.02000	0.01469	0.06833	0.09498	0.01475	0.02426
E *					0.18061	0.18193	0.02980	0.02729	0.11047	0.11050	0.03402	0.03326
F *					0.09306	0.15914	0.04262	0.04140	0.09014	0.07858	0.06037	0.06234
G		Female			0.20872	0.16235	0.03929	0.02043	0.11041	0.06111	0.04017	0.03676
H					0.14653	0.05596	0.01633	0.01123	0.06149	0.04412	0.02122	0.02541
I *					0.14035	0.11894	0.02082	0.02626	0.10211	0.12066	0.03068	0.01529
J					0.20973	0.21903	0.03041	0.02093	0.16031	0.11446	0.02907	0.06331
K *					0.21039	0.20159	0.04625	0.03847	0.06376	0.14237	0.03468	0.04136
M *					0.15723	0.21903	0.02033	0.02095	0.11181	0.07850	0.01829	0.02405

Balanced ANOVA (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$)

Factor	Type	Levels	Values
Eyes	fixed	2	Binocular, Monocular
Gas	fixed	2	14.1% oxygen, 21% oxygen
Gender	fixed	2	Female, Male
Light level	fixed	3	Low photopic, Upper mesopic, Mid-mesopic
Colour axis	fixed	8	60, 70, 145, 175, 240, 250, 325, 355

Source	DF	SS	MS	F	P
Eyes	1	0.013048	0.013048	40.98	**0.000**
Gas	1	0.001630	0.001630	5.12	**0.024**
Gender	1	0.002053	0.002053	6.45	**0.011**
Light level	2	0.690669	0.345335	1084.73	**0.000**
Axis	7	0.498096	0.071157	223.51	**0.000**
Eyes*Gas	1	0.000028	0.000028	0.09	0.768
Eyes*Gender	1	0.000562	0.000562	1.77	0.184
Eyes*Light level	2	0.007400	0.003700	11.62	**0.000**
Eyes*Axis	7	0.005362	0.000766	2.41	0.019
Gas*Gender	1	0.000077	0.000077	0.24	0.622
Gas*Light level	2	0.003107	0.001554	4.88	**0.008**
Gas*Axis	7	0.002368	0.000338	1.06	0.386
Gender*Light level	2	0.002879	0.001439	4.52	0.011
Gender*Axis	7	0.005087	0.000727	2.28	0.026
Light level*Axis	14	0.424527	0.030323	95.25	**0.000**
Eyes*Gas*Gender	1	0.000000	0.000000	0.00	0.970
Eyes*Gas*Light level	2	0.000084	0.000042	0.13	0.877
Eyes*Gas*Axis	7	0.000808	0.000115	0.36	0.924
Eyes*Gender*Light level	2	0.000791	0.000395	1.24	0.289
Eyes*Gender*Axis	7	0.001078	0.000154	0.48	0.847

Eyes*Light level*Axis	14	0.003790	0.000271	0.85	0.614
Gas*Gender*Light level	2	0.000423	0.000212	0.67	0.514
Gas*Gender*Axis	7	0.001085	0.000155	0.49	0.845
Gas*Light level*Axis	14	0.003847	0.000275	0.86	0.600
Gender*Light level*Axis	14	0.004486	0.000320	1.01	0.444
Eyes*Gas*Gender*Light level	2	0.000075	0.000037	0.12	0.889
Eyes*Gas*Gender*Axis	7	0.003210	0.000459	1.44	0.185
Eyes*Gas*Light level*Axis	14	0.001673	0.000120	0.38	0.982
Eyes*Gender*Light level*Axis	14	0.002307	0.000165	0.52	0.924
Gas*Gender*Light level*Axis	14	0.003389	0.000242	0.76	0.713
Eyes*Gas*Gender*Light level*Axis	14	0.006105	0.000436	1.37	0.161
Error	960	0.305627	0.000318		
Total	1151	1.995671			

S = 0.0178427 R-Sq = 84.69% R-Sq(adj) = 81.64%

Study 2

Summary Data Set – measuring axis length in CIE 1931 (x, y) colour space

Subject exposure order: * = hypoxia precedes hyperoxia					Colour direction (°)							
Subject & Order	**Light level**	**Gender**	**Eyes**	**F_IO_2 (%)**	**60**	**70**	**145**	**175**	**240**	**250**	**325**	**355**
A	ND 1.0	Male	2	14.1	0.03088	0.02384	0.00974	0.01352	0.01876	0.01930	0.00987	0.01577
B					0.02884	0.02420	0.00681	0.00733	0.02363	0.01978	0.00402	0.00832
C *					0.06775	0.04306	0.00566	0.00854	0.05820	0.04467	0.00839	0.00739
D *					0.02213	0.03181	0.00947	0.00907	0.02641	0.03085	0.01266	0.00860
E					0.06661	0.05028	0.01332	0.04001	0.05192	0.05313	0.01729	0.01494
F *					0.02376	0.02038	0.01540	0.01055	0.02103	0.02339	0.01484	0.01495
G		Female			0.04362	0.03327	0.00754	0.00895	0.03382	0.03469	0.00931	0.01005
M					0.04169	0.03361	0.00709	0.00785	0.03022	0.03538	0.00689	0.00756
I *					0.04229	0.03236	0.03991	0.05485	0.02491	0.05417	0.02714	0.00548
J *					0.06715	0.06277	0.01480	0.01223	0.03786	0.05094	0.01015	0.01948
K *					0.06205	0.07268	0.01017	0.01549	0.06694	0.02815	0.01631	0.01579
L					0.04521	0.02779	0.01065	0.01045	0.03510	0.02884	0.00978	0.00789
A	ND 1.0	Male	1	14.1	0.04285	0.04925	0.01330	0.01621	0.02296	0.01961	0.01054	0.01159
B					0.01925	0.02007	0.00701	0.00468	0.02551	0.02639	0.00689	0.00789
C *					0.06164	0.07191	0.00767	0.00991	0.06837	0.04257	0.00913	0.01163
D *					0.03932	0.05412	0.00782	0.00788	0.03225	0.02839	0.01260	0.00828
E					0.06526	0.06480	0.01627	0.01361	0.04372	0.02329	0.01530	0.01694
F *					0.03361	0.06927	0.04891	0.07284	0.04122	0.05107	0.04579	0.02986
G		Female			0.03845	0.04415	0.01385	0.00948	0.04308	0.02354	0.01071	0.00894
M					0.06022	0.04398	0.00599	0.00729	0.04873	0.04302	0.01046	0.00897
I *					0.04714	0.04308	0.03259	0.01740	0.10102	0.04324	0.01326	0.03002
J *					0.04927	0.09936	0.01542	0.01366	0.06560	0.06288	0.01526	0.04902
K *					0.06499	0.12305	0.02551	0.01761	0.06327	0.05706	0.02411	0.02940
L					0.05597	0.05577	0.01281	0.01519	0.05718	0.05238	0.01847	0.01306
A	ND 1.0	Male	2	100	0.02256	0.02797	0.01277	0.01162	0.01986	0.01807	0.01046	0.00869
B					0.01783	0.02258	0.00734	0.00638	0.02355	0.01876	0.00361	0.00563
C *					0.04654	0.04460	0.00721	0.00662	0.03701	0.02694	0.00691	0.00965
D *					0.02041	0.02743	0.00743	0.00813	0.02632	0.02082	0.00847	0.01100
E					0.04499	0.04843	0.00741	0.01093	0.03631	0.03500	0.00902	0.01183
F *					0.01099	0.01164	0.00907	0.00958	0.01716	0.01859	0.01062	0.01200
G		Female			0.02362	0.03479	0.00767	0.01303	0.03295	0.02360	0.00773	0.00739
M					0.03916	0.03572	0.00634	0.00887	0.02444	0.01683	0.00889	0.00840
I *					0.03990	0.07689	0.01265	0.06101	0.03300	0.01845	0.00680	0.03791
J *					0.03863	0.03005	0.00596	0.00854	0.03752	0.02142	0.00847	0.00745
K *					0.05097	0.05686	0.01324	0.01148	0.03727	0.03060	0.00839	0.01405
L					0.03856	0.03758	0.00868	0.01067	0.03108	0.03239	0.00651	0.01130

Subject & Order	Light level	Gender	Eyes	F_IO_2 (%)	60	70	145	175	240	250	325	355
A	ND 1.0	Male	1	100	0.02887	0.02369	0.00913	0.01079	0.01832	0.02287	0.00961	0.01304
B					0.02957	0.01734	0.00676	0.00749	0.02386	0.01587	0.00843	0.00864
C *					0.07662	0.05075	0.00552	0.01065	0.04734	0.04705	0.01027	0.00897
D *					0.02027	0.03344	0.00600	0.00733	0.01676	0.04414	0.00814	0.00823
E					0.04132	0.04500	0.01074	0.01077	0.03799	0.03263	0.01030	0.00903
F *					0.02680	0.02834	0.01316	0.01345	0.02047	0.02129	0.01443	0.01789
G		Female			0.03941	0.03389	0.01213	0.00931	0.02498	0.03008	0.00955	0.00864
M					0.05056	0.04628	0.00676	0.00874	0.03609	0.03981	0.00696	0.00963
I *					0.04812	0.03732	0.01230	0.00695	0.03197	0.03090	0.00898	0.00749
J *					0.05443	0.03955	0.00734	0.00785	0.03701	0.03039	0.00814	0.00486
K *					0.04504	0.04401	0.00959	0.00735	0.03392	0.02745	0.00847	0.00897
L					0.05882	0.04973	0.01082	0.01243	0.04271	0.04202	0.01081	0.00948
A	ND 2.0	Male	2	14.1	0.10605	0.09943	0.03312	0.02325	0.08873	0.09300	0.03119	0.02438
B					0.10452	0.07678	0.00633	0.01153	0.06560	0.04424	0.01187	0.01149
C *					0.20932	0.16082	0.01330	0.01387	0.12563	0.08902	0.01706	0.01687
D *					0.13806	0.15212	0.02069	0.01332	0.07557	0.06943	0.02019	0.01974
E					0.18536	0.21903	0.02430	0.01626	0.07713	0.09582	0.02640	0.01836
F *					0.08417	0.11020	0.02244	0.02424	0.06793	0.06289	0.03063	0.02996
G *		Female			0.04309	0.03264	0.01394	0.01674	0.07174	0.06751	0.01886	0.01916
M					0.06845	0.09740	0.01332	0.01173	0.07298	0.06481	0.01164	0.01293
I					0.10626	0.16989	0.01238	0.02750	0.12124	0.06646	0.01654	0.01820
J *					0.15740	0.14431	0.04080	0.02106	0.13347	0.12672	0.02864	0.03864
K					0.15721	0.11923	0.01869	0.02910	0.08954	0.10813	0.01901	0.02881
L *					0.13845	0.11226	0.01287	0.01361	0.04309	0.05650	0.01176	0.02077
A	ND 2.0	Male	1	14.1	0.19944	0.21869	0.03467	0.03921	0.09933	0.12308	0.03217	0.03496
B					0.10665	0.06623	0.01897	0.01273	0.08040	0.04992	0.01592	0.01574
C *					0.16800	0.17216	0.01964	0.02603	0.10520	0.14967	0.03016	0.02523
D *					0.15404	0.06390	0.02454	0.00898	0.07576	0.11763	0.02164	0.01996
E					0.17159	0.18589	0.03203	0.02485	0.12366	0.08587	0.02856	0.02910
F *					0.08841	0.14776	0.02577	0.03361	0.06693	0.08154	0.03341	0.04447
G *		Female			0.18867	0.19506	0.02738	0.02853	0.09170	0.07797	0.01931	0.02280
M					0.19792	0.06822	0.01884	0.02001	0.09933	0.11899	0.01476	0.01930
I					0.21039	0.21741	0.04084	0.03437	0.11539	0.09696	0.02223	0.04046
J *					0.21039	0.01401	0.05210	0.03830	0.11646	0.12525	0.03856	0.05074
K					0.21039	0.21903	0.02229	0.01654	0.14808	0.13175	0.03304	0.01946
L *					0.12697	0.14068	0.01803	0.01822	0.08418	0.07459	0.02529	0.01843
A	ND 2.0	Male	2	100	0.04358	0.16579	0.02023	0.02160	0.06876	0.05665	0.02852	0.03036
B					0.10803	0.06938	0.00767	0.00770	0.05287	0.03753	0.00854	0.01278
C *					0.04647	0.12511	0.01537	0.01086	0.10460	0.08060	0.01220	0.01448
D *					0.11056	0.13051	0.01730	0.01807	0.07195	0.08767	0.01479	0.01786
E					0.08896	0.21869	0.01678	0.02059	0.09871	0.06829	0.02086	0.02212
F *					0.07523	0.06657	0.02471	0.00971	0.06121	0.06533	0.03139	0.02016
G *		Female			0.06630	0.06448	0.01675	0.01729	0.05277	0.07026	0.01898	0.01915
M					0.14931	0.09679	0.01473	0.01111	0.08503	0.07206	0.01113	0.01577
I					0.08950	0.09430	0.01023	0.01002	0.07301	0.07065	0.01326	0.01809
J *					0.13359	0.10690	0.01839	0.00875	0.05579	0.06772	0.01480	0.01756
K					0.15981	0.14951	0.01735	0.01485	0.08809	0.06688	0.01429	0.01455
L *					0.10325	0.06879	0.00534	0.01450	0.04399	0.04291	0.01247	0.01354
A	ND 2.0	Male	1	100	0.09612	0.12191	0.02416	0.02361	0.06979	0.07307	0.02375	0.02791
B					0.09020	0.06286	0.01099	0.01327	0.08413	0.06789	0.01358	0.00911
C *					0.21039	0.12086	0.01409	0.01590	0.11886	0.07437	0.01874	0.02266
D *					0.07462	0.11018	0.01406	0.01179	0.07391	0.07994	0.01536	0.01960
E					0.11206	0.10886	0.02841	0.02685	0.07407	0.08237	0.02101	0.02693
F *					0.05667	0.16868	0.02780	0.02854	0.06830	0.06837	0.03056	0.03606
G *		Female			0.15181	0.17891	0.02100	0.02333	0.07553	0.09131	0.02676	0.02380
M					0.16823	0.14820	0.01900	0.01934	0.10069	0.08942	0.01584	0.01019
I					0.20652	0.14363	0.02348	0.01887	0.12377	0.09060	0.01823	0.08253
J *					0.12291	0.18986	0.02140	0.01732	0.10419	0.03806	0.01773	0.01910
K					0.08559	0.21232	0.02316	0.01998	0.09096	0.11306	0.01883	0.02710
L *					0.18399	0.13937	0.01481	0.01227	0.08382	0.08157	0.01981	0.02064

Balanced ANOVA (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$)

Factor	Type	Levels	Values
Eyes	fixed	2	Binocular, Monocular
Gas	fixed	2	14.1% oxygen, 100% oxygen
Gender	fixed	2	Female, Male
Light level	fixed	3	Upper mesopic, Mid-mesopic
Colour axis	fixed	8	60, 70, 145, 175, 240, 250, 325, 355

Source	DF	SS	MS	F	P
Eyes	1	0.019095	0.019095	41.05	**0.000**
Gas	1	0.019048	0.019048	40.95	**0.000**
Gender	1	0.005193	0.005193	11.16	**0.001**
Light level	1	0.279610	0.279610	601.16	**0.000**
Axis	7	0.696152	0.099450	213.82	**0.000**
Eyes*Gas	1	0.000562	0.000562	1.21	0.272
Eyes*Gender	1	0.002264	0.002264	4.87	0.028
Eyes*Light level	1	0.005314	0.005314	11.42	**0.001**
Eyes*Axis	7	0.008379	0.001197	2.57	0.013
Gas*Gender	1	0.000059	0.000059	0.13	0.721
Gas*Light level	1	0.000269	0.000269	0.58	0.447
Gas*Axis	7	0.007987	0.001141	2.45	0.017
Gender*Light level	1	0.000054	0.000054	0.12	0.734
Gender*Axis	7	0.006795	0.000971	2.09	0.043
Light level*Axis	7	0.203316	0.029045	62.45	**0.000**
Eyes*Gas*Gender	1	0.000000	0.000000	0.00	0.981
Eyes*Gas*Light level	1	0.000262	0.000262	0.56	0.453
Eyes*Gas*Axis	7	0.000152	0.000022	0.05	1.000
Eyes*Gender*Light level	1	0.002776	0.002776	5.97	0.015
Eyes*Gender*Axis	7	0.003974	0.000568	1.22	0.289
Eyes*Light level*Axis	7	0.003387	0.000484	1.04	0.401
Gas*Gender*Light level	1	0.000548	0.000548	1.18	0.278
Gas*Gender*Axis	7	0.002877	0.000411	0.88	0.519
Gas*Light level*Axis	7	0.003939	0.000563	1.21	0.295
Gender*Light level*Axis	7	0.002060	0.000294	0.63	0.729
Eyes*Gas*Gender*Light level	1	0.000189	0.000189	0.41	0.524
Eyes*Gas*Gender*Axis	7	0.002050	0.000293	0.63	0.732
Eyes*Gas*Light level*Axis	7	0.003165	0.000452	0.97	0.450
Eyes*Gender*Light level*Axis	7	0.004491	0.000642	1.38	0.211
Gas*Gender*Light level*Axis	7	0.001938	0.000277	0.60	0.760
Eyes*Gas*Gender*Light level*Axis	7	0.003423	0.000489	1.05	0.394
Error	640	0.297673	0.000465		
Total	767	1.587000			

S = 0.0215665 R-Sq = 81.24% R-Sq(adj) = 77.52%

Appendix 6 Useful Field of View® Data

Study 1 – measuring presentation time (ms)				Normoxia			Hypoxia		
Subject	Light level	Gender	Eyes	Processing Speed	Divided Attention	Selective Attention	Processing Speed	Divided Attention	Selective Attention
A	Direct View	Male	2	16.67	16.67	23.33	16.67	16.67	26.67
B				16.67	16.67	50.00	16.67	16.67	33.33
C				16.67	16.67	36.67	16.67	16.67	56.67
D				16.67	16.67	80.00	16.67	16.67	86.67
E				16.67	16.67	83.33	16.67	16.67	113.33
F				16.67	16.67	60.00	16.67	16.67	46.67
G		Female		16.67	16.67	70.00	16.67	16.67	76.67
H				16.67	16.67	40.00	16.67	16.67	36.67
I				16.67	16.67	106.67	16.67	16.67	86.67
J				16.67	16.67	30.00	16.67	16.67	46.67
K				16.67	16.67	63.33	16.67	16.67	86.67
L				16.67	16.67	60.00	16.67	16.67	56.67
A	ND 1.0	Male	2	16.67	16.67	20.00	16.67	16.67	46.67
B				16.67	16.67	16.67	16.67	16.67	33.33
C				16.67	16.67	20.00	16.67	16.67	140.00
D				16.67	16.67	66.67	20.00	20.00	40.00
E				16.67	16.67	46.67	16.67	16.67	86.67
F				16.67	16.67	66.67	16.67	16.67	50.00
G		Female		16.67	16.67	90.00	16.67	16.67	40.00
H				16.67	16.67	36.67	16.67	16.67	40.00
I				16.67	16.67	160.00	16.67	16.67	56.67
J				16.67	16.67	23.33	16.67	16.67	36.67
K				16.67	16.67	16.67	16.67	16.67	40.00
L				16.67	16.67	63.33	16.67	16.67	16.67
A	ND 1.0	Male	1	16.67	16.67	33.33	16.67	16.67	16.67
B				16.67	16.67	36.67	16.67	16.67	23.33
C				16.67	16.67	16.67	16.67	16.67	100.00
D				16.67	16.67	36.67	16.67	20.00	63.33
E				16.67	16.67	153.33	16.67	16.67	110.00
F				16.67	16.67	43.33	16.67	23.33	110.00
G		Female		16.67	16.67	76.67	16.67	16.67	123.33
H				16.67	16.67	20.00	16.67	16.67	23.33
I				16.67	16.67	60.00	16.67	16.67	153.33
J				16.67	16.67	36.67	16.67	16.67	30.00
K				16.67	16.67	36.67	16.67	16.67	26.67
L				16.67	36.67	56.67	16.67	16.67	50.00
A	ND 2.0	Male	2	23.33	23.33	36.67	16.67	16.67	30.00
B				16.67	16.67	26.67	23.33	23.33	93.33
C				20.00	23.33	90.00	16.67	23.33	53.33
D				16.67	36.67	113.33	16.67	33.33	153.33
E				16.67	20.00	133.33	16.67	16.67	213.33
F				16.67	16.67	63.33	16.67	16.67	86.67
G		Female		16.67	23.33	213.33	16.67	16.67	133.33
H				16.67	16.67	30.00	20.00	20.00	40.00
I				16.67	16.67	163.33	16.67	16.67	90.00
J				16.67	16.67	26.67	16.67	16.67	70.00
K				16.67	23.33	66.67	16.67	60.00	110.00
M				16.67	16.67	23.33	23.33	23.33	86.67
A	ND 2.0	Male	1	16.67	20.00	33.33	16.67	20.00	43.33
B				16.67	16.67	66.67	16.67	23.33	36.67
C				16.67	16.67	133.33	16.67	16.67	16.67
D				16.67	23.33	53.33	33.33	33.33	110.00
E				23.33	26.67	200.00	23.33	26.67	303.33
F				16.67	23.33	23.33	20.00	23.33	73.33

G				16.67	16.67	100.00	16.67	16.67	190.00
H				33.33	33.33	63.33	26.67	26.67	43.33
I		Female		16.67	16.67	153.33	23.33	33.33	200.00
J				16.67	16.67	63.33	26.67	26.67	50.00
K				16.67	30.00	30.00	53.33	53.33	83.33
M				16.67	20.00	116.67	23.33	30.00	50.00

Study 2 – measuring presentation time (ms)				Hyperoxia			Hypoxia		
Subject	**Light level**	**Gender**	**Eyes**	**Processing Speed**	**Divided Attention**	**Selective Attention**	**Processing Speed**	**Divided Attention**	**Selective Attention**
A	ND 1.0	Male	2	16.67	16.67	16.67	16.67	16.67	16.67
B				16.67	16.67	16.67	16.67	16.67	16.67
C				16.67	16.67	40.00	16.67	16.67	26.67
D				16.67	16.67	16.67	16.67	20.00	23.33
E				16.67	16.67	16.67	16.67	16.67	50.00
F				16.67	16.67	26.67	16.67	16.67	20.00
H		Female		16.67	16.67	16.67	16.67	16.67	16.67
I				16.67	16.67	63.33	16.67	16.67	63.33
J				16.67	16.67	16.67	16.67	16.67	26.67
K				16.67	16.67	30.00	16.67	16.67	16.67
L				n/a	n/a	n/a	n/a	n/a	n/a
M				16.67	16.67	43.33	16.67	16.67	26.67
A	ND 1.0	Male	1	16.67	16.67	16.67	16.67	16.67	16.67
B				16.67	16.67	43.33	16.67	16.67	26.67
C				16.67	16.67	16.67	16.67	16.67	36.67
D				16.67	16.67	53.33	16.67	16.67	20.00
E				16.67	16.67	53.33	20.00	56.67	76.67
F				16.67	16.67	53.33	16.67	16.67	23.33
H		Female		16.67	16.67	16.67	16.67	16.67	26.67
I				16.67	16.67	36.67	16.67	16.67	73.33
J				16.67	16.67	26.67	16.67	16.67	26.67
K				16.67	16.67	60.00	16.67	23.33	43.33
L				n/a	n/a	n/a	n/a	n/a	n/a
M				16.67	16.67	36.67	16.67	16.67	66.67
A	ND 2.0	Male	2	16.67	23.33	23.33	16.67	23.33	26.67
B				16.67	16.67	23.33	16.67	20.00	20.00
C				16.67	16.67	66.67	23.33	23.33	113.33
D				16.67	16.67	103.33	16.67	53.33	176.67
E				16.67	20.00	83.33	20.00	43.33	256.67
F				16.67	20.00	36.67	16.67	16.67	36.67
G		Female		16.67	20.00	50.00	16.67	16.67	123.33
H				20.00	20.00	30.00	16.67	16.67	50.00
I				16.67	16.67	26.67	20.00	20.00	63.33
J				16.67	16.67	36.67	16.67	20.00	33.33
K				16.67	16.67	16.67	16.67	16.67	66.67
M				16.67	16.67	23.33	16.67	23.33	23.33
A	ND 2.0	Male	1	16.67	16.67	23.33	16.67	16.67	20.00
B				16.67	16.67	43.33	16.67	23.33	46.67
C				16.67	16.67	16.67	16.67	16.67	73.33
D				30.00	30.00	66.67	23.33	23.33	56.67
E				16.67	26.67	196.67	16.67	90.00	156.67
F				16.67	23.33	50.00	16.67	16.67	73.33
G		Female		16.67	23.33	103.33	16.67	23.33	133.33
H				16.67	16.67	23.33	23.33	23.33	63.33
I				16.67	20.00	156.67	20.00	163.33	220.00
J				16.67	23.33	36.67	16.67	30.00	53.33
K				20.00	23.33	43.33	16.67	46.67	53.33
M				16.67	16.67	16.67	23.33	33.33	63.33

Appendix 7 FDT Sensitivity Data and ANOVA

M = male; F = female A = air; O = 100% O_2; H = hypoxia (14.1% O_2 in N_2)

Direct viewing (100 cd m^{-2}) – measuring contrast threshold (dB)

Subject	Order	Gas	FDT test area																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
M1	AHO	A	29	28	28	27	27	28	27	27	25	27	30	31	27	26	26	29	27
		O	23	28	31	27	24	25	27	27	28	27	27	24	27	23	29	27	29
		H	23	28	28	27	24	28	27	27	25	27	27	28	27	22	26	29	24
	Hypocapnia		22	31	29	30	24	31	27	30	28	27	30	31	27	23	26	26	30
M2	HOA	A	24	28	25	23	30	31	27	24	25	29	31	28	24	26	22	27	21
		O	26	28	31	23	27	31	27	26	28	24	24	25	24	20	24	27	26
		H	31	25	28	22	27	29	24	26	31	26	27	24	22	26	23	23	21
	Hypocapnia		26	32	23	22	30	26	30	30	35	29	27	25	24	26	23	23	21
M3	OAH	A	23	23	24	20	26	25	29	27	27	19	29	27	29	17	23	24	19
		O	23	25	24	20	26	31	30	23	31	22	24	23	21	20	19	24	18
		H	22	25	20	20	29	25	22	27	28	22	23	28	26	20	26	24	20
	Hypocapnia		18	28	24	26	21	31	30	27	28	19	23	29	24	16	23	23	16
M4	AOH	A	29	30	30	26	27	27	27	27	30	29	30	31	30	27	29	26	30
		O	29	30	27	26	27	30	30	27	30	26	27	31	27	29	29	29	27
		H	26	30	30	29	30	30	30	27	30	29	30	27	27	26	31	29	27
	Hypocapnia		43	30	30	31	27	30	27	24	32	29	27	31	30	31	32	31	27
M5	OHA	A	29	32	35	29	33	33	31	32	30	31	30	30	30	29	23	26	29
		O	27	36	24	24	33	32	32	24	27	29	24	24	30	23	27	26	27
		H	24	27	30	24	27	24	32	29	24	29	24	27	23	9	23	24	27
	Hypocapnia		23	35	27	26	23	35	27	35	30	31	29	29	22	23	29	20	23
M6	HAO	A	27	30	27	29	27	29	29	30	30	26	30	30	24	26	29	23	27
		O	29	30	30	27	30	33	35	30	30	26	35	30	30	26	29	26	30
		H	23	30	24	23	27	30	24	27	35	26	27	24	24	26	26	24	19
	Hypocapnia		31	33	35	26	30	36	35	27	32	29	33	36	30	29	29	32	27
F1	OAH	A	23	23	23	23	24	24	30	23	30	29	27	28	30	29	29	26	27
		O	26	30	27	29	24	29	30	27	35	26	29	31	24	29	29	29	27
		H	23	35	30	23	24	29	24	23	30	29	27	31	27	31	31	29	27
	Hypocapnia		24	24	29	26	30	30	30	25	32	29	31	31	27	26	24	24	31
F2	AOH	A	29	25	30	20	27	31	30	27	28	27	30	31	27	31	29	26	24
		O	27	31	35	26	27	31	27	30	28	27	31	29	30	24	29	29	27
		H	26	31	27	27	23	31	27	30	31	27	29	31	27	26	26	26	24
	Hypocapnia		26	29	30	26	23	31	30	27	31	23	30	28	30	22	29	22	24
F3	HAO	A	23	25	28	23	27	31	27	24	31	27	23	31	22	29	26	30	24
		O	23	31	31	22	27	31	27	24	31	27	30	31	24	24	23	30	24
		H	26	23	28	18	17	25	24	21	31	22	29	28	24	26	31	23	21
	Hypocapnia		26	28	29	22	27	31	30	21	31	27	30	25	32	22	26	30	21
F4	OHA	A	23	28	25	27	30	28	24	29	31	27	29	25	27	29	29	27	30
		O	26	25	31	27	27	31	27	27	31	41	23	28	27	23	29	29	30
		H	31	31	28	22	30	25	27	30	31	22	24	24	23	22	29	27	30
	Hypocapnia		23	31	35	22	27	25	24	30	25	27	23	30	27	23	26	23	27
F5	AHO	A	29	23	23	23	24	25	27	20	27	22	24	31	23	24	23	16	24
		O	24	31	28	19	23	25	30	24	23	23	31	28	27	29	22	23	27
		H	23	25	23	23	16	21	23	20	20	22	23	25	24	23	23	20	12
	Hypocapnia		22	27	25	20	27	29	23	21	31	23	32	24	27	13	23	23	21
F6	HOA	A	19	31	28	22	24	28	28	26	31	29	25	28	27	23	27	27	27
		O	23	28	28	23	30	31	25	27	28	30	31	28	30	27	30	30	24
		H	23	30	23	23	23	25	23	21	25	24	21	22	20	16	8	23	21
	Hypocapnia		31	30	31	30	27	22	27	30	28	27	25	35	30	27	27	23	30

Viewing through the ND 1.0 filter (~10 cd m^{-2}) – measuring contrast threshold (dB)

Subject	Order	Gas	FDT test area																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
M1	AOH	A	14	19	21	17	16	20	20	21	21	19	20	21	16	18	19	20	20
		O	16	20	21	17	19	20	20	21	22	23	23	24	20	16	23	26	26
		H	12	15	21	17	16	17	20	21	20	12	17	19	20	16	18	22	21
	Hypocapnia		12	21	21	17	20	21	24	21	25	17	20	25	24	18	18	22	26
M2	HAO	A	16	20	19	16	23	23	24	12	19	16	22	20	19	22	15	16	16
		O	19	24	18	12	22	21	24	17	22	15	24	19	19	16	16	16	14
		H	16	22	18	16	17	21	20	12	21	16	22	21	13	16	14	17	14
	Hypocapnia		20	22	22	19	24	23	22	21	25	21	24	24	20	22	23	22	19
M3	OAH	A	10	19	19	12	16	19	20	17	16	16	25	23	19	10	12	12	10
		O	11	16	14	12	16	19	14	11	17	16	19	19	13	12	10	15	11
		H	6	19	19	14	11	19	12	17	18	16	16	19	17	11	12	12	9
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M4	AHO	A	16	23	22	20	18	20	21	19	20	16	29	22	26	17	23	23	20
		O	20	20	22	20	24	20	21	23	23	20	21	21	21	23	23	23	20
		H	16	20	20	24	21	24	24	19	20	16	26	21	32	26	24	23	22
	Hypocapnia		26	27	20	20	21	27	24	20	23	23	24	25	27	24	24	23	23
M5	HOA	A	20	24	20	20	24	22	26	20	24	23	21	17	19	12	17	12	16
		O	23	23	24	20	27	29	27	29	20	23	20	24	20	11	16	20	12
		H	20	27	16	17	27	20	24	17	19	22	20	19	19	11	7	7	12
	Hypocapnia		23	23	24	17	22	23	26	23	24	22	20	20	19	17	16	20	13
M6	OHA	A	17	20	8	12	18	12	24	20	24	16	26	27	21	16	20	18	19
		O	20	22	21	16	20	24	24	20	22	19	26	24	21	20	23	23	20
		H	20	23	21	16	21	24	24	19	22	19	26	22	21	20	24	18	14
	Hypocapnia		18	22	26	12	21	24	21	20	23	19	24	24	24	15	24	20	19
F1	OHA	A	16	20	19	17	18	20	24	19	24	19	21	22	20	20	23	24	19
		O	16	23	20	20	20	20	24	19	22	19	21	22	26	20	23	23	24
		H	11	19	20	16	20	20	21	19	22	19	21	24	21	20	24	26	19
	Hypocapnia		16	20	20	20	19	20	26	20	24	19	21	25	21	18	24	23	20
F2	HAO	A	18	22	22	23	12	22	22	21	23	16	22	25	22	19	19	20	21
		O	19	22	27	19	20	21	24	24	25	17	24	25	27	19	20	20	24
		H	23	22	22	20	17	22	22	21	25	20	24	25	24	20	23	23	24
	Hypocapnia		20	21	24	20	15	18	23	20	25	12	22	25	20	22	16	20	21
F3	HOA	A	26	25	19	14	20	24	20	16	22	19	29	21	15	20	23	27	20
		O	20	24	20	12	21	27	19	15	20	19	26	23	16	24	24	20	17
		H	17	21	16	13	23	25	20	22	23	23	20	19	14	22	20	19	17
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F4	OAH	A	13	19	19	18	19	21	20	19	19	19	20	21	20	16	19	22	18
		O	16	20	21	13	23	20	16	18	21	19	22	24	16	18	20	20	16
		H	11	16	20	12	16	18	20	19	19	18	20	19	20	14	17	20	19
	Hypocapnia		8	17	17	11	19	20	20	18	18	12	19	21	19	16	18	22	13
F5	AHO	A	19	21	18	15	12	25	22	15	21	16	24	11	16	19	7	17	12
		O	22	21	21	17	20	27	24	26	21	27	27	25	20	20	22	20	21
		H	19	19	21	17	17	21	29	17	25	18	25	21	17	22	12	15	19
	Hypocapnia		16	23	21	19	15	25	23	10	22	20	24	21	24	23	19	20	21
F6	AOH	A	19	21	17	20	16	24	22	19	20	19	23	22	20	16	12	19	21
		O	17	21	21	19	20	21	25	24	21	21	22	28	20	20	23	24	26
		H	19	25	23	22	22	21	25	24	25	17	23	22	22	19	22	19	21
	Hypocapnia		31	30	31	30	27	22	27	30	28	27	25	35	30	27	27	23	30

Viewing through the ND 2.0 filter (~1 cd m^{-2}) – measuring contrast threshold (dB)

Subject	Order	Gas	FDT test area																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
M1	HOA	A	5	4	11	4	16	3	10	8	11	12	14	10	14	7	10	15	8
		O	7	3	11	2	3	11	14	11	13	8	8	12	7	8	7	15	8
		H	3	4	8	7	13	10	8	13	11	8	13	14	12	3	6	12	16
	Hypocapnia		2	5	8	2	3	11	8	7	10	8	10	14	8	0	11	12	6
M2	AOH	A	2	8	2	1	1	2	0	1	2	4	9	5	5	0	1	3	5
		O	10	11	6	2	11	11	8	5	11	8	12	12	9	3	7	7	4
		H	3	9	6	0	3	10	8	5	8	4	11	4	0	3	10	3	5
	Hypocapnia		1	6	12	3	6	14	8	6	17	11	11	4	0	5	1	5	2
M3	AHO	A	2	11	8	3	5	14	11	11	8	7	8	10	6	1	0	3	4
		O	2	4	4	0	8	6	6	10	3	4	6	8	8	0	5	2	0
		H	0	4	6	0	6	4	8	15	8	2	4	5	2	0	4	3	0
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
M4	OAH	A	9	15	8	12	11	14	14	11	14	16	11	12	11	8	12	10	7
		O	10	14	13	12	11	5	8	6	8	8	10	16	7	12	13	7	14
		H	7	13	8	7	8	13	13	14	14	8	11	15	9	12	10	12	10
	Hypocapnia		12	12	9	6	14	12	8	9	11	7	12	6	11	8	10	7	11
M5	HAO	A	8	8	12	4	15	13	10	5	6	8	12	9	7	5	2	5	0
		O	12	14	14	4	13	14	14	11	8	16	11	10	11	3	8	12	3
		H	3	6	8	0	10	9	10	5	4	6	8	2	1	0	3	3	1
	Hypocapnia		8	14	8	7	17	17	13	11	11	12	17	14	11	10	11	3	6
M6	OHA	A	3	8	10	2	5	12	4	3	8	8	13	8	0	0	5	5	3
		O	11	12	13	8	13	16	12	12	13	8	13	14	10	12	14	11	15
		H	7	4	8	2	8	8	8	7	10	4	8	10	2	2	3	6	0
	Hypocapnia		10	13	9	4	9	12	12	9	14	11	11	13	8	2	11	7	5
F1	AOH	A	3	8	9	5	7	10	9	6	8	9	11	12	12	6	10	8	7
		O	4	7	8	7	6	8	10	7	8	8	11	11	8	7	9	12	10
		H	1	4	8	7	2	8	8	7	7	10	8	8	6	7	6	6	8
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F2	OAH	A	4	8	10	4	4	11	12	8	18	6	10	9	8	3	3	8	7
		O	8	10	8	6	7	14	9	8	11	14	11	14	11	11	7	8	9
		H	7	8	9	5	6	17	6	9	9	5	11	12	12	7	9	8	10
	Hypocapnia		6	10	9	4	2	9	4	7	6	4	7	11	6	3	15	6	9
F3	HOA	A	12	11	14	7	13	13	14	8	11	13	13	19	14	7	10	7	13
		O	10	14	8	12	8	12	8	13	14	16	14	19	8	9	10	10	9
		H	3	8	14	0	16	12	8	4	11	22	15	12	6	8	14	11	5
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F4	HAO	A	4	4	2	3	5	6	1	2	4	6	11	7	6	2	2	1	8
		O	7	9	6	3	6	4	8	4	5	6	8	11	4	4	4	4	8
		H	3	4	6	3	9	4	2	4	2	3	5	4	4	3	5	3	8
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F5	AHO	A	1	5	4	0	0	11	0	2	3	3	5	1	8	2	0	0	4
		O	11	11	8	1	8	12	8	8	14	5	14	11	4	3	7	7	8
		H	1	4	0	0	1	6	0	0	0	2	1	0	0	0	0	3	1
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
F6	OHA	A	15	11	14	11	10	15	18	13	14	3	9	14	12	15	12	12	12
		O	6	16	18	6	13	11	8	8	10	11	9	12	13	6	13	13	14
		H	10	8	6	14	10	13	9	12	4	9	10	12	13	3	10	13	14
	Hypocapnia		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Balanced ANOVA for retinal eccentricity (main effects α = 0.05; interactions α = 0.01)

Factor	Type	Levels	Values
Gender	fixed	2	F, M
Respiratory exposure order	fixed	3	H-O, O-H, x-A-x
Gas	fixed	3	A, H, O
Eccentricity	fixed	3	Centre, Inner, Outer

Direct viewing

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	0.988	0.988	0.23	0.636
Order	2	122.765	61.382	14.05	**0.000**
Gas	2	53.707	26.854	6.15	**0.004**
Eccentricity	2	143.728	71.864	16.45	**0.000**
Gender*Order	2	26.251	13.125	3.01	0.058
Gender*Gas	2	9.111	4.556	1.04	0.359
Gender*Eccentricity	2	5.698	2.849	0.65	0.525
Order*Gas	4	22.787	5.697	1.30	0.280
Order*Eccentricity	4	67.181	16.795	3.85	**0.008**
Gas*Eccentricity	4	12.835	3.209	0.73	0.572
Gender*Order*Gas	4	41.934	10.484	2.40	0.061
Gender*Order*Eccentricity	4	6.215	1.554	0.36	0.839
Gender*Gas*Eccentricity	4	13.756	3.439	0.79	0.538
Order*Gas*Eccentricity	8	10.404	1.300	0.30	0.964
Gender*Order*Gas*Eccentricity	8	39.228	4.903	1.12	0.363
Error	54	235.845	4.368		
Total	107	812.434			

S = 2.08986 R-Sq = 70.97% R-Sq(adj) = 42.48%

ND 1.0 filter

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	43.536	43.536	7.80	**0.007**
Order	2	12.673	6.336	1.13	0.329
Gas	2	15.304	7.652	1.37	0.263
Eccentricity	2	202.024	101.012	18.09	**0.000**
Gender*Order	2	29.238	14.619	2.62	0.082
Gender*Gas	2	9.453	4.727	0.85	0.435
Gender*Eccentricity	2	2.245	1.122	0.20	0.819
Order*Gas	4	18.713	4.678	0.84	0.507
Order*Eccentricity	4	7.424	1.856	0.33	0.855
Gas*Eccentricity	4	12.176	3.044	0.55	0.703
Gender*Order*Gas	4	14.061	3.515	0.63	0.644
Gender*Order*Eccentricity	4	14.154	3.539	0.63	0.641
Gender*Gas*Eccentricity	4	5.992	1.498	0.27	0.897
Order*Gas*Eccentricity	8	11.985	1.498	0.27	0.974
Gender*Order*Gas*Eccentricity	8	11.593	1.449	0.26	0.976
Error	54	301.563	5.584		
Total	107	712.134			

S = 2.36315 R-Sq = 57.65% R-Sq(adj) = 16.09%

ND 2.0 filter

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	0.03	0.03	0.00	0.966
Order	2	9.14	4.57	0.28	0.756
Gas	2	100.75	50.37	3.10	0.053
Eccentricity	2	117.02	58.51	3.61	**0.034**
Gender*Order	2	84.89	42.44	2.62	0.082
Gender*Gas	2	22.83	11.41	0.70	0.499
Gender*Eccentricity	2	3.15	1.58	0.10	0.908
Order*Gas	4	33.50	8.38	0.52	0.724
Order*Eccentricity	4	5.22	1.30	0.08	0.988
Gas*Eccentricity	4	3.52	0.88	0.05	0.994
Gender*Order*Gas	4	33.68	8.42	0.52	0.722
Gender*Order*Eccentricity	4	15.37	3.84	0.24	0.916
Gender*Gas*Eccentricity	4	30.09	7.52	0.46	0.762
Order*Gas*Eccentricity	8	52.43	6.55	0.40	0.914
Gender*Order*Gas*Eccentricity	8	15.26	1.91	0.12	0.998
Error	54	876.30	16.23		
Total	107	1403.18			

S = 4.02837 R-Sq = 37.55% R-Sq(adj) = 0.00%

Balanced ANOVA for field quadrant (main effects $\alpha = 0.05$; interactions $\alpha = 0.01$)

Factor	Type	Levels	Values
Gender	fixed	2	F, M
Respiratory exposure order	fixed	3	H-O, O-H, x-A-x
Gas	fixed	3	A, H, O
Field quadrant	fixed	4	SNQ, STQ, INQ, ITQ

Direct viewing

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	7.335	7.335	1.59	0.211
Order	2	152.777	76.388	16.59	**0.000**
Gas	2	99.641	49.821	10.82	**0.000**
Quadrant	3	22.113	7.371	1.60	0.197
Gender*Order	2	44.115	22.058	4.79	0.011
Gender*Gas	2	23.266	11.633	2.53	0.087
Gender*Quadrant	3	28.613	9.538	2.07	0.111
Order*Gas	4	15.869	3.967	0.86	0.491
Order*Quadrant	6	7.036	1.173	0.25	0.956
Gas*Quadrant	6	8.984	1.497	0.33	0.922
Gender*Order*Gas	4	38.056	9.514	2.07	0.094
Gender*Order*Quadrant	6	12.968	2.161	0.47	0.829
Gender*Gas*Quadrant	6	4.713	0.785	0.17	0.984
Order*Gas*Quadrant	12	37.548	3.129	0.68	0.765
Gender*Order*Gas*Quadrant	12	28.777	2.398	0.52	0.894
Error	72	331.438	4.603		
Total	143	863.248			

S = 2.14553 R-Sq = 61.61% R-Sq(adj) = 23.74%

ND 1.0 filter

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	56.563	56.563	8.31	**0.005**
Order	2	129.860	64.930	9.54	**0.000**
Gas	2	54.782	27.391	4.02	**0.022**
Quadrant	3	3.901	1.300	0.19	0.902
Gender*Order	2	102.386	51.193	7.52	**0.001**
Gender*Gas	2	5.876	2.938	0.43	0.651
Gender*Quadrant	3	11.269	3.756	0.55	0.649
Order*Gas	4	28.804	7.201	1.06	0.384
Order*Quadrant	6	87.004	14.501	2.13	0.060
Gas*Quadrant	6	4.343	0.724	0.11	0.995
Gender*Order*Gas	4	8.148	2.037	0.30	0.878
Gender*Order*Quadrant	6	52.194	8.699	1.28	0.279
Gender*Gas*Quadrant	6	7.860	1.310	0.19	0.978
Order*Gas*Quadrant	12	12.217	1.018	0.15	1.000
Gender*Order*Gas*Quadrant	12	16.470	1.373	0.20	0.998
Error	72	490.281	6.809		
Total	143	1071.958			

S = 2.60949 R-Sq = 54.26% R-Sq(adj) = 9.16%

ND 2.0 filter

Source	**DF**	**SS**	**MS**	**F**	**P**
Gender	1	2.92	2.92	0.23	0.632
Order	2	17.40	8.70	0.69	0.504
Gas	2	132.01	66.01	5.24	**0.007**
Quadrant	3	17.47	5.82	0.46	0.709
Gender*Order	2	174.38	87.19	6.93	**0.002**
Gender*Gas	2	1.44	0.72	0.06	0.944
Gender*Quadrant	3	17.49	5.83	0.46	0.709
Order*Gas	4	20.20	5.05	0.40	0.807
Order*Quadrant	6	7.52	1.25	0.10	0.996
Gas*Quadrant	6	4.83	0.80	0.06	0.999
Gender*Order*Gas	4	133.30	33.33	2.65	0.040
Gender*Order*Quadrant	6	71.37	11.90	0.94	0.469
Gender*Gas*Quadrant	6	8.68	1.45	0.11	0.994
Order*Gas*Quadrant	12	8.35	0.70	0.06	1.000
Gender*Order*Gas*Quadrant	12	15.81	1.32	0.10	1.000
Error	72	906.44	12.59		
Total	143	1539.62			

S = 3.54816 R-Sq = 41.13% R-Sq(adj) = 0.00%

Appendix 8 Contrast Acuity Data and ANOVA

Summary Data Set

Measuring contrast (%)

Subject	Light level	Gender	F_IO_2	Exposure order	Retinal eccentricity (degrees of visual angle)						
					-5	-2.5	-1.25	0	+1.25	+2.5	+5
A	12 cd m^{-2}	Male	21%	OHA	7.1	11.5	8.6	7.6	7.6	12.1	9.3
B				HAO	13.2	14.5	13.4	11.4	8.8	17.3	12.3
C				AOH	8.1	12.8	12.6	9.5	8.2	14.8	10.3
D				OAH	11.6	12.7	16.3	15.4	9.6	10.5	11.1
E				HOA	10.4	12.3	16.3	12.4	12.4	16.2	10.4
F				AHO	7.1	7.3	4.1	6.4	3.9	6.1	4.3
G		Female		HOA	8.7	6.4	8.1	6.6	8.0	9.0	8.6
H				OAH	13.3	9.5	13.5	9.4	10.7	19.1	16.5
I				AOH	10.6	10.5	7.7	10.2	6.0	9.4	20.5
J				HAO	7.7	7.7	6.9	8.8	6.6	9.2	11.3
K				OHA	6.5	9.3	7.2	7.5	6.8	7.9	6.6
L				AHO	8.4	9.6	7.5	10.7	7.9	11.2	11.5
A	12 cd m^{-2}	Male	14.1%	OHA	7.4	9.6	13.6	11.0	10.2	24.7	16.2
B				HAO	10.5	10.8	14.4	15.1	16.6	13.6	11.8
C				AOH	9.5	11.0	8.5	8.5	7.3	10.5	6.9
D				OAH	15.6	10.4	13.4	12.9	17.4	8.7	10.4
E				HOA	9.7	8.5	8.5	11.0	11.9	11.8	12.3
F				AHO	5.4	6.5	6.3	7.2	4.7	6.2	6.7
G		Female		HOA	9.9	9.5	7.8	13.0	7.4	8.6	10.8
H				OAH	37.0	16.0	28.5	24.0	29.7	41.0	40.7
I				AOH	9.5	6.8	9.3	9.8	12.3	14.0	11.1
J				HAO	7.2	9.2	8.3	11.4	7.7	8.0	10.7
K				OHA	6.7	12.5	6.7	6.6	5.8	9.5	7.0
L				AHO	10.9	16.0	11.0	15.1	13.8	11.6	6.7
A	12 cd m^{-2}	Male	100%	OHA	9.5	11.1	8.7	7.7	9.0	11.6	10.9
B				HAO	12.4	10.1	11.2	7.8	11.5	12.4	11.5
C				AOH	11.5	8.6	9.4	9.3	8.4	8.6	8.2
D				OAH	9.1	12.6	12.9	15.6	7.7	10.7	8.4
E				HOA	7.4	9.1	10.4	8.9	11.1	8.0	9.2
F				AHO	6.3	3.7	7.7	6.9	6.6	7.8	5.2
G		Female		HOA	8.9	6.8	6.6	7.7	5.4	8.7	7.1
H				OAH	14.7	20.8	13.9	16.0	18.8	23.5	19.2
I				AOH	7.1	6.5	8.3	9.5	10.0	8.7	10.2
J				HAO	8.2	9.2	9.8	8.6	7.8	6.7	7.4
K				OHA	8.3	6.1	7.1	7.8	7.3	8.3	6.0
L				AHO	6.8	8.2	8.2	8.2	7.2	13.3	10.3
A	1 cd m^{-2}	Male	21%	OHA	39.4	26.0	29.5	46.9	30.2	36.2	15.0
B				HAO	26.5	35.5	26.0	29.1	25.1	37.1	34.1
C				AOH	36.7	30.2	21.5	39.5	27.8	31.1	19.8
D				OAH	36.7	22.6	41.7	69.3	52.1	31.5	41.6
E				HOA	21.6	28.3	28.0	48.0	22.2	24.9	28.1
F				AHO	11.0	12.7	10.9	12.8	11.2	13.1	11.7
G		Female		HOA	13.7	19.2	12.6	17.1	15.7	19.0	19.3
H				OAH	67.1	33.8	38.2	33.0	31.5	41.3	31.6
I				AOH	22.6	27.8	25.4	31.1	15.6	26.7	13.2
J				HAO	14.5	22.7	22.1	34.3	26.1	20.4	13.2
K				OHA	15.8	22.4	16.7	20.3	12.3	18.0	15.6
M				AHO	19.2	20.1	26.2	36.8	29.2	33.9	33.7

Subject	Light level	Gender	F_IO_2	Exposure order	Retinal eccentricity (degrees of visual angle)						
					-5	-2.5	-1.25	0	+1.25	+2.5	+5
A	1 cd m^{-2}	Male	14.1%	OHA	27.5	40.6	20.1	28.3	28.5	31.3	21.2
B				HAO	45.0	50.4	47.9	56.3	42.4	37.1	38.6
C				AOH	40.0	17.8	26.7	39.4	25.3	25.5	34.7
D				OAH	28.5	35.4	39.2	68.3	40.2	53.5	17.7
E				HOA	20.2	42.7	28.2	52.6	26.9	31.5	52.0
F				AHO	14.2	19.4	13.2	12.5	8.6	15.9	13.8
G		Female		HOA	17.9	23.0	21.1	23.1	23.1	12.1	20.4
H				OAH	60.6	69.6	50.9	90.9	86.8	163.1	92.0
I				AOH	20.8	23.3	15.8	21.0	33.8	28.7	25.3
J				HAO	20.0	17.7	15.3	26.4	14.2	24.7	23.5
K				OHA	22.7	22.7	20.7	22.7	22.5	20.2	16.8
L				AHO	19.6	30.8	26.9	30.7	23.1	26.5	19.3
A	1 cd m^{-2}	Male	100%	OHA	18.5	25.1	19.0	21.9	24.4	30.5	14.8
B				HAO	24.7	35.9	47.6	39.2	24.8	28.0	25.5
C				AOH	20.2	25.2	26.0	28.9	19.5	32.6	20.4
D				OAH	30.8	38.3	60.3	58.1	43.6	34.6	31.5
E				HOA	14.7	20.3	20.7	42.0	28.3	14.9	19.8
F				AHO	10.6	13.7	9.4	20.2	12.8	15.0	9.2
G		Female		HOA	15.9	17.8	10.3	16.7	14.1	14.9	13.8
H				OAH	42.8	35.0	35.2	41.2	42.2	25.4	33.5
I				AOH	18.9	21.2	16.9	20.1	25.5	28.8	25.2
J				HAO	23.6	26.6	28.3	43.9	29.9	16.4	16.1
K				OHA	13.9	21.6	20.7	20.9	23.6	22.7	13.2
L				AHO	19.2	21.0	23.6	24.1	25.7	32.3	18.9
A	0.1 cd m^{-2}	Male	21%	OHA	19.9	19.6	26.1	21.5	23.4	35.3	25.0
B				HAO	20.9	29.9	33.4	32.0	22.6	20.0	19.3
C				AOH	35.4	35.6	20.5	36.5	24.4	17.2	25.2
D				OAH	30.6	19.5	27.9	43.3	29.1	23.0	22.2
E				HOA	29.6	20.4	17.7	21.3	24.7	23.3	20.7
F				AHO	19.2	19.1	18.1	20.3	15.0	20.5	12.9
G		Female		HOA	21.7	15.9	21.7	20.9	21.7	12.3	14.9
H				OAH	50.2	47.4	48.7	38.5	49.7	41.3	55.7
I				AOH	21.9	22.1	19.2	20.3	21.8	18.9	20.1
J				HAO	18.5	17.6	15.9	21.6	23.4	14.4	19.7
K				OHA	16.7	17.0	20.6	17.9	20.7	17.2	17.2
L				AHO	20.6	20.4	29.9	20.5	17.9	20.4	29.2
A	0.1 cd m^{-2}	Male	14.1%	OHA	24.5	20.5	30.8	37.4	30.4	29.7	28.2
B				HAO	25.5	42.3	30.8	48.9	39.8	32.9	35.6
C				AOH	26.9	26.6	32.4	41.3	30.6	35.9	33.1
D				OAH	30.2	27.8	25.5	37.2	21.1	28.6	28.1
E				HOA	26.0	36.4	25.3	25.5	27.6	27.1	24.8
F				AHO	13.2	14.3	18.7	17.9	14.4	20.9	17.6
G		Female		HOA	30.5	35.2	32.5	33.1	21.7	21.4	17.2
H				OAH	48.9	27.0	34.6	26.2	46.9	36.5	42.0
I				AOH	28.7	21.0	17.6	19.0	18.0	24.3	20.8
J				HAO	23.9	31.4	26.9	33.8	42.4	36.0	19.6
K				OHA	11.6	19.9	28.8	22.1	22.0	18.2	15.0
L				AHO	21.8	35.8	25.0	24.7	33.9	17.1	23.5
A	0.1 cd m^{-2}	Male	100%	OHA	23.9	19.6	20.1	21.1	15.8	31.8	22.7
B				HAO	27.4	35.3	28.3	23.8	27.4	17.0	22.6
C				AOH	16.7	20.5	21.6	30.8	21.4	26.4	23.1
D				OAH	21.0	19.6	20.7	20.3	38.0	17.1	22.9
E				HOA	26.1	11.3	18.1	19.1	20.2	22.6	28.9
F				AHO	14.9	9.9	17.2	13.0	13.7	11.8	13.4
G		Female		HOA	19.3	14.5	14.6	17.1	13.9	15.4	13.0
H				OAH	34.7	37.2	27.1	22.0	34.2	24.5	46.7
I				AOH	19.3	20.1	22.9	20.9	18.7	13.7	20.3
J				HAO	16.9	11.4	13.7	20.5	21.6	19.8	26.2
K				OHA	18.4	20.6	14.6	20.0	18.4	17.7	13.5
L				AHO	18.4	15.7	21.5	17.2	21.7	23.5	14.3

Balanced ANOVA (main effects α = 0.05; interactions α = 0.01)

Factor	Type	Levels	Values
Gender	fixed	2	F, M
Respiratory exposure order	fixed	3	H-O, O-H, x-A-x
Gas	fixed	3	A, H, O
Eccentricity	fixed	7	-5, -2.5, -1.25, 0, +1.25, +2.5, +5

Direct viewing

Source	DF	SS	MS	F	P
Gender	1	0.00021	0.00021	0.01	0.932
Order	2	1.26037	0.63018	22.12	**0.000**
Gas	2	0.24317	0.12158	4.27	**0.016**
Eccentricity	6	0.18446	0.03074	1.08	0.379
Gender*Order	2	0.17094	0.08547	3.00	0.053
Gender*Gas	2	0.09087	0.04543	1.59	0.207
Gender*Eccentricity	6	0.07070	0.01178	0.41	0.869
Order*Gas	4	0.06037	0.01509	0.53	0.714
Order*Eccentricity	12	0.07599	0.00633	0.22	0.997
Gas*Eccentricity	12	0.09767	0.00814	0.29	0.991
Gender*Order*Gas	4	0.09451	0.02363	0.83	0.509
Gender*Order*Eccentricity	12	0.07518	0.00626	0.22	0.997
Gender*Gas*Eccentricity	12	0.06974	0.00581	0.20	0.998
Order*Gas*Eccentricity	24	0.20239	0.00843	0.30	0.999
Gender*Order*Gas*Eccentricity	24	0.18326	0.00764	0.27	1.000
Error	126	3.59015	0.02849		
Total	251	6.46998			

S = 0.168800 R-Sq = 44.51% R-Sq(adj) = 0.00%

ND 1.0 filter

Source	DF	SS	MS	F	P
Gender	1	0.10943	0.10943	3.06	0.083
Order	2	2.71102	1.35551	37.92	**0.000**
Gas	2	0.32332	0.16166	4.52	**0.013**
Eccentricity	6	0.58019	0.09670	2.71	**0.017**
Gender*Order	2	0.25390	0.12695	3.55	0.032
Gender*Gas	2	0.02027	0.01013	0.28	0.754
Gender*Eccentricity	6	0.05574	0.00929	0.26	0.954
Order*Gas	4	0.08150	0.02038	0.57	0.685
Order*Eccentricity	12	0.19488	0.01624	0.45	0.937
Gas*Eccentricity	12	0.10124	0.00844	0.24	0.996
Gender*Order*Gas	4	0.08212	0.02053	0.57	0.682
Gender*Order*Eccentricity	12	0.08882	0.00740	0.21	0.998
Gender*Gas*Eccentricity	12	0.06241	0.00520	0.15	1.000
Order*Gas*Eccentricity	24	0.39725	0.01655	0.46	0.984
Gender*Order*Gas*Eccentricity	24	0.34513	0.01438	0.40	0.994
Error	126	4.50356	0.03574		
Total	251	9.91078			

S = 0.189057 R-Sq = 54.56% R-Sq(adj) = 9.48%

ND 2.0 filter

Source	DF	SS	MS	F	P
Gender	1	0.03111	0.03111	1.80	0.183
Order	2	0.91874	0.45937	26.52	**0.000**
Gas	2	0.64041	0.32020	18.49	**0.000**
Eccentricity	6	0.06479	0.01080	0.62	0.711
Gender*Order	2	0.34363	0.17181	9.92	**0.000**
Gender*Gas	2	0.00041	0.00021	0.01	0.988
Gender*Eccentricity	6	0.05947	0.00991	0.57	0.752
Order*Gas	4	0.03065	0.00766	0.44	0.778
Order*Eccentricity	12	0.10111	0.00843	0.49	0.920
Gas*Eccentricity	12	0.08684	0.00724	0.42	0.954
Gender*Order*Gas	4	0.07094	0.01774	1.02	0.398
Gender*Order*Eccentricity	12	0.16682	0.01390	0.80	0.647
Gender*Gas*Eccentricity	12	0.07865	0.00655	0.38	0.969
Order*Gas*Eccentricity	24	0.17945	0.00748	0.43	0.990
Gender*Order*Gas*Eccentricity	24	0.18959	0.00790	0.46	0.986
Error	126	2.18223	0.01732		
Total	251	5.14485			

S = 0.131603 R-Sq = 57.58% R-Sq(adj) = 15.50%

Appendix 9 Publications

Four peer-reviewed papers have been published that report work undertaken during the course of this thesis. They are reproduced here in order of publication.

Connolly, D. M., and Hosking, S. L. (2006). Aviation-related respiratory gas disturbances affect dark adaptation: A reappraisal. *Vision Research*, 46: 1784-1793.

Connolly, D., and Hosking, S. (2007). Quantitative correlation of hyperventilation and flicker sensitivity. *Optometry and Vision Science*, 84: 529-534.

Connolly, D. M., Barbur, J. L., Hosking, S. L., and Moorhead, I. R. (2008). Mild hypoxia impairs chromatic sensitivity in the mesopic range. *Investigative Ophthalmology and Vision Science*, 49: 820-827.

Connolly, D. M., and Hosking, S. L. (2008). Oxygenation and gender effects on photopic frequency-doubled contrast sensitivity. *Vision Research*, 48: 281-288.

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Aviation-related respiratory gas disturbances affect dark adaptation: A reappraisal

Desmond M. Connolly [a,b,*], Sarah L. Hosking [b]

[a] QinetiQ Centre for Human Sciences, Cody Technology Park, Ively Road, Farnborough, Hampshire GU14 0LX, UK
[b] Aston Academy of Life Sciences, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK

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Abstract

This study examined the time course of early scotopic threshold sensitivity during dark adaptation under mild to moderate hypoxia, moderate hypocapnia and hyperoxia, measuring detection time displacement relative to normoxia. Cone rod inflection and early rod adaptation were highlighted using progressively dimmer green flash stimuli. Early scotopic sensitivity was significantly delayed by hypoxia and hastened by hypocapnia and hyperoxia. Effects of respiratory disturbance on dark adaptation include temporal shifts of early scotopic sensitivity while human rod photoreceptors appear functionally hypoxic when breathing air at one atmosphere. At night, supplementary oxygen may benefit aircrew visual sensitivity, even at ground level.
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Keywords: Dark adaptation; Threshold sensitivity; Retina; Hypoxia; Hyperventilation

1. Introduction

Flying in darkness increases the likelihood of some types of accidents that may be related to a scarcity of external visual cues, even in good weather (Wilson, 1999). At night, aircraft cockpits are generally illuminated at mesopic ambient light levels and contain an increasing variety of sophisticated electro-optical displays that may challenge aircrew visual performance (Harris, 2004; Wickens, 2003). Monochrome displays demand optimal contrast discrimination while the use of colour is proliferating. Viewing a dim external scene, aircrew must adjust cockpit lighting and display luminance to minimum levels that maintain visibility while avoiding glare (Rash & Manning, 2003). Ambient and retinal illumination may fluctuate rapidly while displays may be head-down, head-up, head or helmet-mounted, monocular or binocular, or, in the future, projected directly onto the retina (Melzer & Moffitt, 1997; Wickens, Ververs, & Fadden, 2004).

This paper concerns human visual sensitivity during aviation at night under conditions of respiratory disturbance. The fractional oxygen concentration of atmospheric air may be regarded as constant at 21% by volume while atmospheric pressure falls almost exponentially with increasing altitude. Thus, hypoxia is an inevitable consequence of breathing air at ambient pressure during ascent. Hyperventilation is also common during flight and aircrew may breathe oxygen rich mixtures to combat incipient hypoxia. Hence, hypoxia, hypocapnia, and hyperoxia are frequently encountered in aviation (Harding, 2000). It has long been known that mild hypoxia compromises threshold sensitivity during dark adaptation (McFarland & Evans, 1939) and that hypocapnia enhances visual sensitivity (Wald, Harper, Goodman, & Krieger, 1942). However, little is known of the impact of respiratory disturbance upon mesopic visual performance.

To investigate this, stable and reproducible mesopic adaptation states must be established regardless of respiratory disturbance. Mesopic adaptation may be achieved by establishing scotopic threshold sensitivity during dark adaptation before light adapting to a controlled mesopic ambient

* Corresponding author. Tel.: +44 1252 394472; fax: + 44 1252 392097.
E-mail address: dmconnolly@qinetiq.com (D.M. Connolly).

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condition. This preliminary work aimed to establish a procedure of light and dark adaptation for subsequent mesopic studies by demonstrating, under respiratory challenge, the delay time to achievement of scotopic sensitivity (cone rod inflection) following offset of a bleaching light. The respiratory conditions were anticipated to affect threshold sensitivity. However, there were also significant effects on the delay time to rod vision that do not appear to have been reported before.

The requirement for aircrew to fly using night vision during World War II prompted early interest in the psychophysics of dark adaptation under hypoxia. It was shown that 'dark adaptation curves were elevated progressively with increased altitude or diminished partial pressure of O_2' (McFarland & Evans, 1939). The hypoxic impairment of rod sensitivity could be corrected rapidly with 100% oxygen only to recur just as quickly with further hypoxia. This effect cannot be due to delayed regeneration of photochemical sensitivity in a retina that is already dark-adapted. Subsequent studies confirmed the alveolar partial pressure of oxygen to be the determining variable in elevating threshold sensitivity (Ernest & Krill, 1971; Hecht, Hendley, Frank, & Haig, 1946; Kobrick & Appleton, 1971; McDonald & Adler, 1939; McFarland & Forbes, 1940; Sheard, 1946; Wald et al., 1942). The view prevailed that the timing of cone rod inflection and rate of photochemical adaptation were unaffected by hypoxia. Fischer and Jongbloed (1935) had apparently recorded a slight delay of dark adaptation at 3000 m and a marked delay at 6000 m that they assessed as impaired "regeneration of the photosensitive substance of the retina," but this was subsequently regarded as erroneous (McFarland, Evans, & Halperin, 1941).

Notwithstanding that cerebral blood flow is directly proportional to and exquisitely sensitive to arterial carbon dioxide tension, experimental hypocapnia enhances visual sensitivity and contrast discrimination (Alpern & Hendley, 1952; Otis, Rahn, Epstein, & Fenn, 1946; Wald et al., 1942). However, there appear to have been no studies investigating the effect of sustained hypocapnia, and its accompanying respiratory alkalosis, on the progress of threshold sensitivity during dark adaptation. Studies investigating the effect of supplementary oxygen under normobaric conditions have generally failed to demonstrate any psychophysical benefit over normoxia, although hyperoxia has been shown to enhance threshold sensitivity at altitudes as low as 1500 m (Pretorius, 1970).

In this study, a hypobaric chamber was used to investigate the delay time to early scotopic sensitivity under respiratory disturbances envisaged for subsequent studies of mesopic vision. Hypoxia was generated by breathing air at ambient pressures equivalent to altitudes of 3048 and 4572 m (10,000 and 15,000 ft, respectively; International Civil Aviation Organisation Standard Atmosphere, 1964). The former imposes a degree of mild hypobaric hypoxia at which significant effects may have implications for the use of supplementary oxygen by aircrew. The latter was chosen to impose moderate hypoxia without provoking undue secondary hypocapnia. To examine the influence of hypocapnia, moderate voluntary hyperventilation was investigated at ground level and together with hypoxia at 4572 m. The effect of hyperoxia was investigated breathing 100% oxygen at ground level. The chosen visual stimulus and study method highlighted the rapid progression of early scotopic threshold sensitivity and, by inference, the timing of cone rod inflection.

2. Method

2.1. Subjects

The work adhered to the principles of the Declaration of Helsinki and the study protocol was approved in advance by an independent Local Research Ethics Committee. All subjects provided written informed consent before participating. They were five healthy male volunteers with a mean age of 27 years (range 22–35 years) recruited from a pool of experienced hypobaric chamber subjects to minimise familiarisation and training requirements. Comprehensive medical and ophthalmic screening ensured fitness to participate and excluded previous or current visual deficit. Experienced medical examiners undertook a detailed ophthalmic history, external and fundoscopic examination, and assessed near and distant visual acuity, accommodation, convergence, visual fields, ocular movements and alignment, pupillary reactions and colour perception (Ishihara plates). The subjects were non-smokers who ate and drank normally during the study. They acted as their own controls, each undertaking five experiments at approximately the same time of day over a period of two weeks. All had undergone hypoxia familiarisation training, all were experienced with breathing from pressure demand oxygen regulators through aircrew oxygen masks and all were trained in the hyperventilation technique used in the study. The subjects were familiar with the subjective nature of decompression and it was impractical to disguise the ground level exposures as hypobaric without compromising experimental timings. However, they were unaware as to whether they were breathing air or 100% oxygen and the order of the conditions was semi-randomised.

2.2. Equipment

The hypobaric chamber was screened to exclude external light and internal lighting provided mean illumination of 1100 lux at subject eye level. Ground level barometric pressure ranged from 98.75 to 101 kPa (740–758 mmHg) during the study. Breathing gas was externally supplied air or 100% oxygen, each delivered at ambient chamber pressure through dedicated Normalair (now Honeywell Normalair-Garrett, HNGL) Mk 17F pressure demand breathing regulators. Both regulators fed their gas via a Douglas tap to a common mask tube hose that supplied the subject's mask. Subjects wore standard UK Royal Air Force Type P/Q Series (Normalair-Garrett, now HNGL) aircrew oxygen masks that prevented rebreathing of expired gas. These were modified to provide an access port to the mask cavity, allowing continuous respiratory gas analysis with an Innovision A/S Anais 2000 mass spectrometer. A standard Friedmann Visual Field Analyser Mk II (Friedmann, 1980) with an established xenon flash tube was used to provide a consistent visual stimulus. The dark adaptation mode intended for clinical examination was not used. Instead, the stimulus was tailored using the internal green and neutral density (ND) filters and the black stimulus plate. Non-invasive monitoring of oxygen saturation, heart rate, and blood pressure were undertaken using a Kontron pulse oximeter with finger probe and an Ohmeda Finapres blood pressure monitor with finger cuff. Analogue outputs from both devices were calibrated and recorded, together with the mass spectrometer data, using an ADInstruments PC-based data recording and analysis system employing Powerlab/Chart software.

2.3. Respiratory conditions and visual stimuli

Breathing air at ambient pressure during hypobaric decompression achieves inspired and alveolar gas tensions (P_AO_2, P_ACO_2) that mimic

those produced at altitude. They are estimated by measuring end tidal gas tensions ($P_{ET}O_2$, $P_{ET}CO_2$) breath by breath using the mass spectrometer. Decompressing the hypobaric chamber to ambient pressures of 69.7 kPa (523 mmHg) and 57.2 kPa (429 mmHg) achieved altitude exposures equivalent to 3048 and 4572 m, respectively. Voluntary hyperventilation maintained $P_{ET}CO_2$ close to 3.33 kPa (25 mmHg) for the hypocapnia exposures. Hyperoxia was imposed breathing 100% oxygen at ground level. All control adaptations were conducted breathing air normally at ground level.

The chosen visual stimulus was intended to measure threshold retinal sensitivity by repeatedly challenging cones and rods at multiple retinal locations. The Friedmann Analyser's green filter and q3 stimulus pattern were used, providing a tetrad of spot flash stimuli with a half time of 1.3 ms for each flash. The three peripheral q3 stimuli are located at approximately 4, 7, and 11 o'clock and subtend a visual angle of approximately 15° relative to a central stimulus. The Analyser's ND filters were used to control stimulus intensity, starting at the 3.0 ($\log_{10}$) setting. Progressively dimmer stimuli were achieved by increasing filter strength in 0.2 $\log_{10}$ unit steps until the maximum 4.6 setting was achieved. Subjects positioned the oxygen mask against the Analyser chin rest and looked straight ahead to maintain the direction of gaze perpendicular to the centre of the stimulus plate. No fixation point was provided to reduce the likelihood of repeatedly stimulating exactly the same retinal locations.

2.4. Procedure

All dark adaptations were completed using the same procedures with subjects seated at rest in the hypobaric chamber. Normoxic control adaptations always preceded those under respiratory conditions to avoid post-exposure respiratory 'noise' affecting control data. Continuous respiratory mass spectrometry was maintained throughout with calibration conducted before and after each adaptation, at altitude when indicated, using a variety of gas mixtures of known composition. For the hypobaric exposures, all chamber decompressions and recompressions were controlled to last 3 min. For the hyperventilation exposures, breath-by-breath feedback of mass spectrometry data allowed subjects to control their breathing rate and depth to maintain $P_{ET}CO_2$ close to 3.33 kPa (25 mmHg).

Before commencing dark adaptation, 15 min were allowed for respiratory adaptation to approximate a respiratory steady state. The last 5 min of respiratory adaptation coincided with bilateral light adaptation using a light box with a luminance of 785 cd m^{-2}. All dark adaptations were conducted using the right eye and a natural pupil, with the left eye patched. The flash was triggered manually at the initial stimulus intensity (ND 3.0) every 5 s until the subject acknowledged having seen any of the four spots of light. This time was logged, the stimulus intensity decreased by 0.2 $\log_{10}$ units (by increasing ND filter setting to 3.2) and the flash stimulus presented every 5 s until seen again. This process continued for at least 20 min or until the subject perceived the dimmest stimulus that could be presented (ND 4.6). Subjects had no cues for stimulus presentation. At the end of the control adaptation chamber lighting was restored and the subject rested for 10 min. Using this regime, dark adaptations under altered respiratory conditions began approximately 30 min after completing normoxic control adaptations.

2.5. Analysis

Cardiovascular and respiratory parameters were measured beat by beat and breath by breath throughout dark adaptation. Mean and standard deviation (*SD*) values were derived and compared to expected values. The 25 control dark adaptations under normoxia allowed variability in stimulus detection time, within and between subjects, to be examined using one way analysis of variance (ANOVA). For each dark adaptation conducted under respiratory disturbance, the detection time displacement relative to the normoxic detection time was determined for all stimulus intensities. Negative (leftward) displacement (decreased detection time) represents a hastening of adaptation to dark (or increased light sensitivity for a given time in darkness) while positive (rightward) displacement (increased detection time) represents delayed adaptation and reduced visual sensitivity. The displacements for the five subjects (four degrees of freedom) were analysed for statistical significance to a 95% confidence limit ($p < 0.05$) using the paired t test against the null hypothesis that a sample population experiencing no effect from the imposed respiratory condition would have a mean displacement of zero. Notwithstanding the risk of Type I error when conducting multiple t tests, with only five subjects a single rogue value may oblige loss of significance at a 95% confidence level, resulting in Type II error. It is considered that the results presented here are more vulnerable to Type II than Type I error and are therefore unlikely to overstate the impact of respiratory gas disturbance. Accordingly, Bonferroni correction was considered but has not been applied to the data. Applying Bonferroni correction on the basis that five independent studies have been conducted using the same subjects, then results presented here with $p < 0.01$ would still retain statistical significance at a 95% confidence interval. For convenience, the logarithmic stimulus intensity scale on the graphs shown is given in Analyser ND filter settings. As a representational baseline all graphs use the same mean control curve derived from all 25 control adaptations. Each subject's displacement from his own control detection time is represented relative to the mean control curve and the mean displacement curve illustrates the net effect of the condition across all five subjects.

3. Results

3.1. Respiratory and cardiovascular responses

The end tidal gas tensions and arterial oxygen saturations (S_aO_2) measured during dark adaptation under normoxia and under the imposed respiratory conditions are shown in Table 1. Normally, at 4572 m, mild hypocapnia results from gentle hyperventilation secondary to hypoxia (Harding, 2000). In this study, only one subject developed hypocapnia averaging 3.87 kPa (29 mmHg). This is attributed to the general familiarity of our subjects with hypobaric and breathing system studies. The results breathing air at 3048 m were as expected. The mean and *SD* values indicate tight control of $P_{ET}CO_2$ at the target level of 25 mmHg during the voluntary hyperventilation exposures. Hyperventilation at 4572 m resulted in secondary elevation of $P_{ET}O_2$ and S_aO_2 rose accordingly to 94% ($p < 0.05$). Breathing 100% oxygen at ground level elevated mean S_aO_2 to 99.1 ± 1.0% from 98.1 ± 1.0% during the related control period on air ($p < 0.01$). The results indicate that all five

Table 1
End-tidal gas tensions and arterial oxygen saturations achieved during dark adaptations conducted under normoxia and under the imposed respiratory conditions

		Normoxia	Hypoxia at 4572 m (15,000 ft)	Hypoxia at 3048 m (10,000 ft)	Hypocapnia at ground level	Hypocapnia at 4572 m (15,000 ft)	Hyperoxia (100% O_2) at ground level
$P_{ET}O_2$ (mmHg)	Mean (*SD*)	112.2 (1.3)	44.2 (4.9)	63.5 (5.7)	133.2 (2.3)	61.7 (5.6)	706.6 (12.9)
$P_{ET}CO_2$ (mmHg)	Mean (*SD*)	41.0 (2.0)	40.1 (8.5)	42.7 (3.8)	24.8 (1.3)	24.5 (0.5)	37.4 (2.8)
S_aO_2 (%)	Mean (*SD*)	97.6 (0.8)	77.7 (7.5)	89.8 (1.9)	98.1 (1.3)	94 (1.7)	99.1 (1.0)

Gas tensions are shown as measured in mmHg (1 kPa = 7.501 mmHg). The results confirm that the intended respiratory conditions were achieved.

conditions produced the intended respiratory challenges. Heart rate increased by a mean of 10 beats per minute during the hypoxia exposures at 4572 m ($p < 0.01$) but was unaffected by the other respiratory conditions.

3.2. Variation in dark adaptation under normoxia

Following a highly variable period until detection of the first stimulus, the five stimuli at the 3.0–3.8 Analyser settings were perceived at clearly discrete intervals, typically a minute or two apart. In contrast, the 3.8, 4.0, and 4.2 stimuli were always seen in quick succession, usually separated by only 10 or 15 s. The 4.4 stimulus was then seen within a minute or two followed by a longer and highly variable delay for the 4.6 stimulus. It is concluded that transition from mesopic to scotopic threshold sensitivity, that is, the cone–rod inflection point, always occurred between detection of the 3.6 and 3.8 stimuli. Using this stimulus method, the initial scotopic sensitivity curve is characterised by a steeply falling threshold that was obvious during every adaptation, almost invariably exceeding 0.4 $\log_{10}$ units in the first 30 s. Thereby, it indicated the approximate timing of the inflection point, that is, in the few seconds prior to detection of the 3.8 stimulus.

There was considerable variability in dark adaptation under normoxia. The *SD* of detection times taken from all 25 normoxic control adaptations ranged from 85 to 93 s for all stimulus intensities, except that for the brightest (3.0, cone) stimulus it was 103 s and for the dimmest (4.6, rod) stimulus, 172 s. Within subjects, mean SD detection times were 37–47 s for cone stimuli and 63–70 s for rods, except that for the dimmest stimulus it was 147 s. Variability in dark adaptation within subjects is considerable and appears greater for rods than for cones. The variance ratio between individuals exceeded F_{max} for the brightest cone stimulus, while, for all cone stimuli, one way ANOVA produced F_0 values that easily exceeded F_{crit} at the 0.05 level, indicating statistically significant variability in cone sensitivity between our subjects. The coefficients of variation for cone stimuli, across all control adaptations, diminished with decreasing stimulus intensity, from 23.9% (ND3.0), 18.4% (3.2), 15.9% (3.4) to 13.3% (ND3.6). F_0 values approached but did not exceed F_{crit} at any rod stimulus intensity with coefficients of variation ranging from 10.9 to 13.9%. Variability between subjects exceeded that within subjects and was greater for cones than for rods. Such marked variance within and between subjects obliged a test of paired control/condition data for the respiratory conditions.

3.3. Dark adaptation under respiratory disturbance

The effects of respiratory disturbance that achieved statistical significance in hastening or delaying stimulus detection are summarised in Table 2. Statistically significant data points are represented by solid symbols in all Figures.

The effect upon dark adaptation of hypoxia at 4572 m is represented in Fig. 1. Hypoxia significantly delayed dark adaptation at the majority of stimulus intensities. The slope of the cone curve is shallower under hypoxia, consistent with the expectation of elevated absolute cone sensitivity. The effect on rod adaptation is particularly pronounced, such that the onset and rapid early progression (first 30 s) of scotopic sensitivity are delayed by several minutes. Two subjects failed to perceive the dimmest stimulus despite continuing with adaptation well beyond 20 min. Hence, while not apparent in Fig. 1, detection of the dimmest stimulus was also substantially delayed and consistent with the expectation of elevated absolute rod sensitivity.

The effect of hyperventilation at ground level is represented in Fig. 2. In contrast to hypoxia, hyperventilation displaces the curve to the left, hastening both cone and rod thresholds and achieving statistical significance ($p < 0.05$) at three stimulus intensities. Hyperventilation also hastens dark adaptation at 4572 m (Fig. 3) where its effect is sufficient to abolish that of the concomitant hypoxia (Fig. 1).

Table 2
Statistically significant mean detection time displacements during dark adaptation under respiratory disturbance for five subjects

Light intensity (Analyser setting)	Hypoxia at 4572 m (15,000 ft)		Hypocapnia at ground level		Hypocapnia at 4572 m (15,000 ft)		Hyperoxia (100% O_2) at ground level	
	Mean (s)	p	Mean (s)	p	Mean (s)	p	Mean (s)	p
3.0	19		33		−81		71	<0.05
3.2	58		−87	<0.05	87	<0.05	−46	
3.4	78	<0.05	−90	<0.05	56	<0.05	16	
3.6	128	<0.05	72		−17		25	
3.8	161	<0.05	93		55		83	<0.01
4.0	160	<0.05	111		55		83	<0.01
4.2	202	<0.01	120	<0.05	50		69	
4.4	272	<0.05	116		14		134	<0.01
4.6	*	*	159		9		181	

Positive values represent a delay and negative values a hastening of detection time relative to normoxia.

* Two subjects failed to achieve sensitivity to the 4.6 stimulus despite over 20 min of hypoxic dark adaptation at 4572 m. Statistical significance has been calculated using unadjusted paired t tests and statistically significant data points are represented by solid symbols in all Figures. Applying Bonferroni correction to the data on the basis that five independent studies were conducted using the same subjects, then values of $p < 0.01$ retain statistical significance to a 95% confidence interval, continuing to support the contention that hypoxia delays and hyperoxia hastens early scotopic sensitivity.

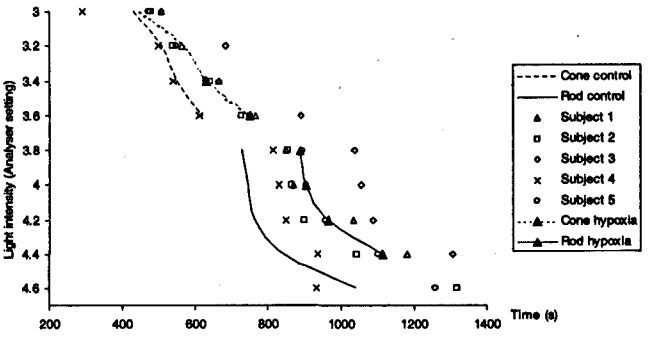


Fig. 1. Effect of hypoxia when breathing air at 4572 m (15,000 ft) on the rate of dark adaptation of five subjects showing detection time displacement relative to a control dark adaptation breathing air at ground level. Mean displacement data points achieving statistical significance are shown by solid symbols (see Table 2). The detection time delay for stimulus 4.2 was highly significant ($p < 0.01$). Subject 4, least affected by hypoxia at this altitude, was the subject who became most hypocapnic during this exposure (mean $P_{ET}CO_2 = 29$ mmHg). Subjects 1 and 3 failed to achieve sensitivity to the 4.6 stimulus despite continuing with dark adaptation well beyond 20 min.

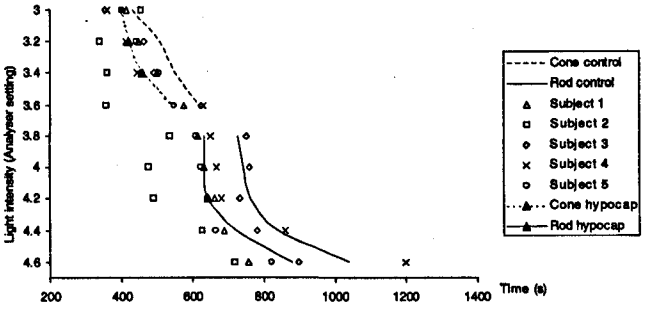


Fig. 2. Effect of voluntary hyperventilation breathing air at ground level on the rate of dark adaptation of five subjects showing detection time displacement relative to a control dark adaptation breathing air normally. End-tidal carbon dioxide tension was maintained close to 3.33 kPa (25 mmHg) throughout adaptation. Mean displacement data points achieving statistical significance are shown by solid symbols (see Table 2).

This effect of hyperventilation, to alter the response to hypoxia at 4572 m, is highly significant ($p < 0.01$ for six stimuli). It is noteworthy that subject 4, least affected by hypoxia at 4572 m (Fig. 1), was also the subject who developed mild secondary hypocapnia ($P_{ET}CO_2$ of 29 mmHg).

The effect of breathing 100% oxygen at ground level is represented in Fig. 4. The onset and progression of scotopic sensitivity are hastened and the effect is highly significant for three out of four early rod stimuli ($p < 0.01$), clearly suggesting a leftward shift of the cone rod inflection point and early scotopic sensitivity curve in comparison with normoxia.

Mean adaptation curves under hyperoxia, normoxia and hypoxia are compared in Fig. 5, which includes the curve for hypoxia at 3048 m. The trend of hypoxia to delay rod adaptation remained apparent at 3048 m but the effect was less marked than that at 4572 m and statistical significance was not achieved at any stimulus intensity. This is likely to have resulted from a lack of statistical power when using only 5 subjects. Nonetheless, the appearance of the rod curves in Fig. 5 suggests a relationship between oxygen tension and delay time to scotopic sensitivity across the range of oxygen tensions under investigation.

The relationship between $P_{ET}O_2$ and stimulus detection time is shown in Fig. 6 for each of the nine stimulus intensities. The appearance of the rod stimulus curves suggests that the effect of hypoxia to delay scotopic sensitivity is rapidly progressive if oxygen tension falls from ~100 mmHg, that is, with any reduction from normal oxygen tension. The effect of hyperoxia to shorten the time course of rod adaptation, relative to normoxia, is also apparent. In contrast, the 'flat' cone curves suggest that hyperoxia has little influence on late cone adaptation.

4. Discussion

4.1. Hypoxia

Past studies of dark adaptation under hypoxia involved relatively slow and infrequent measurements of threshold stimulus intensity, which would be unlikely to capture a

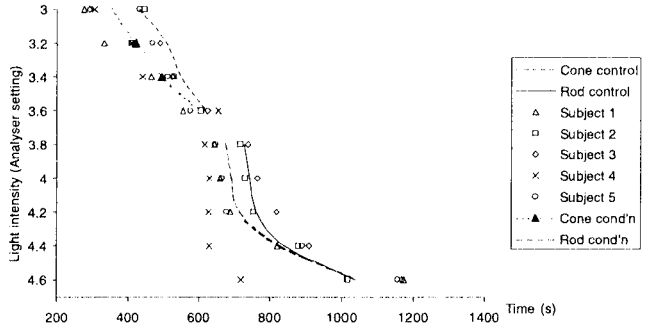


Fig. 3. Effect of voluntary hyperventilation breathing air at 4572 m (15,000 ft) on the rate of dark adaptation of five subjects showing detection time displacement relative to a control dark adaptation breathing air normally at ground level. End-tidal carbon dioxide tension was maintained close to 3.33 kPa (25 mmHg) throughout adaptation. Mean displacement data points achieving statistical significance are shown by solid symbols (see Table 2). Hyperventilation alters the response to hypoxia at this altitude (compare with Fig. 1), appearing to shift the early components of the cone and rod threshold sensitivity curves to the left. The alteration in response at 4572 m was highly statistically significant for six stimulus intensities ($p < 0.01$). However, hypoxia appears to offset the response to hyperventilation, in comparison to the effect of hyperventilation at ground level (Fig. 2), by limiting the leftward shift and elevating the later phases of cone and rod adaptation.

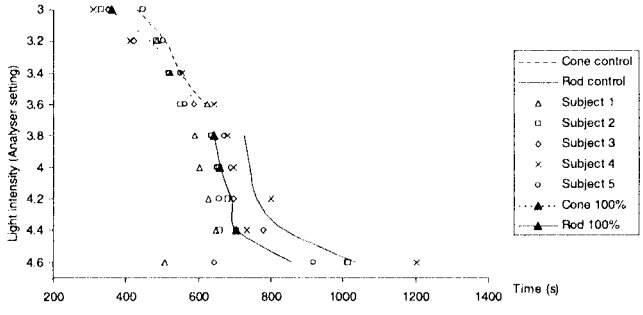


Fig. 4. Effect of breathing 100% oxygen at ground level on the rate of dark adaptation of five subjects showing detection time displacement relative to a control dark adaptation breathing air at ground level. Mean displacement data points achieving statistical significance are shown by solid symbols (see Table 2). All those on the rod portion of the curve were highly significant ($p < 0.01$) suggesting a pronounced effect of hyperoxia to hasten early scotopic sensitivity.

data point close to the moment of cone rod inflection. The prominence of this feature would then be lost when taking mean curves over a number of exposures. In many studies the inflection point is barely perceptible or has been inferred in the absence of appropriate data points. This study highlighted the rapid progression of early scotopic threshold sensitivity using fixed stimulus intensities and measuring time as the dependent variable. In almost every adaptation, early scotopic sensitivity progressed by over 0.4 $\log_{10}$ units during the 30 s following detection of the 3.8 stimulus. This brief early portion of the rod curve is translated laterally by several minutes under respiratory disturbance, suggesting a temporal effect that dictates when rapid progression of scotopic sensitivity can occur. Contrary to earlier studies, hypoxia appears to delay cone rod inflection and early scotopic adaptation, shifting the initial rod curve to the right. Hence, past studies may have averaged mixtures of pre- and post-inflection thresholds, further camouflaging the true inflection point and masking the effects on the early rod curve. The appearance of the lateral shift of the early rod curve is quite distinct from the hypoxic elevation of later threshold sensitivity that was clearly demonstrated in early studies. Hence, hypoxia has both horizontal (temporal) and vertical (sensitivity) effects that are distinguishable at the beginning and end, respectively, of the rod curve.

Recovery of rod sensitivity in the dark requires deactivation of the photoproducts of light absorption, depolarisation of the photoreceptor cell membrane and regeneration of visual pigment (Lamb, 1990; McBee, Palczewski, Baehr, & Pepperberg, 2001). Examining each of these in turn, the processes controlling the phototransduction cascade

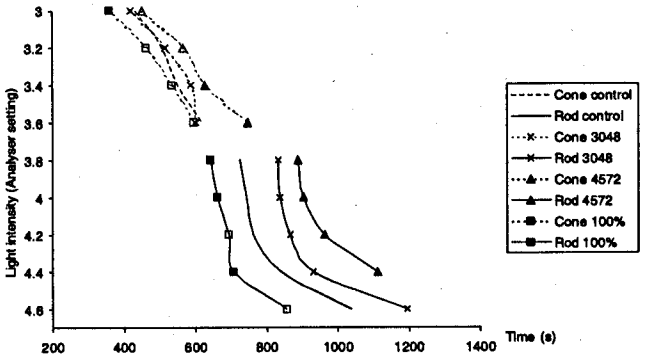


Fig. 5. Comparison of the effects upon dark adaptation of hypoxia breathing air at 3048 m (10,000 ft, crosses) and 4572 m (15,000 ft, triangles) with 100% oxygen at ground level (squares) showing detection time displacements relative to normoxic control adaptations. Statistically significant displacements are shown by solid symbols. Although the data at 3048 m did not achieve statistical significance, the collective appearance of the rod curves suggests that the time taken to achieve early scotopic threshold sensitivity is dependent upon oxygen tension across the range of conditions studied.

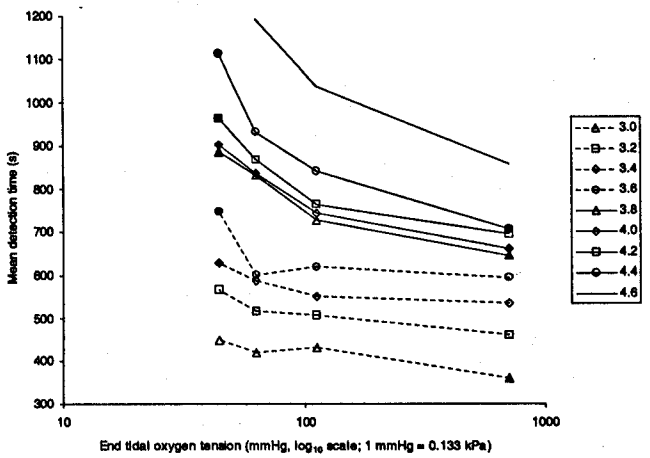


Fig. 6. Relationship between end tidal oxygen tension and stimulus detection time for cones (broken lines) and rods (full lines). Relative to normoxia (112 mmHg), hyperoxia (707 mmHg) appears to compress the time course of early scotopic adaptation but does not influence the later stages of cone adaptation. Cone adaptation appears unaffected by hypoxia until $P_{ET}O_2$ falls below 64 mmHg (3048 m, 10,000 ft). However, rod adaptation is compromised by very mild hypoxia and rods may even be functionally hypoxic when breathing air under normobaric conditions (see Section 4).

reactions in rod outer segments are heavily dependent upon the availability of metabolic energy, for example for rhodopsin phosphorylation, synthesis of cyclic guanosine monophosphate, to support enzyme activity and for chromophore transport (Picaud, 2003). The substantial increase in photoreceptor ion pump activity in the dark is known to be impaired by even mild hypoxia (Steinberg, 1987), suggesting that the availability of metabolic energy in the photoreceptor is critically dependent upon the ongoing supply of oxygen. Finally, hypoxia impairs regeneration of rhodopsin in vitro (Ostroy, Gaitatzes, & Friedmann, 1993). A recent review of dark adaptation kinetics suggested that the impact of hypoxia may be explained by effects on photoreceptor circulating current and/or slowing of 11-*cis* retinal synthesis in the pigment epithelium (Lamb & Pugh, 2004). In support, the current study suggests that hypoxia may impair scotopic sensitivity in vivo, at least in part, by delaying photoproduct deactivation and/or regeneration of photochemical sensitivity.

The age-related impairment of dark adaptation, assessed using a comparable method of fixing stimulus intensity until detected, exhibits a similar appearance to that of hypobaric hypoxia (Jackson, Owsley, & McGwin, 1999). In that study, subjects in their 70 s achieved mean inflection

times two and a half minutes slower than subjects in their 20 s. Using a young subject sample, the results of the current study compare unfavourably, with hypoxia delaying the onset of scotopic sensitivity by almost 2 min at 3048 m and almost 3 min at 4572 m.

4.2. Hyperventilation

Hypocapnia hastens dark adaptation sufficiently to negate the effect of hypobaric hypoxia at 4572 m. Hyperventilation at 4572 m increased mean $P_{ET}O_2$ to 8.23 kPa (61.7 mmHg) from 5.89 kPa (44.2 mmHg) when breathing normally, thereby elevating mean S_aO_2 from 78 to 94%. However, the effect of hyperventilation appears independent of any coexisting change in choroidal oxygen tension or oxygen saturation. First, the increase in oxygen tension/saturation with hyperventilation might be expected to normalise the dark adaptation curve, in comparison to the effect of hypoxia alone, but not to hasten it further. Additionally, the magnitude of the increase in $P_{ET}O_2$ and S_aO_2 is relatively slight, giving values comparable to hypoxia at 3048 m (Fig. 5), yet the adaptation curves are quite different. Finally, hyperventilation at ground level also caused a slight increase in $P_{ET}O_2$ (with a marginal effect on S_aO_2) yet its effect on dark adaptation was even more pronounced than hyperventilation at altitude.

The mechanism whereby hypocapnia affects visual sensitivity remains conjectural although the resulting respiratory alkalosis is most obviously implicated, as hypocapnia is well known to increase nerve cell excitability. Wald et al. (1942) found that the effect of hyperventilation to enhance sensitivity was lost when adding 2% carbon dioxide to the breathing gas. Also, artificially induced metabolic alkalosis does not affect absolute visual sensitivity, in contrast to the effect of hypocapnia, further implying that it is the fall in P_ACO_2 that is significant (Alpern & Hendley, 1952). Charged acid base species in blood are prevented from crossing into brain extracellular fluid by the blood brain barrier and the pigment epithelium similarly controls ion flux between the choroid and the subretinal space (Strauss, 2005). In contrast, carbon dioxide is highly soluble, diffuses rapidly and is the only acid–base species that freely crosses the blood brain barrier. Arterial hypocapnia may tend to reduce choroidal flow (Harris, Arend, Wolf, Cantor, & Martin, 1995; Samuel & Beaugié, 1974) but should still be expected to generate a carbon dioxide tension gradient promoting outer retinal alkalosis. In the brain, however, hypocapnia appears not to increase intracellular pH in vivo due to a compensatory increase in the production of metabolic acids and stimulation of glycolysis (Mellergård & Siesjö, 1998). A similar effect of hypocapnia on the outer retina may be of considerable benefit to photoreceptors during dark adaptation.

The retinal pH gradient and changes induced by light appear generally similar in mammals and lower vertebrates, while changes in pH are known to affect retinal cell function and sensitivity (Barnes, 1998). Light induces outer retinal alkalinization superimposed on a gradient of decreasing pH from ganglion cells to photoreceptors. In the cat the most acidic pH was recorded in the dark near the outer nuclear layer, close to the site of photoreceptor mitochondrial activity and cytoplasmic glycolysis (Yamamoto, Borgula, & Steinberg, 1992). At low levels of illumination, arterial hypoxaemia also acidifies the extracellular space at the outer nuclear layer, suggesting that rods are sensitive to mild hypoxia during dark adaptation, with a consequent increase in glycolytic activity (Yamamoto & Steinberg, 1992). Müller cells appear to have a role in removing acid from this region through elimination of carbon dioxide (Barnes, 1998). Respiratory alkalosis may help to offset the acidification that occurs in the dark and with hypoxia, to the benefit of the underlying metabolic processes, through enhanced elimination of H^+ through the choroid via the bicarbonate buffer system. Notwithstanding the foregoing, the mechanism by which hypocapnia might exert a temporal effect to shorten the delay time to the onset of scotopic vision is unknown.

4.3. Hyperoxia

The choroid endeavours to meet the requirements of the outer retina by maintaining the highest possible oxygen tension and delivering oxygen in solution (Alm & Bill, 1970, 1972). Oxygen delivery to the outer retina follows a steep gas tension gradient that becomes even steeper when rod oxygen consumption increases in darkness (Linsenmeier, 1986). Tissue oxygen tension falls to extremely low levels near photoreceptor inner segments (Ahmed, Braun, Dunn, & Linsenmeier, 1993; Stefansson, Wolbarsht, & Landers, 1983; Yu & Cringle, 2002) and may compromise oxidative metabolism in inner segment mitochondria despite increased oxygen flux from the inner retina (Wangsa-Wirawan & Linsenmeier, 2003). The oxygen requirements of the dark-adapted eye appear vulnerable to reductions in choroidal oxygen tension induced by decreasing choroidal flow (Yancey & Linsenmeier, 1989). Some animal studies report a possible slight reduction in choroidal blood flow with hyperoxia but breathing 100% oxygen does not appear to have the same effect in humans (Kergoat & Faucher, 1999). Hence, the increase in arterial oxygen tension when breathing normobaric 100% oxygen, from ~100 to ~700 mmHg, should enhance the delivery of oxygen from the choroid to the photoreceptors. In the cat, breathing 100% oxygen does not appear to increase photoreceptor oxygen consumption by comparison with normoxia (Linsenmeier & Yancey, 1989), although the choroidal oxygen tension measured in that study was lower than might be expected. On the other hand, hyperoxia causes slight alkalinization of the outer nuclear layer in the dark that suggests some suppression of rod photoreceptor glycolysis, predicting a slight increase in oxygen consumption in comparison to normoxia (Yamamoto & Steinberg, 1992). In that study, electroretinogram effects suggested that 'the tissue was hypoxic prior to the addition of oxygen even though the P_aO_2 before and after

the hyperoxic episode was within the normoxic range.' In the current study, 100% oxygen hastens rod adaptation to dark at one atmosphere, implying that human rod photoreceptors may also be functionally hypoxic when breathing air under normal conditions.

This may explain the biological imperative to locate reisomerisation of 11-*cis* retinal within the retinal pigment epithelium. Thereby, the process of chromophore regeneration has preferential call upon the oxygen flux from the choroid at a time when the photoreceptors are functionally hypoxic. If rhodopsin regeneration remained within the photoreceptor, then competition from numerous other processes might further delay the onset and progression of early scotopic vision. However, the effect of hyperoxia to advance scotopic sensitivity is perhaps less likely through hastened regeneration of 11-*cis* retinal in the pigment epithelium than it is through enhanced photoproduct deactivation and reintegration of chromophore in photoreceptor outer segments.

From the practical perspective, 'fast adapters' breathing oxygen may achieve optimal visual sensitivity long before their mildly hypoxic 'slow adapter' counterparts. The inflection point achieved most quickly breathing 100% oxygen occurred almost four and a half minutes sooner than the slowest breathing air at 3048 m and nine and a half minutes sooner than the slowest at 4572 m.

4.4. Conclusions and relevance to aviation

Hypoxia impairs visual sensitivity and delays rod adaptation to dark while hypocapnia and hyperoxia enhance visual sensitivity and hasten dark adaptation. The delay time to the onset and rapid progression of early scotopic sensitivity is affected by both oxygen and carbon dioxide tension. The effects on the rod adaptation curve include distinct early (temporal) and later (sensitivity) components. Hyperoxia, breathing 100% oxygen at one atmosphere, hastens the onset and early progression of scotopic sensitivity, suggesting that rod photoreceptors are functionally hypoxic when breathing air normally. The mechanism whereby hypocapnia enhances visual sensitivity may be related to enhanced elimination of H^+ near the photoreceptors. In practice, hyperventilation counters the effect of hypoxia, hastens scotopic vision and may be regarded as protective of visual sensitivity in dim light. Recent reviews have emphasised the rate-limited kinetics of dark adaptation and retinoid metabolism (Lamb & Pugh, 2004; McBee et al., 2001). The availability of oxygen and effects of pH may warrant further consideration in this context.

Some subjects appear to adapt to dark conditions much slower than others, while age and altitude impose further delays. The benefit of hyperoxia to hasten and enhance scotopic sensitivity is apparent even at ground level. Accordingly, aircrew should consider using supplementary oxygen if they are reliant upon viewing scotopic external scenes (less than full moonlight) when flying at night. Clearly, this will be most relevant to aircrew flying unpressurised aircraft at low level, especially over elevated terrain, without the benefit of night vision devices. The effects of respiratory disturbance on mesopic visual performance remain to be explored and may be of more direct relevance to the majority of contemporary aircrew flying at night with the benefit of modern electro-optical displays. Work is in progress to investigate this using the procedure of retinal bleach and dark adaptation reported here.

Acknowledgments

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References

Ahmed, J., Braun, R. D., Dunn, R., Jr., & Linsenmeier, R. A. (1993). Oxygen distribution in the macaque retina. *Investigative Ophthalmology and Visual Science, 34*(3), 516–521.

Alm, A., & Bill, A. (1970). Blood flow and oxygen extraction in the cat uvea at normal and high intraocular pressures. *Acta Physiologica Scandinavica, 80*, 19–28.

Alm, A., & Bill, A. (1972). The oxygen supply to the retina, I. Effects of changes in intraocular and arterial blood pressures, and in arterial PO_2 and PCO_2 on the oxygen tension in the vitreous body of the cat. *Acta Physiologica Scandinavica, 84*, 261–274.

Alpern, M., & Hendley, C. D. (1952). Visual functions as indices of physiological changes in the acid–base balance of the blood. *American Journal of Optometry, 29*, 301–314.

Barnes, S. (1998). Modulation of vertebrate retinal function by pH. In K. Kaila & B. R. Ransom (Eds.), *pH and Brain Function* (1st ed., Ch. 25, pp. 491–505). New York: Wiley-Liss.

Ernest, J. T., & Krill, A. E. (1971). The effect of hypoxia on visual function: Psychophysical studies. *Investigative Ophthalmology, 10*, 323–328.

Fischer, F.P., & Jongbloed, J. (1935). Untersuchungen über die Dunkeladaptation bei herabgesetztem Sauerstoffdruck der Atmungsluft. *Archiv für Augenheilkunde*, 109, 432. This reference was not available to the authors but is reported in McFarland, Evans and Halperin (1941).

Friedmann, A. I. (1980). Outline of visual field analyser. Mark II. *Documenta Ophthalmologica Proceedings Series, 22*, 65–67.

Harding, R.M. (revised Gradwell, D.P.) (2000). Hypoxia and hyperventilation. In J. Ernsting, A. N. Nicholson, & D. J. Rainford (Eds.), *Aviation Medicine* (3rd ed., Ch. 5, pp. 43–58). Oxford: Butterworth-Heinemann.

Harris, D. (2004). Head-down flight deck display design. In D. Harris (Ed.), *Human factors for civil flight deck design* (1st ed., pp. 69–102). Aldershot: Ashgate Publishing Ch. 4.

Harris, A., Arend, O., Wolf, S., Cantor, L. B., & Martin, B. J. (1995). CO_2 dependence of retinal arterial and capillary blood velocity. *Acta Ophthalmologica Scandinavica, 73*, 421–424.

Hecht, S., Hendley, C. D., Frank, S. R., & Haig, C. (1946). Anoxia and brightness discrimination. *Journal of General Physiology, 29*, 335–351.

Jackson, G. R., Owsley, C., & McGwin, G., Jr. (1999). Aging and dark adaptation. *Vision Research, 39*, 3975–3982.

Kergoat, H., & Faucher, C. (1999). Effects of oxygen and carbogen breathing on choroidal haemodynamics in humans. *Investigative Ophthalmology and Visual Science, 40*, 2906–2911.

Kobrick, J. L., & Appleton, B. (1971). Effects of extended hypoxia on visual performance and retinal vascular state. *Journal of Applied Physiology, 31*(3), 357–362.

Lamb, T. D. (1990). Dark adaptation: a re-examination. In R. F. Hess, L. T. Sharpe, & K. Nordby (Eds.), *Night vision. Basic, clinical and applied aspects* (1st ed., pp. 177–222). Cambridge: Cambridge University Press Ch. 5.

Lamb, T. D., & Pugh, E. N., Jr. (2004). Dark adaptation and the retinoid cycle of vision. *Progress in Retinal and Eye Research, 23*, 307–380.

Linsenmeier, R. A. (1986). Effects of light and darkness on oxygen distribution and consumption in the cat retina. *Journal of General Physiology, 8*, 521–542.

Linsenmeier, R. A., & Yancey, C. M. (1989). Effects of hyperoxia on the oxygen distribution in the intact cat retina. *Investigative Ophthalmology and Visual Science, 30*(4), 612–618.

McBee, J. K., Palczewski, K., Baehr, W., & Pepperberg, D. R. (2001). Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Progress in Retinal and Eye Research, 20*, 469–529.

McDonald, R., & Adler, F. H. (1939). Effect of anoxemia on the dark adaptation of the normal and of the vitamin A-deficient subject. *Archives of Ophthalmology, 22*, 980–988.

McFarland, R. A., & Evans, J. N. (1939). Alterations in dark adaptation under reduced oxygen tensions. *American Journal of Physiology, 127*, 37–50.

McFarland, R. A., Evans, J. N., & Halperin, M. H. (1941). Ophthalmic aspects of acute oxygen deficiency. *Archives of Ophthalmology, 26*, 886–913.

McFarland, R. A., & Forbes, W. H. (1940). The effects of variations in the concentration of oxygen and of glucose on dark adaptation. *Journal of General Physiology, 24*, 69–98.

Mellergård, P., & Siesjö, B. K. (1998). Cerebral energy metabolism and pH. In K. Kaila & B. R. Ransom (Eds.), *pH and brain function* (1st ed., Ch. 5, pp. 67–91). New York: Wiley-Liss.

Melzer, J. E., & Moffitt, K. (Eds.). (1997). *Head mounted displays: designing for the user*. New York: McGraw-Hill.

Ostroy, S. E., Gaitatzes, C. G., & Friedmann, A. L. (1993). Hypoxia inhibits rhodopsin regeneration in the excised mouse eye. *Investigative Ophthalmology and Visual Science, 34*(2), 447–452.

Otis, A. B., Rahn, H., Epstein, M. A., & Fenn, W. O. (1946). Performance as related to composition of alveolar air. *American Journal of Physiology, 146*, 207–223.

Picaud, S. (2003). Retinal biochemistry. In P. L. Kaufman & A. Alm (Eds.), *Adler's physiology of the eye* (10th ed., pp. 382–408). St. Louis, Missouri: Mosby.

Pretorius, H. A. (1970). Effect of oxygen on night vision. *Aerospace Medicine, 41*, 560–562.

Rash, C. E., & Manning, S. D. (2003). On the flight deck, lighting must satisfy a variety of needs. *Human Factors and Aviation Medicine, 50*(5), 1–12.

Samuel, J. R., & Beaugié, A. (1974). Effect of carbon dioxide on the intraocular pressure in man during general anaesthesia. *British Journal of Ophthalmology, 58*, 62–67.

Sheard, C. (1946). Effects of increased intrapulmonary pressure on dark adaptation. *Journal of Aviation Medicine, 17*, 525–532.

Stefansson, E., Wolbarsht, M. L., & Landers, M. B. (1983). In vivo O_2 consumption in rhesus monkeys in light and dark. *Experimental Eye Research, 37*, 251–256.

Steinberg, R. H. (1987). Monitoring communications between photoreceptors and pigment epithelial cells: effects of 'mild' systemic hypoxia. *Investigative Ophthalmology and Visual Science, 28*, 1888.

Strauss, O. (2005). The retinal pigment epithelium in visual function. *Physiological Reviews, 85*, 845–881.

Wald, G., Harper, P. V., Goodman, H. C., & Krieger, H. P. (1942). Respiratory effects upon the visual threshold. *Journal of General Physiology, 25*, 891–902.

Wangsa-Wirawan, N. D., & Linsenmeier, R. A. (2003). Retinal oxygen: Fundamental and clinical aspects. *Archives of Ophthalmology, 121*, 547–557.

Wickens, C. D. (2003). Aviation displays. In P. S. Tsang & M. A. Vidulich (Eds.), *Principles and practice of aviation psychology* (1st ed., pp. 147–200). London: Lawrence Erlbaum Ch. 5.

Wickens, C. D., Ververs, P. M., & Fadden, S. (2004). Head-up displays. In D. Harris (Ed.), *Human factors for civil flight deck design* (1st ed., Ch. 4, pp. 69–102). Aldershot: Ashgate Publishing.

Wilson, D. R. (1999). Darkness increases risks of flight. *Human Factors and Aviation Medicine, 46*(6), 1–8.

Yamamoto, F., Borgula, G. A., & Steinberg, R. H. (1992). Effects of light and darkness on pH outside rod photoreceptors in the cat retina. *Experimental Eye Research, 54*, 689–697.

Yamamoto, F., & Steinberg, R. H. (1992). Effects of systemic hypoxia on pH outside rod photoreceptors in the cat retina. *Experimental Eye Research, 54*, 699–709.

Yancey, C. M., & Linsenmeier, R. A. (1989). Oxygen distribution and consumption in the cat retina at increased intraocular pressure. *Investigative Ophthalmology and Visual Science, 30*(4), 600–611.

Yu, D-Y., & Cringle, S. J. (2002). Outer retinal anoxia during dark adaptation is not a general property of mammalian retinas. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 132*(1), 47–52.

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TECHNICAL REPORT

Quantitative Correlation of Hyperventilation with Flicker Sensitivity

DESMOND CONNOLLY, MD, and SARAH HOSKING, PhD, FAAO

QinetiQ, Human Protection and Performance Enhancement Group, Cody Technology Park, Farnborough, United Kingdom (DC), and School of Life and Health Sciences, Aston University, Birmingham, United Kingdom (DC, SH)

ABSTRACT

Purpose. The relationship between hyperventilation and the associated increase in flicker sensitivity is poorly defined but may be relevant to display viewing. This exploratory study investigates the potential for quantifying the relationship between the severity of hypocapnia and critical flicker frequency (CFF).

Method. Repeated ascending (fusion) and descending (flicker) measurements were made while breathing normally (normocapnia), and at four levels of progressive, mild to moderate hypocapnia that were induced by voluntary hyperventilation and controlled using continuous respiratory mass spectrometry. The mesopic stimulus was a 2.6°-Gaussian blob viewed through a 5.2-mm-diameter artificial pupil.

Results. Five discrete respiratory conditions were generated. The influences of intersubject variability and severity of hypocapnia upon mean CFF were examined using two-way analysis of variance, demonstrating a statistically significant effect of target end-tidal partial pressure of carbon dioxide [$F(4,40) = 4.63$, $p = 0.005$]. The relationship between decreasing mean end-tidal partial pressure of carbon dioxide and increasing mean CFF was consistent with a linear correlation (Pearson $R = -0.949$, $p = 0.013$).

Conclusions. The results support a close relationship between the respiratory partial pressure of carbon dioxide and flicker sensitivity. However, the absolute magnitude of the underlying increase in flicker sensitivity with hypocapnia is small and the effect is unlikely to be relevant in aviation.

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Key Words: flicker sensitivity; flicker threshold; critical flicker frequency; hyperventilation; hypocapnia; alkalosis

Hyperventilation is alveolar ventilation in excess of metabolic need. The alveolar partial pressure of carbon dioxide (P_{ACO_2}) falls, promoting arterial hypocapnia, cerebral vasoconstriction, and respiratory alkalosis. Healthy aircrew may hyperventilate if inexperienced (during demanding training sorties) or when stressed (combat), and the resulting symptoms may interfere with the flying task.[1] Numerous factors predispose to hyperventilation in aviation, yet aircrew may be unaware that they are hyperventilating, and the incidence of significant in-flight hyperventilation is unclear.[2] Nonetheless, case histories are suggestive and aircrew training in high-performance aircraft may be most at risk.[3]

Rubinstein and Thurman reported the effect of voluntary hyperventilation to increase the frequency at which an intermittent visual stimulus is perceived to flicker.[4,5] Subsequent studies support an increase in critical flicker frequency (CFF) during hyperventilation.[6–9] Granger and Ikeda undertook the most extensive study of this phenomenon to date, examining flicker sensitivity as a function of stimulus size and intensity (controlled retinal illumination) during 30 s of hyperventilation.[8] The resulting increase in flicker sensitivity was greater for larger, brighter stimuli, and could be demonstrated across three orders of magnitude of stimulus luminance. The basic form of the relationship between flicker sensitivity and log luminance was preserved, but the curves were displaced towards higher critical frequencies under hyperventilation. Examining the effect of prior temporal adaptation, Turner confirmed the effect of hyperventilation to elevate flicker thresholds measured using both ascending (fusion) and descending (flicker) techniques.[9] In one study, flicker sensitivity was reported to decrease with hyperventilation, but this was attributed to taking measurements of CFF during the apnoeic pause that immediately follows a period of hyperventilation.[10] In sum, it may be inferred that hyperventilation has the potential to interfere with display viewing, or to distract attention, by increasing sensitivity to flicker, with potential relevance to aircrew.

There appear to have been no recent studies of the relationship between hyperventilation and flicker sensitivity. Existing studies

have used few subjects and single, relatively poorly defined hyperventilation conditions. Specifically, we found no reports quantifying a reduction in P_{ACO_2} with a corresponding effect on sensitivity to flicker, and none appear to have investigated the effect of graduated hypocapnia. Related work, examining the effects of respiratory disturbance on mesopic visual performance, provided an opportunity for an exploratory study to address these themes. Accordingly, we report a limited study that examined the potential for quantitative correlation of mild to moderate hypocapnia with CFF, using a simple mesopic stimulus. Physiological gas pressures were measured most conveniently in mm Hg and are reported here similarly, with the SI unit in parenthesis (kPa).

METHODS

Subjects

The work adhered to the Declaration of Helsinki, the study was approved in advance by an independent Local Research Ethics Committee, and all subjects provided written informed consent before participating. Comprehensive medical and ophthalmic screening included a detailed ophthalmic history, external and fundoscopic examination, assessment of near and distant visual acuity, accommodation, convergence, visual fields, ocular movements and alignment, and pupillary reactions. Any history of migraine or epilepsy was exclusive. Subjects were familiar with breathing through aircrew oxygen masks. In a previous study of hyperventilation and visual sensitivity, subjects controlled their end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) easily, to within 1 mm Hg (0.13 kPa) of a target of 25 mm Hg (3.33 kPa), suggesting that prior training in the technique was unnecessary.[11] It was inferred from that study that 12 subjects should provide 80% power to a 95% confidence interval. After recruitment, one female volunteer became unavailable due to relocation, leaving 11 (six men, five women) healthy, nonsmokers aged from 22 to 36 years. Subjects were trained to undertake repeated ascending (fusion) and descending (flicker) measurements, with an emphasis on accuracy over speed.

Equipment

A cloth helmet supported a military aircrew oxygen mask that was adjusted to ensure a secure face seal. The masks were modified to allow continuous mask cavity gas sampling with an Airspec QP9000 mass spectrometer. This was calibrated immediately before and after each experiment using known gas mixtures to ensure a linear response across the range of $P_{ET}CO_2$ under investigation, with a measurement error of <1% or 0.5 mm Hg (0.07 kPa). Respired partial pressures of oxygen were also monitored and recorded. Blood pressure, heart rate, and oxygen saturation were monitored noninvasively using an Ohmeda Finapres blood pressure monitor and Kontron pulse oximeter.

A simple vision test allowed subjects to make careful flicker measurements while maintaining control of hyperventilation. The Geedev flickermeter comprises a control box and a stimulus presentation tube with an exit aperture of internal diameter 5.2 mm. The test eye is positioned against an eye cup, excluding ambient light, and views the stimulus foveally. The stimulus is a yellow, Gaussian profile light source, with $1/e$ diameter subtending approximately 2.6° of visual angle. It flickers with a 1:1 light:dark duty cycle, modulated sinusoidally, with a fixed, time-averaged luminance of 3.8 cd m^{-2}. Flicker rate is controlled in 0.1 cps steps using a dial on the control box. Mean luminance was measured using a Spectra-Pritchard photometer. An additional aperture was used in front of the objective lens of the photometer to match the output aperture of the flickermeter and the input aperture of the photometer. As the aperture restricted the light reaching the photometer and thus made the photometer self-calibration void, the aperture–photometer combination was calibrated using a Gamma Scientific standard (calibrated) lamp.

Respiratory Conditions

The P_{ACO_2} determines the arterial partial pressure (P_{aCO_2}) and is reflected closely by the $P_{ET}CO_2$ in normal subjects. Normocapnia is generally accepted to be ~40 mm Hg (5.33 kPa).[12] Measurements of $P_{ET}CO_2$ were made breathing normally at rest and at four levels of increasingly severe hyperventilation, aiming for target $P_{ET}CO_2$ of 35, 30, 25, and 20 mm Hg (4.66, 3.99, 3.33, and 2.66 kPa). Randomization of the hypocapnic conditions would require extended rest periods between exposures to allow full recovery of $P_{ET}CO_2$ and blood/tissue pH to normal levels, greatly extending the duration of each experiment. Furthermore, the severity and duration of the initial respiratory effort required to attain each target level of hypocapnia would vary considerably between individuals and conditions. Instead, hyperventilation was intentionally continuous and progressive, minimizing its overall duration and the respiratory effort required to drop $P_{ET}CO_2$ to successive target levels. This avoided protracted recovery times, excessive fatigue, and carry over of acid–base disturbances from more-severe to less-severe conditions, while gathering the required vision data over the shortest possible time period.

Procedure

Ambient illumination was maintained at 1 to 2 lux and the resting eye was patched. Most subjects viewing a uniform field luminance of 3.8 cd m^{-2} have a natural pupil diameter exceeding that of the flickermeter exit aperture,[13,14] and the Geedev flicker stimulus is presented against a dark background. Nonetheless, there is wide variation between subjects, with one formula predicting a pupil diameter of only 3.5 mm in response to a field luminance of 3.8 cd m^{-2}.[15] Accordingly, normocapnic pupil size was measured from images captured, at the moment of completion of simulated Geedev flicker measurements, using an infrared, digital video camera under representative ambient lighting. Ten measurements were made in each of nine participating subjects. Results are shown in Table 1, illustrating that subjects' pupil diameters were consistently greater than the flickermeter exit aperture. Accordingly, measurements of flicker frequency were made using the dominant eye and a natural pupil, viewing through the flickermeter exit aperture to control retinal illumination.

Data recording began with 5 min of restful breathing before taking 10 alternating ascending (fusion) and descending (flicker) measurements over the subsequent 5 min. Care was taken to avoid cued, timed, or habituated measurements.[16] Subjects then hyper-

TABLE 1.
Mean horizontal pupil diameter of nine subjects using the Geedev flickermeter

Subject	A	B	C	D	E	F	G	H	I	Mean (n = 90)	SD (n = 90)
Mean pupil diameter (mm)	6.08	6.22	5.89	5.65	6.89	6.28	7.61	6.74	7.56	6.55	0.70
Standard deviation (mm)	0.37	0.10	0.20	0.36	0.11	0.20	0.22	0.26	0.13		

ventilated to lower $P_{ET}CO_2$ to 35 mm Hg (4.66 kPa), adjusting rate and depth of ventilation using real-time displays of mask cavity carbon dioxide tension (digital), and of each breath against target $P_{ET}CO_2$ (graphical). Vision testing commenced 5 min after first achieving target $P_{ET}CO_2$. This was repeated for target $P_{ET}CO_2$ of 30, 25, and 20 mm Hg (3.99, 3.33, and 2.66 kPa). At $P_{ET}CO_2$ of 25 and 20 mm Hg (3.33 and 2.66 kPa), mild symptoms included paresthesiae of lips and fingers, light-headedness, and irritability. These were tolerated well and resolved rapidly at the end of the experiment. Subjects easily maintained the respiratory effort necessary to hold $P_{ET}CO_2$ steady at each target level of hypocapnia, whereas the extra effort required to drop $P_{ET}CO_2$ to successive levels was required only briefly. All subjects remained calm and fully alert throughout.

Analysis

$P_{ET}CO_2$ was measured for every breath. Mean $P_{ET}CO_2$ was calculated for each subject for each minute at each target $P_{ET}CO_2$ to assess variation within and between subjects. Paired t-tests were used to compare control of $P_{ET}CO_2$ before and during vision testing. The distribution of the normocapnia flicker data was examined for normality and variability, and mean CFF was calculated from the 10 measurements for each subject, and across the sample group, for each respiratory condition. The influences on mean CFF of differences in individual sensitivity and of target levels of $P_{ET}CO_2$ were tested using two-way analysis of variance (ANOVA). To assess the consistency of any changes in CFF with decreasing $P_{ET}CO_2$ within, as opposed to between, subjects, the

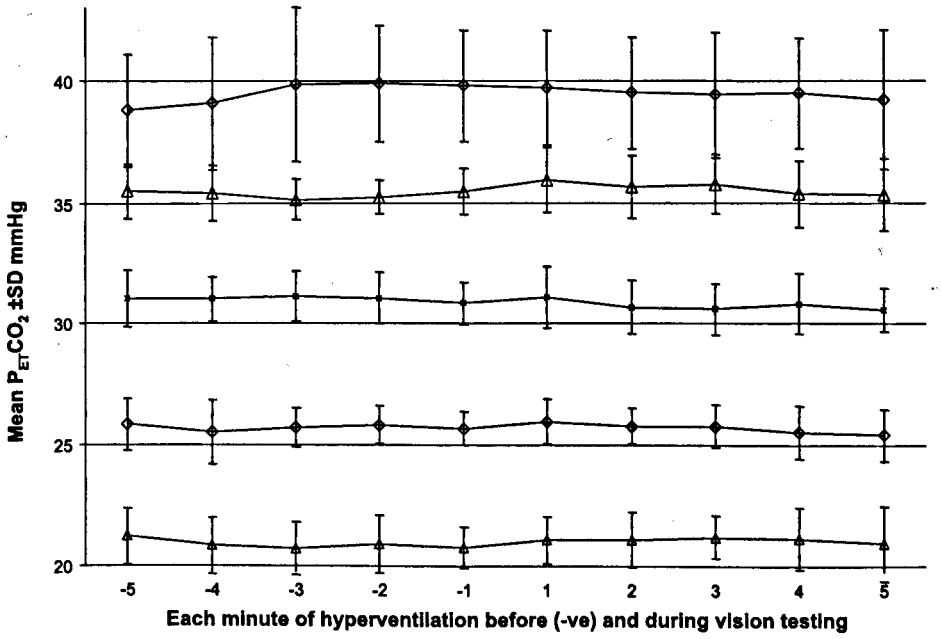


FIGURE 1.
Mean $P_{ET}CO_2$ between subjects for the 10 min at each target level of hypocapnia. The 5 min of hyperventilation before starting measurements of CFF are represented by negative values counting down on the abscissa. The 5 min from commencement of vision testing are then shown by positive values counting up. SD error bars have been used to emphasize the discrete nature of the five respiratory conditions.

direction and statistical significance of differences in mean CFF, between all possible combinations of two levels of hypocapnia, were examined using t-tests on data paired by individual subject. Finally, the relationship between group mean $P_{ET}CO_2$ and group mean CFF was examined using Pearson correlation.

RESULTS

One subject began hyperventilating involuntarily when wearing the mask, which is not uncommon. For the rest, group mean $P_{ET}CO_2$ during restful breathing was 39.4 (SD ± 2.2) mm Hg (5.24 ± 0.29 kPa), consistent with normocapnia. Group mean $P_{ET}CO_2$ for each of the 5 min before and 5 min during vision testing at each level of $P_{ET}CO_2$ are shown in Figure 1, indicating that five stable and discrete respiratory conditions were imposed. Mean $P_{ET}CO_2$ was calculated for each subject for the 5-min periods immediately before and after starting vision testing at each target $P_{ET}CO_2$. Paired t-tests on these data found no significant differences ($\alpha = 0.1$). Thus, vision testing did not compromise control of hyperventilation.

No normocapnia data were obtained from the subject who hyperventilated upon donning the mask, and another admitted difficulty making accurate flicker measurements, particularly under more severe hypocapnia, with rushed responses that suggested a criterion shift in reporting flicker. Those two subjects' data were excluded from further analysis. The normocapnia flicker data of the nine remaining subjects (90 measurements) were consistent with a normal distribution (Anderson–Darling test statistic 0.496, $p = 0.209$, $\alpha = 0.1$).

For each set of 10 measurements, each subject's mean CFF is shown against his or her mean $P_{ET}CO_2$ during those measurements in Figure 2, suggesting a general trend, more pronounced in some subjects than others, for CFF to increase as $P_{ET}CO_2$ falls. Regression lines for eight of the nine sets of data in Figure 2 exhibit slopes that support this trend. Between subjects, the mean increase in CFF between normocapnia and 20 mm Hg is only 1.2 cps. The variability of flicker sensitivity between subjects was far greater than within subjects, with each individual's results appearing to cluster at each level of hyperventilation when compared with the group as a whole. Two-way ANOVA distinguished the influences on mean CFF of individual sensitivity [$F(8,36) = 46.13$, $p < 0.001$] and target level of hypocapnia [$F(4,40) = 4.63$, $p = 0.005$].

A total of 10 two-tailed t-tests were conducted upon individuals' data paired for all possible combinations of two levels of hypocapnia. Those achieving statistical significance ($p < 0.05$) were from normocapnia to 20 mm Hg ($p = 0.01$); from normocapnia to 30 mm Hg ($p = 0.024$); from 35 to 30 mm Hg ($p = 0.043$); and from 35 to 20 mm Hg ($p = 0.024$). Transitions from normocapnia to 25 mm Hg and from 35 to 25 mm Hg approached statistical significance ($p < 0.1$). All support an increase in CFF with decreasing carbon dioxide tension, confirming the trend within individuals across the range of $P_{ET}CO_2$ studied. Only one of the 10 paired comparisons, from normocapnia to 35 mm Hg, suggested a decrease in mean CFF that was negligible (0.02 cps).

The results of two-way ANOVA and the consistency of the trend, within subjects, for CFF to increase with progressive hypocapnia together suggest that the net effect, between subjects, may be represented appropriately by plotting group mean CFF against group mean $P_{ET}CO_2$ (Fig. 3). The Pearson correlation coefficient of the regression line for these data is $R = -0.949$ ($p = 0.013$), suggesting a linear relationship between decreasing $P_{ET}CO_2$ and increasing CFF.

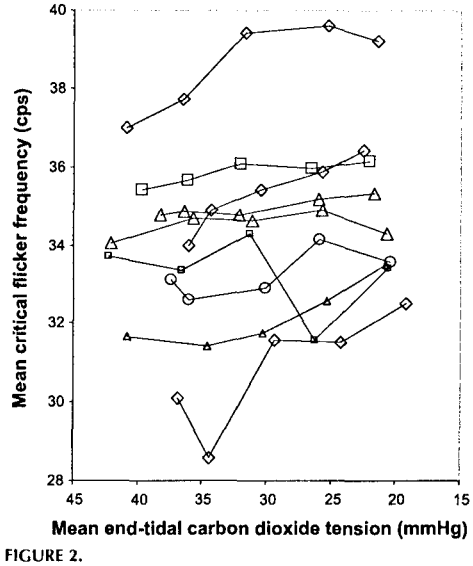


FIGURE 2.
Mean CFF at mean $P_{ET}CO_2$ for nine subjects. For eight of the nine sets of data, regression lines support a trend for CFF to increase with progressive hypocapnia. For each respiratory condition, the variability was considerably greater between than within subjects.

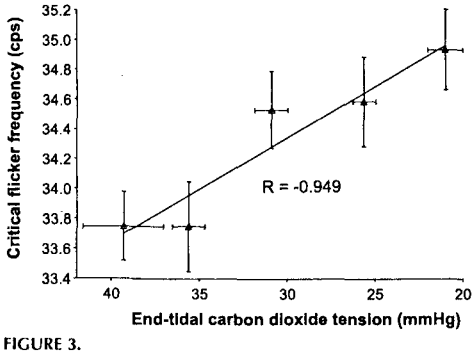


FIGURE 3.
Correlation of group mean CFF against group mean $P_{ET}CO_2$. Horizontal error bars are SD of the mean $P_{ET}CO_2$ between subjects ($n = 9$). Vertical error bars are SE of the mean of all 90 flicker and fusion measurements at each target level of hypocapnia, and reflect the wide variability between rather than within subjects.

DISCUSSION

Each target $P_{ET}CO_2$ may be expected to generate a discrete acid–base disturbance, with the magnitude of the resulting increase in whole blood pH bearing an approximately log linear relationship to the reduction in CO_2 tension, as modified by the concomitant change in plasma bicarbonate concentration.[12] In this study, each pH response during vision testing will be determined by the prevailing CO_2 tension rather than the duration of any preceding milder hyperventilation. It is possible that fatigue and symptomatic distraction may have influenced later measurements, but the linear nature of the group mean data (Fig. 3) suggests that any such effects are far outweighed by the response to changes in ventilation.

CFF measurements were unhurried and taken with care by the subjects whose data were analyzed, with none aware of any differences in judgment of CFF during the experiment. Even so, in adopting a method of limits approach, we cannot exclude the possibility of a criterion shift in the reporting of CFF that may have been related to the degree of hypocapnia. Nonetheless, we believe this to be unlikely and suggest that the results support a quantitative correlation between the severity of hypocapnia and the threshold for perception of flicker. The trend is apparent despite the small absolute magnitude of the effect and the substantial variability in underlying flicker sensitivity, particularly between subjects. However, for the mesopic stimulus used here, the scale of the effect can be regarded as unlikely to have any significance in aviation.

Sensitivity to flicker increases with retinal illumination and may be expected to change with fluctuations in pupil diameter. Relatively little has been published on the physiological effects of hypocapnia on pupil size. However, Gavriysky specifically examined the pupillogram under brief spells of moderate hyperventilation ($PaCO_2$ ~22 to 24 mm Hg). No effect on resting pupil size was reported, but hyperventilation increased latency time (delayed constriction), reduced constriction amplitude, and prolonged time to maximal constriction.[17] This tendency to pupillary dilatation under hypocapnia could enhance flicker sensitivity by increasing retinal illumination. From Table 1, this is unlikely to have confounded the current study. However, it remains possible that hypocapnic mydriasis might further enhance sensitivity to flicker under conditions where pupil size is uncontrolled, such that our results may understate the impact of hyperventilation on net flicker sensitivity, particularly for brighter and larger stimuli.

Hypoxia is known to reduce sensitivity to flicker.[18–20] The effect is apparent long before secondary hyperventilation occurs and is therefore independent of carbon dioxide tension. Hypocapnia is accompanied by an increase in alveolar oxygen tension that, ignoring water vapor, is approximated by the percentage fractional inspired oxygen concentration as a proportion of the change in $PaCO_2$. Nonetheless, the magnitude of the effect when breathing air (20.95% oxygen) at one atmosphere is negligible, being comparable to day-to-day fluctuations from changing meteorological conditions (barometric pressure). This cannot account for the observed effect of hyperventilation on flicker sensitivity as the resulting impact on arterial oxygen tension, hemoglobin oxygen saturation, and tissue oxygen delivery will be minimal.

Effects of hypoxia and hypercapnia to reduce flicker sensitivity have been attributed to acidosis,[21] and only those acid–base disturbances of respiratory, rather than metabolic, origin affect flicker sensitivity.[6] Arterial pH reaches a steady state soon after establishing a new $PaCO_2$.[12] The blood–brain and blood–retinal barriers are impermeable to hydrogen and bicarbonate ions but allow free and rapid diffusion of carbon dioxide, the only acid–base species so blessed. Hence, similarly, rapid cerebral and retinal tissue acid–base disturbances will result from hypocapnia and are most likely to mediate the effect on flicker sensitivity reported here.

Hyperventilation diminishes the a-wave and exaggerates the b-wave of the electroretinogram, reflecting outer retinal (photoreceptor) and inner retinal (bipolar cell) activity respectively.[22] The observation that retinal ganglion cell firing rate loses its "flickering" quality close to the electroretinogram and sensory fusion frequencies has also been advanced in support of a retinal mechanism for the effect of hyperventilation on flicker sensitivity.[8] Turner concluded that temporal adaptation to flicker was a central phenomenon that was preserved with hyperventilation, further supporting a retinal site of action.[9] However, alkalosis enhances neuronal excitability, alters synaptic transmission, and modifies nerve cell function,[23,24] whereas the rate at which the after-effects of hyperventilation on visual evoked responses resolve suggests an association with correcting acid–base balance.[17,25] Hypocapnia is most likely to modulate net flicker sensitivity through both retinal and central mechanisms. It is possible that this increase in flicker sensitivity may be accompanied by changes in other temporally-related aspects of vision, such as temporal integration, visual persistence, and motion perception.

About 50% of normal observers detect flicker up to 72 Hz on a cathode ray tube display with a mean luminance of 80 cd m^{-2} whereas 5% may do so at 87 Hz. This natural variability in flicker sensitivity between subjects was preserved during hyperventilation in the current study, and CFF is known to be greater, and to increase more with hyperventilation, using brighter and larger stimuli.[8] It remains possible that the most sensitive subjects may still be susceptible to flicker, under conditions of moderate to severe hypocapnia, when confronted with the most provocative visual stimuli. Accordingly, any future study of this phenomenon at photopic luminance should employ stimuli that are representative of the envisaged "worst case." If possible, CFF should be measured with and without control of retinal illumination and the pupillary response to hyperventilation should be documented. Consideration should also be given to the use of a breathing gas circuit, for optimal control of CO_2 tension, and to measurement of blood acid–base parameters.

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REFERENCES

1. Hinshaw HC, Rushmer RF, Boothby WM. The hyperventilation syndrome and its importance in aviation. J Aviat Med 1943;14: 100–4.

2. Gibson TM. Hyperventilation in aircrew: a review. Aviat Space Environ Med 1979;50:725–33.
3. Gibson TM. Hyperventilation in flight. Aviat Space Environ Med 1984;55:412–4.
4. Rubinstein B, Thurman PO. Influence of hyperventilation on the fusion frequency of intermittent visual stimuli. Skand Arch Physiol 1935;72:26–34.
5. Simonson E, Brozek J. Flicker fusion frequency; background and applications. Physiol Rev 1952;32:349–78.
6. Alpern M, Hendley CD. Visual functions as indices of physiological changes in the acid-base balance of the blood. Am J Optom Arch Am Acad Optom 1952;29:301–14.
7. Jorgensen V. The effect of hyperventilation on flicker fusion frequencies with foveal images of different sizes. Acta Ophthalmol (Copenh) 1955;33:615–21.
8. Granger GW, Ikeda H. Effect of hyperventilation on foveal critical flicker frequency. Vision Res 1961;1:287–300.
9. Turner P. The modification of critical flicker fusion frequency by an adapting stimulus of flickering light. Vision Res 1965;5:463–70.
10. Baer DJ. Hyperventilation effects on the critical flicker frequency of smokers and nonsmokers. J Gen Psychol 1967;76:201–6.
11. Connolly DM, Hosking SL. Aviation-related respiratory gas disturbances affect dark adaptation: a reappraisal. Vision Res 2006;46: 1784–93.
12. Lumb AB. Nunn's Applied Respiratory Physiology. Boston: Butterworth-Heinemann; 2000.
13. Wyszecki G, Stiles WS. Color Science: Concepts and Methods, Quantitative Data and Formulae, 2nd ed. New York: Wiley; 1982.
14. Boff KR, Lincoln JE. Engineering Data Compendium: Human Perception and Performance. Wright-Patterson Air Force Base, OH: Harry G. Armstrong Aerospace Medical Research Laboratory; 1988.
15. de Groot SG, Gebhard JW. Pupil size as determined by adapting luminance. J Opt Soc Am 1952;42:492–5.
16. Ginsburg N. Ascending-descending differences in CFF measurement as a function of eccentricity and starting point. J Psychol 1967;67: 161–7.
17. Gavriysky VS. Influence of a twofold voluntary hyperventilation on visually evoked cortical potentials and human pupillogram. Doc Ophthalmol 1991;77:213–24.
18. Seitz CP. The effects of anoxia on visual function; a study of critical flicker frequency. Arch Psychol 1940;257:38.
19. Birren JE, Fisher MB, Vollmer E, King BG. Effects of anoxia on performance at several simulated altitudes. J Exp Psychol 1946;36: 35–49.
20. Scow J, Krasno LR, Ivy AC. The immediate and accumulative effect on psychomotor performance of exposure to hypoxia, high altitude and hyperventilation. J Aviat Med 1950;21:79–81.
21. Simonson E, Winchell P. Effect of high carbon dioxide and of low oxygen concentration on fusion frequency of flicker. J Appl Physiol 1951;3:637–41.
22. Alpern M, Faris J, Eskildsen P, Garnett P. Effect of hyperventilation on the human electroretinogram. Science 1955;121:101–2.
23. Somjen GG, Tombaugh GC pH modulation of neuronal excitability and central nervous system functions. In: Kaila K, Ransom BR, eds. pH and Brain Function. New York: Wiley-Liss; 1998:373–93.
24. Chesler M. Regulation and modulation of pH in the brain. Physiol Rev 2003;83:1183–221.
25. Jensen O, Hari R, Kaila K. Visually evoked gamma responses in the human brain are enhanced during voluntary hyperventilation. Neuroimage 2002;15:575–86.
26. Bauer D, Bonacker M, Cavonius CR. Frame repetition rate for flicker-free viewing of bright VDU screens. Displays 1983;4:31–3.

Desmond Connolly
QinetiQ
Human Protection and Performance Enhancement Group
Cody Technology Park
Farnborough GU14 0LX
United Kingdom
e-mail: dmconnolly@QinetiQ.com

Mild Hypoxia Impairs Chromatic Sensitivity in the Mesopic Range

Desmond M. Connolly,[1,2] *John L. Barbur,*[2] *Sarah L. Hosking,*[2] *and Ian R. Moorhead*[1]

Purpose. The effect of mild hypoxia on chromatic sensitivity in the mesopic range is poorly documented. This study was conducted to examine the effects of mild hypoxia and hyperoxia on red-green (R-G) and yellow-blue (Y-B) chromatic sensitivity thresholds at low photopic (22.3 cd · m^{-2}), borderline upper mesopic (1.67 cd · m^{-2}) and mid-mesopic (0.21 cd · m^{-2}) luminance.

Methods. The Color Assessment and Diagnosis (CAD) test was used to measure binocular and monocular R-G and Y-B chromatic sensitivity by using dynamic luminance contrast noise to isolate the use of color signals. Mild hypoxia was imposed by breathing 14.1% oxygen and was investigated relative to control exposures breathing air (normoxia) at each light level. Subsequently, hyperoxia, breathing 100% oxygen, was assessed relative to hypoxia under the mesopic conditions. A balanced, repeated-measures design allowed assessment of main effects and interactions of light level, viewing condition, gender, breathing gas, and exposure order by using multivariate analysis of variance (MANOVA), with post hoc analysis employing ANOVA and paired *t*-tests.

Results. Light level, number of viewing eyes, and oxygenation state were significant determinants of chromatic sensitivity. One man and one woman introduced orthogonal sources of gender bias. The CAD test revealed minimal deuteranomaly (R-G deficiency) in the man and loss of Y-B sensitivity in the only woman using hormonal contraception.

Conclusions. In the mesopic range, mild hypoxia impairs chromatic sensitivity progressively with reducing luminance. Binocular summation of chromatic signals is consistent and independent of the luminance channel. The CAD test is highly sensitive to mild congenital and acquired color vision deficiencies. (*Invest Ophthalmol Vis Sci.* 2008;49:820–827) DOI:10.1167/iovs.07-1004

In aviation, minimum color vision requirements are essential for distinguishing colors critical for air navigation, precision approach, warning, and emergency purposes.[1,2] However, the increasing use of color is placing ever greater demands on aircrew color vision, not least with regard to in-flight use of maps, manuals, and contemporary electronic flight displays. This trend is likely to continue, as color increases target conspicuity and enhances visual performance in coding or grouping and segmentation tasks.[3] Thus, the use of color coding enhances visual performance and can reduce operator error.[4,5]

Atmospheric air comprises 20.95% oxygen by volume, whereas barometric pressure falls almost exponentially with increasing altitude, and so hypobaric (low pressure) hypoxia (oxygen deficiency) is an inevitable consequence of breathing air at ambient pressure during ascent. Investigators in various studies have examined color vision during hypoxia, to deduce the effects of acute altitude exposure, employing a variety of visual stimuli and methodologies.[6–11] The results have not always been consistent, but it is generally accepted that moderate hypoxia, at equivalent altitudes above 3,048 m (10,000 ft), can produce impairment of color discrimination that varies with the level of light adaptation and may be more pronounced in the visual and retinal periphery, where rod photoreceptor density is high.

Retinal phototransduction processes require considerable metabolic energy, for rhodopsin phosphorylation, synthesis of cyclic guanosine monophosphate, chromophore transport, and support of enzyme activity.[12] In the dark, rod photoreceptor oxygen uptake increases dramatically to support the ion pumps that maintain the "dark current," consuming more oxygen than any function of any other cell.[13,14] In consequence, the retinal partial pressure of oxygen (Po_2) falls to remarkably low levels near photoreceptor inner segments and may even compromise oxidative phosphorylation in inner segment mitochondria.[15–19] Furthermore, breathing 100% oxygen (hyperoxia), rather than air, hastens the onset of scotopic sensitivity during dark adaptation, implying that rods may even be functionally hypoxic in the dark in normal respiratory and barometric conditions.[20]

During night flights, aircraft cockpits and flight decks are illuminated typically at mesopic levels, and so normal color vision is degraded.[21,22] In dim light, rod-driven retinal hypoxia may be anticipated to compromise cone oxygenation and function and to render threshold color sensitivity vulnerable to further exogenous hypoxia—specifically, the hypobaric hypoxia that results from altitude exposure. Progressive reduction in luminance would be expected to exaggerate the impairment. We report the results of two studies designed to investigate the effects of respiratory disturbance on mesopic color sensitivity. In study 1, we compared the effects of mild hypoxia (breathing 14.1% oxygen in nitrogen) with those of normal air (normoxia control) in subjects at low photopic, upper mesopic, and mid-mesopic luminances. In study 2, responses to hyperoxia (breathing 100% oxygen) were compared with those to hypoxia (14.1% oxygen control) at upper and mid-mesopic luminances. For convenience, physiological gas pressures are reported as measured (in millimeters of mercury), with SI units (in kilopascals) in parenthesis in the text.

Methods

Subjects

The work adhered to the principles of the Declaration of Helsinki, the study protocol was approved in advance by an independent Local Research Ethics Committee, and all subjects provided written informed consent before participating. Twelve healthy volunteers (six men and

From [1]QinetiQ, Farnborough, United Kingdom; and the [2]Applied Vision Research Centre, The Henry Wellcome Laboratories for Vision Sciences, City University, London, United Kingdom.

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Corresponding author: Desmond M. Connolly, QinetiQ, Cody Technology Park, Ively Road, Farnborough, Hampshire GU14 0LX, UK; dmconnolly@qinetiq.com.

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six women) completed each experimental condition. However, one female subject withdrew from the study due to relocation and was replaced. The mean age of the seven women was 26.1 years (range, 22–34) and of the six men was 31.7 years (range, 24–39).

Comprehensive medical screening ensured fitness to participate and included a medical questionnaire, detailed physical examination, electrocardiogram, and urinalysis to exclude glycosuria. Ophthalmic screening included a detailed ophthalmic history, external and funduscopic examination, and assessments of near and distant visual acuity, accommodation, convergence, visual fields, ocular movements and alignment, and pupillary reactions. All subjects were assessed as having normal trichromatic color vision by an experienced aviation medical examiner using the first 17 Ishihara pseudoisochromatic plates and by a postdoctoral vision scientist using a Nagel type I anomaloscope. Neither examiner was involved in the subsequent study. The Snellen acuity of all test eyes was 6/6 or better, using untinted corrective spectacles (two women) or contact lenses (one man), as necessary. The ophthalmic prescriptions for these subjects were unchanged throughout the study.

Subjects were nonsmokers who were asked to avoid alcohol for 24 hours before and caffeine on the day of an experiment. Subjects were masked to the presentation order of the breathing gases, and these were randomized such that three men and three women undertook each possible exposure order for each luminance condition. All subjects were familiar with breathing from pressure-demand oxygen regulators through aircrew oxygen masks. The women took a urine test to exclude pregnancy before each experiment.

Respiratory and Monitoring Equipment

Each subject was fitted with a modified U.K. Royal Air Force Aircrew Respirator Mk 5 (AR5), comprising an appropriately sized oxygen mask (over mouth and nose) with an attached polycarbonate visor and butyl rubber cowl, covering the head, neck, and shoulders. The neck seal was removed for comfort, and the helmet suspension harness was not needed. The mask antisuffocation valve was sealed to exclude light, but the microphone was retained for the same reason. The bulk of the visor was cut away to leave a residual scaffold on which matching polycarbonate scaffolds could be mounted. These supported neutral density (ND) gelatin filters (Wratten; Eastman Kodak, Rochester, NY) of either 1 or 2 optical density units. A port to the mask cavity allowed breathing gas composition to be analyzed continuously with a mass spectrometer (MGA 2000; Airspec, Kent, UK). The AR5 allowed ambient air to be blown gently and independently of the breathing gas supply, across the facial aspect of the ND filters, ensuring that they remained free from misting. Hence, normal corneal oxygenation was preserved, regardless of respiratory condition, and subjects could wear normal corrective spectacles and lenses, as required. Thus, the AR5 enabled total, contemporaneous control of visual and respiratory adaptation state.

Each of the three breathing gases (air, oxygen, and the hypoxic mixture) was supplied to its own dedicated, pressure-demand, breathing gas regulator at a nominal supply pressure. The regulators had identical pressure–flow characteristics and imposed minimal breathing resistance, making them indistinguishable to the user, and their breathing gases were delivered, via a selection tap, to a common mask hose. Mask valves prevented rebreathing of expired gas, and the mass spectrometer traces were monitored to ensure that good face–oxygen mask seals were maintained. Mass spectrometer calibrations were conducted immediately before and after each respiratory condition by using various gas mixtures of known composition, giving a measurement error of less than 1% for physiological partial pressures of oxygen and carbon dioxide. Noninvasive monitoring of blood pressure, heart rate, and oxygen saturation were undertaken with a blood pressure monitor (Finapres 2300; Ohmeda, Englewood, NJ) and a pulse oximeter (7840; Kontron Instruments, Ltd.; Watford, UK) with finger probe. Analog outputs from both devices were calibrated and recorded, together with the mass spectrometer data, using a PC-based data recording and analysis system with software (Powerlab/Chart software; ADInstruments, Castle Hill, NSW, Australia).

Vision Test Equipment and Stimuli

Vision testing was conducted binocularly and then monocularly (dominant eye) using the Color Assessment and Diagnosis (CAD) test.[23,24] Randomly interleaved staircases were used with variable step sizes and a four-alternative, forced-choice procedure, to measure threshold chromatic sensitivity along eight color directions in the CIE 1931 (x, y) chromaticity chart. The uniform background field was approximately equivalent to daylight at a color temperature of 6500 K, with x, y chromaticity of 0.305, 0.323, respectively. The directions of chromatic displacement away from background chromaticity were chosen to maximize the output of either the red-green (R-G) or yellow-blue (Y-B) color channels. Thus, the cone-contrast signal generated by the test stimuli in S-cones is approximately zero along the R-G axis whereas the M- and L-cone contrast signals are approximately zero along the Y-B axis.

The CAD test stimulus is generated in the center of a large, uniform background field (30° × 24°) and comprises a square array of 15 × 15 achromatic checkers subtending a horizontal visual angle of ~3.3°. The uniform background provides a steady state of light adaptation while the luminance of each checker scintillates rapidly, independently, and randomly above and below background, to generate achromatic, dynamic, luminance contrast noise. The function of the noise is to mask the detection of residual luminance contrast signals in the "isoluminant" colored stimulus, a condition that does not affect significantly the threshold for detection of the color signal.[25] The color-defined stimulus comprises 5 × 5 checkers (~1.1°) moving diagonally across the checkerboard, along one of four possible directions. The subject's task is to indicate the direction of stimulus movement by pressing one of four buttons arrayed in a square, with each button corresponding to a destination corner on the checkerboard. The chance probability of a correct response is reduced to 1 in 16 by requiring the subject to report the correct direction of stimulus motion twice before the strength of the color signal is reduced according to the interleaved staircase procedure.

The stimulus for the CAD test was generated on a CRT display (Trinitron Multiscan G520 monitor; Sony, Tokyo, Japan) calibrated for both luminance and chromaticity with a luminance photometer (LMT, model 1009; Lichtmesstechnik, Berlin, Germany) and a telespectroradiometer (model CS1000; Minolta, Osaka, Japan). The luminance calibration was repeated automatically at regular intervals by using the internal calibration program. Subjects positioned a notch in the expiratory port of the oxygen mask against a chin rest, viewing the display screen along a matt-black viewing tunnel from a distance of 70 cm. A central fixation point was provided, and the subjects were familiarized with the test before starting the study.

Respiratory and Viewing Conditions

At 1 atmosphere, breathing 14.1% oxygen with a balance of nitrogen simulates mild hypobaric hypoxia by imposing an alveolar partial pressure of oxygen (P_AO_2) of ~60 mm Hg (8 kPa). This approximates the P_AO_2 achieved when breathing air at an altitude of 3,048 m (10,000 ft) and lowers arterial hemoglobin oxygen saturation (S_AO_2) to ~90%. The use of supplementary oxygen by aircrew is generally regarded as unnecessary below this altitude. It is therefore pertinent to establish the possible effects that this "acceptable" level of hypoxia may have on visual performance challenges relevant to aviation. In study 1, the effect of hypoxia on chromatic sensitivity was established relative to normoxia, viewing the display directly (low photopic, 22.3 cd · m $^{-2}$), and, on subsequent visits, through ND 1.0 (borderline upper mesopic, 1.67 cd · m $^{-2}$) and ND 2.0 (mid-mesopic, 0.21 cd · m $^{-2}$) filters. Study 2 repeated the mesopic experiments to investigate the potential benefits on chromatic sensitivity of breathing supplementary 100% oxygen, relative to performance during mild hypoxia. Each respiratory

condition was imposed for 15 minutes, to achieve a respiratory steady state before commencing vision testing.

Experimental Procedure

The subject was seated at rest and adapted to the low ambient light level (1–2 lux). The AR5 was fitted and the subject breathed room air through the mask hose. A halter was worn, supporting the breathing gas supply hose and the blower for the de-mist air supply. The subject was prepared for physiological monitoring, and the mass spectrometer sampling line was connected to the mask. Concurrent visual and respiratory adaptation were then undertaken with the purpose of establishing unambiguous, stable and procedurally reproducible adaptation states, within and between subjects. The process was based on a dark-adaptation regimen that measured time to scotopic sensitivity under analogous conditions of respiratory disturbance, reported previously.[20] Five minutes of light adaptation were undertaken by viewing directly, with natural pupils, a light box with a peak mean luminance of 735 cd · m^{-2}. Subsequently, 15 minutes of dark adaptation commenced with application of the appropriate polycarbonate visor frame and filter, together with an opaque material cover, and gentle blown air was supplied from ambient air to prevent filter misting. After 15 minutes the material cover was removed to allow 5 minutes of light adaptation while viewing the test display either directly or through the relevant ND filter. Five minutes into dark adaptation, the breathing gas supply hose was connected to the mask hose to begin 15 minutes of respiratory adaptation, so that vision testing began on completing 25 minutes of visual adaptation and 15 minutes of concurrent respiratory adaptation. Tests of achromatic spatial contrast sensitivity, in matching background viewing conditions, preceded the CAD tests. The CAD tests commenced after approximately 30 minutes of respiratory exposure, to within a minute or so and occupied a total of ~20 minutes. After binocular testing, an opaque occluder was placed in front of the nondominant eye and the CAD test was repeated monocularly. Further unrelated vision testing occupied another 10 minutes or so, after which the subject rested for 15 minutes before repeating the entire procedure in the alternate respiratory condition.

Analysis

Cardiovascular and respiratory parameters were recorded continuously throughout all exposures. The significance of differences in physiological parameters between respiratory conditions was assessed using paired *t*-tests on within-subject data. Chromatic signals for threshold detection of color-defined motion were measured along each of the eight color directions. For both studies, these thresholds were treated as eight related, dependent variables in multivariate analysis of variance (MANOVA), assessing main effects and interactions of light level, viewing condition (binocular or monocular), gender, breathing gas and respiratory exposure order ($\alpha = 0.05$). Post hoc analysis was conducted by using a variety of ANOVA techniques and paired *t*-tests. All analyses were conducted on computer (Minitab 14 software; Minitab, State College, PA). Mean R-G and Y-B axes were calculated for each respiratory condition in relation to "standard normal" CAD units.[24,26] Mean threshold data are represented in graphs of CIE 1931 (x, y) color space using group mean (x, y) coordinates for each color direction. Increases in individual thresholds may appear small when represented in this way. However, it should be considered that the consequent and disproportionate increases in area of the color ellipses defined by increases in R-G and Y-B axis lengths may represent substantial losses of net color sensitivity.

Cardiorespiratory Parameters

The inspired Po_2 (Pio_2), end tidal Po_2 ($P_{ET}O_2$), end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), peripheral S_AO_2, heart rate, and systolic, diastolic, and mean blood pressures were measured during all exposures and remained stable during vision testing. Mean (±SE) respiratory responses between subjects are shown for both studies in Table 1.

TABLE 1. Respiratory Parameters Imposed during Studies 1 and 2

	Normoxia	Hypoxia
Study 1: Comparing Hypoxia (14.1% Oxygen) with Normoxia Control		
Direct viewing (22.3 cd · m^{-2})		
Gas tension (mmHg)		
Pio_2	156 (0.7)	108 (0.5)
$P_{ET}O_2$	107 (1.2)	60 (0.9)
$P_{ET}CO_2$	36.7 (0.5)	37.5 (0.5)
S_AO_2	97.7 (0.3)	90.6 (0.4)
ND 1.0 filter (1.67 cd · m^{-2})		
Gas tension (mm Hg)		
Pio_2	157 (0.6)	107 (0.5)
$P_{ET}O_2$	109 (1.1)	60 (1.1)
$P_{ET}CO_2$	38.0 (0.7)	38.9 (0.6)
S_AO_2 (%)	97.9 (0.3)	91.3 (0.4)
ND 2.0 filter (0.21 cd · m^{-2})		
Gas tension (mm Hg)		
Pio_2	155 (0.7)	105 (0.6)
$P_{ET}O_2$	108 (1.2)	58 (1.0)
$P_{ET}CO_2$	38.2 (1.1)	38.2 (1.1)
S_AO_2 (%)	98.3 (0.3)	91.0 (0.6)
Study 2: Comparing Hyperoxia (100% Oxygen) with Hypoxia Control (14.1% Oxygen)		
ND 1.0 (1.67 cd · m^{-2})		
Gas tension (mm Hg)		
Pio_2	744 (4.8)	105 (0.6)
$P_{ET}O_2$	681 (4.9)	58 (1.5)
$P_{ET}CO_2$	35.0 (0.8)	39.1 (1.1)
S_AO_2 (%)	99.7 (0.2)	91.5 (0.6)
ND 2.0 filter (0.21 cd · m^{-2})		
Gas tension (mmHg)		
Pio_2	740 (5.4)	105 (0.7)
$P_{ET}O_2$	681 (5.1)	59 (1.4)
$P_{ET}CO_2$	33.2 (0.9)	38.0 (1.2)
S_AO_2 (%)	99.8 (0.1)	91.8 (0.7)

Data are expressed as the mean ± SE (1 kPa = 7.501 mm Hg). The imposed changes in gas tension and resultant S_AO_2 were closely controlled and stable throughout. The hypoxia exposures were highly reproducible.

The respired gas tensions and S_AO_2 data show that the intended respiratory conditions were achieved and were closely controlled. There was no effect of mild hypoxia to induce secondary hyperventilation. However, hyperoxia was accompanied by a slight but unequivocal, consistent, and highly statistically significant ($P < 0.001$) reduction in $P_{ET}CO_2$ by approximately 5 mm Hg (~0.7 kPa). This appears paradoxical but is attributable to the Haldane effect, whereby the enhanced oxygenation of hemoglobin in venous blood reduces carriage of carbon dioxide from the tissues in the carbamino form.[27,28] This has not confounded interpretation of the effects of altered oxygen tension on threshold color sensitivity.

The group mean cardiovascular responses, including derived pulse pressure (systolic minus diastolic), are shown for both studies in Table 2. The results suggest a slight tendency for blood pressures to increase under mild hypoxia, but no increase achieved statistical significance. However, a statistically significant increase in heart rate with hypoxia was consistent with expectations.[20]

RESULTS

Derived mean (±SE) Y-B and R-G axis lengths are shown in Table 3 with reference to the standard normal CAD observer (CAD units). However, the data for one man and one woman

TABLE 2. Cardiovascular Parameters Recorded during Studies 1 and 2

	Normoxia	Hypoxia
Study 1: Comparing Responses to Hypoxia (14.1% Oxygen) with Normoxia Control		
Direct viewing (22.3 cd · m $^{-2}$)		
Blood pressures (mm Hg)		
Systolic	120 (3.9)	128 (7.1)
Diastolic	68 (3.7)	70 (3.8)
Mean	85 (3.6)	89 (4.7)
Pulse	52 (2.9)	58 (4.6)
Heart rate (min $^{-1}$)	65 (1.8)	69 (2.1)
ND 1.0 (1.67 cd · m $^{-2}$)		
Blood pressures (mm Hg)		
Systolic	117 (2.9)	121 (2.9)
Diastolic	69 (2.6)	70 (2.5)
Mean	85 (2.5)	87 (2.3)
Pulse	48 (2.5)	51 (2.4)
Heart rate (min $^{-1}$)	72 (2.9)	75 (3.2)
ND 2.0 (0.21 cd · m $^{-2}$)		
Blood pressures (mm Hg)		
Systolic	117 (4.0)	120 (4.5)
Diastolic	66 (3.5)	65 (3.4)
Mean	83 (3.5)	84 (3.6)
Pulse	51 (2.5)	55 (2.7)
Heart rate (min $^{-1}$)	65 (2.8)	68 (2.9)
Study 2: Comparing Responses to Hyperoxia (100% Oxygen) with Hypoxia Control (14.1% Oxygen)		
ND 1.0 (1.67 cd · m $^{-2}$)		
Blood pressures (mm Hg)		
Systolic	116 (3.8)	118 (2.7)
Diastolic	68 (3.0)	67 (2.6)
Mean	84 (3.1)	84 (2.4)
Pulse	47 (2.0)	51 (2.2)
Heart rate (min $^{-1}$)	63 (3.3)	72 (3.8)
ND 2.0 (0.21 cd · m $^{-2}$)		
Blood pressures (mm Hg)		
Systolic	123 (4.4)	132 (2.4)
Diastolic	69 (4.5)	71 (2.8)
Mean	87 (4.3)	91 (2.4)
Pulse	54 (2.1)	61 (2.5)
Heart rate (min $^{-1}$)	63 (2.7)	72 (3.2)

Date are expressed as the mean ± SE. Cardiovascular status remained stable during all exposures. The slight increase in heart rate during hypoxia was statistically significant for direct viewing in study 1 ($P < 0.01$) and for both exposures in study 2 ($P < 0.001$). The slight tendency for systolic and pulse pressures to increase with hypoxia was not statistically significant. Cardiovascular status remained essentially stable between respiratory conditions.

are presented separately from those of the remaining 10 normal trichromats as these two subjects appear to contribute to gender bias, discussed later. The binocular, photopic CAD data of the 10 normal trichromats, breathing air, are consistent with those of the standard normal CAD observer.[24,26] The mean (±SE) Y-B and R-G thresholds in study 1 are shown in Figure 1, and those in study 2 are in Figure 2. Unless indicated otherwise, the following probabilities are from the initial balanced, repeated-measures MANOVA conducted on the data from all 12 subjects.

A main effect of light level is unambiguous for both study 1 ($P < 0.001$) and study 2 ($P < 0.001$) and is self-evident in Figures 1 and 2 for all viewing and respiratory conditions.

The number of viewing eyes was statistically significant in both study 1 ($P < 0.001$) and study 2 ($P = 0.011$), such that monocular viewing impaired chromatic sensitivity along both the Y-B and R-G axes. The effect is illustrated in CIE 1931 (x, y) color space in Figure 3, using just the normoxia data from study 1. A statistically significant interaction between light level and viewing condition was seen ($P < 0.05$), such that the monocular loss of color sensitivity increases in absolute value with decreasing luminance.

A statistically significant main effect of breathing gas was achieved in both study 1 ($P = 0.001$) and study 2 ($P = 0.002$). Considering study 1 (Fig. 1), hypoxia clearly impairs chromatic sensitivity at the lowest light level, viewing through the ND 2.0 filter. Y-B and R-G axis lengths are compromised progressively by mild hypoxia alone, by monocular viewing alone, and by viewing monocularly when hypoxic. In contrast, there was no unambiguous effect of breathing gas at photopic luminance. At the intermediate, upper mesopic luminance the data for some color directions suggest a tendency in favor of hypoxic impairment. A statistically significant interaction between light level and breathing gas ($P < 0.01$) supports a progressive effect of hypoxia with reducing luminance, reinforced by post hoc paired t-tests on the data obtained at each of the three light levels.

In study 2, a consistent benefit of breathing 100% oxygen over hypoxia on R-G and Y-B sensitivity was apparent at both light levels when viewing binocularly and monocularly (Fig. 4). Although not always achieving statistical significance at $\alpha = 0.05$, post hoc analysis on study 2 data subsets, disaggregated by light level and/or number of viewing eyes, indicate a consistent trend for impairment under hypoxia.

A statistically significant main effect of respiratory exposure order was seen in study 1 ($P = 0.01$) but not study 2 ($P = 0.717$). The respiratory exposures were controlled closely (Table 1) and test–retest variability on the CAD test is small, so neither explains the order effect. The color direction data were examined by exposure order and light level, revealing a slight but consistent tendency for overall chromatic sensitivity to be worse when hypoxia preceded normoxia than vice versa. This suggests that preceding hypoxia may have compounded possible fatigue or inattention in subsequent normoxic exposures, whereas the alerting effect of supplementary oxygen would mitigate such an effect of exposure order in study 2.

A main effect of gender was seen in study 1 ($P = 0.016$) but not in study 2 ($P = 0.1$). Two consistent trends were apparent when comparing thresholds in the male and female participants for all color directions at each luminance, with the data sorted by gender, viewing eyes, and breathing gas: the R-G axes tended to be longer in the men, whereas the Y-B axes were longer in the women. Two systematic and orthogonal sources of gender bias were apparent when considering individual responses, introduced by one male and one female subject. The man had marginally but consistently poorer R-G sensitivity than his peers, whereas the woman had poorer Y-B sensitivity than hers. Post hoc ANOVA conducted on each threshold axis in both studies 1 and 2 revealed consistent effects of gender only along the 60° to 240° Y-B axis. The results for both subjects were removed from the data for study 1, and a further MANOVA was performed. There was no longer a statistically significant effect of gender ($P = 0.291$), but main effects of light level ($P < 0.001$), viewing condition ($P < 0.001$), and breathing gas ($P = 0.004$) remained, together with the interactions identified previously.

DISCUSSION

Both R-G and Y-B thresholds are impaired significantly at low light levels, with Y-B thresholds affected the most. Consistent with previous findings,[21,26] an asymmetry in Y-B thresholds is apparent at the lowest light level, with yellow thresholds,

TABLE 3. R-G and Y-B Chromatic Sensitivity Expressed in CAD Units

Light Level (cd · m^{-2})	Number of Viewing Eyes	Respiratory Condition	R-G Thresholds (CAD Units) 10 Normal Trichromats Mean (SD)	R-G Thresholds (CAD Units) Man with Minimal Deuteranomaly	R-G Thresholds (CAD Units) Woman Using Hormonal Contraception	Y-B Thresholds (CAD units) 10 Normal Trichromats Mean (SD)	Y-B Thresholds (CAD units) Man with Minimal Deuteranomaly	Y-B Thresholds (CAD units) Woman Using Hormonal Contraception
Direct viewing (22.3)	Binocular	Normoxia	1.14 (0.19)	1.79	1.01	0.99 (0.24)	0.77	1.12
		Hypoxia	1.06 (0.18)	1.42	0.95	0.95 (0.20)	0.87	1.02
	Monocular	Normoxia	1.36 (0.30)	1.78	0.90	1.24 (0.27)	0.79	1.30
		Hypoxia	1.37 (0.33)	1.80	1.39	1.25 (0.33)	0.98	1.66
ND 1.0 (1.67)	Binocular	Normoxia	1.99 (0.47)	2.67	1.64	2.64 (0.85)	1.64	3.06
		Hypoxia	2.00 (0.60)	2.94	1.56	2.68 (0.69)	2.09	3.37
	Monocular	Normoxia	2.29 (0.80)	2.42	2.03	3.37 (0.87)	2.19	4.40
		Hypoxia	2.52 (0.99)	2.82	1.95	3.29 (0.83)	2.20	3.49
ND 2.0 (0.21)	Binocular	Normoxia	3.78 (1.20)	6.03	4.68	7.74 (2.11)	9.42	12.82
		Hypoxia	4.32 (1.58)	7.67	4.42	9.21 (3.03)	9.99	11.92
	Monocular	Normoxia	5.07 (1.92)	6.83	4.99	10.45 (2.77)	10.17	10.98
		Hypoxia	6.15 (2.44)	8.64	8.26	10.55 (2.97)	11.41	15.77
ND 1.0 (1.67)	Binocular	100% Oxygen	2.51 (2.13)	2.50	1.75	2.85 (0.90)	1.98	2.86
		Hypoxia	3.03 (1.85)	2.81	3.26	3.54 (1.39)	2.08	5.80
	Monocular	100% Oxygen	2.17 (0.54)	2.45	1.62	3.30 (1.01)	2.10	3.62
		Hypoxia	3.93 (2.89)	2.97	5.37	4.60 (1.63)	3.02	6.21
ND 2.0 (0.21)	Binocular	100% Oxygen	3.48 (1.04)	5.79	3.42	7.77 (2.03)	7.51	8.16
		Hypoxia	4.09 (1.30)	6.43	7.42	9.07 (2.81)	8.68	12.60
	Monocular	100% Oxygen	4.96 (2.25)	5.71	4.34	10.06 (2.77)	8.09	10.20
		Hypoxia	5.58 (1.61)	8.10	10.33	11.35 (3.07)	14.36	10.45

One CAD unit is the mean threshold chromatic sensitivity for the "standard normal CAD observer," viewing binocularly at photopic luminance under normoxic conditions, established by testing a large number of normal trichromatic subjects.[24,26] Note the substantial standard deviations of the normal trichromats, which reflect considerable within- and between-subject variability in response in the different conditions. Where coefficients of variation exceed 35% the outlying (poor performance) data points were contributed inconsistently by half of the 10 trichromatic subjects. Comparison of the trichromats' mean responses with those of the mildly deuteranomalous male and the female with tritan deficiency reveals the consistently orthogonal nature of their confounding influences under almost all conditions, explaining the apparent gender effect seen with initial balanced MANOVA.

corresponding to S-cone decrements, compromised more than the complementary blue thresholds that correspond to S-cone increments (Fig. 5). This suggests an asymmetry for increments and decrements in S-cone signals at low light levels, supporting a greater metabolic demand of the S-cone system and a possible role of hypoxia in the etiology of disease-induced tritan defects.[29,30]

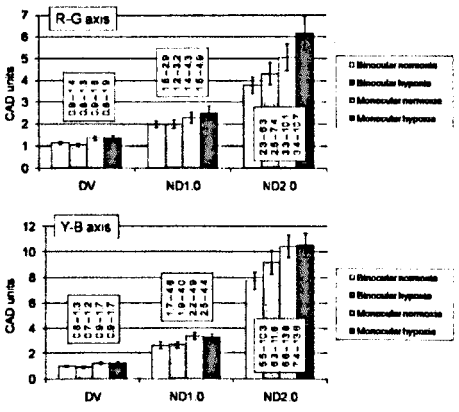


FIGURE 1. Mean ± SE Y-B and R-G thresholds of 10 normal trichromats in study 1, viewing directly (DV; 22.3 cd · m^{-2}) and through ND 1.0 (1.67 cd · m^{-2}) and 2.0 (0.21 cd · m^{-2}) filters. The range for each data set is shown, illustrating the considerable overlap between conditions.

Thresholds for color detection were consistently lower in binocular than in monocular viewing. As the chromatic stimuli were presented against luminance contrast noise, this benefit of binocular viewing relates specifically to improved discrimination of the color signal. This finding conflicts with one recent report[31] but is in accord with others.[32–35] For normal trichromats, R-G thresholds in Table 1 approximate the semiminor axes of corresponding color ellipses and the Y-B thresholds approximate the semimajor axes. Thus, while monocular axes may not appear much longer than binocular axes, they may represent substantial increases in ellipse area and considerable net loss of color sensitivity. Although the difference does not appear significant in Figure 3, the calculated ellipse area of the normal trichromats when viewing monocularly rather than binocularly increased by 67% at 22.3 cd · m^{-2}.

The man with deuteranomaly passed the screening tests for the study but, unfortunately, his Nagel anomaloscope matching range data were not recorded. However, records from an unpublished previous study indicate that he made repeated errors on Ishihara testing some years previously. His baseline R-G threshold of 1.79 CAD units was just within the range consistent with minimal deuteranomaly.[24,26] At photopic and upper mesopic luminance his R-G sensitivity was marginally worse than that of normal trichromats, but his Y-B sensitivity was somewhat better. However, at the lowest light level, his sensitivity to yellow deteriorated dramatically (Fig. 6). Despite

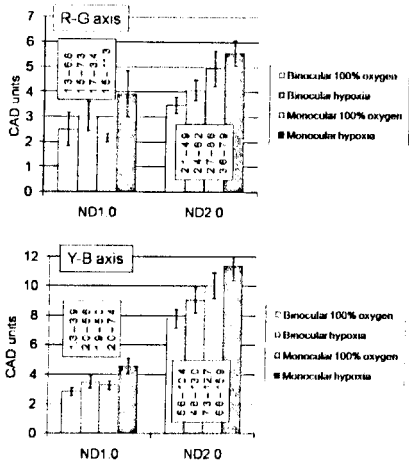


FIGURE 2. Mean ± SE Y-B and R-G thresholds of 10 normal trichromats in study 2, viewing through ND 1.0 (1.67 cd · m $^{-2}$) and ND 2.0 filters (0.21 cd · m $^{-2}$). The range for each data set is shown. Performance breathing 100% oxygen is comparable to that breathing air in study 1. Performance under hypoxia varies from that in study 1 and is markedly worse for the ND 1.0 condition.

near-normal photopic and upper mesopic color sensitivity, he manifests a specific loss of sensitivity to yellow at mid-mesopic luminance. Such responses call into question the suitability of conventional color tests for determining the functional acceptability of color vision at reduced luminance.

The woman with tritan deficiency exhibited the most severe Y-B loss at mid-mesopic luminance, particularly to blue. She was the only woman using hormonal contraception, known to influence color vision.[2] Figure 6 illustrates how the

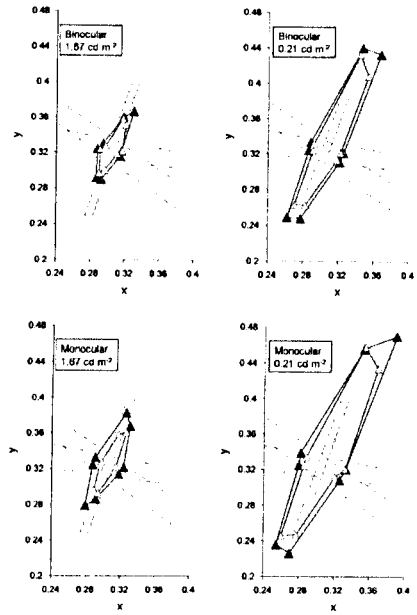


FIGURE 4. Effect of 100% oxygen (*white symbols*) consistently to optimize chromatic sensitivity along all eight color directions in CIE 1931 *x*, *y* color space, relative to mild hypoxia (*gray symbols*), at both upper and mid-mesopic luminance (n = 12). Use of the same *x*, *y* scales allows direct comparison of the data by light level. Small increases in R-G and Y-B axis lengths under hypoxia may represent substantial increases in the corresponding color ellipse area.

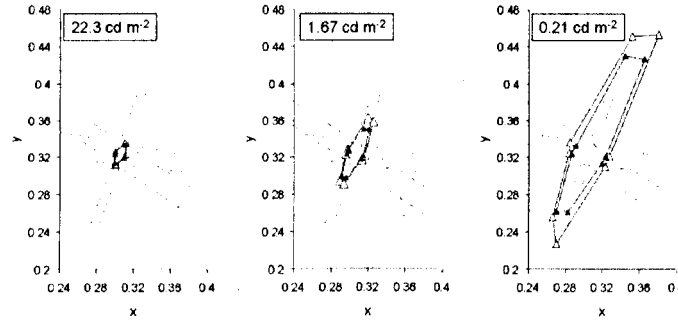


FIGURE 3. Effect of viewing monocularly (*white symbols*) rather than binocularly (*black symbols*) in study 1 to compromise normoxic chromatic sensitivity in CIE 1931 (*x*, *y*) color space at three light levels (n = 12). Mean thresholds are shown, measured along eight color directions from x = 0.305, y = 0.323. The effect of monocular viewing to compromise thresholds in all eight color directions is consistent and independent of the respiratory component of the study. Use of the same *x*, *y* scales allows comparison of the data by light level but tends to mask the discrete R-G threshold data between viewing conditions. Loss of color sense is reflected by the increasing area of the corresponding color ellipses. Monocular viewing increases calculated color ellipse area by 67% at 22.3 cd · m $^{-2}$.

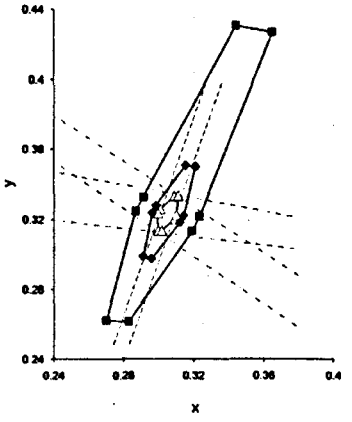


FIGURE 5. Effect of light level on binocular, normoxic mean chromatic thresholds in CIE 1931 (x, y) color space at 22.3 cd · m^{-2} (*triangles*), 1.67 cd · m^{-2} (*diamonds*) and 0.21 cd · m^{-2} (*squares*). The normal mesopic tritanopia is apparent, but there is far greater loss of sensitivity to yellow than to blue at the lowest light level (n = 12).

combined, orthogonal influences of the tritan woman and deuteranomalous man would be likely to introduce a gender bias in relation to the mean performance of the normal trichromats.

The effect of hypoxia on mesopic color sensitivity is well established at an equivalent altitude of 3,048 m (10,000 ft), and so lesser impairment may be anticipated at lower altitudes. However, chromatic sensitivity was preserved at photopic luminance, contrary to previous reports of threshold elevation[36] and loss of color discrimination[37] with this level of hypoxia.

Optimal chromatic sensitivity breathing 100% oxygen in study 2 was similar to that breathing air in study 1 and showed that supplementary oxygen conferred no benefit over normoxia. Hypoxia was associated with far greater loss of chromatic sensitivity in study 2 than in study 1, particularly at 1.67 cd · m^{-2} (Fig. 4), yet the hypoxia exposures are highly reproducible and virtually identical between studies (Table 1). However, familiarity with the challenging study procedure and reduced levels of psychological arousal may have increased susceptibility to cognitive or attentional effects of hypoxia during later experiments. Responses to hypoxia are notoriously variable, within and between subjects, and this may be compounded in dim light if attention wanders. In contrast, 100% oxygen is arousing and appears to maintain normal chromatic sensitivity.

In general, the magnitude of the effect of hypoxia is less than but broadly comparable to that of viewing monocularly. As with viewing monocularly, small increases in hypoxic Y-B and R-G thresholds may result in substantial increases in the area of the corresponding color ellipses. However, the asymmetry in S-cone responses, in conjunction with a progressive effect of hypoxia with decreasing mesopic luminance, and the suggestion of a tilt in the major axis of mesopic color ellipses,[21] suggest that it is inappropriate to extend comparison of ellipse area into the mesopic range, when using the data available herein. Nonetheless, progressive hypoxic loss of color sensitivity may be anticipated with decreasing mesopic luminance at 3,048 m (10,000 ft).

As the effect of hypoxia is progressive with falling light it is therefore more likely due to an ocular rather than central mechanism. A drop in P_AO_2 to ~60 mm Hg (~8 kPa) will reduce ciliary artery Po_2 to ~50 to 55 mm Hg, reducing the choroidal oxygen "pressure head" by ~50%. As the available light decreases, the compound influences of progressive rod-driven retinal hypoxia and the reduced choroidal Po_2 may compromise cone oxygenation and function. An effect of mild hypoxia on cone-mediated vision commences at upper mesopic luminance and is well-established at mid-mesopic luminance (0.21 cd · m^{-2}), supporting an increased retinal vulnerability to hypoxia in dim light. Chromatic sensitivity may be more vulnerable to hypoxia with distance from the fovea, as rod density increases and cone density decreases.

Hypoxia's compromising the acquisition of color-coded information in the mesopic cockpit has implications for aircrew operating in unpressurized cabins at or above 3,048 m (10,000 ft). Besides reduced conspicuity of colored targets, color deficiency is associated with extended search times and higher error rates.[38,39] However, the consequences of reduced color sensitivity go beyond impaired extraction of chromatic information. At mesopic levels, the chromatic signal contributes to "effective" luminance contrast and reaction time,[40,41] so hypoxia may compromise wider aspects of aircrew performance.

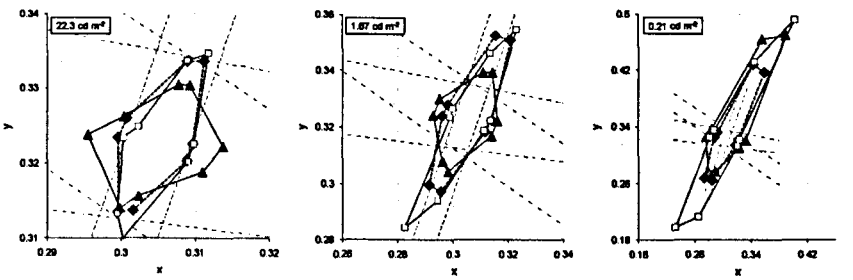


FIGURE 6. Comparison of the chromatic thresholds of the man with minimal deuteranomaly (*gray triangles*) and the woman using hormonal contraception (*white squares*) with the means of the 10 normal trichromats (*black diamonds*) at each light level (binocular, normoxic). The different (x, y) scales are necessary to resolve the detail of the different patterns of response at the different light levels. The protan, deutan, and tritan color confusion axes shown (*dashed lines*) are the same plots in each diagram. Note the mildly but clearly elevated deutan thresholds of the man with minimal deuteranomaly at 22.3 cd · m^{-2} and the consistent loss of Y-B sensitivity of the woman using hormonal contraception.

Acknowledgments

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References

1. Elliott EC, Moorhead IR, Evans AD. A task analysis of minimum colour vision requirements for professional pilots (Abstract). *Aviat Space Environ Med.* 2005;76(3-1):328.
2. Menu J-P, Ivan D, Daumann F-J, et al. *Operational Colour Vision in the Modern Aviation Environment.* Working Group 24 of the Human Factors and Medicine Panel of the North Atlantic Treaty Organization Research and Technology Organization. RTO Technical Report 16. Brussels, Belgium: NATO; 2001.
3. Barbur JL, Forsyth PM, Wooding DS. Colour, effective contrast and search performance. In: Schmid R, Zambarbieri D, eds. *Oculomotor Control and Cognitive Processes.* Amsterdam, The Netherlands: Elsevier Science; 1991:413-430.
4. Macdonald WA, Cole BL. Evaluating the role of colour in a flight information cockpit display. *Ergonomics.* 1988;31(1):13-37.
5. Post DL, Geiselman EE, Goodyear CD. Benefits of color coding weapons symbology for an airborne helmet-mounted display. *Hum Fact.* 1999;41(4):515-523.
6. Kobrick JL. Effects of hypoxia and acetazolamide on color sensitivity zones in the visual field. *J Appl Physiol.* 1970;28(6):741-747.
7. Ernest JT, Krill AE. The effect of hypoxia on visual function: psychophysical studies. *Invest Ophthalmol.* 1971;10(5):323-328.
8. Smith VC, Ernest JT, Pokorny J. Effect of hypoxia on FM 100-Hue Test performance. *Mod Probl Ophthalmol.* 1976;17:248-256.
9. Kobrick JL, Zwick H, Witt CE, Devine JA. Effects of extended hypoxia on night vision. *Aviat Space Environ Med.* 1984;55(3):191-195.
10. Vingrys AJ, Garner LF. The effect of a moderate level of hypoxia on human color vision. *Doc Ophthalmol.* 1987;66(2):171-185.
11. Richalet J-P, Duval-Arnould G, Darnaud B, Kéromès A, Rutgers V. Modification of colour vision in the green/red axis in acute and chronic hypoxia explored with a portable anomaloscope. *Aviat Space Environ Med.* 1988;59(7):620-623.
12. Picaud S. Retinal biochemistry. In Kaufman PL, Alm A, eds. *Adler's Physiology of the Eye.*10th ed. St. Louis, MO: Mosby; 2003:382-408.
13. Steinberg RH. Monitoring communications between photoreceptors and pigment epithelial cells: effects of "mild" systemic hypoxia. *Invest Ophthalmol Vis Sci.* 1987;28(12):1888-1904.
14. Arden GB, Sidman RL, Arap W, Schlingemann RO. Spare the rod and spoil the eye. *Br J Ophthalmol.* 2005;89(6):764-769.
15. Stefansson E, Wolbarsht ML, Landers MB. In vivo O_2 consumption in rhesus monkeys in light and dark. *Exp Eye Res.* 1983;37(3):251-256.
16. Linsenmeier RA. Effects of light and darkness on oxygen distribution and consumption in the cat retina. *J Gen Physiol.* 1986;88(4):521-542.
17. Ahmed J, Braun RD, Dunn R Jr, Linsenmeier RA. Oxygen distribution in the macaque retina. *Invest Ophthalmol Vis Sci.* 1993;34(3):516-521.
18. Yu D, Cringle SJ. Outer retinal anoxia during dark adaptation is not a general property of mammalian retinas. *Comp Biochem Physiol A Mol Integr Physiol.* 2002;132(1):47-52.
19. Wangsa-Wirawan ND, Linsenmeier RA. Retinal oxygen: fundamental and clinical aspects. *Arch Ophthalmol.* 2003;121(4):547-557.
20. Connolly DM, Hosking SL. Aviation-related respiratory gas disturbances affect dark adaptation: a reappraisal. *Vision Res.* 2006;46(11):1784-1793.
21. Walkey HC, Barbur JL, Harlow JA, Makous W. Measurements of chromatic sensitivity in the mesopic range. *Color Res Appl.* 2001;26(suppl 1):S36-S42.
22. Yebra A, Garcia JA, Nieves JL, Romero J. Chromatic discrimination in relation to luminance level. *Color Res Appl.* 2001;26(2):123-131.
23. Barbur JL, Harlow AJ, Plant GT. Insights into the different exploits of colour in the visual cortex. *Proc R Soc B.* 1994;258(1353):327-334.
24. Rodriguez-Carmona ML, Harlow AJ, Walker G, Barbur JL. The variability of normal trichromatic vision and the establishment of the "normal" range. In: *Proceedings of the 10th Congress of the International Colour Association.* Granada; 2005:979-982.
25. Barbur JL. 'Double-blindsight' revealed through the processing of color and luminance contrast defined motion signals. *Prog Brain Res.* 2004;144:243-259.
26. Barbur JL, Rodriguez-Carmona M, Harlow AJ. Establishing the statistical limits of "normal" chromatic sensitivity. In: *Proceedings of the ISCC/CIE Expert Symposium 2006 "75 Years of the CIE Standard Colorimetric Observer",* 16-17 May 2006, Ottawa, Ontario, Canada. CIE x030:2006.
27. Dautrabande L, Haldane J. The effect of respiration of oxygen on breathing and circulation. *J Physiol (Lond).* 1921;55:296-299.
28. Becker HF, Polo O, McNamara SG, Berthon-Jones M, Sullivan CE. Effect of different levels of hyperoxia on breathing in healthy subjects. *J Appl Physiol.* 1996;81(4):1683-1690.
29. Greenstein VC, Hood DC, Ritch R, Steinberger D, Carr RE. S (blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. *Invest Ophthalmol Vis Sci.* 1989;30(8):1732-1737.
30. Cho N-C, Poulsen GL, Ver Hoeve JN, Nork M. Selective loss of S-cones in diabetic retinopathy. *Arch Ophthalmol.* 2000;118(10):1393-1400.
31. Costa MF, Ventura DF, Perazzolo F, Murakoshi M, Silveira LC. Absence of binocular summation, eye dominance, and learning effects in color discrimination. *Vis Neurosci.* 2006;23(3-4):461-469.
32. Verriest G, Van Laetham J, Uvijls A. A new assessment of the normal ranges of the Farnsworth-Munsell 100-hue test scores. *Am J Ophthalmol.* 1982;93(5):635-642.
33. Simmons DR, Kingdom FAA. On the binocular summation of chromatic contrast. *Vis Res.* 1998;38(8):1063-1071.
34. Jiménez JR, Medina JM, Jiménez del Barco L, Díaz JA. Binocular summation of chromatic changes as measured by visual reaction time. *Percept Psychophys.* 2002;64(1):140-147.
35. Forte JD. Binocular summation of color and luminance contrast gratings (Abstract). *J Vis.* 2005;5:795a.
36. Brandl H, Lachenmayr B. Dependence of the sensitivity of the central visual field on hemoglobin-oxygen saturation [in German]. *Ophthalmologe.* 1994;91(2):151-155.
37. Karakucuk S, Oner AO, Goktas S, Siki E, Kose O. Color vision changes in young subjects acutely exposed to 3,000 m altitude. *Aviat Space Environ Med.* 2004;75(4):364-366.
38. Cole BL, Macdonald WA. Defective colour vision can impede information acquisition from redundantly colour-coded video displays. *Ophthalmic Physiol Opt.* 1988;8(2):198-210.
39. Cole BL, Maddocks JD, Sharpe K. Visual search and the conspicuity of coloured targets for colour vision normal and colour vision deficient observers. *Clin Exp Optom.* 2004;87(4-5):294-304.
40. Walkey HC, Barbur JL, Harlow JA, Hurden A, Moorhead IR, Taylor JAF. Effective contrast of colored stimuli in the mesopic range: a metric for perceived contrast based on achromatic luminance contrast. *J Opt Soc Am A Opt Image Sci Vis.* 2005;22(1):17-28.
41. Walkey HC, Harlow JA, Barbur JL. Characterising mesopic spectral sensitivity from reaction times. *Vision Res.* 2006;46(25):4232-4243.

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Oxygenation and gender effects on photopic frequency-doubled contrast sensitivity

Desmond M. Connolly [a,b,*], Sarah L. Hosking [b]

[a] QinetiQ, Cody Technology Park, Ively Road, Farnborough, Hampshire GU14 0LX, UK
[b] Applied Vision Research Centre, The Henry Wellcome Laboratories for Vision Sciences, City University, Northampton Square, London EC1V 0HB, UK

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Abstract

Thresholds to a temporally modulated contrast stimulus were examined across the central visual field, at photopic luminance (100 cd m^{-2}), under aviation-related respiratory disturbances. These were mild hypoxia (14.1% oxygen), hyperoxia (100% oxygen), and hypocapnia (voluntary hyperventilation), with control exposures breathing air at rest. Thresholds were analysed by retinal eccentricity and by visual field quadrant. Hypoxia compromised sensitivity away from fixation ($p < .001$). Gender differences in sensitivity were apparent over the nasal hemifield and in response to 100% oxygen. An unexpected and highly statistically significant effect of oxygen tension (PO_2) exposure order ($p < .001$) implies the existence of short-term retinal 'memory' for recent PO_2.
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Keywords: Temporal contrast sensitivity; Frequency doubling; Hypoxia; Gender

1. Introduction

Mild hypoxia has long been known to impair contrast discrimination under low light conditions (Hecht, Hendley, Frank, & Haig, 1946; McFarland, Halperin, & Niven, 1944). However, there have been few photopic studies of temporal contrast sensitivity and none appear to have examined a temporally modulated contrast stimulus away from the fovea, despite the obvious relevance to aircrew visual performance. Yap, Garner, Legg, and Faris (1995) studied foveal spatial and temporal contrast sensitivity in a decompression chamber and found no effect of hypobaric (low pressure) hypoxia at equivalent altitudes of 2134 and 3658 m (7000 and 12,000 ft). Benedek et al. (2002) imposed more severe hypoxia, equivalent to 5486 m (18,000 ft) and reported enhanced foveal contrast sensitivity at low and medium spatial frequencies. However, the respired gas pressures were not measured and such hypoxia is sufficiently severe to induce secondary hyperventilation in some subjects. This promotes hypocapnia, a reduction in arterial and tissue partial pressures of carbon dioxide that results from the fall in alveolar carbon dioxide tension (P_ACO_2). Unfortunately, mild hypocapnia may be associated with increased visual and contrast sensitivity and could have confounded the results (Otis, Rahn, Epstein, & Fenn, 1946; Wald, Harper, Goodman, & Krieger, 1942).

To establish a reference against which to compare the results of subsequent mesopic studies, we examined photopic (100 cd m^{-2}) contrast thresholds to a temporally modulated stimulus under a variety of respiratory conditions. Vision testing employed the N-30 (threshold) protocol of a standard Frequency Doubling Technology (FDT) perimeter. This was chosen as offering a relatively brief and easily repeatable test, using a dynamic contrast stimulus, while providing threshold information across the central visual field. The test was readily available, easy to administer and undertake consistently in conjunction with altered respiratory conditions, and had the potential to allow variations in threshold sensitivity to be interpreted retinotopically.

* Corresponding author. Fax: +44 1252 392097.
E-mail address: dmconnolly@qinetiq.com (D.M. Connolly).

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Numerous gender differences in ocular and visual function have been reported (Guttridge, 1993), including menstrual cycle-dependent changes in both spatial and temporal contrast sensitivity (Brabyn & McGuiness, 1979; Dunn & Ross, 1985; Johnson & Petersik, 1987), although this is not a consistent finding (Solberg & Brown, 2002). Gender differences in performance on two different perimeter tests have also been reported (Akar et al., 2005; Cohn, DeAgostini, Aron-Rosa, Laloum, & Boller, 1994). Accordingly, the current study was balanced to allow comparison of male and female responses under the various respiratory challenges.

Retinal neuroglobin (Ngb) is concentrated at the sites of greatest mitochondrial density and oxygen consumption, including photoreceptor inner segments, outer and inner plexiform layers, and ganglion cells (Bentmann et al., 2005; Schmidt et al., 2003). Possible roles include local oxygen storage, regulation of mitochondrial oxygen consumption and protection from hypoxia (Hamdane et al., 2003; Hankeln et al., 2005; Sun, Jin, Mao, Zhu, & Greenberg, 2001). Its ligand binding properties appear related to cellular redox state and suggest delayed responses to local fluctuations of PO_2 (Burmester & Hankeln, 2004; Pesce et al., 2002). These characteristics suggest that imposed disturbances of retinal oxygen tension could have extended effects that might confound vision testing under subsequent respiratory conditions. Accordingly, PO_2 exposure order was carefully randomised and balanced by gender, with the aim of excluding it as a confounding factor.

Hypobaric hypoxia was simulated by breathing 14.1% oxygen (balance nitrogen) while the effect of supplementary oxygen was examined breathing 100% oxygen (hyperoxia). Moderate hypocapnia was induced by voluntary hyperventilation sufficient to lower the end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) to 25 mm Hg (3.3 kPa). Normoxic, normocapnic control exposures were conducted breathing air at rest. Physiological pressures are reported as measured, in mmHg, with SI Units in parenthesis (1 kPa = 7.501 mm Hg).

2. Methods

2.1. Subjects

The work adhered to the principles of the Declaration of Helsinki, the study protocol was approved in advance by an independent Local Research Ethics Committee, and all subjects provided written informed consent before participating. The mean age of the 12 subjects (6M, 6F) was 30 y (range 23–37 y) and the age distributions of males and females were similar. The mean (±SD) age of males was 31.8 ± 5.0 y while that of females was 27.7 ± 4.1 y, with no significant difference on two-sample t test ($p = .148$). Comprehensive medical and ophthalmic screening ensured fitness to participate and included a detailed medical and ophthalmic history, external and fundoscopic examination, near and distant visual acuity, accommodation, convergence, visual fields, ocular movements and alignment, pupillary reactions and colour perception (Ishihara plates). Exclusion criteria included any chronic or current systemic illness, any evidence of past or current systemic or ocular abnormality, and any regular systemic or topical medication, other than hormonal contraception. The Snellen acuity of all test eyes was 6/6 or better, with correction in two subjects. Each subject undertook a clinical screening FDT test to exclude abnormal perimetry and all were normal for age. Subjects were non-smokers who were asked to avoid alcohol for 24 h and caffeine on the day of their experiment. Females undertook a urine test to exclude pregnancy before beginning the experiment. All subjects were familiar with breathing from pressure-demand regulators using aircrew oxygen masks, and training in the technique of voluntary hyperventilation was unnecessary (Connolly & Hosking, 2007).

2.2. Equipment

Each subject was fitted with an appropriate size of standard aircrew oxygen mask, which was supported from a cloth helmet. This arrangement prevents inhalation of expired gas, can be worn comfortably for prolonged periods, and integrates well with the FDT apparatus, such that the display test area remains in full view of the test eye. The mask was modified to provide an access port to the mask cavity, allowing continuous respiratory gas analysis using an Airspec MGA 2000 mass spectrometer. This was calibrated immediately before and after each experiment using a variety of gas mixtures of known composition, resulting in a measurement error of less than 1% for physiological pressures of oxygen and carbon dioxide. Blood pressure, heart rate and oxygen saturation were monitored non-invasively and recorded using an Ohmeda Finapres 2300 blood pressure monitor and a Kontron 7840 pulse oximeter with finger probe. Analogue outputs from both devices were calibrated and recorded, together with the mass spectrometer data, using an AD Instruments PC-based data recording and analysis system employing Powerlab/Chart software.

At the start of each test, the FDT perimeter self-calibrates to a background field luminance of 100 cd m^{-2}, and this was confirmed at the start of the study. The sinusoidal contrast stimuli are presented monocularly and comprise vertical gratings of low spatial frequency (0.25 cpd) with high temporal frequency counter-phase flicker (25 Hz), to induce the frequency doubling illusion. The perimeter examines 17 test areas that cover a 40° × 40° square of central visual field (Fig. 1), with each having a maximum horizontal diameter of 10° of visual angle. Test areas are chosen pseudorandomly and thresholds are assessed using a modified staircase technique; subjects respond with a button press when a stimulus is seen. There is little, if any, difference between detection- and resolution-contrast thresholds for the frequency doubling illusion (McKendrick, Anderson, Johnson, & Fortune, 2003). Accordingly, our subjects remained unaware of the illusion and were asked simply to respond to unambiguous perception of a faint 'shimmering' stimulus, so that the earliest perception of the stimulus was determined for all participants under all conditions. Maximum stimulus duration is 720 ms with 400 ms at plateau contrast. The N-30 protocol also examines two additional nasal test areas that are not included in this analysis. Subjects undertook three 'training' FDT tests to counter any learning effect (Heeg, Ponsioen, & Jansonius, 2003; Iester, Capris, Pandolfo, Zingirian, & Traverso, 2000). The 48 subsequent exposures accepted for analysis provided a cumulative total of 5 h of

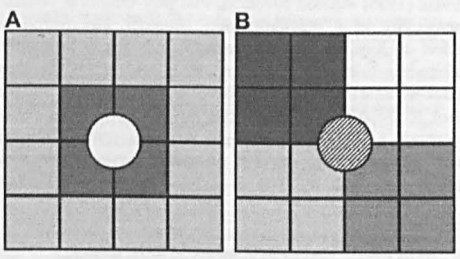


Fig. 1. Data analysed (A) by eccentricity and (B) by field quadrant (central hatched data excluded from analysis).

vision testing, between 12 subjects, generating only 22 fixation errors (distributed equally between males and females), four false positive responses and a single false negative response, with no clear relationship to respiratory condition. All tests were conducted using the dominant eye.

2.3. Respiratory conditions

Control measurements were made breathing air (20.95% oxygen) at rest. Hypoxia was induced by breathing 14.1% oxygen (balance nitrogen) to generate an alveolar partial pressure of oxygen (P_AO_2) of 55–60 mm Hg (7.3–8.0 kPa), equivalent to that expected when breathing air at 3048 m (10,000 ft), and lowering arterial haemoglobin oxygen saturation (S_aO_2) to ~90%. Hyperoxia was imposed breathing 100% oxygen. Each breathing gas was supplied through its own dedicated, pressure-demand aircrew breathing gas regulator. These had matching pressure/flow characteristics so the breathing gases were indistinguishable to the subjects, who remained unaware of the presentation order throughout. The mass spectrometer trace was monitored continuously during each exposure to ensure that an adequate face-mask seal was maintained.

As well as needing to control for delayed effects of altered retinal oxygenation, it was recognised that immediate repeat testing could be associated with a reduction in FDT sensitivity (Artes, Nicolela, McCormick, LeBlanc, & Chauhan, 2003). Accordingly, a wholly balanced design was employed for the normoxia, hypoxia and hyperoxia conditions to control out any possible confounding effects and interactions of PO_2 exposure order and immediate repeat testing. One subject of each gender experienced each possible exposure order of the three PO_2 conditions.

Hyperventilation is alveolar ventilation in excess of metabolic need, inducing hypocapnia by lowering the P_ACO_2. Hypocapnia enhances sensitivity to the perception of flicker (Alpern & Hendley, 1952; Connolly & Hosking, 2007; Granger & Ikeda, 1961) and also, initially, to contrast, until the hypocapnia becomes pronounced (Otis et al., 1946). Moderate hypocapnia was imposed by voluntary hyperventilation sufficient to lower $P_{ET}CO_2$ to 25 mm Hg (3.3 kPa), breathing air from the appropriate regulator. This condition was always undertaken last to avoid any delayed and potentially confounding effect of the resulting acid–base disturbance (respiratory alkalosis) on the balanced order of exposures involving altered PO_2.

2.4. Procedure

Ambient illumination was 1–2 lux at subject eye level. Seated at rest, subjects were prepared for monitoring and the hose supplying the first breathing gas was connected to the mask. Each breathing gas condition was imposed for precisely 15 min, to achieve a respiratory steady state, before commencing vision testing. The perimeter design obscures the test area from view by the resting eye but does not mask all stray light. This resulted in interference through binocular rivalry, apparent as pronounced early dimming of the test field, and obliged the first two experiments to be abandoned and repeated. Subsequent experiments were completed uneventfully with the resting eye occluded by a patch.

Upon completion of the first vision test, the breathing gas supply hose was disconnected and the subject rested for 2–3 min before repeating the process with the next breathing gas. Thus, each exposure occupied 20–25 min. When the randomised hypoxic, normoxic and hyperoxic conditions were complete, the subject was again supplied with air from the appropriate regulator and began hyperventilating until $P_{ET}CO_2$ fell to 25 mm Hg (3.3 kPa). This continued for 15 min, with verbal feedback from the experimenter guiding the subject's ventilatory effort and breath-by-breath monitoring of $P_{ET}CO_2$ being maintained throughout. After 15 min, the subject commenced the final vision test, still hyperventilating. By this stage, typically, a steady ventilatory rhythm was well established such that minimal, if any, prompting was required to maintain a steady $P_{ET}CO_2$ during vision testing. Upon completion of the test, the subject relaxed and was monitored until $P_{ET}CO_2$ rose above 30 mm Hg (4 kPa).

2.5. Analysis

For each test location, FDT perimeter N-30 results are expressed as threshold measurements in dB, according to the manufacturer's proprietary formula (Kogure, Membrey, Fitzke, & Tsukahara, 2000). Fig. 1A illustrates how these data were grouped to allow analysis by eccentricity from fixation. The circular central fixation test area has a radius of 5° of visual angle. Beyond this lies an inner 'ring' of four irregularly-shaped test locations that extend to 10° from fixation along the horizontal and vertical meridians and have their stimuli centred at about 9.6° along any 45° diagonal from fixation. The outer 'ring' of 12 square stimulus areas extends to 20° from fixation along the horizontal and vertical meridians such that the corner test areas have their stimuli centred at just over 21° from fixation. In this paper these three concentric test areas are referred to as 'centre', 'inner' and 'outer', respectively. The corresponding areas of retina differ in their anatomical structure, in their density and distribution of cone and rod photoreceptors, and by the nature of their vascular supply and, therefore, oxygenation. Each individual's means of the four 'inner' and 12 'outer' results were taken as the data points to feed, with the single 'centre' measurement, into group descriptive analysis. Selective comparisons were then undertaken using *t* tests on within-subject data paired by respiratory condition.

Fig. 1B illustrates how the data were examined by field quadrant (ignoring the 'centre' test location). For each respiratory condition, each subject's mean threshold of the four data points in each quadrant fed into the group analysis. First, the normoxia data were checked for a normal distribution before interrogating the complete data set using balanced analysis of variance (ANOVA) to identify main effects and interactions of breathing gas, gender and field quadrant.

After excluding the hyperventilation data, this analysis was repeated specifically to include assessment of main effects and interactions of PO_2 exposure order. The possible exposure orders are HAO, HOA, AHO, AOH, OAH and OHA, where H represents the hypoxia condition, A is the normoxia (air) condition, and O is the 100% oxygen condition. For this analysis, these were arranged into three groups according to whether hypoxia immediately preceded the oxygen condition (H–O, incorporating the HOA and AHO data), oxygen immediately preceded hypoxia (O–H, incorporating OHA and AOH data), or the hypoxia and oxygen conditions were separated in time by the intervening air condition (x–A–x, incorporating OAH and HAO data).

Finally, mean quadrant thresholds were treated as four dependent variables and interrogated using balanced multivariate ANOVA (MANOVA) to identify any influences of the previous factors that might have varied systematically across the visual field. Specific *post hoc* comparisons were conducted using ANOVA.

3. Results

3.1. Cardio-respiratory responses

The cardio-respiratory parameters associated with these experiments are shown in Table 1. The imposed partial pressures of oxygen and carbon dioxide were as intended and well controlled, generating the anticipated changes in S_aO_2, and consistent with the data from a recent hypobaric chamber study (Connolly & Hosking, 2006). Not shown in Table 1, mild hypoxia induced a mean (±SD) $P_{ET}O_2$ of ~59 ± 4 mm Hg (7.9 ± 0.5 kPa), reflecting the imposed P_AO_2. Hyperoxia was associated with a slight but consistent and reproducible fall in $P_{ET}CO_2$ by about 5 mm Hg (0.67 kPa) that is explained by the Haldane Effect, whereby hyper-oxygenated haemoglobin carries less carbon dioxide from the tissues in the carbamino form (Becker, Polo, McNamara, Berthon-Jones, & Sullivan, 1996; Dautra-

Table 1
Group mean (±SD between subjects) cardio-respiratory parameters ($N = 12$) during vision testing for each of the four imposed respiratory conditions

	P_IO_2 (mm Hg)	$P_{ET}CO_2$ (mm Hg)	S_aO_2 (%)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (bpm)
Control (normoxia, normocapnia)	158 ± 1	38 ± 3	97 ± 1	139 ± 20	90 ± 16	66 ± 10
Mild hypoxia (14.1% oxygen)	107 ± 1	38 ± 2	90 ± 2	140 ± 14	89 ± 8	68 ± 10
Hyperoxia (100% oxygen)	739 ± 6	33 ± 3	99 ± 1	139 ± 18	88 ± 16	61 ± 9
Hyperventilation (hypocapnia)	159 ± 1	23 ± 2	99 ± 1	145 ± 20	99 ± 19	63 ± 12

Blood pressures are higher than normal due to an uncorrected hydrostatic pressure effect on measurements made using photoplethysmography; seating height varied between subjects.

bande & Haldane, 1921). When hyperventilating, all subjects maintained a steady state of hypocapnia, close to the target level of 25 mm Hg (3.3 kPa), before and during the vision test. There were no significant effects of the respiratory disturbances on heart rate or blood pressure.

3.2. Eccentricity from the fovea

Group mean threshold sensitivity data for the 'centre', 'inner' and 'outer' test areas are shown for each respiratory condition in Fig. 2, suggesting an effect of hypoxia to degrade threshold sensitivity away from the 'centre', particularly for the 'inner' data and to a lesser extent for the 'outer' data. These effects of hypoxia were analysed relative to normoxic (control) performance using within-subject data paired by respiratory condition. The resulting data sets were normalised by subtraction (Anderson Darling $p > .1$), allowing assessment using paired t tests ($\alpha = 0.05$). The effect of hypoxia was highly statistically significant for the 'inner' data ($p = .001$) and almost achieved statistical significance for the 'outer' data ($p = .051$). Similarly, from Fig. 2, the apparent slight effect of hypocapnia to enhance sensitivity at the 'fovea' was not statistically significant ($p = .207$).

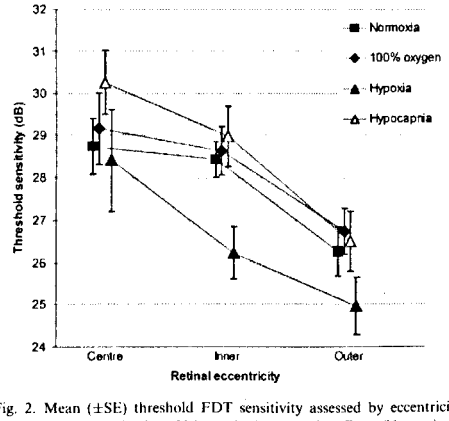


Fig. 2. Mean (±SE) threshold FDT sensitivity assessed by eccentricity from fixation (see Fig. 1A). Using paired t tests, the effect of hypoxia to impair sensitivity, compared to normoxia, is highly statistically significant ($p = .001$) for the 'inner' data and almost achieves statistical significance for the 'outer' data ($p = .051$).

3.3. Field quadrant

The normoxic mean threshold sensitivity of all quadrants for all subjects ($N = 48$) was consistent with a normal distribution (Anderson Darling $p = .28$). Mean (±SD) threshold sensitivity was 26.8 ± 2.1 dB. The entire data set was analysed for main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of breathing gas, gender and field quadrant on threshold sensitivity using balanced ANOVA. Only a main effect of breathing gas achieved statistical significance ($p < .001$), shown in Fig. 3 to be a reduction in threshold sensitivity under mild hypoxia.

Next, the hyperventilation data were excluded and the balanced ANOVA was repeated to include consideration of PO_2 exposure order. The main effect of breathing gas remained ($p < .001$) and an unexpected but equally unambiguous main effect of PO_2 exposure order was also apparent ($p < .001$). No other main effects or interactions were

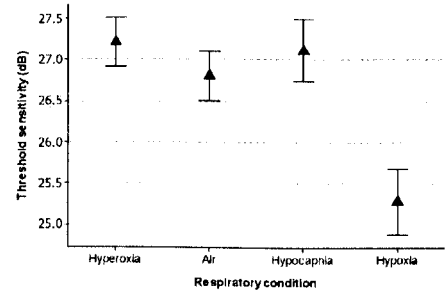


Fig. 3. Mean (±SE) threshold FDT sensitivity for all field quadrants by respiratory condition. Using balanced ANOVA, the highly statistically significant ($p < .001$) main effect of breathing gas is shown to be an impairment of threshold sensitivity under hypoxia.

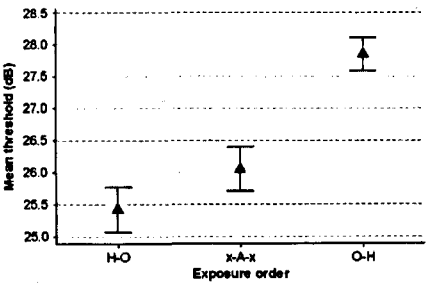


Fig. 4. Mean (±SE) threshold FDT sensitivity for all field quadrants, grouped by PO_2 exposure order, according to whether 100% oxygen immediately preceded hypoxia (O–H); hypoxia immediately preceded 100% oxygen (H–O); or the normoxia (air) condition was interposed between them (x–A–x). The statistically highly significant main effect of PO_2 exposure order is apparent ($p < .001$).

seen. The main effect of exposure order is represented in Fig. 4, indicating greatest overall sensitivity during the experiments in which exposure to 100% oxygen immediately preceded exposure to hypoxia, and poorest sensitivity when hypoxia immediately preceded 100% oxygen, with intermediate results when these conditions were separated by the normoxic control. For each exposure order, the mean threshold sensitivity achieved with each breathing gas is shown in Fig. 5, illustrating the consistent benefit of supplementary oxygen over hypoxia, regardless of exposure order, but also the clear effect of exposure order to influence the overall sensitivities achieved with each breathing gas. In brief, prior hypoxia diminishes the benefit of subsequent oxygen, while prior 100% oxygen mitigates the impairment under subsequent hypoxia.

Having excluded a main effect of field quadrant, the four sets of quadrant data were examined as dependent variables using balanced MANOVA, for main effects ($\alpha = 0.05$) and interactions ($\alpha = 0.01$) of breathing gas, gender and exposure order. Again unexpectedly, only a main effect of gender achieved statistical significance ($p = .007$). This is represented in Fig. 6, indicating greater male sensitivity over the nasal hemifield. Accordingly, the data for all four quadrants were examined in detail for trends in response by gender and are shown in their entirety in Fig. 7. Most obviously, and regardless of respiratory condition, male sensitivity is greater than female sensitivity over the nasal hemifield. Relative to normoxia, hypoxia impairs male and female sensitivity in all four quadrants. Also relative to normoxia, female sensitivity is slightly but consistently enhanced by 100% oxygen in all quadrants, while male sensitivity is not. However, both males and females exhibit a clear benefit from 100% oxygen, in all quadrants, when compared to hypoxic performance. Finally, hypocapnia appears to enhance male and female sensitivity on the nasal side of the field, but not the temporal, in comparison to the normoxic, normocapnic control exposures. Each of these apparent trends was subject to specific *post hoc* analysis using one-way ANOVA ($\alpha = 0.05$).

First, the effect of gender was examined in each of the four field quadrants individually, using the data from all four respiratory conditions, achieving statistical significance only in the superior ($p = .02$) and inferior ($p = .049$) nasal quadrants and supporting greater male sensitivity over just the nasal hemifield. Secondly, the female hyperoxia data for all four quadrants were examined relative to normoxia, supporting a slight benefit of supplementary oxygen to enhance female sensitivity across the visual field ($p = .004$). The same analysis on the male data was not significant ($p = .487$). Thirdly, the hyperoxia data for all subjects and field quadrants was compared to

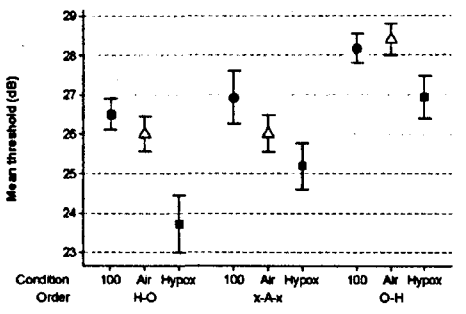


Fig. 5. Mean (±SE) threshold FDT sensitivity for the data in Fig. 4 broken down by breathing gas (100 = 100% oxygen; air = 20.95% oxygen; hypox = 14.1% oxygen). Labeling of exposure order is as for Fig. 4. Each data point represents the mean of 64 individual FDT test area thresholds (4 quadrants of each of 4 subjects).

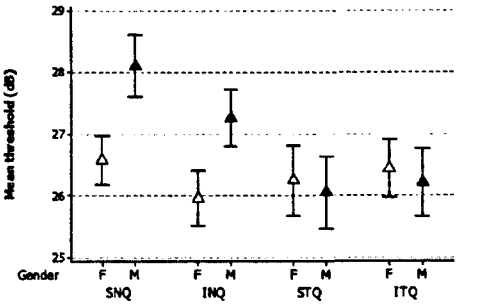


Fig. 6. Mean (±SE) threshold FDT sensitivity for males (black triangles) and females (white triangles) by field quadrant, for the conditions involving alterations of PO_2 (superior nasal, SNQ; inferior nasal, INQ; inferior temporal, ITQ; superior temporal, STQ). From balanced MANOVA, the statistically significant main effect of gender ($p = .007$) appears to result from consistently greater male sensitivity over the nasal hemifield.

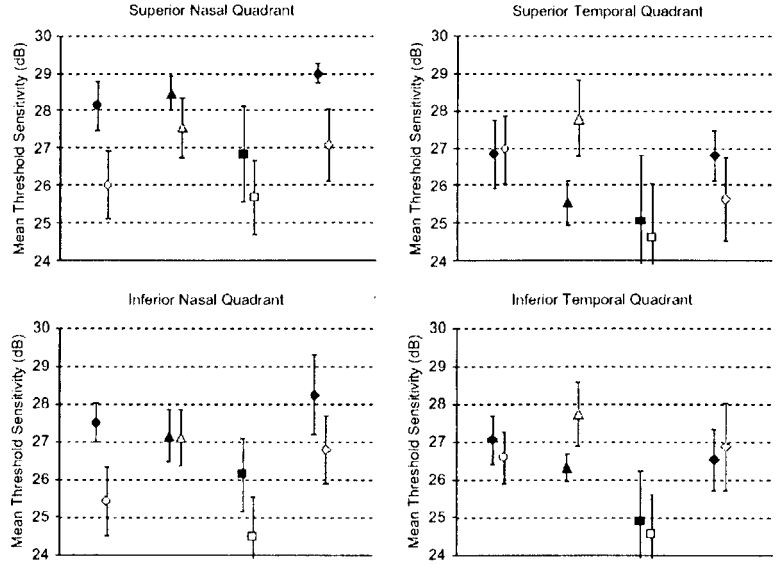


Fig. 7. Mean (±SE) threshold FDT sensitivity for males (solid symbols) and females (open symbols) by field quadrant and respiratory condition (circles = normoxia; triangles = 100% oxygen; squares = hypoxia; diamonds = hypocapnia).

the hypoxia data, demonstrating a highly statistically significant benefit of 100% oxygen ($p < .001$). Finally, the hypocapnia data for the nasal quadrants of both genders were examined relative to normocapnia (normoxia) and were not significantly different ($p = .119$).

4. Discussion

4.1. Oxygenation state

Mild hypoxia, equivalent to breathing air at only 3048 m (10,000 ft), degrades temporal contrast sensitivity beyond the fovea under good viewing conditions. The impairment is unambiguous, so greater and lesser losses may be anticipated at higher and lower altitudes, respectively. Sensitivity appears unaffected at the fovea, supporting the findings of Yap et al. (1995). However, thresholds appear much more vulnerable away from fixation. The geometric centres of the four 'inner' test areas lie just over 9° from the centre of the test display, suggesting significant hypoxic impairment at the boundary of the macula (Sharma & Ehinger, 2003).

The retinotopic sensitivity to hypoxia suggests a retinal, rather than central, mechanism. The imposed hypoxia was mild, causing only slight reduction of S_aO_2. Accordingly, any tendency for inner retinal tissue PO_2 to fall should be compensated comfortably by autoregulation of inner retinal blood flow. In contrast, the outer retina relies on a high choroidal PO_2 to drive oxygen diffusion down a steep gas tension gradient to the photoreceptors. Breathing 14.1% oxygen, this choroidal oxygen 'pressure head' will fall from >100 mm Hg to <60 mm Hg, and is much more likely to compromise the outer retina. The basal oxygen consumption of the numerous rods in the periphery might be sufficient to compromise the sparser cones under hypoxic conditions. Foveal sparing may then be explained by the paucity of rods. Repeating the study using the smaller test areas of FDT Matrix perimetry might provide better spatial resolution of the influence of hypoxia with eccentricity from the fovea. However, the Matrix perimeter employs counter-phase flicker at 18 Hz, and while the adaptation and temporal tuning characteristics of FDT stimuli and spatially uniform flicker stimuli are indistinguishable at 25 Hz and above, this is not so at lower temporal frequencies (Anderson & Johnson, 2002). Thus, it might be preferable to use spatially uniform flicker stimuli to isolate specific mechanisms.

Relative to hypoxia, 100% oxygen enhances temporal contrast sensitivity in all quadrants, so supplementary oxygen should benefit sensitivity at 3048 m (10,000 ft). Modest enhancement of the fractional inspired oxygen concentration to ~32% is likely to preserve normal sensitivity at 3048 m (10,000 ft) by maintaining P_AO_2, and hence choroidal PO_2, equal to that at ground level.

This study employed a natural pupil to assess the impact of respiratory disturbance in relation to aircrew vision dur-

ing flight, that is, net of any effect on pupil size. FDT perimetry may be unaffected by pupil sizes greater than 2 mm diameter (Johnson & Sample, 2003), although other studies indicate that performance is affected by retinal illumination and, therefore, pupil size (Kogure et al., 2000; Swanson, Dul, & Fischer, 2005). Studies conflict over the effect of hypoxia on pupil size, with none supporting a meaningful effect of acute, brief and mild challenges to constrict the pupil. In particular, the study by Ernest and Krill (1971) suggests that moderate hypoxia, using breathing gas mixtures, does not affect resting pupil size. In the current study, neither the retinotopic pattern of response nor the modifying effect of PO_2 exposure order is explained by an effect of pupil size. Nonetheless, it remains possible that pupil size has influenced the results under hypoxia.

4.2. Gender

Right-handed, right-eye-dominant females have exhibited reduced sensitivity over the left hemifield using static Humphrey (24-2) perimetry, with no difference between genders for both eyes combined, or between eyes for either gender (Cohn et al., 1994). The finding was independent of the ovarian cycle. Decreased left hemifield sensitivity has also been documented in right-handed females during the luteal phase of the ovarian cycle, but not during the follicular phase, using short wavelength automated perimetry (Akar et al., 2005). Other (non-physiological) female sample populations have exhibited reduced nasal field sensitivity during the luteal phase of the ovarian cycle (Apaydin, Akar, Akar, Zorlu, & Özer, 2004; Yucel et al., 2005).

The current study adds FDT to the perimeter tests showing a gender difference in sensitivity across the vertical meridian, favouring males, when accounting for ocular dominance. The females in the current study included three, presumed anovulatory, right-eye-dominant females using oral contraception, tested on days 7, 13 and 17; two left-eye-dominant, cycling females tested on days 14/26 and 15/33; and one right-eye-dominant, oligomenorrhoeic female tested on day 38/49. Thus, they exhibit no obvious trend in relation to stage of the ovarian cycle. Instead, the results indicate an underlying difference between males and females in relation to hemifield sensitivity of the dominant eye. The basis of this difference is unexplained, but it is not an isolated finding and may warrant further study. Compared to normoxia, hyperoxia enhanced female sensitivity across all field quadrants. This was unexpected, is also unexplained and requires validation.

4.3. Concluding observations

The benefit of 100% oxygen extends well beyond exposure to the gas, while a less dramatic deficit persists after exposure to mild hypoxia. Thus, changes in PO_2 influence visual sensitivity for at least 20–25 min after exposure, implying the existence of 'retinal memory' for recent PO_2 and warranting further work to establish its duration. Notwithstanding the premise under which we controlled for exposure order, it remains entirely speculative that the mechanism for this unexpected observation is attributable to Ngb.

Future research into visual performance under conditions of disturbed PO_2 should consider carefully the timings of respiratory challenges and their exposure order, while past studies should be interpreted with this in mind. In the current study, the normoxic control exposures may have been influenced by prior hypoxia or hyperoxia, confounding paired comparison with hypocapnia data. Thus, it should not be inferred from this study that hypocapnia does not influence visual sensitivity.

Unpressurised light aircraft, helicopters and gliders may be operated at altitudes up to and over 3048 m (10,000 ft) without the benefit of supplementary oxygen. The findings presented here may be relevant to daytime flight in poor visibility, low contrast conditions (bad weather), especially at low level over elevated terrain, and to visual performance on contrast-dependent display-based tasks (such as those generating monochrome imagery). Additionally, much of the imagery and information presented on helmet-mounted aircrew displays will fall well outside the central 10° diameter area spared by hypoxia in this study and may be vulnerable to mild hypoxia. Furthermore, the effect of hypoxia may be considered likely to extend further into the retinal periphery and risk compromising peripheral motion sensation. Hypoxic impairment continues well beyond the duration of exposure. Thus, any visual deficit occurring at modest altitude will tend to persist following descent. From a practical perspective, if supplementary oxygen is indicated during flight it should be initiated early in the climb, to avoid establishing any persistent effect of mild hypoxia, and discontinued late in the descent to maintain its benefit ('ON' early and 'OFF' late).

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References

Akar, Y., Zulauf, M., Yucel, I., Akar, M. E., Erdem, U., & Trak, B. (2005). Menstrual-cycle dependent differences between right and left visual hemifields in perimetry. *Current Eye Research, 30*, 723–727.

Alpern, M., & Hendley, C. D. (1952). Visual functions as indices of physiological changes in the acid–base balance of the blood. *American Journal of Optometry, 29*, 301–314.

Anderson, A. J., & Johnson, C. A. (2002). Mechanisms isolated by frequency-doubling technology perimetry. *Investigative Ophthalmology and Visual Science, 43*, 398–401.

Apaydin, K. C., Akar, Y., Akar, M. E., Zorlu, G. C., & Özer, H. O. (2004). Menstrual cycle dependent changes in blue-on-yellow visual field analysis of young diabetic women with severe non-proliferative diabetic retinopathy. *Clinical and Experimental Ophthalmology, 32*, 265–269.

Artes, P. H., Nicolela, M. T., McCormick, T. A., LeBlanc, R. P., & Chauhan, B. C. (2003). Effects of blur and repeated testing on sensitivity estimates with frequency doubling perimetry. *Investigative Ophthalmology and Visual Science, 44*, 646–652.

Becker, H. F., Polo, O., McNamara, S. G., Berthon-Jones, M., & Sullivan, C. E. (1996). Effect of different levels of hyperoxia on breathing in healthy subjects. *Journal of Applied Physiology, 81*(4), 1683–1690.

Benedek, K., Kéri, S., Grósz, A., Tótka, Z., Tóth, E., & Benedek, G. (2002). Short-term hypobaric hypoxia enhances visual contrast sensitivity. *NeuroReport, 13*, 1063–1066.

Bentmann, A., Schmidt, M., Reuss, S., Wolfrum, U., Hankeln, T., & Burmester, T. (2005). Divergent distribution in vascular and avascular mammalian retinae links neuroglobin to cellular respiration. *Journal of Biological Chemistry, 280*, 20660–20665.

Brabyn, L. B., & McGuiness, D. (1979). Gender differences in response to spatial frequency and stimulus orientation. *Perception and Psychophysics, 26*, 319–324.

Burmester, T., & Hankeln, T. (2004). Neuroglobin: A respiratory protein of the nervous system. *News in Physiological Science, 19*, 110–113.

Cohn, H., DeAgostini, M., Aron-Rosa, D., Laloum, L., & Boller, F. (1994). Sex differences in the left and right hemifields of normal subjects with computerised static perimetry. *British Journal of Ophthalmology, 78*, 837–841.

Connolly, D. M., & Hosking, S. L. (2006). Aviation-related respiratory gas disturbances affect dark adaptation: A reappraisal. *Vision Research, 46*, 1784–1793.

Connolly, D. M., & Hosking, S. L. (2007). Quantitative correlation of hyperventilation with flicker sensitivity. *Optometry and Vision Science, 84*, 529–534.

Dautrabande, L., & Haldane, J. (1921). The effect of respiration of oxygen on breathing and circulation. *Journal of Physiology (London), 55*, 296–299.

Dunn, C., & Ross, H. (1985). Gender, the menstrual cycle and visual contrast sensitivity. *Journal of Physiology, 267*, 19P [Abstract in proceedings of the physiological society].

Ernest, J. T., & Krill, A. E. (1971). The effect of hypoxia on visual function. Psychophysical studies. *Investigative Ophthalmology, 10*, 323–328.

Granger, G. W., & Ikeda, H. (1961). Effect of hyperventilation on foveal critical flicker frequency. *Vision Research, 1*, 287–300.

Guttridge, N. M. (1993). Changes in ocular and visual variables during the menstrual cycle. *Ophthalmic and Physiological Optics, 14*, 38–48.

Hamdane, D., Kiger, L., Dewilde, S., Green, B. N., Pesce, A., Uzan, J., et al. (2003). The redox state of the cell regulates the ligand binding affinity of human neuroglobin and cytoglobin. *The Journal of Biological Chemistry, 278*, 51713–51721.

Hankeln, T., Ebner, B., Fuchs, C., Gerlach, F., Haberkamp, M., Laufs, T. L., et al. (2005). Neuroglobin and cytoglobin in search of their role in the vertebrate globin family. *Journal of Inorganic Biochemistry, 99*, 110–119.

Hecht, S., Hendley, C. D., Frank, S. R., & Haig, C. (1946). Anoxia and brightness discrimination. *Journal of General Physiology, 29*, 335–351.

Heeg, G. P., Ponsioen, T. L., & Jansonius, N. M. (2003). Learning effect, normal range, and test-retest variability of frequency doubling perimetry as a function of age, perimetric experience, and the presence or absence of glaucoma. *Ophthalmic and Physiological Optics, 23*, 535–540.

Iester, M., Capris, P., Pandolfo, A., Zingirian, M., & Traverso, C. E. (2000). Learning effect, short-term fluctuation, and long-term fluctuation in frequency doubling technique. *American Journal of Ophthalmology, 130*, 160–164.

Johnson, N., & Petersik, J. T. (1987). Preliminary findings suggesting cyclic changes in visual contrast sensitivity during the menstrual cycle. *Perceptual and Motor Skills, 64*, 587–594.

Johnson, C. A., & Sample, P. A. (2003). Perimetry and visual field testing. In P. L. Kaufman & A. Alm (Eds.), *Adler's physiology of the eye: Clinical application* (10th ed.). St. Louis: Mosby.

Kogure, S., Membrey, W. L., Fitzke, F. W., & Tsukahara, S. (2000). Effect of decreased retinal illumination on frequency doubling technology. *Japanese Journal of Ophthalmology, 44*, 489–493.

McFarland, R. A., Halperin, M. H., & Niven, J. I. (1944). Visual thresholds as an index of physiological imbalance during anoxia. *American Journal of Physiology, 142*, 328–349.

McKendrick, A. M., Anderson, A. J., Johnson, C. A., & Fortune, B. (2003). Appearance of the frequency doubling stimulus in normal subjects and patients with glaucoma. *Investigative Ophthalmology and Visual Science, 44*, 1111–1116.

Otis, A. B., Rahn, H., Epstein, M. A., & Fenn, W. O. (1946). Performance as related to composition of alveolar air. *American Journal of Physiology, 146*, 207–223.

Pesce, A., Bolognesi, M., Bocedi, A., Ascenzi, P., Dewilde, S., Moens, L., et al. (2002). Neuroglobin and cytoglobin. Fresh blood for the vertebrate globin family. *EMBO Reports, 3*, 1146–1151.

Schmidt, M., Giessl, A., Laufs, T., Hankeln, T., Wolfrum, U., & Burmester, T. (2003). How does the eye breathe? *The Journal of Biological Chemistry, 278*, 1932–1935.

Sharma, R. K., & Ehinger, B. E. J. (2003). Development and structure of the retina. In P. L. Kaufman & A. Alm (Eds.), *Adler's physiology of the eye: Clinical application* (10th ed.). St. Louis: Mosby.

Solberg, J. L., & Brown, J. M. (2002). No sex differences in contrast sensitivity and reaction time to spatial frequency. *Perceptual and Motor Skills, 94*, 1053–1055.

Sun, Y., Jin, K., Mao, X. O., Zhu, Y., & Greenberg, D. A. (2001). Neuroglobin is up-regulated by and protects neurons from hypoxic ischemic injury. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 15306–15311.

Swanson, W. H., Dul, M. W., & Fischer, S. E. (2005). Quantifying effects of retinal illuminance on frequency doubling perimetry. *Investigative Ophthalmology and Visual Science, 46*, 235–240.

Wald, G., Harper, P. V., Goodman, H. C., & Krieger, H. P. (1942). Respiratory effects upon the visual threshold. *Journal of General Physiology, 25*, 891–902.

Yap, M. K. H., Garner, L. F., Legg, S., & Faris, J. (1995). Effects of exposure to simulated altitudes on visual fields, contrast sensitivity, and dazzle recovery. *Aviation Space and Environmental Medicine, 66*, 243–246.

Yucel, I., Akar, M. E., Dora, B., Akar, Y., Taskin, O., & Özer, H. O. (2005). Effect of the menstrual cycle on standard achromatic and blue-on-yellow visual field analysis of women with migraine. *Canadian Journal of Ophthalmology, 40*, 51–57.