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Interferon- α -induced deficits in novel object recognition are rescued by chronic
exercise

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Abstract

The anti-viral drug interferon-alpha (IFN- α) is widely-known to induce psychiatric and cognitive effects in patients. Previous work has shown that physical exercise can have a positive effect against brain insult. We investigated the effects of a clinically-comparable treatment regime of IFN- α on cognitive function in male Wistar rats and assessed the impact of chronic treadmill running on the deficits generated by IFN- α . We found that IFN- α induced significant impairments in performance on both spatial novelty and object novelty recognition. Chronic forced exercise did not protect against IFN- α -induced learning deficits in reactivity to spatial change, but did restore the capacity for novel object recognition in IFN- α -treated animals.

Keywords: Interferon-alpha; forced exercise; object learning; hippocampus; cytokine.

Introduction

Interferons are a family of multi-functional, pleiotropic proteins belonging to the cytokine family of proteins. They are produced by the body in response to viral infections and tumors; key functions include antiviral activities, inhibition of cell growth and control of apoptosis. While IFN- α is currently a mainstay of treatment for a variety of viral diseases, cancers and other disorders (comprehensively reviewed in [1]), there are considerable problems regarding the emergence of psychiatric adverse events during patient treatment. Although systemic effects such as myalgia and chills normally regress after about two weeks of treatment and do not tend to interfere with treatment compliance [2], serious psychiatric and cognitive phenomena emerge later on in the course of treatment and can even necessitate treatment cessation [3].

Clinical studies have documented a wide range of IFN- α -related psychiatric disturbances including depression [4-7], psychoses [8] and aggressive tendencies [9]. Few have investigated the effects of IFN- α on neurocognitive function, but significant findings have been made: both numerical working memory function [10] and verbal recall abilities [11] have been found to be significantly impaired in IFN- α patients, independent of depressive symptoms. Further, prefrontal cortical hypometabolism has been detected using PET imaging in patients receiving low-dose IFN- α treatment [12]. In contrast, Capuron *et al.*, [13], found no effect of four weeks high-dose IFN- α on spatial working memory accuracy or spatial planning ability.

Suggestions of IFN- α -generated cognitive dysregulation are also found in the animal literature. Mice that over-express IFN- α in astrocytes have significantly impaired spatial learning abilities and deficits in the induction of hippocampal LTP [14]. One might hypothesize that systemic IFN- α administration could exert similar effects on spatial learning behaviors in rodents.

There has been a lack of consensus in the literature on the ability of systemic administration of human IFN- α in rodents to provoke a CNS syndrome that mimics the patient condition [15-19]. However, our laboratory has recently described a clinically-relevant animal model of IFN- α affective dysregulation [20]: our procedure involves sustained (>3 weeks) systemic administration of IFN- α with a dose and regime similar to that used in humans. Here, we first set about investigating cognitive effects of IFN- α in this model using the object recognition (OE) task which has long been used to investigate animals' ability to encode spatial representations [21].

Chronic physical activity has been found to confer significant benefits in terms of brain wellbeing. In both clinical and analogous animal populations, exercise may act as a neuroprotectant in aging, Parkinson's disease (PD) traumatic brain injury (TBI), cerebral ischemia/stroke and kainic acid-induced neurodegeneration (for recent examples and discussions see [22-30]). Even in pathology-free animals, exercise can alter several cognition-related parameters, as indexed by variety of behavioral (watermaze [31, 32], object recognition [32], DNMS [33], radial arm maze [34]) and neurobiological (LTP [32], neurogenesis [35]) measures. On a molecular level, alterations in a number of plasticity-related, synaptic, anti-apoptotic and immune response gene expression profiles are seen following chronic wheel-running in rats [36]. In the study presented here, we also evaluated the efficacy of a chronic, forced treadmill running regime in preventing predicted IFN- α -induced learning deficits.

Materials and Methods

Animals

Thirty-two male Wistar rats (215-330g at the beginning of the experiment, BioResources Unit, Trinity College Dublin; n=8 per group) were housed in groups of

three (standard hard-bottomed, polypropylene cages, cage dimensions - 44 x 28 x 18cm) or four (cage dimensions - 55 x 30 x 25cm) in a temperature-controlled vivarium (20 to 22°C), with a 12:12h light:dark cycle, and allowed free access to food and water. Animals were given at least 5 days to acclimate to their environment and receive gentle handling before any experimental manipulation began. All experiments were performed under a license issued by the Department of Health (Ireland), in strict adherence to national laws and international recommendations for the use and care of experimental animals (European Community; 86/609/EEC).

Exercise Protocol

The protocol was a modified version of that described previously, which was shown to have a beneficial impact on cognitive performance in other spatial learning tasks [32]. Animals were familiarized to motorized treadmills (Exer 3/6 treadmill, Columbus Instruments) over two consecutive days. Briefly, animals were gently placed on the moving treadmill (at a speed of 0.78km/h) and belt speed was gradually increased to the test speed of 1.02 km/h over a 15–30 minute period. The treadmill was equipped with wire loops at one end of the belt through which a mild electric shock could be delivered; this induced the rats to run continuously and were activated at low levels (on average an intensity of 2 on a scale of 0–10; this represents a current of 0.7 mA with an inter-pulse interval of 2 s) throughout all habituation and exercise sessions. During habituation, animals were carefully monitored for signs of fatigue or distress.

Animals were then assigned to treatment groups: saline sedentary, SALSED; IFN- α sedentary, IFNSED; saline exercised, SALRUN; IFN- α exercised, IFNRUN; n = 8 in each initially, but two animals from the saline exercised group were excluded during the experiment due to ill-health. Habituation sessions revealed that the animals tired

significantly after approximately 20 minutes running; therefore, each exercise session consisted of two 20-minute running sessions (belt speed 1.02 km/h), with a 20-minute inter-session rest interval. Exercise sessions were carried out every second day for a total of seven weeks. The exercise sessions took place towards the end of the light period (usually between 18.30 and 20.00h), as near as possible to waking time to introduce minimal disturbance to the animals' sleep cycle. Rats were carefully monitored while exercising to ensure they ran continuously and were not unduly stressed. Control (sedentary) rats were placed in stationary treadmills for the same duration, with shock loops activated as for exercised animals.

Animals were exercised for two weeks prior to initiation of IFN- α treatment to prevent the formation of a negative association between IFN- α administration and exercise.

Drug Treatment

Animals were treated with either approximately 170,000 IU (international units)/kg Roferon-A (human recombinant interferon-alpha 2a, Roche Pharmaceuticals, USA; 0.2 mL, subcutaneously), or vehicle (0.9% NaCl, 0.2 mL, s.c.) three times per week (rat doses if IFN- α were calculated using the mean weight of rats in the IFN- α group; weight was monitored once a week and IFN- α doses adjusted accordingly with weight gain). Roferon-A was supplied in 0.5 mL syringes, containing 3,000,000 IU (3MIU) interferon-alpha 2a (11.5 μ g/0.5 mL). Previous work in our laboratory has shown that treatment with this dose for four weeks reliably induces behavioral effects in rats [20], and is in line with a mid-range human dose (12MIU); IFN- α doses can range from, for example, 3MIU, s.c. three times per week for chronic Hepatitis C virus to 30MIU/m² body surface area for AIDS-related Kaposi's sarcoma [37, 38].

Behavioral Procedures

Object Exploration Task

The OE task was performed towards the end of week six of IFN- α treatment. OE testing for each treatment group began approximately 14 hours after the last exercise session of that group and 24 hours after the last IFN-alpha injection to ensure that the acute effects of the drug did not interfere with behavioral assessment. Each group was tested on separate days, testing was thus carried out across four days.

Habituation and reactions to spatial change and novel object introduction were evaluated by the exploration of distinct objects placed in an open field using a task design modified from Gobbo *et al.*, [39]. The apparatus consisted of a black, circular fiberglass arena (diameter = 200 cm, height = 35 cm) surrounded by black curtains with several extra-maze cues attached. The experimenter stood at the south-eastern side of the arena during testing. Four objects were placed in a square formation at the centre of the arena, approximately 30 cm apart. The objects used included a concrete pillar, a cardboard rectangular box, a metal jug, a white translucent plastic bottle and brown glass bottle; all objects were of similar dimensions and their weight was such that they could not be displaced by the rats.

Animals were initially placed in the centre of the empty arena and allowed to explore freely for six minutes (open field exploration, trial 0). Data for this trial were recorded using the image analyzing software system EthoVision (Noldus Information Technology, Wageningen, Netherlands). Parameters measured were: distance traveled (cm), mean velocity (cm/s) and time spent in a virtually-defined thigmotactic boundary region (15 cm from the arena walls). Animals were then removed from the experimental area and returned to their home cage, while objects were placed in the arena in the aforementioned spatial configuration. Animals were given six trials of six

minutes duration with three-minute inter-trial intervals (ITI's). In trial 4, one of the objects was moved to a new location towards the periphery of the open field (spatial novelty recognition trial). This spatial configuration was maintained for trial 5. In trial 6, the displaced object was replaced by a novel object (object novelty recognition trial); see figure 1A for a pictorial representation of the task protocol. To eliminate any bias of olfactory cues, all objects were manipulated before trials 4 and 6, and the arena and all objects were cleaned with a lightly-scented disinfectant between each animal's set of trials.

Object exploration was evaluated by the time spent sniffing the objects, defined as directed head movement whisker twitching with nose proximity to objects within 2-3cm. Both time spent exploring the moved or novel object specifically and time spent exploring other objects were recorded manually by the experimenter.

Statistical Analyses

Statistics were carried out using Statistical Package for the Social Sciences (SPSS) version 11. Data were assessed for normality prior to analyses using the Shapiro-Wilks' W test. Data are expressed as means $\pm$ standard errors; * $p < 0.05$, * $p < 0.01$, * $p < 0.001$.

Object recognition task Open field exploration (trial 0) was analyzed using one-way ANOVA's to examine the effect of treatment group. Habituation to the objects (trials 1-3) was assessed by means of a mixed-factorial ANOVA with exploration time of all objects (3 levels) as within-subjects factor and treatment group as between-subjects factor. Subsequent one-way ANOVA's were carried out to examine the effect of treatment group within individual trials.

Response to spatial change was assessed by calculating the mean time in contact with either displaced, DO, or non-displaced, NDO objects in trial 4 minus the mean time spent in contact with the same object category in trial 3. Response to introduction of a novel object was assessed by calculating the mean time in contact with either novel, NO, or old, OO objects in trial 6 minus the mean time spent in contact with the same object category in trial 5 (since the novel object was not present in trial 5, the time spent exploring the object to be replaced by the new object was used for calculations). Planned paired samples t-tests (paired variables: DO-NDO or NO-OO) were subsequently performed to assess object-location recognition and novel object recognition performance for each group.

Results

Open Field Exploration

One-way ANOVA analyses did not reveal any significant effect of treatment group on locomotion in the open field (trial 0) in terms of distance traveled, velocity or thigmotaxis; data not shown.

Object Recognition Task

Habituation Over trials 1 – 3 of this task, all groups showed habituation to their environment as expected, with total exploration time decreasing across trials (Fig. 1B); a mixed-factorial ANOVA revealed a significant effect of trial ($F(2, 52) = 9.41, p < 0.001$), but no significant trial x group interaction ($F(2, 52) = 1.36, p = 0.247$), and no significant effect of group ($F(1, 26) = 0.428, p = 0.735$). In addition, no significant effect of treatment group was seen on any of the habituation trials following one-way ANOVA's for each trial.

Recognition of a spatial change For object location recognition paired samples t-tests (DO-NDO) indicated that both saline sedentary ($t = 2.99$, $df = 7$, $p < 0.05$) and saline exercised ($t = 4.00$, $df = 5$, $p < 0.05$) animals showed significant recognition of a spatial change, while neither group of IFN- α animals (sedentary: $t = 1.83$, $df = 7$, $p = 0.11$; exercised: $t = -0.17$, $df = 7$, $p = 0.872$) discriminated between DO and NDO (Figure 2A).

Recognition of a novel object For object recognition paired samples t-tests (NO-OO) revealed that saline sedentary ($t = 4.6$, $df = 7$, $p < 0.01$), saline exercised ($t = 2.73$, $df = 5$, $p < 0.05$) and IFN- α exercised ($t = 4.88$, $df = 7$, $p < 0.01$) animals showed significant preference for the novel object compared to old objects (Figure 2B). IFN- α sedentary animals did not show this preference ($t = 1.82$, $df = 6$, $p = 0.12$).

Discussion

The aims of this study were to evaluate the impact of chronic, systemic IFN- α treatment on cognitive function in the rat, and to assess the potential of chronic forced exercise to prevent the induction of IFN- α -induced cognitive deficits. Previous data indicate the positive prophylactic effects of exercise against neuronal insult (see Introduction). We found that our treatment regime of IFN- α (which has previously been shown to induce an affective-like syndrome in rodents, [20]) had a significant negative impact on spatial learning and novel object recognition performance. Further, exercise training in IFN- α animals rescued the deficits in novel object recognition.

Both groups of IFN- α -treated animals spontaneously explored the open field normally, in terms of distance traveled and velocity, and did not show increased thigmotaxis in comparison to controls. Groups did not differ in terms of the amount of

initial exploration in the task (trial 1), indicating normal visuomotor function and exploratory tendencies. In addition, all groups showed normal habituation to their spatial environment, gradually decreasing exploration of the objects across trials 1-3 (Figure 1A). If such an asymptotic habituation curve reflects normal encoding of the environmental features and spatial configuration, one may predict that alterations to the initial situation should elicit increased exploratory behavior. However, previous studies have shown a dissociation between the capacity for normal habituation and that for recognition of spatial or object novelty [40-42]. The results presented here support such a dissociation: while IFN- α sedentary animals show a normal habituation curve, they fail to detect either a spatial change or the introduction of a novel object.

The decrease in the ability to detect both spatial change and novelty introduction in the IFN- α sedentary animals might imply a generalized decrease in the normal, natural tendency to explore novelty. However, the dichotomous effects of exercise on these parameters in IFN- α exercised animals point to discrete mechanisms underlying the two phenomena: while exercise can restore the ability to recognize novel features of the spatial arrangement, it does not allow the IFN- α animals to notice a configurational change in the spatial arrangement. This result is in agreement with previous results [32] where a similar exercise protocol was found to improve novel object recognition but did not affect spatial learning in the reference memory version of the watermaze. This idea is also supported by lesions studies. In a task similar to that used here, hippocampal lesions have been found to impair performance on the spatial change trial, but not the novel object trial, while entorhinal cortex and subicular lesions impaired both novel object recognition and spatial change recognition [40]. While some data indicate that entorhinal cortical lesions alone do not

impair performance in a continuous recognition (spatial location) memory procedure [43] this behavioral testing procedure is very different from the OE task used here. The protocol requires several days of training and testing, unlike the short timespan of the OE protocol. These differences could underlie the conflicting lesion reports regarding the role of entorhinal cortex in spatial learning and memory. Moreover, Hargreaves *et al.*, [44] show that spatial information is represented by medial entorhinal cortex and nonspatial by lateral entorhinal cortex. These results are in support of the concept that both medial and lateral entorhinal function (representing spatial and nonspatial information, respectively) could be impaired in IFN- α rats.

The data presented here suggest that chronic IFN- α treatment has a negative impact on the function of the extended hippocampal formation circuit (entorhinal cortex, subiculum and hippocampus proper) as measured by recognition of spatial and object novelty in the OE task. Concurrent, chronic forced exercise may ameliorate IFN- α -induced deficits in recognition of a novel object by somehow modulating entorhinal cortical and subicular function to a degree. However, the persistent impairments in recognition of a spatial change in IFN- α exercised animals indicate that, along with overriding deficits in hippocampus proper, deficits in entorhinal cortical and subicular function may also contribute. It is possible that the forced exercise regime used here may not have an entorhinal cortex-/subiculum-specific effect and that the OE task may only be sensitive to these effects; any ameliorative effects on hippocampal function may not be strong enough to produce significant results in this task. Any suggested mechanism underlying an apparent entorhinal cortex-/subiculum-specific effect of exercise would be purely speculative at this stage. It remains to be seen whether this IFN- α treatment regime would induce deficits in other tasks dependent

on the hippocampal formation or in the *in vivo* electrophysiological profile/synaptic plasticity potential of the hippocampus.

It is interesting to note that in clinical studies on the CNS effects of IFN- α examinations of cognitive status are rarely conducted, with most focusing on the affective standing of the individual. These novel results from our animal model suggest that it could be beneficial to patients to incorporate monitoring of cognitive status during IFN- α treatment into clinical protocol. This is especially important in light of earlier findings that cognitive impairments in IFN- α -treated individuals seem to emerge independently of depressive symptoms [10, 11]. The results of this study strengthen the case for the neuroprotective properties of exercise and provide an interesting first insight into the cognitive deficits induced by IFN- α in an animal model.

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Figure legends

Figure 1 Neither IFN- α nor exercise affects habituation in the OE task

Pictorial representation of the task protocol (A). X marks the experimenter's position. All groups habituate normally to the spatial layout of the environment, decreasing total exploration time across trials 1-3 (B). Data are expressed as mean $\pm$ SEM; n = 6-8 for each group.

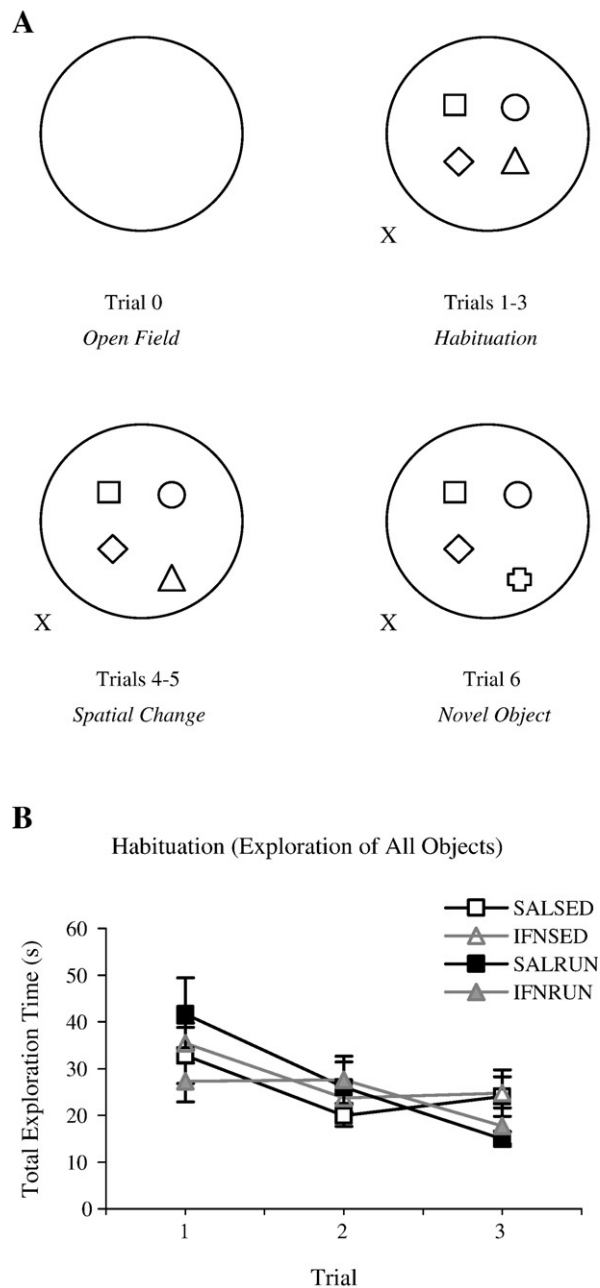


Figure 2 IFN- α impairs both recognition of spatial novelty and object novelty; exercise prevents only the deficits in novelty recognition.

Only SALSED and SALRUN animals show reactivity to the spatial change (A; paired t-tests, $p < 0.05$ for both groups). While IFNSED animals are also impaired in reactivity to object novelty, SALSED, SALRUN and IFNRUN animals show significant preference for the novel object (B; paired samples t-test, $p < 0.05$ for each group). Data are expressed as mean change in time (s) $\pm$ SEM; $n = 6-8$ for each group; DO: Displace Object, NDO: Non-Displaced Objects, NO: New Object, OO: Old Object.

