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Research report

Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety

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ABSTRACT

Voluntary wheel running in rodents is associated with a number of adaptive behavioral and physiological effects including improved learning, reduction in stress-associated behaviors, neurogenesis, angiogenesis, increases in neurotrophic factors, and changes in several signaling molecules. Exercise has also been reported to reduce anxiety-like behaviors. However, other studies have failed to find an anxiolytic effect of exercise. The inconsistencies in the literature may contribute to the scarcity of data examining the physiological correlates of the anxiolytic effect of exercise. Here we show that 2 weeks of voluntary exercise in male C57 mice is associated with reduced anxiety as measured with acoustic startle, stress-induced hyperthermia, social interaction, light-enhanced startle, and some, but not all, measures in the open field. A great deal is known about the neural circuits underlying anxiety. Given the consistency of the anxiolytic effect of voluntary exercise across several measures, it is now possible to begin a systematic analysis of the physiological basis of the anxiolytic effect of exercise.

1. Introduction

A number of human studies support the idea that physical exercise can reduce the signs and symptoms of anxiety [7,10,33,38,46,51,63,70,77] and specifically benefit the treatment of PTSD, panic disorder and phobia [24,93]. Despite the importance of these findings, relatively little is known about the physiological basis of the anxiolytic effect of exercise. This is particularly surprising in light of the fact that in animals exercise is associated with a variety of adaptations in the brain including neurogenesis and increased neuronal survival [8,25,96–99], angiogenesis [5,55,87], increased vascular flow [87], increased expression of neurotrophins [21,37,73,74,100,101], changes in gene expression [91] and signaling molecules [84], and changes in serotonin [40,42], norepinephrine [22] and GABA [18]. However, few of these effects have been directly linked to anxiolytic effects of exercise.

This lack of understanding of the physiological basis of the anxiolytic effect of exercise may be due in part to the inconsistent effects that exercise has in animal models of anxiety. In studies allowing animal voluntary access to a running wheel there are reports of an anxiolytic effect of voluntary exercise [4,17,18,21,42,43], no effect of exercise [79] or increased anxiety following exercise [9,95]. While these inconsistencies may be due to any number of experimental variables, they point to the need for continued assessment of the putative anxiolytic effects of exercise in animal models. We have shown that voluntary exercise in mice is associated with lower acoustic startle amplitude [32]. Specifically, C57BL/6J mice given free access to a running wheel for 2 weeks showed lower acoustic startle amplitude than mice not given access to a running wheel. Higher acoustic startle amplitude is often observed in clinically anxious individuals [57,68] and in rodents, treatments that increase anxiety (e.g., bright lights, anxiogenic drugs) increase startle amplitude [107] while treatments that reduce anxiety (e.g., anxiolytic drugs) decrease startle amplitude. Therefore, the reduction in acoustic startle amplitude in exercising mice [32] may be consistent with the reports indicating that voluntary exercise in mice is anxiolytic [4,17,18,21,42,43]. However, given the inconsistent effects of voluntary exercise across experiments, we sought to examine the generality of the putative anxiolytic effect of voluntary exercise in mice by examining anxiety across a battery of tests [9] that includes acoustic startle. To this end, in separate experiments, mice were given free access to either a functioning running wheel or a non-functioning (i.e., locked) running wheel for 2 weeks. They were then tested for acoustic startle amplitude, open field behavior, stress-induced hyperthermia, social interaction and light-enhanced startle.

2. Materials and methods

2.1. Animals

Eight weeks old, male C57BL6/J mice were obtained from Jackson Laboratories in Bar Harbor, Maine. Mice were housed in groups of four in standard acrylic cages

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(24 cm (W) × 45 cm (D) × 20 cm (H)) located in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved conventional animal facility. Mice were maintained on a 12 h light/dark cycle (lights on at 07:00 h) with food and water available at all times. A 7-day acclimation period was given to mice before introduction of the running wheels. All procedures were approved by the University of Vermont Animal Care and Use Committee.

2.2. Voluntary wheel running

Mice were given ad lib access to a running wheel (Superpet mini run-a-round, measuring 11.4 cm in diameter) for 2 weeks prior to the start of behavioral testing. For half of the cages, the wheels were locked preventing running (non-exercising control) and for the remaining cages the wheels were functional.

2.3. Behavioral testing

All behavioral testing occurred during the light cycle and between the hours of 9:00 and 15:00. All procedures except prepulse inhibition of startle were carried out in naive groups of mice to eliminate the possibility of carry over effects between tests of anxiety [71]. Prepulse inhibition was examined in mice following the open field test.

2.3.1. Acoustic startle

Acoustic startle amplitude is a sensitive measure of fear and anxiety [107]. In rodents, treatments that increase anxiety (e.g., bright lights, anxiogenic drugs) increase startle amplitude [107] while treatments that reduce anxiety (e.g., anxiolytic drugs) decrease startle amplitude [13]. We have previously shown in a between subjects design [32] that mice given 2 weeks of access to a running wheel show lower startle amplitude than non running controls. In order to determine whether running reduces startle amplitude, as opposed to startle amplitude increasing in non-running controls, we ran a within subjects design in which mice were tested for acoustic startle amplitude both before and after 2 weeks of voluntary exercise.

The startle tests were conducted in eight sound attenuating cubicles measuring 58 cm (W) × 32 cm (D) × 55 cm (H). Each cubicle was lined with black, sound absorbing foam with no internal source of light. Each cubicle contained a stabilimeter device consisting of a load cell platform onto which the behavioral chamber was mounted (MED-ASR-PRO1, Med Associates, Georgia, VT). The chamber was constructed of clear acrylic, cylindrical in shape, 12.5 cm in length, with an inner diameter of 5 cm. The floor of the chamber consisted of a removable grid composed of 6 steel rods 3.2 mm in diameter, and spaced 6.4 mm apart. Startle responses were transduced by the load cell, amplified, and digitized over a range of 0–4096 units. Startle amplitude was defined as the largest peak to trough value within 100 ms after the onset of the startle stimulus. Startle stimuli were 20 ms bursts of white noise provided through a Radio Shack Supertweeter located 10 cm behind the behavioral chamber. Data collection and the control and sequencing of all stimuli were controlled by Med-Associates startle reflex hardware and software.

In the first startle experiment mice had access to a locked (n = 16) or functioning (n = 16) running wheel for 2 weeks. Running distance and time spent running were recorded at 12 h intervals (at 7:00 and 19:00 h) over the 2 weeks. Mice were then tested for acoustic startle on each of three consecutive days (i.e., days 15–17). Mice continued to have free access to the running wheels on the test days. On each of the three test days mice were transported to the lab in the home cage and placed individually in the startle apparatus. After a 5 min acclimation period, mice were presented with the first of 30 startle stimulus alone trials. Ten stimuli of each intensity level (95, 100, and 105 dB) were presented in a pseudo-random order (the constraint being that each intensity occur within each block of three trials) with a mean inter-trial interval (ITI) of 60 s. A total of three startle sessions were used to obtain stable measure of acoustic startle amplitude. Mean startle amplitude was computed for each mouse for each startle stimulus intensity within each startle test (i.e., for each of the three pre and post running tests).

The second startle experiment used a within subjects design to examine the effect of wheel running on acoustic startle. All mice had access to locked running wheels for 2 weeks prior to the first of three tests for acoustic startle. Groups were then randomly divided into exercising (n = 16) and non-exercising controls (n = 16). For the exercising groups the wheels were then unlocked and for the non-exercising groups the wheels remained locked. Two weeks later the mice were again tested for acoustic startle on each of three consecutive days.

The third startle experiment examined prepulse inhibition of startle across a range of prepulse stimulus frequencies in an effort to determine whether the ability to process auditory stimuli is altered in exercising mice. Changes in the ability to process auditory stimuli could contribute to lower startle amplitude. To behaviorally assess the ability to process auditory stimuli, we examined prepulse inhibition (PPI) of startle in exercising and non-exercising mice. PPI is the degree to which the acoustic startle response is reduced when the startle-eliciting stimulus is preceded by a brief non-startle eliciting stimulus [53,56]. PPI is thought to reflect the ability to "gate" sensory information [36,88,89] and has been used as a means of behaviorally assessing hearing in mice [108–112]. For example, mice with high frequency sensorineuronal hearing loss show poor PPI to high frequency auditory prepulse stimuli, but not low frequency prepulse stimuli.

Exercising (n = 12) and non-exercising (n = 14) mice were given 30 startle-eliciting noise bursts (105 dB SPL) 20 msec in duration at a 1 min inter-trial interval. On prepulse trials, the prepulse stimulus preceded the startle stimulus by 100 ms. The prepulse was a 20 ms, 70 dB pure tone or either 4, 8, 12 or 16 kHz. Four tone frequencies were used in order to span the range of mouse hearing. Twenty-five of the 30 startle stimuli were preceded by a prepulse (5 at each frequency). The remaining 5 trials were startle stimulus alone trials. PPI was calculated by dividing startle amplitude on prepulse trials by startle amplitude on startle stimulus alone trials. Thus, 100% indicates that the prepulse had no effect on startle amplitude whereas 0% indicates that the prepulse completely inhibited startle.

2.3.2. Open field

In the open field test anxiety is generally associated with overall lower levels of activity, thigmotaxis and decreased exploratory behavior [1,11]. Open field is a common test for anxiety and is sensitive to both anxiolytic and anxiogenic drugs. Given its wide use and clear ethological validity, we assessed whether 2 weeks of access to a running wheel would be associated with reduced anxiety-related behavior in the open field. The open field consisted of a square, opaque acrylic container (42 cm × 42 cm × 25 cm) located in a dimly lit room. The floor of the container was divided into 9 cm × 14 cm squares. Open field behavior was recorded using a digital video monitoring system (MED-VFC-NIR-M, Med Associates, Georgia, VT) that quantified overall activity by accumulating the change in contrasting video pixels over the recording session at a sample rate of 60 Hz. The digital video also served as a record of behavior in the open field and was used to score the time spent in center of the open field, the number of center crossings, the frequency of grooming, rearing, and the number of attempts to escape the open field which were defined as jumps against the side wall. All scoring of behavior was done offline by an observer blind to the animal's group assignment.

Following 2 weeks of access to a running wheel, mice (n = 12 exercising and n = 14 non-exercising) were transported to the lab in their home cage. A single mouse was placed in the open field and its behavior recorded for 6 min. Following this, the mouse was removed and returned to its home cage. The open field was thoroughly cleaned and dried prior to running the next mouse.

2.3.3. Rota-rod

In order to assess motor behavior in exercising and non-exercising mice, mice (n = 16 exercising and n = 16 non-exercising) were placed on a rota-rod (ENV-576, Med Associates, Georgia, VT) programmed to accelerate from 4 to 40 rpm over a 5 min period. Each mouse was given three consecutive trials with a 10 min inter-trial interval.

2.3.4. Stress-induced hyperthermia

In mice, core body temperature appears to be a sensitive measure of stress and anticipatory anxiety [75,114]. Borsini et al. [6] observed that among group housed mice, mice taken last from the cage had higher core body temperatures than mice taken first. The increase in temperature has been likened to an 'emotional fever' and is thought to be related to stress and anticipatory anxiety associated with handling cage mates and so has been termed stress-induced hyperthermia (SIH) [75]. Consistent with this interpretation, anxiolytic drugs decrease this SIH [6,45,65] while anxiogenic drugs increase SIH [6]. In an extension of this effect, Van Der Heyden [94] showed that within singly housed mice, repeating the rectal temperature measurement at 10 min intervals led to a robust hyperthermia that reached asymptote within 30 min. This effect was also blocked by anxiolytic drugs [76,94] and has been offered as a method for examining anticipatory anxiety in individual mice. To examine whether 2 weeks of voluntary exercise would decrease SIH, groups of mice (n = 15 exercising and n = 15 non-exercising) were individually housed 24 h prior to temperature measurement. Mice from exercising groups continued to have access to a functioning running wheel while individually housed. Core body temperature was measured with a Thermalert Monitoring Thermometer (PhysiTemp TH-5, Clifton, New Jersey) equipped with a mouse rectal temperature probe (PhysiTemp RET-3, 0.16 mm tip diameter). Temperature measurements were made in the colony room to decrease potential effects of transport stress. For temperature measurement a mouse was placed into a small acrylic restraining tube (4 cm × 12 cm) and lightly restrained by holding the tail. The temperature probe was lubricated with peanut oil and then inserted 5 mm into the rectum and held in place for 30 s. At 30 s, the temperature was recorded to the nearest 0.1 °C. The mouse was then placed back into its home cage (still individually housed). Ten minutes later, the mouse's temperature was again assessed. SIH was defined as an increase in core body temperature from the first temperature measurement to the second measurement.

2.3.5. Social interaction

The social interaction test is an ethologically relevant test in which the time spent by pairs of rodents in social interaction (sniffing, grooming or following one another) is taken as a measure of emotionality [29,31]. Rodents that make more frequent social contacts or spend more time contacting are thought to be less anxious. Consistent with this, anxiolytic drugs increase the amount of social interaction [28] and anxiogenic drugs decrease the amount of social interaction [30].