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Acute Forced Exercise Increases *Bdnf IV mRNA* and Reduces Exploratory Behavior in C57BL/6J Mice

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Abstract

Acute exercise has been shown to improve memory in humans. Potential mechanisms include increased *Bdnf* expression, noradrenergic activity, and modification of glutamate receptors. Because mice are commonly used to study exercise and brain plasticity, it is important to explore how acute exercise impacts behavior in this model. C57BL/6J mice were assigned to 3 groups: control, moderate-intensity running, and high-intensity running. Control mice were placed on a stationary treadmill for 30 min and moderate- and high-intensity mice ran for 30 min at 12 m/min and 15–17 m/min, respectively. Mice were sacrificed immediately after running and the hippocampus removed. Total *Bdnf*, *Bdnf* exon IV, and glutamate receptor subunits were quantified with qPCR. Total and phosphorylated GluR1 (Ser845 and Ser831) protein was quantified following immunoblotting. Utilizing the same protocol for control and high-intensity running, object location memory was examined in a separate cohort of mice. Anxiety-like behavior was assessed in the open field task (OFT) in a third cohort of mice that were separated into 4 groups: control–saline, control–DSP-4, acute exercise–saline, and acute exercise–DSP-4. DSP-4 was used to lesion the central noradrenergic system. We observed higher *Bdnf IV* mRNA in high-intensity runners compared to controls, but no effects of acute exercise on memory. In the OFT, runners traveled less distance and spent more time grooming than controls. DSP-4 did not attenuate the effects of exercise. CONCLUSION: A single bout of exercise increases *Bdnf IV* mRNA in an intensity-dependent manner; however, high-intensity running reduces exploratory behavior in C57BL/6J mice.

Keywords

Brain-derived neurotrophic factor; Acute Exercise; Anxiety; AMPA; Hippocampus

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Competing interest
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Introduction

A preponderance of evidence supports that exercise training and chronic physical activity are beneficial for brain health (for reviews, see [1–3]). The hippocampus is thought to be particularly sensitive to physical activity, as both functional and structural adaptations have been reported following periods of physical activity, including enhanced adult neurogenesis [4–7] (for review, see [8]), increased branching of neuronal dendrites [9,10], and an increase in the amplitude and a reduction in the threshold of long-term potentiation (LTP) [6,11]. Physical activity also improves hippocampus-dependent learning [12–17]. The underlying mechanisms of the response to *chronic* physical activity has received much attention, however, little is known about the response to a single acute bout of exercise. As chronic physical activity is an accumulation of single acute bouts of exercise, we posit that if exercise is to be used as a strategy to enhance learning and memory, it is advantageous to understand the response to each individual bout of acute exercise.

Meta-analyses report that acute exercise is effective at improving performance on memory tasks [18–20], though the mechanisms remain largely unknown. One possibility is that acute exercise-induced elevations in circulating catecholamines act to facilitate plasticity (reviewed in [21]), as it has been shown that norepinephrine (NE) promotes spike-timing dependent synaptic plasticity [22]. Indeed, exercise of sufficient intensity and duration increases circulating catecholamines (epinephrine and NE) and central NE [23,24] (for review, see [21]). Norepinephrine can activate Gs-dependent signaling cascades that mediate post-translational modifications to the GluR1 subunit of the AMPA-type glutamate receptor (AMPAR) [25], the ionotropic glutamate receptor at excitatory synapses. The threshold for the induction of LTP is lowered following phosphorylation of specific sites on the C-terminal tail of the GluR1 subunit, which can regulate the concentration and conductance of synaptic AMPARs [26–28] (for reviews, see [29,30]). Two such residues in the C-terminal tail of GluR1, serine 845 (Ser845), and to a lesser extent, serine 831 (Ser831), are phosphorylated in the hippocampus following NE signaling, which reduces the threshold for LTP and learning [25]. Phosphorylation of Ser831 on GluR1 by CAMKII increases ion channel open probability and channel conductance [31,32], while phosphorylation of Ser845 by PKA increases perisynaptic insertion and decreases AMPAR internalization [28,33]. Similarly, stimulation that reduces synaptic strength is associated with a rapid dephosphorylation of Ser845 [34]. We have previously shown that continuous exposure to a voluntary running wheel for one month resulted in an increase in the expression of GluR1 and phosphorylation of Ser845 [35]. However, we observed no change in Ser845 phosphorylation 15 minutes after a single 45-minute bout of treadmill exercise [35]. Hu et al. [25] found elevated Ser845 phosphorylation 15 minutes following a peripheral injection of epinephrine, suggesting that the phosphorylation of Ser845 is induced rapidly.

Alternatively, exercise training may induce structural and functional plasticity in the hippocampus by increased transcription and translation of brain derived neurotrophic factor (BDNF) [8]. BDNF is critical for the maintenance of optimal brain health and plays an integral role in functional and structural plasticity in the hippocampus throughout the lifespan (for review, see [36]). BDNF is important for structural plasticity, including hippocampal neurogenesis [37–40] and dendritic/synaptic development [41–43], as well as

functional adaptations at the synaptic [44–51] and behavioral [51–57] levels. BDNF has been shown to be necessary for the benefits of exercise training on structural and functional plasticity [16,58,59], however, little is known about the influence of acute exercise on *BDNF* transcription.

Alternative splicing of the nine exons of the *BDNF* gene can produce 22 possible mRNA transcripts [60]. Each transcript has a different subcellular localization and transport capability [61,62] and the large number of transcripts allow for differential temporal and regional control of translation, mRNA longevity, and distribution. *BDNF* transcript IV (*Bdnf IV*) is of particular interest, as it appears to be uniquely sensitive to neuronal stimuli *in vitro* [63,64] and *in vivo* [65,66]. We previously demonstrated an increase in total *Bdnf* and *Bdnf IV* mRNA expression 15 minutes following a 45-minute bout of treadmill exercise [35] - an effect that was independent of exercise intensity. The exercise protocol utilized in our previous investigation was long (45 minutes) and employed a rest period (15 minutes) prior to determination of *Bdnf* expression, so the threshold of exercise duration and intensity is yet to be determined.

Although research in humans suggests acute exercise can improve memory [18,20], this has not been adequately investigated in rodents. Since rodents are commonly used as models to understand the mechanisms that drive behavioral adaptations in humans, it is important to understand the behavioral responses to acute exercise in this model. Several studies have reported that chronic exercise improves both spatial [14,58,67] and non-spatial [13,67] memory in rodents, yet the influence of acute exercise has been mostly neglected. In addition, the novelty of an acute bout of exercise may induce unique responses related to both the physical and psychological stress of the exercise. Beyond the novelty of exercise, there is evidence that exploratory behavior may be reduced after a night with access to a voluntary running wheel, even after three weeks of running wheel exposure [68]. This suggests that behavioral tests performed immediately after acute exercise may be associated with reduced exploratory behavior, which could impact performance on several tests of learning and memory. Here we investigate the influence of a single, 30-minute bout of acute treadmill exercise on phosphorylation and abundance of GluR1 protein and *Bdnf* mRNA and spatial memory. Furthermore, exercise is known to stimulate the release of central and peripheral catecholamines [23,69,70], which influence anxiety-like behavior [71]. We therefore also used a pharmacological approach to reduce the output of the central noradrenergic system prior to acute exercise, to examine the impact on anxiety-like behavior.

Materials and Methods

Mouse model.

Three-month-old male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) were used in this investigation. This mouse strain is commonly used to study the impact of exercise on brain phenotypes and, in our lab, displays adequate treadmill running ability [35,72]. All mice were group housed and cared for by University of Maryland veterinary staff. Mice were kept on a 12hr light/12hr dark cycle and provided standard rodent chow. The University of Maryland Institutional Animal Care and Use Committee approved all protocols.

Overview and Treadmill Protocol: Mice (N=35) were randomly separated into three groups: 1) treadmill without exercise (CON; n=12), 2) moderate-intensity acute treadmill exercise (MOD; n=11), 3) high-intensity acute treadmill exercise (HI; n=12). All procedures were performed 4 hours into the light phase, beginning at approximately 10:00am. For three days leading up to the experiment, mice were placed on the stationary treadmill for five minutes per day, during which the electrical stimulus grid at the end of the treadmill belt was activated and mice were allowed to explore the treadmill-testing environment [35]. During active treadmill running, the stimulus grid provides a weak foot shock, which causes an involuntary muscle contraction that encourages running. Tactile stimulation to the tail was used to encourage mice to run prior to touching the stimulus grid. On day four, mice underwent their group-determined intervention, one at a time. This was performed in a counterbalanced order so that the initial animal for each day was from a different group than the previous day. Mice in the CON group were placed on the stationary treadmill for 36 minutes with the electrical stimulus grid activated. MOD and HI group mice were placed on the treadmill and underwent a six-minute warm up, where the first minute was a no-exercise treadmill exposure; thereafter the treadmill belt began to move at 5 m/min, increasing 1 m/min every minute for five minutes[35]. The treadmill speed was then incrementally increased to the group-appropriate speed and the mouse ran for 30 minutes at this pace. The MOD group ran for 30 minutes at 12 m/min at 0% grade; this stimulus has been reported to be ~75% of VO_{2max} in adult C57BL/6J mice [73]. The HI group ran for 30 minutes at a speed ranging from 15–17 m/min at 0% grade, depending on running ability; this speed has been reported to be ~80% of VO_{2max} in adult C57BL/6J mice [73].

Tissue Processing:

Mice were sacrificed by decapitation under isoflurane anesthesia immediately following group-specific treadmill exposure. The hippocampus from each hemisphere was removed and immediately frozen in liquid nitrogen. For western blot analysis, the hippocampus was sonicated in 1% SDS, boiled for 10 minutes [25,35], and stored at −80°C. Protein concentration was determined by spectrophotometry using the BCA Protein Assay (Pierce®, Rockford, IL, USA). For RNA extraction, samples were homogenized in a glass Dounce tissue homogenizer followed by RNA isolation using TRI Reagent (Life Technologies, Grand Island, NY, USA). RNA quantity and purity were assessed by UV spectroscopy.

Western Blotting:

Twenty-five μg of protein was loaded onto 7.5% SDS polyacrylamide gels and electrophoresed, followed by transfer to nitrocellulose membranes and immunoblotting. Nitrocellulose membranes were incubated with a monoclonal rabbit anti-phospho-GluR1 (Ser845: 1:5000, Millipore, Temecula, CA, USA; or Ser831: 1:1000, Millipore, Temecula, CA, USA) antibody, stripped with a 100 mM glycine-HCl (pH 3.0) solution, and re-probed with a polyclonal rabbit anti-GluR1 antibody (1:1000, Millipore, Temecula, CA, USA; [35]). Although the short time between the initiation of the exercise bout and sacrifice (30 minutes) is unlikely to produce changes in total GluR1 translation, we also immunoblotted for total GluR1 protein followed by stripping and re-probing for the neuronal nuclear marker NeuN with a polyclonal rabbit anti-NeuN antibody (1:1000, Millipore, Temecula, CA, USA). Appropriate fluorescent secondary antibodies were used for detection [goat anti-rabbit

IgG Cyanine3 (Invitrogen, Waltham, MA, USA) and goat anti-rabbit IgG Alexafluor488 (Invitrogen, Waltham, MA, USA)]. Proteins were visualized with a Typhoon TRIO Variable Imager (GE Healthcare, Pittsburgh, PA, USA) using fluorescence acquisition mode with an emission filter for Alexa 488. Band intensities were quantified with ImageQuant TL (GE Healthcare, Pittsburgh, PA, USA) utilizing Rubber Band function for background subtraction. Levels of phosphorylation, expressed as the ratio of phospho-GluR1 divided by total GluR1 intensity from the same lane, were used for statistical analysis. Total GluR1 divided by NeuN intensity from the same lane was used for statistical analysis to determine total GluR1 protein expression.

To confirm that peripheral epinephrine (via stimulating central NE release) increases hippocampal GluR1 Ser845 phosphorylation, a subsample of mice were injected with saline (10ml/kg; n=3) or epinephrine (0.5mg/kg at 10 ml/kg; n=4) and sacrificed by decapitation under isoflurane anesthesia, 15 minutes post-injection. Hippocampi were dissected, sonicated in lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, protease inhibitor cocktail], and immunoblotted as described above.

Gene Expression:

One μg of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time quantitative PCR (qPCR) was used to assess mRNA expression of total *Bdnf* (exon IX), *Bdnf* transcript IV (*Bdnf IV;* exon IV), *GluR1*, *NR2A*, *NR2B*, *Gapdh*, and *ActB* (*Gapdh & ActB*; expression controls; primer sequences listed in supplemental table 1). Primer:probe assays were purchased pre-made [*GluR1 (Gria1), NR2A (Grin2a), NR2B (Grin2b), Gapdh, ActB*] or designed (*Bdnf IX, Bdnf IV*) for the mRNA sequence of each gene using PrimeTime qPCR Assay designer (Integrated DNA Technology, Coralville, IA, USA). All primer pairs, except *Bdnf* total, spanned exons to prevent amplification of genomic DNA. Because *Bdnf* total is represented by amplification of only exon IX, this primer pair did not span exons. Efficiency for each primer:probe assay was confirmed prior to use. qPCR data were normalized to the geometric mean of *Gapdh* and *ActB* using the −ΔΔCt method [74,75] and expressed as fold induction ($2^{-\Delta\Delta Ct}$) of mRNA expression compared to the control group (1.0-fold induction).

Object Location Memory Task: A subset of three-month-old male C57BL/6J mice (N=30) were tested on the object location memory task immediately following the acute bout of exercise or no-exercise treadmill exposure. The three-day treadmill familiarization approach was the same as described above. Mice in the treadmill control group (n=15) sat on the stationary treadmill for 36 minutes while mice in the exercise group (n=15) ran on the treadmill at 15 to 17 m/min for 30 minutes following a six-minute warm-up. The procedures for the object location task were adapted from Barker and Warburton [76]. On the two days immediately prior to testing, mice were exposed to the testing arena (43×43×21.5 cm open field box) for five minutes/day to acclimate them to the procedure and environment. On the test day, immediately following the treadmill exposure, mice were placed in the testing apparatus for the first phase of the task (familiarization), in which they were allowed to explore the arena containing two identical objects (small, Duplo blocks®) for five minutes

followed by a fifteen-minute inter-trial interval in their home cages. Subsequently, mice were returned to the testing box for the second phase (test) in which they were allowed to explore the arena containing one object from the first exposure and a third object that was identical to the initial objects, but moved to a different location for five minutes. The left/right position of the new object was counterbalanced between mice. Behavior during the familiarization and test phases was monitored using EthoVision XT 11 Behavioral Tracking Software (Noldus, Leesburg, VA, USA), which provides automatic tracking, analysis, and storage of animal activity and behavior. Object interaction (time spent interacting with objects and number of interactions; defined as nose within 2 cm of object) and total distance traveled were recorded for each mouse.

Open Field Behavior Task: An additional subset of three-month-old male C57BL/6J mice (N=36) were used to assess anxiety-like behavior and the influence of noradrenergic signaling on behavior following acute exercise. Mice were separated into four groups: 1) Control – Saline (CON-SAL; n=9), 2) Control – DSP-4 (CON-DSP-4; n=10), Acute Exercise – Saline (EX-SAL; n=8), and Acute Exercise – DSP-4 (EX-DSP-4; n=9). Mice underwent the same treadmill familiarization and running as described above and ran at a treadmill speed between 15 and 17 m/min depending on running ability. Immediately after the acute bout of exercise or no-exercise treadmill exposure, performance on the open field task was assessed. Mice were placed in the testing apparatus (43×43×21.5 cm field box) and allowed to explore for 15 minutes while behavior was monitored using the EthoVision XT 11 Behavioral Tracking Software. Total distance traveled, time spent in central and peripheral zones, and time spent grooming were recorded and separated into five-minute blocks (0–5 minutes, 5–10 minutes, 10–15 minutes).

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4):

DSP-4 is a neurotoxin that specifically lesions the locus coeruleus (LC) noradrenergic (NA) system and reduces tissue levels of NE in regions innervated by the LC [77–85]. DSP-4 easily crosses the blood brain barrier and irreversibly disrupts central NA signaling while leaving non-LC NA neurons and serotonergic and dopaminergic systems unaffected (for review, see [86]). We have previously demonstrated that DSP-4 does not influence treadmill-running performance [35]. DSP-4 (Sigma Aldrich) was prepared in 0.9% saline and a single 50 mg/kg dose was delivered by IP injection in a volume of 10 ml/kg; this dose is frequently used in both rats and mice and is effective in depleting hippocampal NE [86]. Control mice received a single IP injection of 0.9% saline in a volume of 10 ml/kg. Solutions were prepared for five animals, were protected from light exposure and injected within 15 minutes of preparation. Mice received injections seven days prior to treadmill familiarization (i.e., 10 days prior to experimental treadmill day). This dose of DSP-4 and the interval between injection and task performance results in >90% reduction in hippocampal NE in C57BL/6J mice [85].

Statistical Analysis:

To determine differences in GluR1 protein expression/phosphorylation and mRNA expression among groups, we used a one-way analysis of variance with Tukey's *post hoc* comparisons when $p<0.05$. Object location memory performance was analyzed with a repeated measures ANOVA and Sidak's multiple comparison test when appropriate. Open

field behavior data were analyzed using a three-way repeated measures ANOVA (treadmill exposure x drug treatment x time). Upon no significant main or interaction effects of treadmill exposure and/or drug treatment, data were collapsed across the drug condition and a two-way repeated measures ANOVA (treadmill condition x time) was performed followed by Sidak's multiple comparison tests when appropriate. P values for post-hoc contrasts are presented as "adjusted p values" to indicate that they have been corrected for multiple comparisons.

Results

To confirm that peripheral epinephrine (via stimulating central NE release) increases hippocampal GluR1 Ser845 phosphorylation, a subsample of mice (N=7) were injected with saline (10ml/kg; n=3) or epinephrine (0.5mg/kg at 10 ml/kg; n=4) and sacrificed 15 minutes post-injection. The IP injection of epinephrine was sufficient to induce phosphorylation of Ser845 ($t_{(5)}$= 3.048; p=0.03) but did not influence Ser831 phosphorylation or GluR1 protein expression (Fig. 1).

To determine how acute exercise influences GluR1 phosphorylation in the hippocampus, mice (N=35) were randomly separated into three groups: 1) treadmill without exercise (CON; n=12), 2) moderate-intensity acute treadmill exercise (MOD; n=11), or 3) high-intensity acute treadmill exercise (HI; n=12). One protein sample from the CON group and one protein sample from the HI group were compromised during protein isolation. We found no significant difference in the phosphorylation of Ser845, Ser831, or the total level of GluR1 protein following acute high- or moderate-intensity exercise (Fig. 2). Similarly, we found no effect of acute high- or moderate-intensity exercise on *GluR1*, *NR2A*, or *NR2B* mRNA levels (Fig. 3). In contrast, we observed an effect of acute exercise intensity on the levels of *Bdnf IV* mRNA ($F_{(2, 32)}$=3.79; p=0.03; Fig. 4a). High-intensity acute exercise induced higher mRNA levels compared to controls (adjusted p=0.03), but no effect on total *Bdnf* mRNA (Fig. 4b). Together, this demonstrates a modest impact of acute exercise on markers for synaptic plasticity.

To ask if acute exercise affects memory, a cohort of three-month-old male C57BL/6J mice (N=30) were tested on a novel object location task immediately following acute exercise. We found no significant effect of acute exercise on total time spent exploring the two objects during either the familiarization or test phase of the task (Fig. 5a). During the test phase, there was no significant difference between controls and exercisers in time spent with the newly moved object relative to time spent with either of the other objects (i.e., discrimination ratio; Fig. 5b). However, we observed a significant main effect of acute exercise on total distance moved ($F_{(1,28)}$=14.06; p=0.008; Fig. 5c). There was a tendency for an interaction between acute exercise and phase of test on total distance moved ($F_{(1,28)}$=3.684; p=0.07). Mice exposed to high-intensity treadmill running moved significantly less (total distance traveled) during the familiarization phase compared to controls (Fig. 5c; p=0.0003). This difference was not observed during the test phase. We observed a significant main effect of acute exercise on the number of interactions with the objects ($F_{(1,28)}$=4.553; p=0.04; Fig. 5d) and an interaction effect between exercise and phase of test ($F_{(1,28)}$=6.938; p=0.01; Fig. 5d). The exercisers interacted with the two objects less

frequently than controls during the familiarization phase (Fig. 5D; p=0.003). Again, the difference was not observed during the test phase.

Because the influence of acute exercise on activity during the object location task was observed during the familiarization phase (immediately following exercise), but not the test phase (15–20 minutes following exercise), we used an additional cohort of three-month-old male C57BL/6J mice (N=36) in the open field task to analyze behavior in five minute intervals. Utilizing a three-way repeated measures ANOVA [treadmill condition (acute exercise vs. stationary treadmill) X drug (DSP-4 vs. saline) X time (0–5 minutes vs. 5–10 minutes vs. 10–15 minutes], we observed no main effect of drug and no interaction effects for drug x time, drug x treadmill condition, or drug x treadmill condition x time for any dependent variable of interest. We therefore collapsed across the drug condition. All statistical data presented for the open field task were analyzed by a two-way repeated measures ANOVA (treadmill condition x five-minute time interval) with Sidak's multiple comparisons when appropriate.

We observed a significant main effect of treadmill condition ($F_{(1,34)} = 25.12$; $p < 0.0001$), a main effect of time ($F_{(2,68)} = 29.91$; $p < 0.0001$) and an interaction between treadmill condition and time ($F_{(2,68)} = 12.81$; $p < 0.0001$) on total distance traveled (Fig. 6a). *Post hoc* analysis revealed that exercise mice traveled less distance during the first five-minute interval (adjusted $p<0.0001$), the second five-minute interval (adjusted $p=0.04$), and the final five minute interval (adjusted $p=0.03$) compared to control mice (Fig. 6a). Concerning the effect of time, *post hoc* analysis revealed that control animals moved significantly less distance during the second (adjusted $p<0.0001$) and third (adjusted $p<0.0001$) five-minute time intervals compared to the first five minutes of the task. There were no significant differences between time intervals for total distance traveled in mice that performed 30 minutes of exercise (Fig. 6a). We observed a significant main effect of treadmill condition on time spent self-grooming during the open field task ($F_{(1,34)} = 53.93$; $p<0.0001$) but no main effect of time or interaction effect (Fig. 6B). There was a main effect of time ($F_{(2,68)} = 4.755$; $p = 0.01$) and an interaction between treadmill condition and time ($F_{(2,68)} = 4.478$; $p = 0.01$) for frequency of entries into the center of the testing arena, but no main effect of treadmill condition (Fig. 6C). *Post hoc* analysis revealed that in exercise mice, the number of entries was lower during the second (adjusted $p=0.005$) and third (adjusted $p=0.009$) five-minute time intervals compared to the first five minutes of the task. Further, during the 10–15-minute time interval, exercise mice had fewer entries into the center of the arena compared to control mice (adjusted $p=0.03$). There were no main effects of treadmill condition or time on the percent of total time spent in the center of the testing arena. There was an interaction between treadmill condition and time ($F_{(2,68)} = 3.556$; $p = 0.03$; Fig. 6D). *Post hoc* analysis revealed that in exercise mice, less time was spent in the center of the testing arena during the final five-minute time interval compared to the first time interval (adjusted $p=0.03$).

We observed no significant difference between EX-SAL and EX-DSP-4 in running performance, indicated by number of stimulus grid touches (Fig. 7a). However, we did observe a significant negative correlation between the total number of stimulus grid touches and distance traveled during the first five-minute block of the open field task ($p=0.005$; Fig.

7b). Better running performance, indicated by fewer stimulus grid touches, was associated with higher activity in the open field task during the first five-minute block. This correlation was no longer observed during the second and third five-minute blocks (data not shown). There was no correlation between running performance and time spent self-grooming or entries into the center of the testing arena during any five-minute time block (data not shown).

Discussion

A single 30-minute bout of high-intensity treadmill exercise was sufficient to increase mRNA expression of *Bdnf* transcript IV, suggesting that signaling pathways known to be engaged by chronic exercise [3,8] can be initiated by single exercise bouts. However, a single 30-minute bout of acute exercise did not influence the phosphorylation status of the GluR1 subunit of the AMPAR, and therefore differs significantly from the response to peripheral injection of catecholamines [25]. To ask if acute exercise improves memory performance in mice, as observed in humans [18,20], mice performed a low stress, one-trial memory task immediately following an acute bout of exercise. However, instead of revealing the expected effect on memory retention, following an acute bout of exercise, the behavior of mice appeared to reflect enhanced anxiety. Indeed, acute exercise significantly reduced locomotor activity and significantly increased time spent self-grooming in the open field task, measures often used as indicators of anxiety-like behavior.

We hypothesized that, like acute psychological stress and peripheral injections of epinephrine [25], acute forced treadmill exercise would increase phosphorylation of GluR1 at Ser845 in the hippocampus, potentially via the release of catecholamines and central noradrenergic signaling. However, while an IP injection of epinephrine induced the expected increase in the phosphorylation of Ser845 on GluR1 [25], 30 minutes of high- or moderate-intensity acute exercise did not. Thus the effect of acute exercise may differ from the effects of acute psychological stress and chronic exercise, which has been shown to increase phosphorylation of Ser845 [35,87]. Alternatively, exposure to the treadmill environment alone may have elevated Ser845 phosphorylation in controls, and therefore masked the effect of acute exercise. Without a cage-control group that did not undergo the acclimation or experimental day treadmill exposure, we cannot rule out the possibility that the three days of acclimation to the treadmill environment or the 36-minute treadmill exposure in the stationary controls produced novelty-induced arousal. This arousal may have activated the noradrenergic system and stimulated hippocampal β_2 adrenergic receptors [88]. Indeed, novelty exploration increases neuronal activity in the LC and increases the release of NE in the hippocampus [89].

The lack of an effect of acute exercise on GluR1 phosphorylation may also reflect that 30 minutes of exercise was not sufficient to elevate peripheral epinephrine/central NE and/or engage the signaling pathways that target GluR1 phosphorylation. Pagliari and Peyrin [23] found that cortical NE increases in response to treadmill running after ~40 minutes of exercise in the rat, while Goekint et al. [90] observed no influence of 60 minutes of treadmill running on extracellular hippocampal NE. In contrast, Dishman et al. [91] reported that 15

minutes of treadmill running or immobilization stress decreased NE levels in the LC and hippocampus, likely through release and metabolism of the neurotransmitter.

Our data demonstrate that an acute bout of treadmill exercise does not stimulate rapid transcription of glutamate receptor subunits in mouse hippocampus. Potentially, multi-day treadmill exposures, or chronic voluntary wheel running, may be necessary to increase mRNA expression of *GluR1*, *NR2A*, or *NR2B*. Alternatively, it is possible that there was insufficient time for activity-dependent transcription of glutamate receptors between the start of exercise and sacrifice. The literature reporting the effects of exercise training on glutamate receptor expression is inconsistent. Prior research has reported an increase in *NR2B* mRNA expression following short-term exposure to a voluntary running wheel [11,92]. Molteni et al. [92] reported that three days of voluntary running increased both NR2B, NR2A, and to a much lesser extent, GluR1 expression in rat hippocampus. NR2A remained significantly different than controls after seven days of wheel running but was no longer significantly different after one-month of exposure. Ni et al. [93] found that GluR1 mRNA expression was not influenced by six days of daily treadmill running in healthy Sprague-Dawley rats.

Numerous studies have demonstrated that exercise increases protein and mRNA expression of Bdnf in rodent hippocampus [58,92,94–99] and we previously demonstrated that a single 45-minute bout (followed by 15 minutes of rest) of high-intensity exercise increased expression of total *Bdnf*, while both high- and moderate-intensity exercise increased *Bdnf IV* [35]. Here we show that 30 minutes of high-intensity, but not moderate-intensity, treadmill running increased only *Bdnf IV*. Bdnf stimulates hippocampal neurogenesis [37–40,100], synaptic plasticity [44–51], and promotes memory [51–57] – all effects that have also been observed following exercise training [8,101,102]. Blocking Bdnf activity prevents exercise-induced improvements in spatial memory and expression of plasticity-associated genes [58]. The finding that an acute 30-minute bout of exercise increased the transcription of *Bdnf IV* gene suggests that these pathways can be induced with short bouts of acute exercise, albeit forced and highly stressful in our investigation.

Acute exercise caused an increase in *Bdnf IV* but not total *Bdnf* expression, suggesting a compensatory decrease in another *Bdnf* transcript may compensate for the rapid activation of *Bdnf IV* transcription. We previously demonstrated that 45 minutes of exercise did not influence the expression of *Bdnf* transcripts I, II, III or VI [35]. Indeed, expression of *Bdnf IV* is rapidly initiated in response to exercise, similar to an immediate early gene, while the other *Bdnf* transcripts have a slower pattern of transcription [63,64,103]. Hippocampal *Bdnf IV* expression is also known to increase in response to acute immobilization stress [104], fear conditioning [65], and exercise training [16,105]. It is important to note that our exercise protocol was likely stressful, as aspects of the protocol were unpredictable and uncontrollable. In this sense, our protocol may mimic aspects of fear conditioning and immobilization stress.

There is not yet a consensus, either in research approach or experimental findings, concerning the influence of acute exercise on *Bdnf* mRNA expression. For example, Oliff et al. [106] reported that six hours of voluntary wheel running increased total *Bdnf* but not *Bdnf IV* mRNA expression in rat hippocampus (i.e., hilus, CA1, and CA3). Importantly,

these investigators allowed acclimation of mice to the running wheel for three nights followed by a 10-day washout period. However, mice that underwent the three days of wheel acclimation, but no acute wheel exposure after the washout, had significantly elevated *Bdnf IV* in all hippocampal regions examined. This demonstrates that acclimation protocols, commonly used in acute exercise studies, can influence hippocampal *Bdnf* expression. Rasmussen et al. [107] reported significantly greater total *Bdnf* mRNA in the hippocampus two- and six-hours post-treadmill running to exhaustion but not immediately after exercise. Similar to Oliff et al. [106], Rasmussen et al. [107] used an acclimation protocol that included running on the treadmill for multiple days before the acute treadmill running.

We hypothesized that an acute bout of high-intensity exercise would lower the threshold for memory formation and/or improve memory performance, as has been shown with epinephrine injections [25] and three weeks of voluntary wheel running [16]. However, mice exposed to high-intensity acute exercise showed significantly less exploratory behavior (e.g., frequency of interaction with objects and significantly less distance traveled) during the familiarization phase of the novel object location task, a memory task known to be dependent on the hippocampus [76]. The absence of active exploration of the novel objects likely prevented learning during the task and interfered with our ability to assess memory. Potentially, catecholamines elevated by our acute exercise protocol contributed to the reduced exploratory behavior and negatively impacted performance in the novel object location task. We speculate that the elevation in catecholamines was transient and returned to baseline by the test phase when exploratory behavior was similar between runners and controls. Another possibility is that the mice were fatigued following the bout of treadmill exercise. Although the treadmill exercise was not exhaustive, even moderate fatigue induced by exercise may cause a reduction in activity [108].

A similar behavioral profile was observed in the open field task, a task commonly used to examine anxiety-like behavior in rodents. Mice that performed an acute bout of exercise showed significantly less activity, indicated by total distance travelled, during the task and spent significantly more time self-grooming. Moreover, exercise mice had a pattern of reduced entries during the final five minutes of the open field task. These behaviors are often indicative of anxiety-like behavior [109,110], yet we are cautious in our interpretation of these behaviors as anxiety.

Self-grooming is a complex behavior that has been shown to be increased with high levels of anxiety and stressful situations, and is reduced by benzodiazepines [110]. However, grooming behavior is controlled by many brain regions/circuits and influenced by numerous pharmacological manipulations [110]. Alternatively to increased anxiety, increased grooming could represent reduced vigilance and more internally-directed behavior [111], and so we are cautious in our interpretation of increased grooming following forced exercise. Percent of time spent in the center of the testing arena and number of entries into the testing arena, both common indicators of anxiety-like behavior [109], were not impacted by exercise other than a small yet significant difference in number of entries into the center of the arena during the 10–15 minute interval.

The majority of published research on exercise and anxiety has investigated the influence of chronic exercise. This research has reported primarily anxiolytic effects of exercise (reviewed in [113]); however, a few studies have reported anxiogenic effects [114–116]. Salam et al. [117] provided C57BL/6J mice with access to a voluntary running wheel for two weeks prior to exposure to the open field task and found behaviors suggestive of both reduced and increased anxiety. They reported that runners spent significantly more time in the center of the testing box and entered the center more frequently than sedentary mice. These behaviors are indicative of less anxiety; however, they also observed significantly less activity and more grooming behavior in runners, which is indicative of higher levels of anxiety and is similar to what we observed. Some investigations on the effects of chronic exercise on anxiety offer insight into rodent behavior immediately following a bout of exercise. For example, Duman et al. [68] reported that three weeks of voluntary wheel running in C57BL/6J mice increased anxiety-like behavior (i.e., reduced activity) in the open field task if the task was initiated the morning after a night of voluntary wheel running. In contrast, if the task was initiated 24 hours after the last exposure to the voluntary running wheel, they observed anxiolytic-like behavior. Further, Fuss et al. [108] reported reduced anxiety-like behavior in the dark-light box immediately after an acute five-hour bout of voluntary wheel running. Although mice exposed to running spent more time in the bright area, they showed reduced activity indicated by the number of exits from the dark compartment to the light compartment. These results are consistent with our findings and suggest that tests dependent on exploratory and/or locomotor activity are compromised when performed immediately after an acute bout of exercise. Fuss et al. [108] further observed that when mice were returned to cages with running wheels following behavioral testing, mice that underwent five hours of acute exercise immediately before behavioral testing were less active compared to mice that did not undergo the acute bout of exercise before behavioral testing. This further suggests that the reduction in activity following acute exercise may be due to fatigue. Fatigue following the acute exercise could explain the reduction in exploratory behavior observed in both the object location task and the open field task. Indeed, there was a significant negative correlation between number of stimulus pad touches during treadmill running, our indirect measure of running ability, and total distance travelled during the first 5 minutes of the open field task. Mice that experienced more stimulus pad touches presumably struggled more with the intensity of running and may have experienced more fatigue following the exercise.

An alternative explanation for the observed anxiety-like behaviors during the novel object location and open field tasks may be in response to the release of adrenal stress hormones and central noradrenergic signaling. The reduced locomotor behavior observed in the object location and open field tasks is similar to what we observed in the home cage following an IP injection of epinephrine (unpublished observation). Administration of selective NE reuptake inhibitors used as antidepressants (e.g., reboxetine) are initially anxiogenic [118], but become anxiolytic after chronic administration by reducing stress-induced cortical NE release [119]. Potentially, acute and chronic exercise mimic acute and chronic treatment with NE reuptake inhibitors by increasing extrasynaptic NE, which is acutely anxiogenic but becomes anxiolytic with chronic exposure. The role of NE in anxiety is complex and is associated with both anxiolytic and anxiogenic behavior depending on the type of acute stress stimulating the NE release [71]. It is important to recognize that our treadmill protocol

was forced and likely highly stressful. Forced treadmill running, in contrast to voluntary wheel running, is uncontrollable and associated with higher levels of stress hormones, such as corticosterone and NE, compared to voluntary wheel running [120–123]. It is possible that we would have observed a different behavioral profile if voluntary wheel running was utilized.

We hypothesized that the anxiogenic-like behaviors (e.g., reduced exploratory behavior, increased self-grooming) observed following acute exercise would be attenuated with pre-treatment with the selective neurotoxin for the LC- noradrenergic system, DSP-4. However, we did not observe an effect of the drug on any measure of behavior in the open field task. DSP-4 treatment has been previously shown to reduce overall activity in the open field task, which can be attenuated with chronic mild stress [124]. Potentially, three days of treadmill acclimation and the 36 minutes of stationary treadmill exposure was the optimal level of stress to attenuate the effect of DSP-4 treatment on exploratory behavior in the control mice and may explain why we did not observe a significant reduction in activity in mice that received DSP-4 alone. This would suggest that an inverted-U effect of LC-derived NE may exist, with both low and high levels resulting in reduced exploratory activity. Other stress hormones (e.g., corticosterone) and amygdala activity, independent of LC innervation, may be sufficient to cause the behavioral profile that we observed. Moreover, high levels of exogenous catecholamines can bypass the LC to exert behavioral effects. Bennett et al. [83] reported that peripheral injections of epinephrine following DSP-4 treatment can attenuate impairments in active avoidance induced by DSP-4. Our exercise protocol may have been psychologically and physically stressful enough to raise peripheral catecholamines and bypass the LC noradrenergic system; however, peripheral catecholamines were not assayed.

We were primarily interested in the influence of forced exercise, and not the influence of the novel treadmill environment. We hypothesized that the novel environment alone may induce plasticity and designed our study to observe the effects of the treadmill exercise beyond the effects of novelty. We did not envision an occlusion of plasticity markers by the novelty; however, not having a home-cage control prevents us from being able to determine if our lack of observed effect on GluR1 phosphorylation and total *Bdnf* was due to the novel environment masking the effects of acute exercise. In addition, a limitation of this investigation is that we were unable to measure the tissue and extracellular content of NE in response to exercise and DSP-4. Although there is ample evidence to support that DSP-4 reduces tissue content of NE [77–85], the possibility of an ineffective drug treatment or increased extracellular content of NE [86] following treatment allows for uncertainty.

Summary:

We show that a single acute bout of exercise does not increase GluR1 phosphorylation but increases the level of mRNA of the important plasticity-promoting gene, *Bdnf,* in a transcript- and intensity-dependent manner. Furthermore, following acute exercise, locomotor and exploratory behavior are reduced and self-grooming behavior increased. Exercise does not increase anxiety or incidents of anxiety attacks in humans and generally appears to be anxiolytic in rodents [113,125,126], so we are hesitant to conclude that the acute bout of exercise is actually anxiogenic. Our data suggest that tasks with low intrinsic

motivation and/or dependent on locomotor or exploratory behavior may give results that can be interpreted as an anxious phenotype. Careful consideration should be used when selecting the appropriate behavioral task to assess memory or anxiety following acute exercise exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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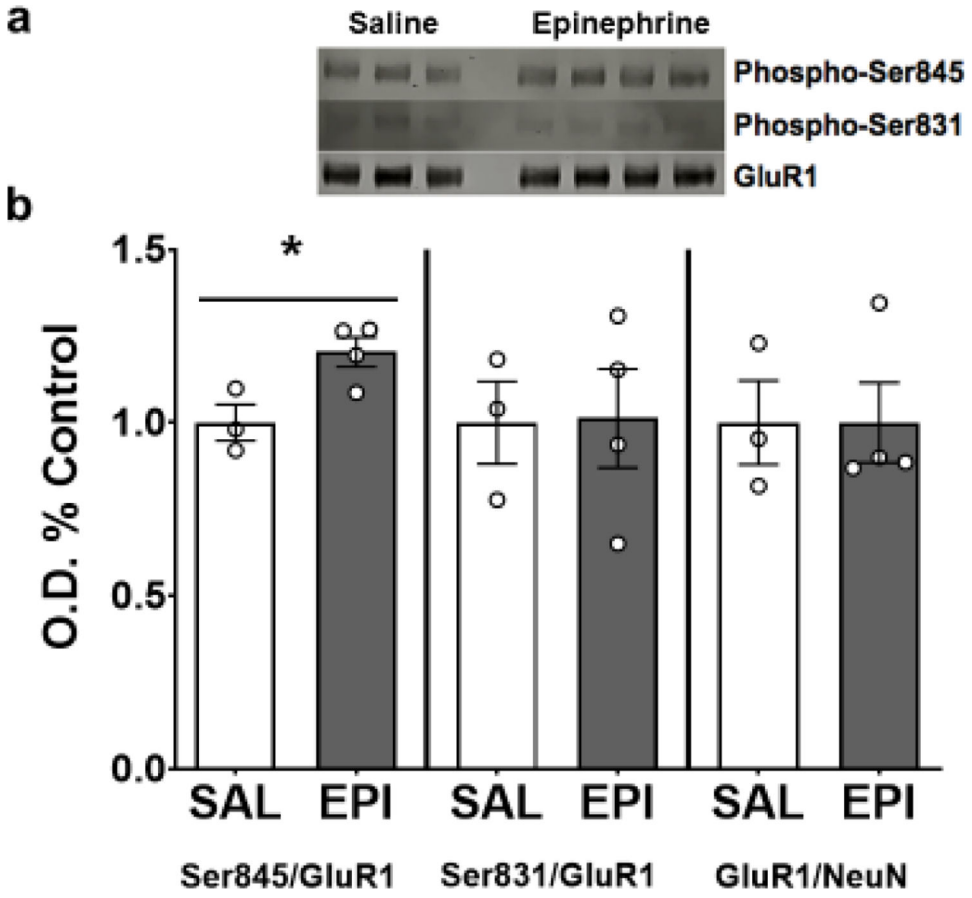

Figure 1. Intraperitoneal injection of epinephrine induces Ser845 phosphorylation of GluR1 in the hippocampus.

(A) Immunoblot of 3 saline controls (lanes 1–3) and 4 epinephrine experimental subjects (lanes 5–8). (B) Intraperitoneal injection of epinephrine increased the ratio of Ser845 phosphorylated over total GluR1 protein but had no effect on Ser831 phosphorylation or total GluR1. Data depicted as average +/− SEM. * indicates $p<0.05$.

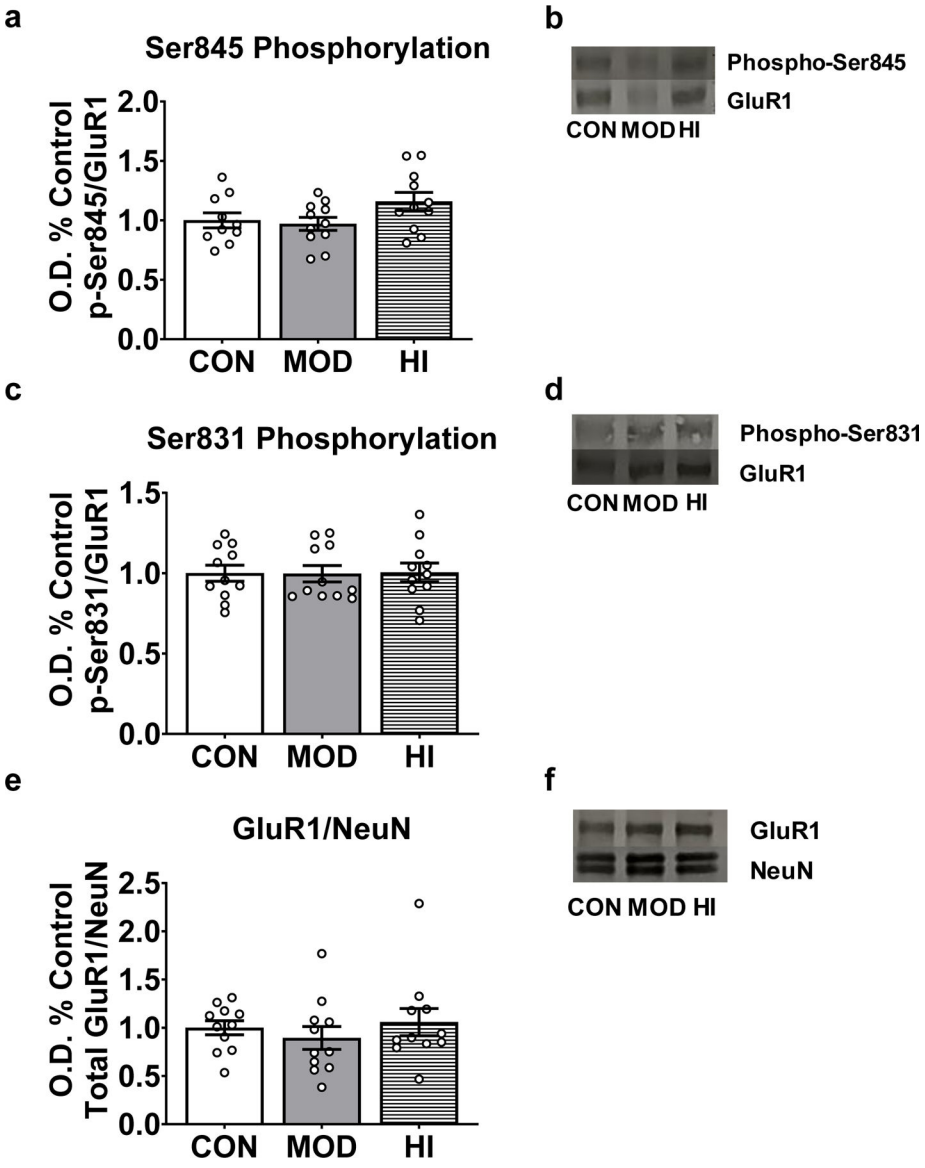

Figure 2. Acute exercise does not affect GluR1 phosphorylation.

No significant effect of acute exercise on Ser845 phosphorylation (A), Ser831 phosphorylation (B) or total GluR1 protein level (C). (D-F): Representative immunblots for each condition. Data depicted as average +/− SEM.

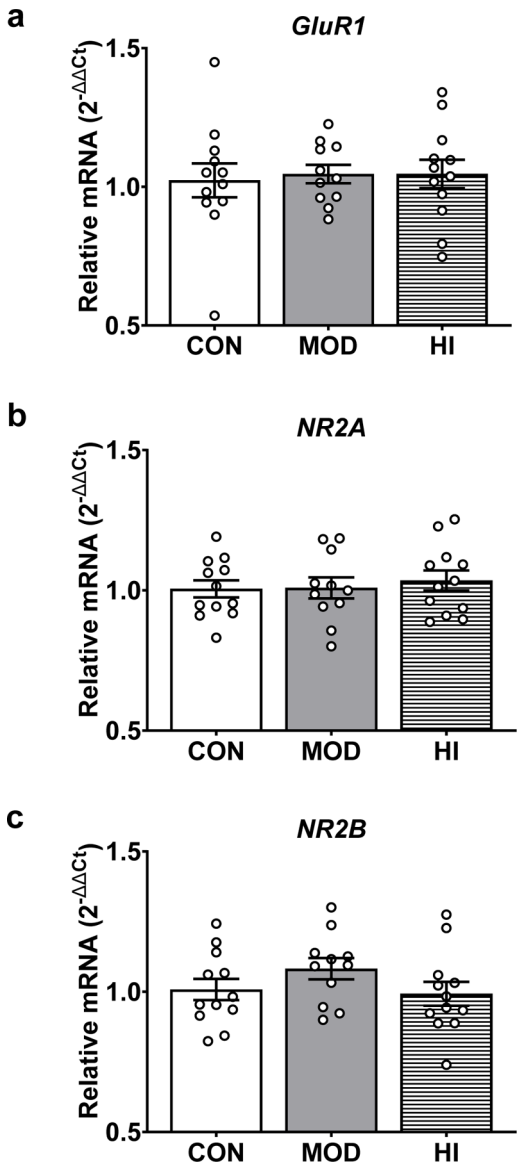

Figure 3. Acute exercise does not affect glutamate receptor subunit mRNA levels in the mouse hippocampus.

qPCR revealed no significant effect of acute exercise on *GluR1* (A), *NR2A* (B), or *NR2B* (C) mRNA levels. Target mRNA levels are presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh* mRNA. Data depicted as average +/− SEM.

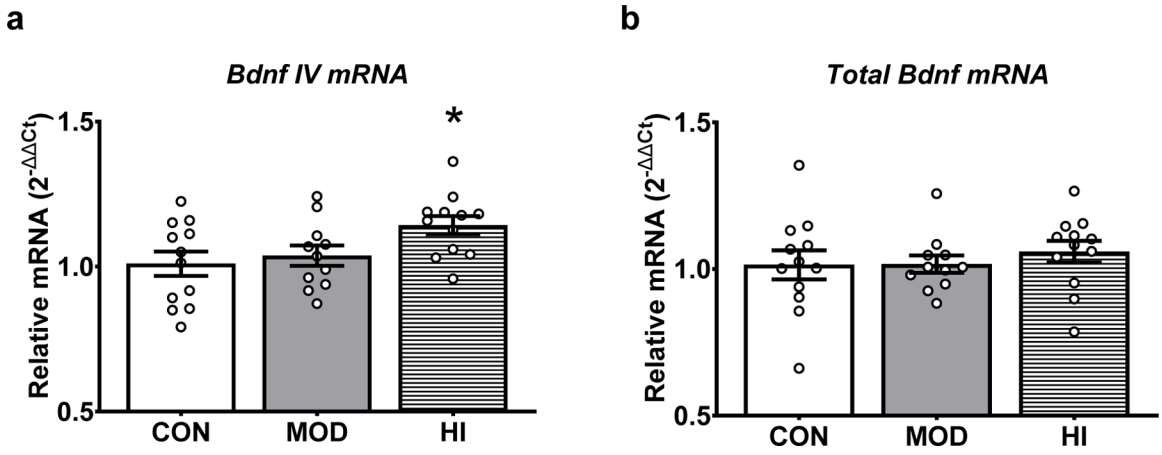

Figure 4. High-intensity exercise increases transcript-specific *Bdnf* expression.

(A) Acute exercise significantly affects *Bdnf IV* mRNA levels in hippocampus. High-intensity exercise resulted in greater *Bdnf IV* mRNA relative to controls. (B) No significant effect of acute exercise on total *Bdnf* mRNA expression. Target mRNA levels are presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh.* Data depicted as average +/− SEM. * indicates $p<0.05$ versus control.

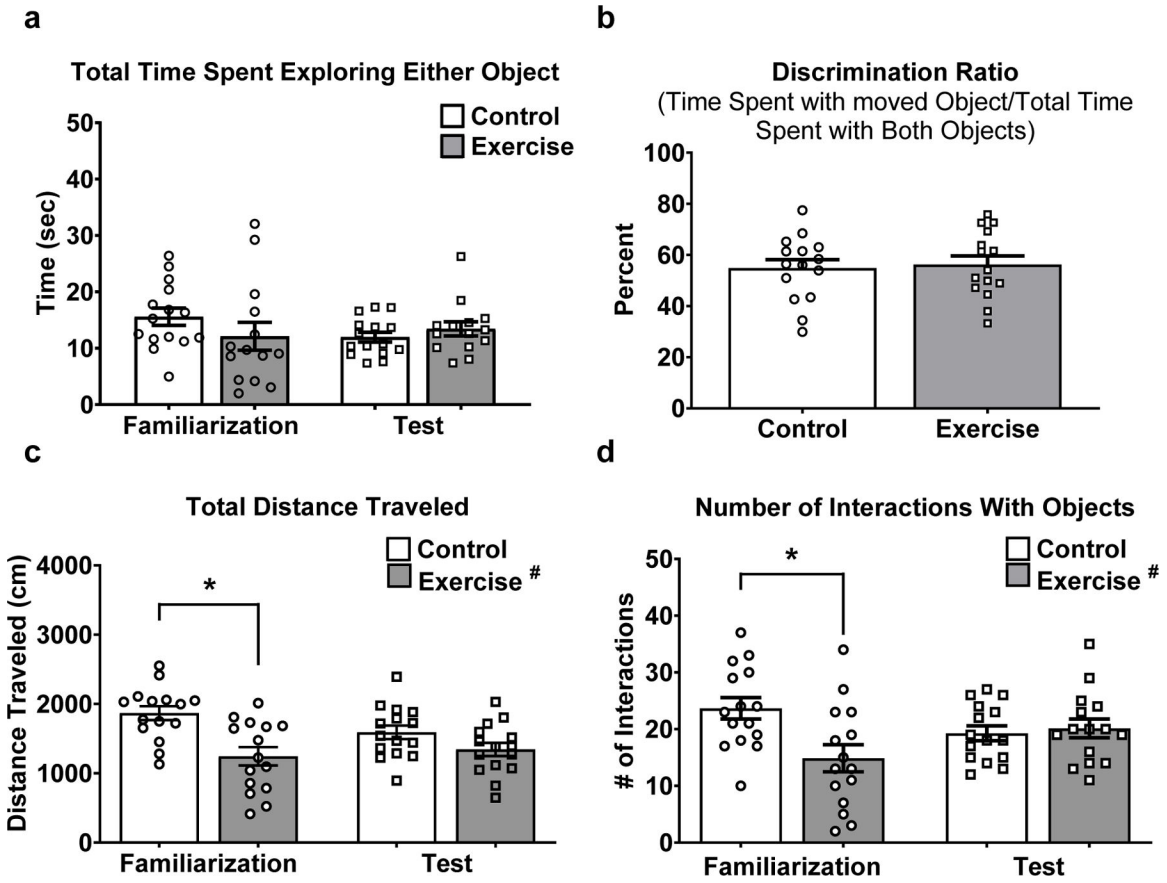

Figure 5. Acute exercise does not influence object recognition, but reduces exploratory behavior.

(A) No significant effect of acute exercise, phase of test, or interaction on the time spent exploring the objects. (B) No significant effect of acute exercise on % time spent exploring the moved object relative to the time spent exploring both objects during the test phase. (C) Mice that performed high-intensity treadmill running traveled less distance than treadmill controls during the familiarization phase. (D) A significant effect of acute exercise and an interaction between acute exercise and test phase on the number of interactions with the objects. Mice that performed high-intensity treadmill running had fewer interactions with the objects during the familiarization phase relative to the treadmill controls. Data depicted as average +/− SEM. * indicates $p<0.05$ after Sidak correction for multiple comparisons

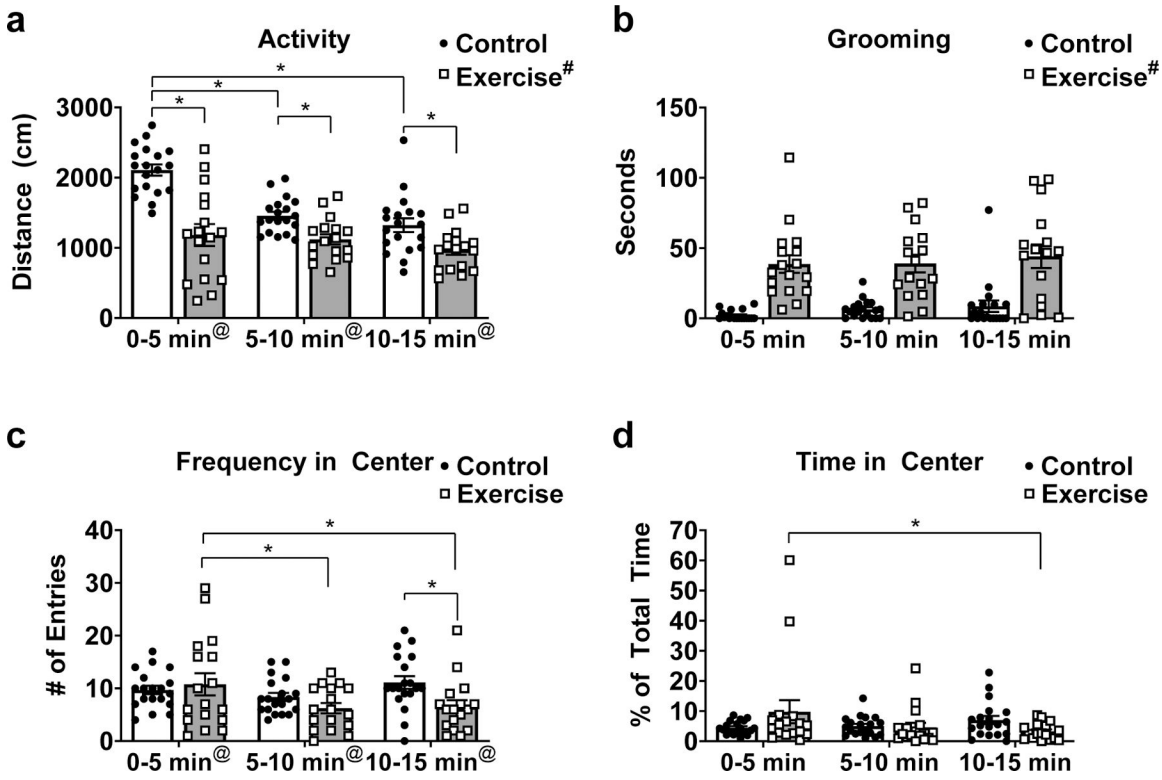

Figure 6. High-intensity acute exercise induces anxiety-like behavior in the initial 5 minutes of open field task.

(A) Mice that performed high-intensity treadmill running traveled less total distance compared to sedentary controls at each time point. There was an interaction between treadmill condition and time, which demonstrated that total distance traveled was lower at 5–10 minutes and 10–15 minutes compared to 0–5 minutes in sedentary controls. (B) Mice that performed high-intensity treadmill running spent significantly more time self-grooming compared to sedentary controls during each time interval. (C) There was a significant effect of time on number of entries into the center of the testing arena and an interaction between time and treadmill condition. Fewer numbers of entries were observed at 5–10 minutes and 10–15 minutes compared to 0–5 minutes in the exercised mice. Mice that performed high-intensity treadmill running had fewer entries into the center of the arena during the 10–15 minute time interval compared to controls. (D) There was a significant interaction between time and treadmill condition for time spent in the center of the arena. In mice exposed to high-intensity treadmill running, there was less time spent in the center of the arena during the 10–15 minute time interval compared to the 0–5 minute time interval. Data depicted as average +/− SEM. # indicates significant main effect of acute exercise. @ indicates significant main effect of time. * indicates $p<0.05$ after Sidak correction for multiple comparisons.

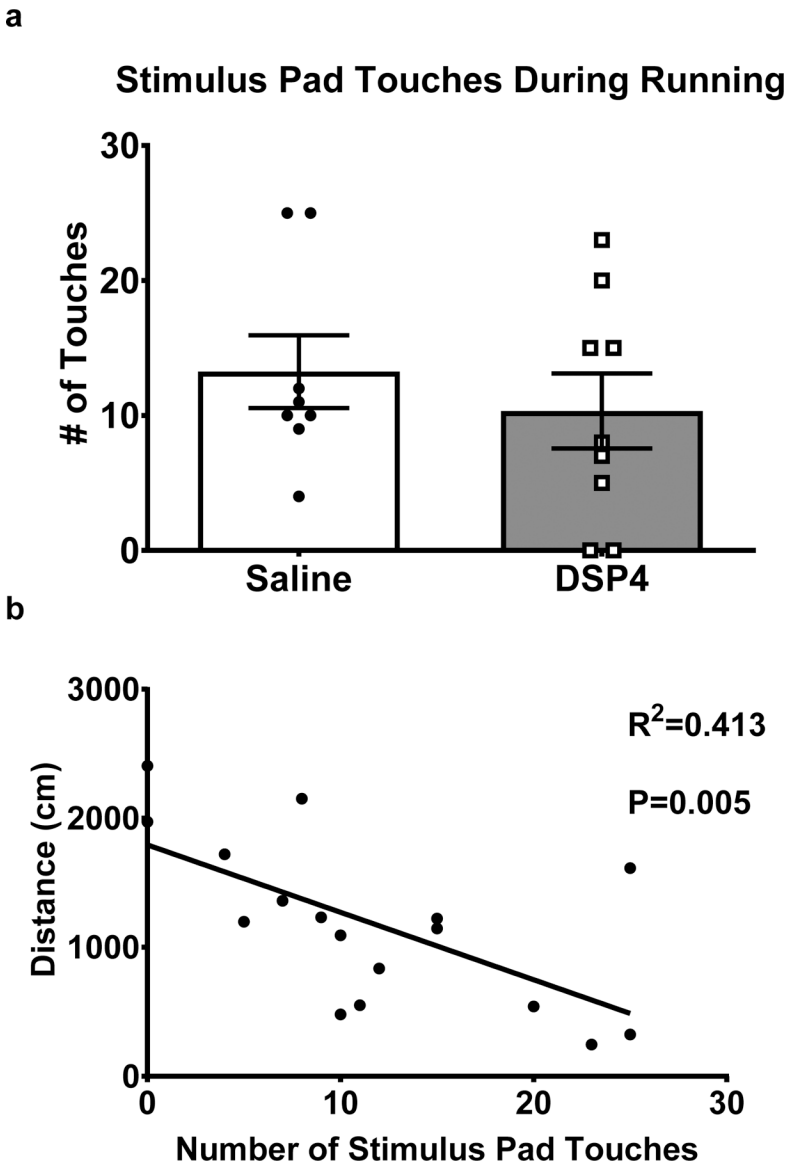

Figure 7. Negative correlation between number of stimulus grid touches and distance travelled in open field task.

(A) No significant difference between DSP-4 treated and saline treated mice in stimulus grid touches during running. (B) A significant correlation between the total number of stimulus grid touches and distance traveled during the first five-minute block of the open field task.