Exercise Enhances the Behavioral Responses to Acute Stress in an Animal Model of PTSD

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ABSTRACT

Introduction: This study examined the effects of endurance exercise on the behavioral response to stress and patterns of brain derived neurotrophic factor (BDNF), neuropeptide Y (NPY) and δ-opioid receptor (phosphor-DOR) expression in the hippocampus. Methods: Animals ran on a treadmill at 15 m·min⁻¹, 5 min·day⁻¹ gradually increasing to 20 min·day⁻¹, 5 days·week⁻¹ for 6 weeks. Following training one group of animals were exposed to a predator scent stress-(PSS) protocol for 10 min. Outcome measurements included behavior in an elevated plus-maze (EPM) and acoustic startle response (ASR) 7 days after exposure to stress. Immunohistochemical technique was used to detect the expression of the BDNF, NPY and phospho-DOR in the hippocampus 8 days after exposure. Results: Sedentary animals exposed to PSS were observed to have a greater incidence of extreme behavior responses including higher anxiety, less total activity in the EPM and a greater amplitude in the ASR than unexposed and/or trained animals. Exercise trained animals exposed to PSS developed a resiliency to the stress, reflected by significantly greater total activity in the EPM, reduced anxiety, and reduced ASR compared to the sedentary, exposed animals. Exercise in the absence of stress significantly elevated the expression of BDNF and phospho-DOR, while exposure to PSS resulted in a significant decline in the expression of NPY, BDNF and phospho-DOR. Trained animals that were exposed maintained expression of BDNF, NPY and phospho-DOR in most subregions of the hippocampus. Conclusion: Results indicated that endurance training provided a mechanism to promote resilience and/or recovery from stress. In addition, exercise increased expression of BDNF, NPY and DOR signaling in the hippocampus that was associated with the greater resiliency seen in the trained animals. Key Words: Post-Traumatic Stress Disorder, Training, BDNF, NPY, DOR
INTRODUCTION

Traumatic experiences that generally stem from exposure to serious threats of injury or death resulting in extreme fear, helplessness or horror has been used to define post-traumatic stress disorder (PTSD) (21). Symptoms of PTSD often include recurring and unwanted recollections or dreams of the event that are brought upon by exposure to specific cues or flashbacks, avoidance, negative cognition and mood, and hyperarousal (21). The consequence of PTSD are concerning. Individuals diagnosed with PTSD have significant issues in social and family interactions, which often manifests itself in aggressive behavior, depression, and poor physical health (21, 26). Symptoms of PTSD have been associated in a dose-response fashion with the incidence of type 2 diabetes (32), and patients diagnosed with PTSD are also at greater risk for cardiometabolic and autoimmune diseases (25). Although PTSD can occur in all population groups, the experiences of combat make the soldier especially vulnerable to PTSD. The prevalence of PTSD among returning veterans from recent conflicts in Iraq and Afghanistan have been reported to range from 10% - 17% (34). Issues with PTSD may not surface on initial return from combat, but symptoms appear to increase during the twelve months following deployment as soldiers begin to reintegrate into society (34). Considering that symptoms of PTSD appear to be delayed upon the soldier’s return to society, it may provide an opportunity for a potential strategy to be employed to minimize or prevent the symptoms of PTSD from manifesting.

A number of studies have shown the beneficial effects of exercise on psychological health and function. In humans, exercise has been shown to improve symptoms of depression, decrease anxiety and enhance mood and coping capacity in response to stress (3, 4). The benefits associated with exercise and resilience to stress may be related in part to specific neuroendocrine
adaptations. Acute exercise has been shown to increase β-endorphins (16), which have been suggested to play an important role in mediating depression and providing a sense of well-being (16). In addition, fit individuals appear to respond to multiple stress challenges more effectively than unfit individuals, with the more fit individuals experiencing a reduced stress response, reflected by lower plasma cortisol concentrations to both mental and physical challenges (37). Delta opioid receptor (DOR) expression has also been reported to be dependent upon changes in stress (36). Whether DORs are effected by exercise is not well understood, and similarly changes in the expression of these receptors during periods of depression or anxiety that accompany PTSD is not clear.

Neuropeptide Y (NPY) is widely distributed in the central nervous system and has been suggested to have a number of physiological roles including regulating the emotional response to various stressors (20). Evidence has demonstrated that NPY can facilitate the containment of negative consequences following stress and has anxiolytic-like effects (20). Recent research has reported that NPY regulation is associated with behavioral resilience to stress in rodent models of PTSD (7). There are only a limited number of studies that have examined the effect of exercise on changes in NPY concentrations. Rämson and colleagues (30) reported significant elevations following two weeks of high volume, low intensity exercise in rowers. However, whether exercise related elevations in NPY concentrations can modulate behavioral responses still remains unknown.

Brain-derived neurotrophic factor (BDNF) is part of the neurotrophin family suggested to have a potential role in exercise-induced anti-depressive effects (17, 23). BDNF has been demonstrated to have an important role in neuronal remodeling and modulating synaptic plasticity and neurotransmitter release (5). Kozlovksy and colleagues (23) have reported that
PTSD-like behavioral responses are associated with a down-regulation of BDNF mRNA, suggesting that individuals who are experiencing extreme behavioral responses to stress may have an impaired synaptic stabilization and increased sensitivity to an extreme stress response. Exercise has been shown to increase BDNF concentrations (17, 24), and has also been suggested to be a mechanism for the depression relieving effects of exercise (26).

Several recent studies have demonstrated the successful use of an animal model for studying PTSD (7, 12, 23, 28). In this model, populations of exposed rodents are classified according to the degree of their individual behavioral response using standardized "cut-off behavioral criteria" (CBC) creating three distinct groups; "extreme behavioral response" (EBR) and "minimal behavioral response" (MBR) at the extremes, and a middle group of "partial responders" (PBR) (10, 11). Therefore, the purpose of this study was to examine the effectiveness of 6-weeks of endurance exercise in preventing PTSD-like behavioral changes in rodents exposed to a predator-scent stress (PSS). A secondary purpose was to investigate the mechanisms underlying the beneficial effects of exercise on stress and resilience by examining the effects of exercise on BDNF, NPY, and phospho-DOR expression in the hippocampus.

MATERIALS AND METHODS

Animals:

Adult male Sprague-Dawley rats weighing 200-250 gm (n = 101) were habituated to housing conditions for at least seven days. All animals were housed four per cage in a vivarium with stable temperature and a reversed 12-h light/dark cycle, with unlimited access to food and water. Animals were handled once daily. All testing was performed during the dark phase in dim red light conditions. This study was performed according to the principles and guidelines of the
Exercise protocol:

All animals went through a five-day adaptation period to a treadmill during which they were allowed to explore the equipment. The treadmill was turned on for only 15 min at low speeds (5.0 to 8.3 m·h⁻¹). This procedure had the purpose of excluding animals which were intolerant to the treadmill and refused to run, thereby providing a homogenous group of rats willing to exercise. The animals which presented problems adapting to the treadmill or refused to run were excluded. During the study rats were run on the treadmill at a speed of 15 m·min⁻¹, 5 min·day⁻¹ gradually increasing to 20 min·day⁻¹, 5 days/week, for 6 weeks.

Predator-Scent Stress (PSS)

The animal model of PTSD used in this study has been previously established as a valid and effective method of examining biomolecular and physiological parameters of specific response patterns of stress (9). This model has also been established as an effective approach to determine the effects of various interventions on the behavioral response to stress (9).

Following the 6-week training program, animals were exposed to the PSS protocol. The PSS protocol consisted of placing the experimental animal on well-soiled cat litter (in use by the cat for 2 days, sifted for stools) for 10 minutes in a closed environment. Control animals were exposed to fresh, unused litter for the same amount of time. The situational reminder consisted of placing animals on fresh, unused cat litter for 10 minutes.
Assessment Schedule

Behavioral responses were assessed in the elevated plus-maze, acoustic startle response and contextual freezing. All results were recorded and analyzed using an EthoVision automated tracking system (Noldus Information Technology, The Netherlands). Performance in the elevated plus-maze and acoustic startle response occurred 7-days following the initial exposure to the PSS. The contextual freezing measures were performed on days 8 following initial exposure. The delay in performing these measures from the PSS is based upon findings that extreme behavioral changes, which remain constant after 7 days of exposure represent ‘chronic symptoms’ (12) and persist over a prolonged duration (10, 12). Following behavioral assessments, all animals were sacrificed and the brains were removed for analysis.

Behavioral Measures

Elevated Plus-Maze (EPM)

Behavioral assessments performed in the EPM have been previously described (7, 11). The EPM is a plus-shaped platform with two opposing open and two opposing closed arms (open only towards the central platform and surrounded by 14-cm high opaque walls on three sides). Rats were placed on the central platform facing an open arm and allowed to explore the maze for 5 min. Each session was videotaped and subsequently scored by an independent observer. Arm entry was defined as entering an arm with all four paws. Behaviors assessed were: time spent (duration) in open and closed arms and on the central platform; number of open and closed arm entries; and total exploration (entries into all arms). Total exploration was calculated as the number of entries into any arm of the maze in order to distinguish between impaired exploratory behavior, exploration limited to closed arms (avoidance) and free exploration. "Anxiety Index", an index that integrates the EPM behavioral measures, was calculated as follows:
Anxiety Index values range from 0-1 where an increase in the index expresses increased anxiety-like behavior as described by Cohen and colleagues (8) and demonstrated in several subsequent studies (7, 13).

Acoustic Startle Response

Startle response was measured using two ventilated startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). The SR-LAB calibration unit was used routinely to ensure consistent stabilimeter sensitivity between test chambers and over time. Each Plexiglas cylinder rests on a platform inside a sound-proofed, ventilated chamber. Movement inside the tube is detected by a piezoelectric accelerometer below the frame. Sound levels within each test chamber are measured routinely using a sound level meter to ensure consistent presentation. Each test session started with a 5-min acclimatization period to background white noise of 68 dB, following by 30 acoustic startle trial stimuli in six blocks (110 dB white noise of 40 ms duration with 30 or 45 sec inter-trial interval). Behavioral assessment consisted of mean startle amplitude (averaged over all 30 trials) and percent of startle habituation to repeated presentation of the acoustic pulse. Percent habituation -- the percent change between the response to the first block of sound stimuli and the last – was calculated as follows:

$$\text{Percent Habitation} = 100 \times \left[ \frac{(\text{average startle amplitude in Block 1}) - (\text{average startle amplitude in Block 6})}{(\text{average startle amplitude in Block 1})} \right]$$
Classification According to Cut-Off Behavioral Criteria (CBC)

This classification model is based upon the degree to which individual behavior is affected by a stressor. It is based upon the premise that in the natural environment, such extremely compromised behavior in response to the priming trigger may compromise behaviors essential for survival, and is thus inadequate and maladaptive, representing a pathological degree of response (10).

Verification of overall effect of PSS exposure

The data must demonstrate that the stressor had a significant effect on overall behavior of exposed vs. unexposed populations at the time of assessment.

Application of the CBC to the data

Two behavioral measures were used to define CBC: fearful behavior on the EPM and non-habituated exaggerated startle reaction. The validity of these measures as a stress responses has been previously demonstrated (10, 11, 12), and each measure is well-defined and straightforward to score. To maximize the resolution and minimize false positives, extreme responses to both of these paradigms performed in sequence were required for ‘inclusion’ into the extreme behavioral response (EBR) group, whereas a negligible degree of response was required for inclusion into the minimal behavior response (MBR) group. Animals whose behavioral responses were not clear were defaulted into the partial behavioral response (PBR).

Definition of CBC for each paradigm

Extreme behavioral response

1. Five minutes (entire session) spent in closed arms and no entries into the open arms on the EPM.
2. Mean amplitude of the startle response (at 110 db) exceeds 800 units and the startle response shows no habituation over time.

**Minimal behavioral response**

1. 0-1 min spent in closed arms and ≥ 8 open-arm entries on the EPM.
2. Mean amplitude of the startle response (at 110 db) does not exceed 700 units and habituation is demonstrated.

**Partial behavioral response**

Animals that were not part of EBR or MBR were deemed to be ‘partial behavior responders (PBR).

**Contextual Freezing Measurement**

Freezing behavior was scored during the situational reminder/cue exposure and was defined as an absence of all movement (except for respiration) (22). Total cumulative freezing time (total seconds spent freezing during each assessment period) was measured and calculated as a percentage of total time. Freezing behavior was recorded using an overhead video camera and scored for immobility using the recorded images. The videotape and the recorded images were both scored by a trained observer unaware of the treatment conditions.

**Immunofluorescence:**

**Tissue preparation**

Twenty-four hours following the behavioral tests, animals were deeply anesthetized (ketamine and xylazine mixture) and perfused transcardially with cold 0.9% physiological saline followed by 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer (pH 7.4). Brains were quickly removed, postfixed in the same fixative for 12 h at 4 °C, and were cryoprotected
overnight in 30% sucrose in 0.1 M phosphate buffer at 4 °C. Brains were frozen on dry ice and stored at −80 °C. Serial coronal sections (10 µm) at the level of dorsal hippocampus were collected for each animal, using a cryostat (Leica CM 1850) and mounted on coated slides.

Sliced sections were air dried and incubated in frozen methanol (2 min) and in 4% Paraformaldehyde (4 min). After three washes in phosphate buffer saline (PBS) containing Tween 20 (PBS/T) (Sigma-Aldrich), the sections were incubated for 60 min in a blocking solution in (normal goat or horse serum in PBS) and then overnight at 4 °C with the primary antibodies against NPY, BDNF and phosphorylated DOR (1:250 each; Abcam). After three washes in PBS/T, sections were incubated in DyLight-488 labeled goat-anti-rabbit IgG or Dylight-594 goat anti-mouse IgG (1:250; KPL,MD ,UDA) in PBS containing 2% normal goat or house serum for 2 h. Sections were washed, mounted with mounting medium (Vectastain Vector laboratories, USA). Control staining was performed in the absence of the primary antibodies. Additionally, secondary fluorescent labels were swapped to check cross-reactivity and sections were incubated without any primary antibodies to check for any non-specific binding of the secondary antibodies.

Quantification:

A computer-assisted image analysis system (Leica Application Suite V3.6, Leica, Germany) was used for quantitative analysis of the immunostaining and 50× objective lens were employed to assess the number of NPY, BDNF and DOR positive cells in the hippocampus, divided into three (counted separately) areas: CA1 subfield, CA3 subfield and dentate gyrus (DG). The regions of interest were outlined and computer-aided estimation was used to calculate the number of NPY-IR, BDNF-IR and phospho-DOR cells in the pyramidal layer of CA1 and CA3, and in the granular layer of the DG. Seven representative sections of the hippocampus
were chosen (between Bregma -2.30 and Bregma -3.60) from each animal, from each group. The sections were analyzed by two observers blinded to the treatment protocol. Standard technique was used to estimate the number of NPY, BDNF and phospho-DOR cells profiles per unit area for each investigated hippocampal structure.

**Statistical analyses:**

The statistical analyses were performed using a one-way analysis of variance (ANOVA). In the event of a significant F ratio, a Bonferroni post-hoc analysis was used for pairwise comparisons. All data are reported as mean ± SD. The prevalence of the behavioral characteristics among the rodents were tested using cross-tabulation and nonparametric \( \chi^2 \) tests. An alpha level of \( p < 0.05 \) was used to determine statistical significance. Data were analyzed using SPSS v22 software (SPSS Inc., Chicago, IL).

**RESULTS:**

**Elevated plus-maze:**

Comparisons between the groups for time spent in open arms indicated a significant effect (\( F(3, 97) = 5.8, p<0.0015 \)). Post-hoc analyses indicated that animals that exercised for 6-weeks and exposed to PSS spent significantly greater time in the open arms than those animals that were subjected to PSS and not exercised (\( p<0.035 \)). In addition, animals that were exposed to PSS spent significantly less time compared to unexposed animals that did not exercise (\( p < 0.0015 \)) (see figure 1A). Comparisons of open arm entries (see figure 1b) also showed a significant interaction between groups (\( F(3, 97) = 5.90, p < 0.0015 \)). Sedentary animals that were exposed to PSS exhibited significantly (\( p < 0.000 \)) fewer entries than animals that were unexposed and did not exercise. In addition, a trend (\( p < 0.09 \)) towards a greater number of
entries were seen in the trained animals exposed to PSS compared to sedentary animals that were exposed. No other significant differences were noted.

A significant difference was also noted between groups in the total activity performed on the EPM ($F(3, 97) = 14.26, p < 0.000$). Animal exposed to PSS and did not exercise had significantly (p’s < 0.001) less total activity than all other groups (see Figure 1C). In addition, trained animals that were unexposed had significantly greater activity ($p = 0.050$) on the EPM than animals that were unexposed and did not exercise. Based upon the integrated behavioral measures a significant difference in the anxiety-index was noted between the groups (see figure 1D) ($F(3, 97) = 6.07, p = 0.001$). Sedentary animals exposed to PSS exhibited significantly greater anxiety ($p = 0.000$) than sedentary animals that were unexposed. In addition, trained animals exposed to PSS appeared to trend ($p = 0.087$) towards a reduced anxiety compared to animals that did not exercise. No other differences were noted.

**Acute startle response and startle habituation:**

Comparisons between the groups in acoustic startle amplitude revealed a significant group difference ($F(3, 97) = 26.08, p = 0.000$) (see figure 2A). Startle amplitude was significantly greater for sedentary animals that were exposed to PSS compared to all other groups (p’s = 0.000). Although no other significant differences were noted, trained animals that were exposed to the PSS tended to have a greater startle amplitude than trained animals that were unexposed ($p = 0.059$). A significant difference was also noted in startle habituation ($F(3, 97) = 17.27, p = 0.000$) (see figure 2B). Sedentary animals exposed to PSS had a significantly lower habituation score than all other groups (p’s = 0.000). No other significant differences between groups were noted.
**Relative prevalence rates according to CBC’s:**

Significant differences in the prevalence rate of animals displaying EBR were noted among groups ($\chi^2=17.13$, df =3, $p<0.001$). The prevalence of EBR in animals exposed to PSS was 30.0% of the total population and differed significantly from the unexposed group (0%) ($\chi^2=10.9$, $p<0.002$) as well as the group of animals that exercised and exposed to the PSS (8%) ($\chi^2=4.13$, $p<0.045$). The prevalence of MBR among the PSS-exposed animals was 3.3%, and differed significantly from animals that were exercised and exposed ($\chi^2=3.9$, $p<0.05$), and from animals that were unexposed ($\chi^2=6.12$, $p<0.015$). There were no significant differences in the prevalence of PBR among the groups.

**Effect of cue-exposure on freezing behavior at Day 8:**

Figure 3 depicts the freezing behavior upon cue-exposure. A significant difference between the groups was seen in freezing behavior ($F (3,97) = 10.31$, $p = 0.000$). Sedentary animals that were exposed to PSS had significantly greater immobility upon cue than all other groups ($p$’s < 0.001). No other significant differences were noted.

**NPY IR expression at Day 8 post-PSS exposure:**

Comparisons between the groups in NPY expression in the CA1, CA3 and DG subregions can be observed in figures 4A – 4C, respectively. Significant differences were noted in all three subregions ($F (3,50) = 16.3$, $p = 0.0000$, $F (1,50) = 11.2$, $p = 0.000$ and $F (1,50) = 10.78$, $p = 0.000$ in CA1, CA3 and DG, respectively). PSS-exposure decreased expression of NPY in the CA1 and CA3 subregions in sedentary animals compared to all other groups. While no other between group differences in the expression of NPY levels was observed in the CA 3 region, NPY expression in the CA1 region appeared to trend higher in trained animals that were
unexposed than that seen in trained animals that were exposed to PSS (p = 0.067) and in sedentary animals that were unexposed (p = 0.064). NPY expression in the DG region was significantly lower in sedentary animals that were exposed to PSS compared to sedentary animals that were unexposed (p = 0.017), trained animals that were unexposed (p = 0.000), and trended towards a difference compared to trained animals that were exposed (p = 0.086).

**BDNF expression at Day 8 post-PSS exposure:**

Comparisons between the groups in BDNF expression can be observed in figure 5. A significant difference was noted between the groups (F (3, 54) = 43.95, p = 0.000). The highest expression of BDNF was observed in the trained animals that were unexposed. These values were significantly greater than all other groups (p’s = 0.000). The lowest expression of BDNF was seen in animals exposed to PSS and these values were lower compared to all other groups (p’s = 0.000). BDNF expression in trained animals exposed to PSS was significantly lower (p = 0.000) compared to trained animals and were unexposed. No differences were noted between trained animals that were exposed compared to animals that were unexposed.

**Phospho-DOR expression at Day 8 post-PSS exposure:**

Comparisons between the groups in DOR expression in the CA1, CA3 and DG subregions can be observed in figures 6A – 6C, respectively. Significant differences were noted in all three subregions (F (3,54) = 42.30, p = 0.0000, F (1,54) = 15.56, p = 0.000 and F (1,54) = 5.40, p = 0.003 in CA1, CA3 and DG, respectively). In the CA1 subregion PSS-exposure without exercise resulted in a significantly lower expression of phospho-DOR than any other group (p’s < 0.000). In addition, trained animals that were either exposed or unexposed to PSS were observed to have significantly greater expression of phospho-DOR than animals that were unexposed and sedentary. In the CA3 subregion animals that were sedentary and exposed to PSS
experienced a significantly lower phospho-DOR expression compared to trained animals that were unexposed (p = 0.000). Phospho-DOR expression in the CA3 subregion was also significantly higher in trained animals that and exposed compared to sedentary animals that were unexposed (p = 0.005). Although a trend towards a difference (p= 0.068) was noted between animals exposed and trained versus animals exposed and not trained, no other differences were noted in this region. Phospho-DOR expression in the DG subregion was significantly reduced in sedentary animals that were exposed compared to trained animals that were not exposed to the PSS (p = 0.001). No other significant differences were noted in differences in Phospho-DOR expression in this DG subregion.

DISCUSSION

The results of this study indicated that 6-weeks of physical exercise attenuated the negative behavioral changes associated with exposure to PSS. Exercise appeared to reduce the prevalence of an extreme behavior response, while increasing the prevalence of a minimal behavior response in animals exposed to PSS. Further, sedentary animals exposed to PSS were observed to be significantly less active when placed in the elevated maze compared to animals that were either unexposed, or animals that were exposed but were endurance trained. In addition, sedentary animals exposed to PSS experienced a significantly elevated startle response, significantly less startle habituation and demonstrated a significantly greater freezing response than animals unexposed, or exposed to PSS and endurance trained.

The pattern of behavior experienced by the animals exposed to PSS was consistent with previous studies using the same model of PTSD in rodents (7, 12, 23). Exposure to PSS reduced total activity by 28% in the animals that were not exercised in comparison to animals unexposed and also were sedentary. These results were consistent with the 27% decrease recently reported
in total activity by others using the same PTSD model (12). Similarly, startle amplitude seen in this study was 2.8-fold higher in the rodents exposed to PSS, which was consistent with the 2.5-fold increase reported by Cohen and colleagues (12). Interestingly, exposure to the PSS in this study resulted in a 29% elevation in anxiety index and a 2-fold increase in freezing response compared to animals unexposed. These changes did not appear to be at the same magnitude as recently reported by some investigators (12), but similar to that observed by others (7). The laboratory assessments of activity, anxiety and stress in the animals that were exposed to the PSS but endurance trained support the beneficial role that exercise has been suggested to have in both human (1, 18) and murine (28) studies on PTSD. One study examining men with combat-related PTSD showed that participation in regular exercise programs resulted in a better perception of quality of life compared to veterans that were sedentary (1). Other investigations that prospectively examined the effects of endurance exercise on patients diagnosed with PTSD reported symptoms of PTSD including depression and anxiety were reduced following the exercise program (18). This is consistent with research demonstrating the anxiolytic effects of endurance exercise on students diagnosed with high anxiety sensitivity (4) and competitive athletes (29). In addition, the lower prevalence of EBR in rodents that were exercised in this study is consistent with the overall anxiolytic benefits of exercise.

The results of this study are also consistent with other rodent models using a different PTSD model (28). Patki and colleagues (28) examined the single-prolonged stress model that incorporates psychological, physical and endocrinological stresses to increase the severity of symptoms that are associated with PTSD. A major difference between these studies is that Patki et al (28) exposed the animals to the stressor prior to the beginning of a 2-week exercise protocol, whereas the present study trained the animals for 6-weeks prior to administering the
PSS. Although both studies reported that exercise was able to reduce adverse behavioral responses associated with PTSD, the present study demonstrated the potential preventative effects, while Patki and colleagues (28) indicated that exercise may also be used post-exposure as a potential treatment option.

The treatment of PTSD often involves a broad array of options (21, 26). The use of pharmaceutical agents has become a popular treatment strategy, especially the antidepressant medications such as selective serotonin reuptake inhibitors. Tricyclic antidepressants and monoamine oxidase inhibitors (21). However, the potential side effects associated with these medications may result in some hesitancy in their use. PTSD is often associated with elevated stress hormones that can modulate function within the brain (26). Specifically, the hippocampus is quite sensitive to both acute and chronic stress. A chronic stress response may elevate glucocorticoid expression in the brain causing the neurons in the hippocampus to undergo reversible remodeling especially in the DG-CA3 regions (26). This remodeling generally involves dendritic atrophy (33), which may result in diminished function in hippocampal-dependent memory tasks (6). In contrast, BDNF is reported to have the opposite effect on neuroplasticity by enhancing neurogenesis and dendritic remodeling (38).

The significant decrease in BDNF expression observed in this study in the rodents that were sedentary and exposed to PSS is consistent with the association previously reported between BDNF expression and PTSD-like behavioral stress response (23, 40). Exercise has been previously shown to be a potent stimulator of BDNF expression in the hippocampus (17, 24). This is supported by the results of the present study which showed a significantly greater expression of BDNF in the trained rats that were unexposed. However, exposure to PSS did appear to attenuate BDNF expression, as significant differences were noted between exposed and
unexposed trained rats. Still, the significant differences between trained and untrained animals exposed to PSS do indicate that exercise can mitigate the down-regulation of BDNF expression during periods of stress. This is consistent with recent research by Fang and colleagues (17).

The results of this study are also consistent with the effects of PTSD on NPY expression (8, 15). Both the expression of NPY and plasma concentrations of NPY are significantly reduced in combat veterans suffering from PTSD (31) or in rats exposed to PSS (8). Previous studies have suggested that BDNF may serve as a regulator of the function and morphological expression of the NPY neuron (2), and provide a greater resiliency to stress by increasing synaptic plasticity and stabilize synaptic connectivity (14). Exercise appeared to be a potent stimulator of maintaining normal expression of NPY, but did not appear to exacerbate its expression compared to unexposed conditions. These benefits appeared in both the CA1 and CA3 subregions of the hippocampus, but not in DG. Differential responses of hippocampal subregions to stress and learning have been previously reported (19), this study appears to be the first to observe this differential effect resulting from an exercise intervention.

Exercise has been reported to have a strong effect on elevating endogenous opioid polypeptide compounds (16). An elevation in β-endorphins is thought to provide relief of depression (27), and is considered to be a potential therapeutic treatment strategy (26). As such, physical activity may alleviate the need for pharmacological intervention as a treatment strategy. The up-regulation of the phospho-DOR in the hippocampus seen in the trained rodents provides additional support for the positive benefits associated with endurance exercise. However, the ability of phospho-DOR expression to be maintained when the animals were exposed to PSS is a unique study outcome. It demonstrates the potential preventative ability that exercise has in minimizing PTSD-like behaviors through elevations in phospho-DOR. Up-regulation of
phospho-DOR may also have attributed to the increased expression of BDNF. Several investigators have reported that phospho-DOR agonists can increase gene expression of BDNF across several regions of the brain (35, 39). The increased expression of phospho-DOR in CA1, but not CA3 or DG subregions during PSS may also be related to differences in the lack of consistency in the phospho-DOR and BDNF relationship recently reported between subregions of the hippocampus (35). This may be suggestive of an attenuation in the signal between phospho-DOR and BDNF following PSS in some regions of the hippocampus.

In summary, the results of this study indicate that 6-weeks of endurance training provides a mechanism to promote resilience and/or recovery from stress. Thus, the increased expression of BDNF, NPY and phospho-DOR following training may increase synaptic plasticity and stabilization of synaptic connectivity, leading to resilience to psychopathology. Although an increase in the expression of BDNF are thought to regulate changes in NPY (2), and an up-regulation of phospho-DOR may stimulate changes in BDNF (35, 39), further research is still warranted to examine the regulatory mechanism associated with these physiological effects. Whether exercise initiated following the appearance of symptoms related to PTSD provides the same effects needs further examination. However, this study provides evidence that prolonged exercise training can provide a non-pharmacological intervention for the treatment or prevention of PTSD.
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Conflict of Interest

The authors declare no conflict of interest. In addition, results of this present study do not constitute endorsement by ACSM
REFERENCES


Figure Legends

Figure 1: Behavioral Performance in Elevated Plus-Maze. A) Comparison between the groups for time spent in open arms; B) Comparison between the groups in the number of open arm entries; C) Comparison between the groups in the total activity; D) Comparison between the groups in the anxiety-index; a = significantly different than UNEX + SED; b = significantly different than UNEX + EX; c = significantly different than PSS + EX; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX + EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.

Figure 2: Acoustic Startle Response. A) Comparisons between the groups in startle amplitude; B) Comparisons between the groups in startle habituation; a = significantly different than UNEX + SED; b = significantly different than UNEX + EX; c = significantly different than PSS + EX; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX + EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.

Figure 3: Freezing Behavior upon Cue-Exposure. a = significantly different than UNEX + SED; b = significantly different than UNEX + EX; c = significantly different than PSS + EX; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX +
EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.

Figure 4: NPY IR Expression at Day 8 Post-PSS Exposure. NPY expression in the CA1 (figure 4A), CA3 (figure 4B) and DG (figure 4C) subregions. a = significantly different than UNEX + SED; b = significantly different than UNEX + EX; c = significantly different than PSS + EX; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX + EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.

Figure 5: BDNF Expression at Day 8 Post-PSS Exposure. a = significantly different than UNEX + SED; b = significantly different than UNEX + EX; c = significantly different than PSS + EX; d = significantly different than PSS + SED; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX + EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.

Figure 6: DOR expression at Day 8 post-PSS exposure. DOR expression in the CA1 (figure 6A), CA3 (figure 6B) and DG (figure 6C) subregions. a = significantly different than UNEX + SED; b
= significantly different than UNEX + EX; c = significantly different than PSS + EX; d = significantly different than PSS + SED; UNEX + SED = rodents that remained sedentary and were not exposed to the predator scent stress; UNEX + EX = rodents that were trained and were not exposed to the predator scent stress; PSS + EX = rodents that were trained and were exposed to the predator scent stress; PSS + SED = rodents that remained sedentary and were exposed to the predator scent stress. All data reported as mean ± SD.
Figure 1
Figure 2

A

B

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Figure 3

![Bar chart showing freezing percentages for different conditions: UNEX+SED, UNEX+EX, PSS+EX, PSS+SED. The chart indicates that PSS+SED has the highest freezing percentage, followed by UNEX+SED, UNEX+EX, and PSS+EX. The bars are labeled with error bars indicating variability.]
Figure 4
Figure 5
Figure 6