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Voluntary physical exercise protects against behavioral and endocrine reactivity to social and environmental stressors in the prairie vole

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Abstract

Physical activity has been shown to combat detrimental effects of stress. The current study examined the potential protective effects of exercise against a combination of social isolation and chronic mild stress (CMS) in a prairie vole model. Female voles were isolated for 4 weeks, with the addition of CMS during the final 2 weeks. Half of the voles were allowed access to a running wheel during this final 2 weeks, while the other half remained sedentary. Animals underwent behavioral tests to assess depressive- and anxiety-behaviors. In a subset of animals, plasma was collected 10 minutes after behavioral testing for corticosterone analysis. In a separate subset of animals, brains were collected 2 hours after behavioral testing for cFos analysis in the paraventricular nucleus (PVN). Voles in the exercise group displayed significantly lower depressive- and anxiety-like behaviors, and also had significantly lower corticosterone levels, compared to animals in the sedentary group. There was no difference in PVN cFos activity between groups. Interestingly, animals that moderately exercised displayed lower levels of depressive-like behavior and attenuated corticosterone reactivity compared to animals in the low and high activity subgroups. Current findings suggest that physical activity can protect against a combination of social and environmental stressors.

Keywords

prairie voles; social isolation; chronic mild stress; exercise; paraventricular nucleus; cFos

Introduction

Research over several decades indicates that stress can affect a person's emotional state and physiological well-being (McCarty, 2016). In attempting to cope with stress, a person may experience emotional and behavioral disturbances (Barbaglia, ten Have, Dorsselaer, Alono, & de Graaf, 2015; Schneiderman, Ironson, & Siegel, 2008; Taylor, Lerner, Sage, Lehman, & Seeman, 2004). In addition, perceived stress activates the hypothalamic-pituitary-adrenal

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Disclosure Statement

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(HPA) axis, resulting in the release of metabolic hormones such as adrenocorticotrophic hormone (ACTH) and cortisol or corticosterone. This is an adaptive response; however, prolonged high levels of stress and hyperactivity of the HPA axis can have damaging effects on emotion and physiology, and are associated with many psychological disorders such as depression, anxiety, and post-traumatic stress disorder (Reviewed by Otte et al., 2016).

Many of the stressors that lead to emotional, behavioral, and physiological disruptions are environmental or social in nature. Environmental stressors such as daily stress from work, school, and home life have been associated with HPA dysfunction, cardiovascular irregularities, and dampened immune reactivity both in humans and animal models (Campeau et al., 2010; Janssens et al., 2016; Viena, Banks, Bardu, Schulman, & Tartar, 2012). For example, ambulance paramedics show a significant elevation in saliva cortisol levels during workdays compared to rest days (Wong, Ostry, Demer, & Davies, 2012). In rodents, environmental stressors such as white noise, novel environment, and/or predator odor elevate several behavioral and physiological variables, including plasma corticosterone, resting heart rate, core body temperature, activation in the hypothalamic paraventricular nucleus (PVN), and anxiety-like behavior (Campeau et al., 2010; Hennessy et al., 2015; Masini, Nyhuis, Sasse, Day, & Campeau, 2011). Further, the unpredictability of stressors may lead to learned helplessness, depressive behavior, and HPA hyperactivity (Reviewed by Liu, Kleiman, Nestor, & Cheek, 2015).

Importantly, human literature has reported that we experience multiple stressors at the same time throughout our lives, and lifetime stress has been associated with poor physical and psychological health (Bergen et al., 2012; Kennedy & Adolphs, 2011; Mahoney et al., 2015; Silver-Aylaiian & Cohen, 2001). In addition to negative consequences as a function of general environmental stressors, social stressors such as objective and perceived isolation are associated with emotional and psychological disturbances, physiological disturbances, and increase the risk for premature mortality (Cacioppo & Cacioppo, 2014; Steptoe, Shankar, Demakakos, & Wardle, 2013). For example, prolonged social isolation has been shown to affect HPA functions, often resulting in an elevation of basal cortisol levels (Doane & Adam, 2010; Steptoe, Owen, Kunz-Ebrecht, & Brydon, 2004). The combination of social and environmental stressors may be especially detrimental to psychological and physiological health, as previous research indicates that the more stress one perceives, the more significant the health problems (Schreier et al., 2016; Silver-Aylaiian & Cohen, 2001; Zannas et al., 2015).

Given previous evidence indicating that social and environmental stressors contribute to poor health outcomes, knowledge can be gained from focusing more specifically on causes, consequences, or potential treatments associated with stress using animal models. The prairie vole is a valuable translational model for the study of social stressors, due to similarities of the social structures between prairie voles and humans. Prairie voles are a socially monogamous rodent species, forming strong pair-bonds, exhibiting biparental care of offspring, living with extended families, and responding negatively to social stressors (Ahern & Young, 2009; Carter, DeVries, & Getz, 1995). Social isolation in this species is associated with behaviors related to depression and anxiety, as well as several HPA axis disruptions (Bosch, Nair, Ahern, Neumann, & Young, 2009; Grippo et al., 2007; Grippo,

Trahanas, Zimmerman, Porges, & Carter, 2009; McNeal et al., 2014; Sun, Smith, Lei, Liu, & Wang, 2014). Prairie voles exposed to both social isolation and additional environmental stressors display disruptions in both behavior and physiological reactivity, compared to socially paired controls (Grippe et al., 2015; McNeal et al., 2017). Further, both brief and prolonged social isolation increase activity in the PVN of the hypothalamus, suggesting that the PVN may mediate some of these stress responses (Maken, Weinberg, Cool, & Hennessy, 2010; Pan, Liu, Young, Zhang, & Wang, 2009).

In searching for a non-pharmacological solution to protect against detrimental consequences of environmental and social stressors, many have turned to exercise. Physical activity has global effects on the body and is associated with physiological fitness, mood elevation, and positive mental health (Reviewed by Schuch et al., 2016). Exercise induces many psychological and physiological changes; however, the specific mechanisms through which exercise protects against behavioral and neuroendocrine consequences of stress require further investigation. Physical activity may have stress-buffering effects via actions on the HPA axis as well as multiple neurotransmitter systems. For example, a 21-day swim training in rats buffered against a series of mild stressors; trained animals had significantly lower plasma cortisol levels and a significant increase in central release of serotonin, dopamine, and norepinephrine compared to the control group (He et al., 2012). In addition, exercise protects against negative behavioral consequences of social isolation (alone) in prairie voles, including both depressive- and anxiety-like behaviors (Grippe et al., 2014). Moreover, exercise attenuates cFos reactivity in the PVN after exposure to mild stressors compared to sedentary conditions (Campeau et al., 2010).

While previous studies have illustrated the effectiveness of physical activity in protecting against consequences of social and environmental stressors individually (Campeau et al., 2010; Gerber et al., 2017; Grippe et al., 2014), the potential protective influence of exercise on a combination of these stressors is not well elucidated. Given the frequency of co-occurrence of social and environmental stressors (Mahoney et al., 2013; Schreier et al., 2016; Zannas et al., 2015), the investigation of factors that may protect against this combination of stressors using an animal model will provide translational value for humans. Therefore, the goal of the current study is to examine the potential protective influence of exercise on negative behavioral and neurobiological consequences of social and environmental stressors in prairie voles. We predicted that voluntary exercise would attenuate depressive- and anxiety-like behaviors in female prairie voles exposed to a combination of chronic social and environmental stressors, compared to sedentary conditions. We also predicted that exercise would attenuate the corticosterone response and PVN activation following a short-term stress challenge.

Methods

Animals

A total of 78 adult, sexually naïve, female prairie voles, descendants of a wild stock caught near Champaign, Illinois, were used in the present study. Animals had a mean ($\pm$ standard error of the mean, SEM) age of 75 ± 3 days and a body weight of 39 ± 0.6 grams. All animals were maintained on a 14/10 h light/dark cycle (lights on at 0630h), with a mean $\pm$

SEM ambient temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $40 \pm 5\%$. Animals were allowed food (Purina rabbit chow) and water ad libitum, unless otherwise noted. Offspring were removed from breeding pairs at 21 days of age and housed in same-sex sibling pairs until the commencement of the study. For all procedures described here, only one animal from each sibling pair was used. All procedures were conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by the Northern Illinois University Institutional Animal Care and Use Committee.

General Experimental Design

The general experimental design is included here and in Figure 1; all specific procedures (including sample sizes when subsets of animals were tested) are detailed in the following sections. All animals were removed from their respective siblings and housed individually for 4 weeks. During the final 2 weeks of the 4-week isolation period, all animals were exposed to chronic mild stress (CMS). Half of the animals were allowed access to a running wheel in the cage during this 2-week period, while the other half remained sedentary in a standard cage. At the conclusion of the isolation/CMS period, a subset of animals in each group was exposed to a 5-minute elevated plus maze (EPM), followed by replacement in their respective housing conditions (isolated/CMS with either exercise or sedentary conditions). Twenty-four hours following this test, all animals were exposed to a 5-minute forced swim test (FST), followed by replacement in their respective housing conditions. Ten minutes following the end of the FST, a subset of animals was euthanized under anesthesia; blood was collected for the analysis of circulating corticosterone, and adrenal glands were dissected and weighed. Two hours following the end of the FST, the remaining animals were euthanized under anesthesia, and brain tissue was dissected for the analysis of cFos immunohistochemistry in the PVN.

Social Isolation

All animals were removed from the home cage and placed into clean cages. Each animal was housed individually for a total of 4 weeks, without visual, olfactory, or auditory cues from its sibling. Cage changes and measuring of body weight prior to, during, and following this period were matched for all animals.

Chronic Mild Stress

During the final 2 weeks of the isolation period, all animals were exposed to CMS using a variation of methods described elsewhere (Grippe, Francis, Beltz, Felder, & Johnson, 2005), with modifications for use in prairie voles (McNeal et al., 2017). Briefly, animals were exposed to a series of mild stressors on an unpredictable schedule for varying durations (see Figure 2 for details), including: (a) continuous overnight illumination; (b) a brief period of water deprivation followed by exposure to an empty water bottle; (c) stroboscopic illumination in a dark room (300 flashes/min); (d) background white noise (90 dB); (e) 40° cage tilt along the vertical axis; (f) damp bedding (100 ml of water poured over approximately half of the bedding in the bottom of the cage; and (g) a foreign object in the cage (a brick, 6 inches length $\times$ 3 inches width $\times$ 2.5 inches height; this final stressor was used in lieu of the paired housing stressor, which is typical in CMS paradigms in rats).

(Willner, 2005) but would interfere with the social housing manipulation in the present study.

Access to Physical Activity

During the 2-week isolation/CMS period, half of the animals had continuous access to a running wheel ($n = 39$; 4.5 in diameter; Super Pet Mouse Silent Spinner Mini Exercise Wheel, Model #100079369, Elk Grove Village, IL) to allow for voluntary physical activity, while the other half ($n = 39$) remained sedentary in a standard cage. In the group with access to running wheels, daily distance traveled was monitored via an odometer adapted for use with the running wheel (Bell F12 Cyclocomputer, Model # 7001115, Van Nuys, CA).

Elevated Plus Maze

Twenty-four hours following the final day of isolation and CMS, behaviors during the EPM were categorized to operationally define an anxiety-like phenotype (Pellow, Chopin, File, & Briley, 1985) in a subset of animals from each group ($n = 16$ from the exercise group; $n = 16$ from the sedentary group). The maze (57cm height) was designed with two opposing open arms of clear Plexiglas (49.5×10cm), two opposing closed arms of black Plexiglas with an open roof (49.5×10×30.5cm), and a center section of clear Plexiglas (10×10cm). Each animal was placed in the center in a brightly-lit room, and allowed to explore freely for 5 minutes. The maze was cleaned thoroughly prior to testing each animal. Each animal was returned to its home cage, in its respective condition (isolated/CMS with either exercise or sedentary conditions), immediately following the test.

Behaviors during the EPM were recorded using a digital video camera, and scored manually by trained observers that were blind to the experimental conditions. The behaviors in the EPM were defined as the following: (a) duration of time spent in each the open, closed, and center sections of the maze; and (b) number of crosses into the center section. The animal was coded as entering a section of the maze when all 4 paws crossed into the respective section. Number of crosses into the center section of the maze was used as an index of general activity; total time spent exploring the open arms of the maze was used as an index of anxiety-relevant behavior (Pellow et al., 1985).

Forced Swim Test

Twenty-four hours following the EPM (or at the equivalent time point for animals that were not exposed to the EPM), behaviors during the FST were categorized to operationally define a depressive phenotype in all animals ($n = 39$ from the exercise group; $n = 39$ from the sedentary group). Each animal was exposed to a 5-minute period of forced swimming using procedures described previously in rodents (Cryan, Valentino, & Lucki, 2005), including prairie voles (Grippio, Wu, Hassan, & Carter, 2008). A clear, cylindrical Plexiglas tank (height 46 cm; diameter 20cm) was filled to a depth of 18 cm with room temperature tap water (25-26° C). Each animal was placed into the tank for 5 minutes. The tank was cleaned thoroughly and filled with clean water prior to testing each animal. Each animal was returned to its home cage, in its respective condition (isolated/CMS with either exercise or sedentary conditions), immediately following the swim period, with access to a heat lamp for 10 minutes.

Behaviors during the swim test were recorded using a digital video camera, and scored manually by trained observers that were blind to the experimental conditions. The behaviors during the FST were defined as the following: (a) swimming: directed movements of the forelimbs and hindlimbs without breaking the surface of the water; (b) struggling: forelimbs breaking the surface of water; (c) climbing: attempts to climb the walls of the tank; and (d) immobility: no limb or body movements (floating) or using limbs solely to remain afloat without corresponding trunk movements. Times spent struggling, climbing and swimming were totaled to provide one index of active coping behaviors; immobility was used as the operational index of depressive behavior (Cryan et al., 2005).

Collection of Plasma

Ten minutes following the end of the FST, blood was collected in a subset of animals ($n = 23$ from the exercise group; $n = 22$ from the sedentary group). Each animal was anesthetized with a combination of ketamine (67 mg/kg, sc; NLS Animal Health, Owings Mills, MD) and xylazine (13.33 mg/kg, sc; NLS Animal Health, Owings Mills, MD). The animal was anesthetized within 1 minute of being removed from the home cage. Blood was sampled within 2 minutes of the anesthetic injection, from the periorbital sinus via a heparinized capillary tube, and was collected during a period not exceeding 1.5 minutes. The blood was placed immediately on ice, and centrifuged at 4° C, at 3500 rpm, for 15 minutes to obtain plasma. Plasma aliquots were stored at -80° C.

Enzyme-Linked Immunosorbent Assay

Plasma levels of corticosterone were determined using a commercially available enzyme-linked immunosorbent assay kits (Enzo Life Sciences, Farmingdale, NY), which has been validated previously by our laboratory for use in prairie voles. The plasma was diluted in assay buffer as necessary (1:500) to give results reliably within the linear portion of the standard curve. The minimum detection limits for this assay is .027 ng/ml; and inter- and intra-assay coefficients of variation are both < 5 %. Cross-reactivity with other steroids or peptides is less than 1.7%.

Collection of Adrenal Glands

Ten minutes following the end of the FST, a subset of animals was euthanized under anesthesia after blood was collected, and adrenal glands were immediately dissected and weighed, for the analysis of adrenal/body weight ratio ($n = 16$ from the exercise group; $n = 16$ from the sedentary group).

Collection of Brain Tissue

Two hours following the end of the FST, a subset of animals was euthanized under anesthesia, and brains were removed for the analysis of cFos immunoreactivity in the PVN ($n = 7$ from the exercise group; $n = 7$ from the sedentary group). The brains were processed with a passive perfusion (i.e., spin immersion) technique described previously (Cushing, Martin, Young, & Carter, 2001; Grippo, Cushing, & Carter, 2007). Briefly, brains were immersed in a fixative solution consisting of 4% paraformaldehyde, and gently agitated for a total of 4 hours. Brains were postfixed for 24 hours in 4% paraformaldehyde, and sunk in

25% sucrose. Tissue was stored in 25% sucrose at 4° C until it was sectioned at 40 µm on a cryostat. Sliced serial brain sections were stored in wells at -20° C, in cryoprotectant antifreeze solution, until assayed for cFos in the PVN.

Immunohistochemical Analysis

Serial brain slices (40 µm) from the PVN were assayed for expression of cFos using standard double-label avidin:biotinylated enzyme complex (ABC) immunohistochemistry. Anti-cFos (Oncogene Science, Cambridge, MA; generated in rabbit) was used at a concentration of 1:100,000, and the target was visualized using nickel-diaminobenzadine (DAB) dissolved in 0.175 M sodium acetate.

Free-floating sections were rinsed 6 times during a 1 hour period with potassium phosphate buffered saline (KPBS). Sections were then incubated in 1% sodium borohydride for 20 minutes at room temperature. After multiple washes in KPBS, sections were incubated in 0.014% phenylhydrazine for 15 minutes at room temperature. Tissue was rinsed 6 times during a period of 1 hour in KPBS. Sections were then incubated in primary antibody for cFos diluted in KPBS + 0.4% Triton X-100 for 1 hour at room temperature, and then incubated for 42 hours at 4° C. Following this incubation period, sections were rinsed 10 times during a period of 1 hour with KPBS. Sections were then incubated in anti-rabbit IgG (BA-1000; Vector Laboratories, Burlingame, CA; 1:600) for 1 hour at room temperature. Sections were rinsed 5 times during a period of 50 minutes with KPBS, and then incubated in A/B solution (Vectastain Elite PK-6100; Vector Laboratories, Burlingame, CA; 45 µl A, 45 µl B per 10 ml KPBS + 0.4% Triton X-100) for 1 hour at room temperature. Sections were rinsed 3 times in KPBS and then 3 times in 0.175 M sodium acetate. cFos was visualized by incubation in DAB and nickel sulfate, dissolved in sodium acetate, for 9 minutes at room temperature, and then sections were rinsed 3 times with sodium acetate and 3 times with KPBS.

Stained sections were mounted on electrostatically-charged slides, air-dried, dehydrated in a series of ethanol dilutions, cleared with Histoclear (National Diagnostics, Atlanta, GA), and then protected with coverslips using Histomount mounting medium (National Diagnostics, Atlanta, GA).

Images were captured using a Nikon Eclipse E 800 microscope, Sensi-cam camera and IPLab software (Scanalytics, Inc., Fairfax, VA). The density of cFos-immunoreactive cell bodies was determined in the PVN with a 10x objective, using a standardized sampling area, according to procedures described previously (Pan et al., 2009; Ruscio, Sweeny, Hazelton, Suppatkul, & Carter, 2007). Measurements within the PVN were taken in a caudal section of the nucleus where the stained cells take a characteristic shape demonstrated in previous studies (Wang, Zhuo, Hulihan, & Insel, 1996; Ruscio et al., 2007). This section is further characterized by the medial-lateral position of the fornix (relative to the third ventricle) and medial and dorsal location of the optic tract (relative to more central and ventral position in more rostral sections). It is approximate to Figure 49 in Paxinos and Watson (2005).

cFos immunoreactivity was determined with a mean optical density method similar to previous studies (Köppen et al., 2016; Liu et al., 2001; Maken et al., 2010; Pan et al., 2009).

Using ImageJ (National Institutes of Health, Bethesda, MD), a standardized oval shape (with the area of 0.083 mm² per hemisphere) was used to determine the density reading of the PVN. Background density readings were obtained from areas adjacent to the PVN and were subtracted from the density readings to determine the final cFos expression. The optical density values were relative to the gray values associated with the sampled area (white: 0; black: 255). Density measurements were taken from sections matched in rostral-caudal orientation to minimize variability. Two to three brain sections were analyzed from each subject, and results were averaged across sections. Density measurement was performed separately for each hemisphere, and the results were averaged between hemispheres. For all subjects, density measures were conducted by two trained, experimentally-blind raters, and the results were averaged between raters. Therefore, density measures were averaged across multiple brain slices, hemispheres, and raters to provide an accurate estimation of optical density in the PVN. Damaged sections were excluded from analysis.

Data Analysis

Data are presented as means $\pm$ SEM for all analyses and figures. Behavioral data from the EPM and FST, and physiological data from corticosterone reactivity, adrenal/body weight ratios, and PVN immunoreactivity were analyzed with single factor analyses of variance (ANOVA) and/or independent groups Student's t-tests, with group (exercise vs. sedentary) as the independent factor. Body weight data were analyzed with a mixed-design ANOVA, with group (exercise vs. sedentary) as the independent factor and time as the repeated measures factor.

Animals with access to running wheels displayed a high degree of individual variability in the daily distance traveled, with a mean range of 0.01 km/day to 16.3 km/day (total group mean, 2.8 ± 0.6 km/day). Therefore, the animals in this group were split into 3 subgroups comprising approximately the bottom 1/3, middle 1/3, and top 1/3 of mean daily distances traveled. These groups were characterized as the low activity subgroup (less than 0.1 km/day), moderate activity subgroup (between 0.1 km/day and 3.5 km/day), and high activity subgroup (greater than 3.5 km/day) for the purpose of within-group comparisons, as described in the Results section. Single-factor ANOVAs were used for these exploratory comparisons, with subgroup (low activity, moderate activity, high activity) as the independent factor; follow-up comparisons were conducted with t-tests with a Bonferroni correction.

A value of $p < 0.05$ was considered to be statistically significant for ANOVAs and individual t-tests; a value of $p < 0.025$ was considered to be statistically significant for Bonferroni-corrected t-tests involving multiple comparisons.

Results

Body Weight

Body weight did not differ between the exercise and sedentary groups at any point during the experiment (see Table 1). A mixed-design ANOVA yielded no significant main effect of either group [$F(1,304) = 1.10$] or time [$F(3,311) = 0.99$], and no significant group by time

interaction [$F(3,311) = 0.21$; $p > 0.05$ for all comparisons]. No follow-up tests and no subgroup comparisons in the exercise group were conducted.

Elevated Plus Maze

Animals in the exercise group spent a greater amount of time in the open arms [$t(30) = 3.16$, $p < 0.004$] and a reduced amount of time in the closed arms [$t(30) = 4.15$, $p < 0.0003$] of the EPM versus the sedentary group, with no difference in duration of time spent in the center section [$t(30) = 0.41$, $p > 0.05$] (see Figure 3A). The number of entries into the center section did not differ significantly [sedentary, 14.7 ± 3.3 entries; exercise, 13.2 ± 2.2 entries; $t(30) = 0.36$, $p > 0.05$]. There were no significant differences in open arm time among the 3 exercise subgroups (see Figure 3B); [$F(2,15) = 1.33$, $p > 0.05$; no follow-up t-tests were conducted].

Forced Swim Test

Physical activity was associated with lower levels of immobility in the FST versus sedentary conditions [$t(76) = 3.28$, $p < 0.002$]. Moderate activity was associated with the lowest levels of immobility, versus either low or high activity (see Figure 4). Comparisons of immobility levels among the exercise subgroups indicated a significant main effect of activity level [$F(2,38) = 10.64$, $p < 0.0002$]. Animals in the moderate activity group displayed significantly lower levels of immobility than the low activity [$t(25) = 3.55$, $p < 0.001$] and high activity groups [$t(28) = 4.20$, $p < 0.0003$]. The low activity and high activity subgroups did not differ significantly in the amount of immobility displayed during the FST [$t(19) = 1.88$, $p > 0.025$].

Corticosterone

Physical activity was associated with lower levels of FST-induced circulating corticosterone, versus sedentary conditions [$t(43) = 9.21$, $p < 0.00001$]. Moderate activity was associated with the lowest levels of corticosterone, compared to low or high activity (see Figure 5). Comparisons of circulating corticosterone levels among the exercise subgroups indicated a significant main effect of activity level [$F(2,22) = 19.13$, $p < 0.00002$]. Animals in the moderate activity group displayed significantly lower levels of corticosterone than both the low activity [$t(14) = 3.19$, $p < 0.007$] and high activity groups [$t(15) = 5.99$, $p < 0.00002$]. The low activity and high activity subgroups did not differ significantly in the amount of circulating corticosterone [$t(11) = 2.38$, $p > 0.025$].

Adrenal Weight to Body Weight Ratio

Physical activity was associated with a reduced adrenal/body weight ratio, versus sedentary conditions. The ratio was $0.0003 \pm 2.9\text{E-}05$ in the exercise group, and was $0.0005 \pm 2.4\text{E-}05$ in the sedentary group [$t(30) = 3.70$, $p < 0.0009$]. There were no significant differences in adrenal/body weight ratio among the 3 exercise subgroups. A one-way ANOVA yielded no significant effect among the 3 subgroups [$F(2,15) = 0.62$, $p > 0.05$; data not shown; no follow-up t-tests were conducted].

Immunohistochemical Analyses

Physical activity was not associated with a change in cFos immunoactivity in the PVN. The optical density of cFos immunoreactivity in the PVN of physically active animals (31.14 ± 2.55 arbitrary unit) was not significantly different from those that remained sedentary [24.10 ± 5.95 arbitrary unit; $t(12) = 1.438$, $p > 0.05$].

Discussion

Exercise not only promotes a healthy lifestyle (van Praag, Fleshner, Schwartz, & Mattson, 2014), but also is beneficial for physiological and psychological well-being (Fragala et al., 2011; Joyner & Green, 2009; Lavie et al., 2015; Simpson, Kunz, Agha, & Graff, 2015; Zschucke, Gaudlitz, & Ströhle, 2013). The present study used the prairie vole model to specifically investigate the potential beneficial effects of exercise in the context of a combination of social and environmental stressors. Voluntary exercise – especially moderate levels of exercise – was shown to prevent negative behavioral and endocrine stress responses to social isolation and CMS; however, exercise did not alter neural activity in the PVN. This study demonstrates for the first time the protective influence of moderate levels of exercise on both behavioral and physiological consequences of stress using the prairie vole model. The findings from the current study have implications for understanding the mechanisms through which exercise serves a protective role against emotion-related disorders and physiological dysfunction associated with environmental and social stress in humans.

The present findings illustrate that voluntary exercise prevents both depressive- and anxiety-like behaviors in prairie voles following a combination of social isolation and CMS. Compared to sedentary animals, prairie voles that were allowed access to a running wheel exhibited lower levels of immobility in the FST, which is hypothesized to be an operational index of helpless behavior (Cryan et al., 2005; Grippo et al., 2008). Similarly, prairie voles in the voluntary exercise condition spent a greater amount of time exploring the open arms of the EPM (versus the sedentary group), which is hypothesized to represent higher levels of exploratory behavior (Pellow et al., 1985). The current findings are consistent with previous studies demonstrating that physical activity may buffer several consequences of social (Benite-Rebeiro, Santos, & Duarte, 2014; Grippo et al., 2014; Ishikawa, Ogawa, Owada, & Ishikawa, 2014; Otsuka, Shiuchi, Chikahisa, & Shimizu, 2015; Schneider et al., 2010) and environmental (Campeau et al., 2010; Masini et al., 2011) stressors independently. The current study is the first to provide evidence that exercise can protect against multiple types of stressors at once in the prairie vole model, expanding the potential behavioral benefits of exercise. This has clinical implications in which exercise may be recommended to highly stressed individuals as a preventative measure for psychological disorders prior to, or in addition to, pharmacological interventions.

Similar to the behavioral responses, animals that experienced social isolation and CMS in the exercise condition displayed a lower corticosterone response following the FST compared to those in the sedentary condition. The current findings are consistent with those of Campeau et al. (2010), in which exercise animals displayed blunted ACTH and corticosterone responses to an acute novel environment compared to sedentary animals. Prairie voles in the exercise group also had a significantly smaller adrenal weight to body

weight ratio, compared to the sedentary condition, suggesting that exercise can protect against enlarged adrenal glands, which has been observed previously in chronically stressed animals (Kim et al., 2008; Liaudat et al., 2015). Taken together, the current findings indicate that voluntary physical activity attenuates both acute and chronic endocrine stress responses. These results have important implications for considering treatment strategies associated with stress. For example, a significant association between high lifetime stress (including social and environmental stress) and DNA demethylation in regions specific to glucocorticoid receptor signaling has been reported in humans, which may have contributed to HPA dysfunctions in individuals exposed to multiple stressors (Zannas et al., 2015). Physical activity has been shown to buffer negative effects of stress at this level; for instance, socially isolated mice that were allowed to exercise had significantly higher glucocorticoid receptor levels and DNA methylation activity in the hippocampus compared to socially isolated sedentary mice (Pan-Vazquez et al., 2015).

The initial goal of this study was to compare the effects of exercise versus sedentary conditions on responses to social isolation and CMS; however, we observed high levels of variability in physical activity level among prairie voles. Therefore, we conducted subgroup analyses to determine whether the level of physical activity was systematically associated with the dependent measures. The current findings indicate that moderate levels of exercise are the most effective in preventing some of the damaging behavioral and endocrine responses to environmental and social stressors. Prairie voles with moderate levels of exercise displayed the lowest levels of immobility in the FST, and also exhibited the lowest concentrations of corticosterone following the FST, compared to animals that engaged in either low or high levels of activity. However, neither behaviors in the EPM nor adrenal to body weight ratios differed among the subgroups. Although it is possible that inadequate statistical power prevented the ability to detect subgroup differences in these measures, the findings also may be due to different mechanisms through which exercise serves a protective role. For instance, moderate exercise may be more beneficial for altering depression-relevant behaviors versus anxiety-relevant behaviors, and for protecting against short-term HPA-axis responses rather than long-term adrenal responsiveness. While some studies in humans have reported that physical activity improved both depressive and anxiety symptoms (De Moor, Beem, Stubbe, Boomsma, & De Geus, 2006; Khanzada, Soomro, & Khan, 2015), a meta-analysis by Wegner et al. (2014) found that anxiolytic benefits of exercise had a small effect size (0.34) compared to the antidepressant benefits, which had a moderate effect size (0.56).

It is not surprising that moderate-level exercise would be more beneficial than low-level exercise, as previous findings indicate that moderate physical activity improves strength, balance, and coordination (Brown et al., 2000), as well as oxygen consumption (Duscha et al., 2005). In addition to physical improvements, moderate exercise has also been shown to alleviate depressive symptoms. Depressed patients were asked to perform aerobic exercise at low level (7.0/kcal/kg/week), moderate-level (17.5/kcal/kg/week), or flexibility exercise (control) for 12 weeks; both exercise groups displayed significantly reduced depression scores compared to the control group, with moderate activity reducing scores 47% and low activity reducing scores 30% from baseline (Dunn, Trivedi, Kampert, Clark, & Chambliss, 2005).

Interestingly, moderate exercise also conferred behavioral and physiological benefits above high-level exercise, with animals in the moderate-level exercise subgroup displaying both lower depressive-like behavior in the FST and lower corticosterone levels versus the high-level exercise subgroup. Further, these responses in the high-level exercise subgroup did not differ statistically from those of the low-level exercise subgroup. There are several reasons why moderate exercise may have been more beneficial than high-level exercise in the present study. First, elevated corticosterone levels may have been a reflection of an increased metabolism in the high-level exercise subgroup. For instance, many sports-related studies have described a positive correlation between cortisol levels and energy expenditure in athletes during exercise (Reviewed by Coyle, 2000), illustrating that glucocorticoids are released to meet metabolic needs when physically active. It is also possible that high physical activity was perceived as a stressor in the current study. High intensity exercise has resulted in hippocampal apoptosis, which may impact HPA functions (Ding, Chang, Xie, Chen, & Ai, 2014) as well as increase anxiety- and depressive-like behaviors (Otsuka et al., 2016). Some animals in the current study may have displayed high levels of exercise as a coping strategy, as others have reported (Cairney, Kwan, Veldhuizen, & Faulkner, 2014; Lalanza et al., 2015). Finally, individual differences in motivation to engage in physical activity and in stress reactivity may have mediated the current findings. In support of this hypothesis, rats in a voluntary exercise group were found to run at a faster speed for a shorter period of time compared to those in a forced exercise group (Leasure & Jones, 2008), suggesting that motivational mechanisms may influence activity level. Therefore, it is possible that the prairie voles in the current study that were most capable of moderating their activity level also demonstrated the most adaptive stress coping strategies.

The specific neural mechanisms through which exercise serves a protective role require further investigation. Although the current study observed a buffering effect of physical activity on short-term corticosterone and long-term adrenal responsiveness, exercise did not significantly decrease cFos immunoreactivity in the PVN. This was contrary to our hypothesis, which was based in part on previous literature demonstrating altered PVN activation following chronic stressors in other rodent models (Reviewed by Herman & Tasker, 2016). Previous evidence on the influence of exercise on PVN activity is mixed. For instance, a study by Saito and Soya (2003) and a study by Otsuka et al. (2016) did not find a change in PVN cFos immunoreactivity in rats that performed acute treadmill running at a low speed (15 m/min) compared to control rats, but an increase in PVN activity was observed in animals that ran at a high speed (25 m/min); it should be noted that animals in these studies were not chronically stressed. The lack of a difference in PVN activation in the present study may be due to the multi-functional, integrative role of the PVN in the context of stress, emotion, endocrine and autonomic regulation. In addition to its role in HPA activation, the PVN also integrates sympathetic and cardiovascular responses, and previous studies have shown that exercise mediates autonomic functions via the PVN (Dishman, Renner, White-Welkley, Burke, & Bunnell, 2000; Mastelari, de Aguiar Corrêa, de Souza, & Martins-Pinge, 2012). Since cFos expression only indicates general neuronal activity, it is possible that PVN activity of sedentary animals reflected HPA functions (e.g., via CRH neurons; Otsuka et al., 2016), and PVN activity of exercise animals reflected autonomic functions (e.g., via glutamatergic neurons; Mastelari et al., 2012). Future studies should

consider using double-labeling techniques to gain a better understanding of specific neural mechanisms involving the PVN as well as additional brain regions. For example, previous studies have observed neuronal changes in the hippocampus (Erickson et al., 2011; Nokia et al., 2016; Sheikhzadeh, Etemad, Khoshghadam, Asl, & Zare, 2015) and the prefrontal cortex (Basso, Shang, Elman, Karmouta, & Suzuki, 2015; Greenwood et al., 2013) following exercise; these two regions have inhibitory projections to the PVN and may mediate HPA stress responses in exercise animals (Herman & Tasker, 2016; Hostinar, Sullivan, & Gunnar, 2014).

In conclusion, exercise serves a protective role against negative behavioral and endocrine consequences of social isolation and CMS in female prairie voles. This study shows for the first time that moderate exercise, compared to low and high levels, was the most effective at protecting against depressive behaviors and short-term corticosterone reactivity in the prairie vole model. The current findings have clinical implications; specifically, moderate physical activity can be recommended to individuals experiencing both social and environmental stress as a strategy to combat negative behavioral and neurobiological consequences. Future studies using animal models may provide additional insight into the mechanisms underlying the beneficial effects of exercise on behavior and physiology.

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Figure 1.
Experimental timeline for the current study.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Water Deprivation		08:30h - 13:30h					
Empty Water Bottle		13:30h - 14:30h					
Continuous Lighting		20:30h → 06:30h		20:30h → 06:30h			
Cage Tilt			17:00h → 16:00h				12:00h - 16:00h
Foreign Object			12:00h - 17:00h				08:30h - 12:00h
Damp Bedding						0830h - 13:00h	
Strobe Light	11:00h - 16:00h					13:00h - 16:00h	
White Noise	08:30h - 11:00 h				12:00h - 15:00h		

Figure 2.

Details of the CMS protocol. One week of stressors is shown here; this schedule was repeated during the second week of CMS.

Figure 3.

Mean (+ SEM) duration of time spent in different locations (open arms, closed arms, center section) during the 5-minute EPM in prairie voles exposed to social isolation and CMS, as a function of either voluntary physical exercise or sedentary conditions (Panel A); and duration of time spent in the open arms in the low-, moderate- and high-level exercise subgroups (Panel B). * $p < 0.05$ vs. the other group(s) in the same panel.

Figure 4.

Mean (+ SEM) duration of immobility during the 5-minute FST in prairie voles exposed to social isolation and CMS, as a function of either voluntary physical exercise or sedentary conditions (Panel A), and in the low-, moderate- and high-level exercise subgroups (Panel B). * $p < 0.05$ vs. the other group(s) in the same panel. Note: the remainder of 300 seconds is comprised of active coping behaviors.

Figure 5. (Mean (+ SEM) plasma corticosterone concentrations 10 minutes following the FST in prairie voles exposed to social isolation and CMS, as a function of either voluntary exercise or sedentary conditions (Panel A), and in the low-, moderate- and high-level exercise subgroups (Panel B). * $p < 0.05$ vs. the other group(s) in the same panel.

Table 1

Body weight (grams) in prairie voles exposed to social isolation and CMS, as a function of either voluntary physical exercise or sedentary conditions.

	Prior to Isolation Period	Following Isolation Period	Following CMS Period	End of Study
Sedentary	39 ± 1	39 ± 1	40 ± 1	40 ± 1
Exercise	39 ± 1	41 ± 1	41 ± 1	41 ± 1

Note: values represent means ± SEM. There were no significant difference between the exercise and sedentary conditions at any time point.