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Exercise: Blocking the Negative Effects of Social Isolation Stress and Moderate Environmental Stressors in an Animal Model

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Abstract

Stress on the body, especially social stress, adversely affects the body's ability to regulate various physiological systems and behavior. This stress may be a mechanism that influences the release of stress hormones and contributes to psychological and physiological disorders. However, exercise has positive effects on the body that may counter these negative effects of social stress. This project involves using an animal model to test the effectiveness of exercise as a treatment for psychological and biological symptoms of stress from social isolation. Prairie voles (rodents) were used because they are socially comparable to humans. Thirty adult, female prairie voles were isolated from a sibling for four weeks. During the final two weeks, the prairie voles were also exposed to mild environmental stressors. Throughout these final two weeks, half of the voles had continuous access to a running wheel while the other half was sedentary. A behavioral test for depression was conducted and blood samples were collected. Prairie voles offered an exercise option showed fewer depressive behaviors in an operational test and lower stress hormone levels in the blood. Of the voles given the exercise wheel option, the group that exhibited a moderate activity level showed lower levels of depressive behaviors and stress hormones compared to the groups that exhibited either low or high activity levels. A moderate level of exercise may therefore be beneficial for blocking the negative effects of social isolation stress. This research has important implications for understanding social stress in humans.

Keywords: social isolation stress, exercise, depression

1. Introduction

Stress is an adaptive response of the body; however, in some circumstances stress is harmful to a person's emotional and physiological health. The body's attempt to adapt to stress can lead to changes in mood and behavior[1,12]. For example, previous research shows that environmental stress, such as job loss or financial difficulty, can lead to mood disorders, including depression[3]. Stress also activates the hypothalamic-pituitary-adrenal (HPA) axis, which releases hormones that facilitate the stress response, including cortisol and corticosterone[26]. However, prolonged exposure to stress is associated with functional changes, which may alter the behavioral, emotional, and physiological responses to stress over time.

Social stress is a common form of stress that may have especially damaging effects on emotional and physiological health. Humans are a highly social species, depending on a network of a close relationships and positive social interactions to promote health[4,5]. Perceived loneliness, or stress from social isolation, contributes to the development of mood disorders, including depression and anxiety, and increases the risk of cognitive decline, obesity, elevated blood pressure, heart disease, and changes in brain activity[4,5,18].

Given the negative consequences associated with social stress, research focused on mechanisms and prevention strategies will provide valuable insight that can improve the quality of life for humans. Prairie voles (*Microtus ochrogaster)* are rodents with a similar social structure to humans, and therefore serve as a useful model for studying

behavioral and neurobiological mechanisms of social stress[2,14]. Prairie voles are among only 3-5% of mammals that are socially monogamous[19]. They also form long-term social bonds, live in large family groups, and engage in biparental care[6]. Previous studies have found that the disruption of social bonds in prairie voles leads to similar behavioral and physiological consequences as in humans[12,18,25]. Behavioral dysfunction as a result of social stress includes depressive- and anxiety- related behaviors, including anhedonia (the loss of the feeling of pleasure), helplessness, and reduced exploration[13]. Prairie voles also experience similar physiological effects as humans following social stressors, including autonomic nervous system imbalance, changes in heart rate, exaggerated stress hormone levels, and alterations in brain tissue[2,12].

Previous research has discussed the translational value of the prairie vole model for understanding mechanisms and consequences of social stress[12,25]. This species may also be an ideal model for investigating treatment and prevention strategies associated with social stress. For example, exercise has positive effects on the body that may block the negative consequences of social isolation stress. Physical activity has been shown to change central nervous system (CNS), cardiovascular, and endocrine functions, elevating mood and promoting physiological fitness[7,24]. Studies with rats show that exercise may improve cognitive function and increase serotonin, dopamine, and norepinephrine levels in the brain, potentially preventing depression[15,22]. Exercise has also been shown to prevent some depressive- and anxiety-like behaviors in socially isolated prairie voles[13]. Due to these benefits in the central and peripheral nervous systems, exercise may be an effective, non-pharmacological strategy that can improve responses to social and environmental stress.

Given previous evidence supporting the benefits of exercise on mood and physiological health, this study used the prairie vole model to determine whether exercise can protect against the negative behavioral and physiological consequences of a combination of social isolation and mild environmental stress. Compared to sedentary conditions, voluntary exercise was predicted to prevent behaviors associated with depression, as well as short-term corticosterone reactivity following a behavioral stressor.

2. Methodology

2.1 Animals

Thirty adult, female prairie voles, descendants of a wild stock caught near Champaign, Illinois, were used in this study. All animals were maintained on a 14/10 h light/dark cycle (lights on at 6:30am), with a mean ± standard error of the mean (SEM) ambient temperature of 25 ± 2°C, relative humidity of 40 ± 5%, and ad libitum food (Purina rabbit chow) and water access. Animals were housed with a female sibling until the beginning of the experiment; only one animal from each sibling pair was used for the experiments described here. 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.

2.2 General Study Design

All prairie voles were socially isolated from a female sibling for 4 weeks. During the final 2 weeks, the prairie voles were also exposed to a series of daily mild environmental stressors. Additionally during these 2 weeks, half of the prairie voles (n = 15) had continuous access to an exercise wheel and the other half (n = 15) remained sedentary. After this period, a behavioral test for depression was conducted and blood was collected for analysis of corticosterone.

2.3 Social Isolation

Each prairie vole was removed from its home cage and housed in an individual cage for 4 weeks (without visual, olfactory, or auditory cues from the respective sibling).

2.4 Chronic Mild Stress

During the final 2 weeks of the isolation period, all prairie voles were exposed to a chronic mild stress (CMS) procedure, based on methods described previously[17]. Briefly, animals were exposed to a series of mild stressors on an

unpredictable schedule for varying durations (see Table 1). This procedure maximizes the unpredictable nature of the mild stressors in an attempt to imitate everyday hassles in humans.

Table 1. Detailed 7-day CMS procedure; this schedule was repeated during the second week. Note that a foreign object stressor was included here – in place of a paired housing stressor which is typical in other CMS paradigms[27] – so as to not interfere with the isolation housing manipulation in the current study.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Removal of water bottle		8:30am -- 1:30pm					
Empty water bottle		1:30pm -- 2:30pm					
Continuous overnight light		8:30pm --	6:30am		8:30pm --	6:30am	
40º cage tilt			5:00pm --	4:00pm			12:00pm -- 4:00pm
Foreign object (brick) in cage			12:00pm -- 5:00pm				8:30am -- 12:00pm
Damp bedding						8:30am -- 1:00pm	
Background strobe light	11:00am -- 4:00pm					1:00pm -- 4:00pm	
Background white noise, continuous	8:30am -- 11:00am						
Background white noise, random intermittent					12:00pm -- 3:00pm		

2.5 Exercise And Sedentary Conditions

During the 2-week isolation/CMS period, half of the animals had 24-hour access to a running wheel (4.5 in diameter; Super Pet Mouse Silent Spinner Mini Exercise Wheel, Model #100079369, Elk Grove Village, IL), while the other half remained sedentary in a standard cage. In the exercise group, daily distance traveled was monitored via an odometer adapted for use with the running wheel (Bell F12 Cyclocomputer, Model # 7001115, Van Nuys, CA).

2.6 Forced Swim Test

Forty-eight hours following the end of the isolation/CMS period, a 5-minute forced swim test (FST) was conducted to test for depressive behaviors, using procedures described previously[13]. Briefly, behaviors were scored as: actively swimming (directed movements of the fore-limbs and hind-limbs without breaking the surface of the water), struggling (forelimbs breaking the surface of water), climbing (attempts to climb the walls of the tank), or immobility (no limb or body movements or using limbs solely to remain afloat without corresponding trunk movements). Immobility, a maladaptive, helpless response, is hypothesized to represent an operational index of depressive behavior[8].

2.7 Collection Of Blood And Analysis Of Plasma

Ten minutes following the end of the FST, blood was collected in anesthetized prairie voles [ketamine; 67 mg/kg, sc; NLS Animal Health, Owings Mills, MD; xylazine (13.33 mg/kg, sc; NLS Animal Health, Owings Mills, MD)], placed immediately on ice, and centrifuged to obtain plasma, using procedures described previously[18]. Plasma levels of corticosterone were determined with a commercially available enzyme-linked immunosorbent assay kit (Enzo Life Sciences, Farmingdale, NY; 1:500 dilution in assay buffer), according to the kit instructions as described previously[18].

2.8 Statistical Analyses

All data were analyzed with independent groups Student's *t*-tests. A value of $p < 0.05$ was considered to be statistically significant. A Bonferroni correction was applied to all multiple comparisons. All data are presented as mean ± SEM.

3. Data

3.1 Daily Activity Measurements

Prairie voles with access to running wheels ran a mean (± SEM) distance of 1.74 ± 0.37 miles/day. Prairie voles displayed a high degree of individual variability in the daily distance traveled, with a range of 0.006 miles/day to 10.1 miles/day. Therefore, in addition to the planned analyses involving exercise vs. sedentary conditions, the animals in the exercise group were split into 3 subgroups of daily distance traveled, comprising approximately the bottom 1/3 (low activity subgroup, less than 0.06 miles/day, $n = 5$), middle 1/3 (moderate activity subgroup, between 0.06 and 2.2 miles/day, $n = 5$), and top 1/3 (high activity subgroup, greater than 2.2 miles/day, $n = 5$), for the purpose of exploratory, within-group comparisons, as described below.

3.2 Forced Swim Test

The prairie voles in the voluntary exercise condition displayed significantly lower levels of immobility in the FST compared to the sedentary prairie voles [Figure 1, left panel; $t(20) = 2.60$, $p < 0.009$]. Among the three subgroups of low, moderate, and high activity, the moderate activity level subgroup displayed significantly lower levels of immobility during the FST, when compared to both the low and high activity level subgroups (Figure 1, right panel; moderate vs. low, $t(8) = 3.88$, $p < 0.002$; moderate vs. high, $t(8) = 6.00$, $p < 0.0001$]. The immobility levels of the low and high activity subgroups did not differ statistically [low vs. high, $t(8) = 2.24$, $p > 0.05$].

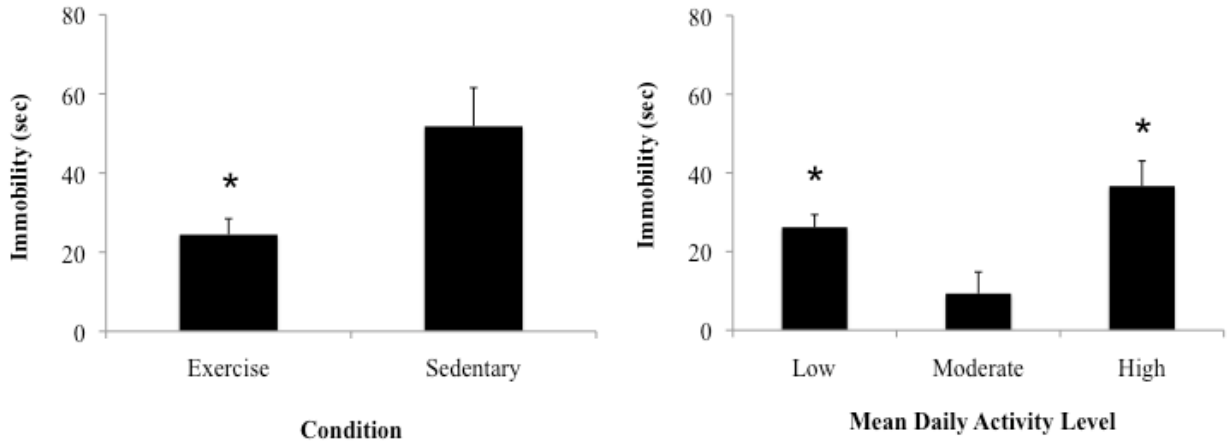

Figure 1. Immobility during FST

Figure 1. Mean (+ SEM) immobility level during the 5-minute FST in prairie voles as a function of exercise or sedentary conditions (left panel); and as a function of low (less than 0.06 miles/day), moderate (0.06 – 2.2 miles/day), and high (greater than 2.2 miles/day) levels of activity in the exercise group (right panel). *$p < 0.05$ vs. either sedentary group or moderate activity subgroup.

3.3 Corticosterone

The prairie voles in the voluntary exercise condition displayed statistically lower levels of the stress hormone, corticosterone, in the blood compared to sedentary prairie voles, following the FST (Figure 2, left panel; $t(20) = 6.48$,

$p < 0.0001$). Among the three subgroups of low, moderate, and high activity, prairie voles in the moderate activity subgroup displayed significantly lower levels of corticosterone in the blood compared to both the low and high activity subgroups (Figure 2, right panel; moderate vs. low, $t(8) = 5.36$, $p < 0.0003$; moderate vs. high, $t(8) = 3.56$, $p < 0.004$]. The corticosterone levels of the low and high activity subgroups did not differ statistically [low vs. high, $t(8) = 0.77$, $p > 0.05$].

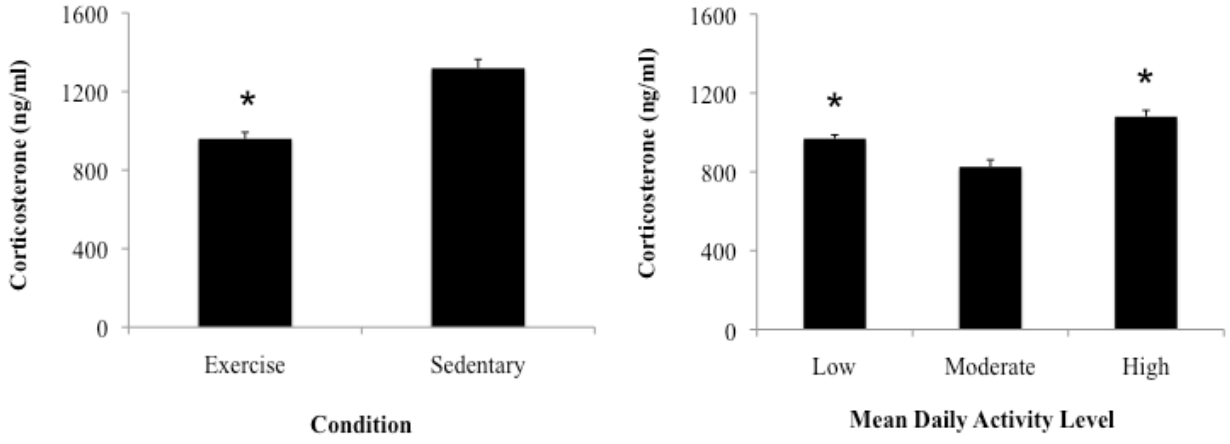

Figure 2. Corticosterone levels after FST

Figure 2. Mean (+ SEM) circulating corticosterone levels 10 minutes following the FST in prairie voles as a function of exercise or sedentary conditions (left panel); and as a function of low (less than 0.06 miles/day), moderate (0.06 – 2.2 miles/day), and high (greater than 2.2 miles/day) levels of activity in the exercise group (right panel). *$p < 0.05$ vs. either sedentary group or moderate activity subgroup.

4. Conclusion

Exercise can boost mood and enhance overall physiological health[7,24]. To extend these previous findings, the present study used a prairie vole model to test the utility of exercise for preventing negative emotional and physiological disturbances associated with social and environmental stressors. The current study demonstrates that voluntary exercise is protective against both behavioral and physiological reactivity to a combination of social isolation and mild environmental stressors in the prairie vole. Further, this study provides evidence that moderate levels of exercise may be more beneficial than either very low or very high levels of exercise in the context of social and environmental stress.

The present study investigated behaviors in the FST, as well as corticosterone reactivity following the FST, to gain an integrative understanding of the stress response to social isolation and CMS. Prairie voles that were allowed access to an exercise wheel spent significantly less time immobile during the FST than did sedentary prairie voles. Reduced immobility has been hypothesized to represent lower levels of depressive behaviors[8], and in the current study may be indicative of a more adaptive stress-coping response in the exercise group. In combination with the behavioral response, this study also revealed that exercise attenuates physiological stress reactivity in prairie voles, indicated by a reduced corticosterone response following the FST in the exercise group. These findings are consistent with previous research supporting the hypothesis that physical activity can prevent mood dysfunction[13,15,22], and lower stress responses[9,11].

The protective benefits of exercise on behavior and endocrine function may be operating through several mechanisms. Exercise requires various aspects of CNS control to regulate cardiovascular, respiratory, and hormonal function, as well as behavior[9]. Voluntary physical activity may influence metabolic and chemical pathways in the brain that attenuate stress responses. This altered brain activity may be associated with changes in the regulation of serotonin and norepinephrine, which can be beneficial for preventing mood disturbances. For example, previous studies have shown that long-term running wheel use is successful at preventing learned helplessness behavior during the FST in rats; this effect is due to the ability of physical activity to buffer against increased production and release

of serotonin, which can reduce exaggerated serotonin responses to stress[16]. Further, exercise mitigates stress responses in CNS regions that are responsible for controlling peripheral sympathetic nervous system functioning, which may reduce oxidative stress[9]. Additionally, certain proteins that protect the body from stress are increased and work more efficiently following physical activity[9]. Previous studies have begun to identify these mechanisms through which exercise can protect against depression and stress, but additional research will further elucidate the many ways that physical activity can influence the body.

In addition to the primary results of this study, the exploratory analyses in the exercise condition indicate that moderate levels of activity were associated with the greatest protection of both depressive behaviors in the FST and corticosterone reactivity. This interesting finding may suggest that different levels of physical activity confer differential benefits in the context of behavioral and physiological reactivity to stress. It is reasonable to hypothesize that a moderate activity level may have a greater effect than low activity, as a low level of exercise may not have strong enough positive effects on the central or peripheral nervous systems to block the consequences of social and environmental stress. This view is supported by studies that have shown a moderate level of exercise to be beneficial at preventing depression and stress[21,23]. For example, moderate intensity exercise in humans with major depressive disorder reduced scores on the Hamilton Rating Scale for Depression, while a low level of exercise was similar to the placebo control of flexibility exercise, indicating that a moderate activity level is more beneficial than a low activity level at improving depression symptomatology[10].

Perhaps more surprising than the benefit of moderate activity over low activity is the observation that moderate activity also improved depressive behavior and corticosterone reactivity when compared to a high level of activity. It is possible that, while a moderate level of activity has positive effects on the brain and/or body, a high activity level may serve as an additional stressor. This hypothesis is supported by a study suggesting that excessive exercise and overtraining syndrome are associated with poor mental health[21]. Another study, conducted on female swimmers, found that athletes in an overtraining condition showed significantly higher resting levels of cortisol and more symptoms of depression, compared to a control group of active females[20]. Future investigation is needed to determine whether a high activity level served as an additional stressor in prairie voles, rendering this level of activity less capable of preventing behavioral and physiological responses to social and mild environmental stress.

In conclusion, the present study demonstrates that exercise protects against negative emotional and physiological disturbances as a function of social isolation and CMS in prairie voles. The data also indicate that a moderate activity level is more effective than low or high activity levels at preventing depressive-like behavior and short-term corticosterone reactivity. Therefore, strategies that encourage moderate activity levels may be effective, non-pharmacological treatment and prevention measures for the consequences of social and environmental stress in humans. Continued research using translational animal models will provide a better understanding of the specific protective effects of exercise in the context of stress.

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