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Wheel access has opposing effects on stress physiology depending on social environment in female prairie voles (*Microtus ochrogaster*)

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Abstract

Physical exercise and chronic social stress are both known to impact general health and hypothalamic-pituitary-adrenal (HPA) axis function, albeit typically in opposing directions. Therefore, the question we investigated in this study was how these two factors – physical exercise and chronic social isolation – would interact when presented simultaneously in a female rodent model. Adult female prairie voles were separated into four experimental groups: 1) isolated without wheel access, 2) isolated with wheel access, 3) paired without wheel access, and 4) paired with wheel access. Plasma, hair, and adrenal glands were sampled to investigate changes in stress physiology. Our results indicate that, when isolated, wheel access had a mitigating effect on HPA activity. However, in paired animals, wheel access had the opposite effect, as both adrenal mass and increase in hair corticosterone concentrations were greater in paired animals with wheel access. Strong correlations were detected between change in hair corticosterone and adrenal mass, while no correlations were found between plasma corticosterone and either of the other markers. These results imply that the HPA axis is highly sensitive to both the social environment and the physical demands placed on the individual, and that when investigating the effects of chronic isolation, both hair corticosterone and adrenal mass may be more reliable markers than a single plasma corticosterone sample.

Keywords

Chronic social isolation; exercise; hair corticosterone; Prairie vole; *Microtus ochrogaster*; female

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6.0AUTHOR CONTRIBUTIONS

All authors contributed to the study design. NM conducted the behavioral trials and analyses with the assistance of AJG, AD, and WC. AJG, WTW, and MCN conducted the plasma corticosterone assays. MRJ conducted the hair corticosterone assays. All authors contributed to the writing and revising of the manuscript.

5.0CONFLIFT OF INTEREST

None declared.

1.0 INTRODUCTION

Long-term physical activity boosts metabolism, increases muscle mass, decreases body fat, and improves cardiovascular fitness. Not only does exercise promote physical fitness (Bullo et al., 2015; Deuster and Silverman, 2013; Hills et al., 2015), it has also been shown to improve psychological health (Barbour et al., 2007; Carek et al., 2011; McMahon et al., 2017). Depressive symptoms seem to be particularly responsive to exercise, compared to other psychological disorders (Kenkel and Carter, 2016; Lawlor and Hopker, 2001; Rebar et al., 2015; Wegner et al., 2014). The socioenvironmental component of depression has made alleviating depressive symptoms difficult with pharmacotherapy alone (Netz, 2017; Rosenquist et al., 2011; Slavich and Irwin, 2014). Alternative therapeutic strategies incorporate moderate exercise into patient treatment and often result in similar measures of improvement in alleviating depressive symptoms to those observed following pharmacotherapy (Blumenthal et al., 2007; Brenes et al., 2007; Carneiro et al., 2015; Cooney et al., 2013; Danielsson et al., 2013; Ernst et al., 2006; Hoffman et al., 2011; Kvam et al., 2016). Further, in pharmacotherapy treatment-resistant patients, exercise is a proven strategy for reducing depressive symptoms (Mota-Pereira et al., 2011).

Exercise has been shown to improve several behavioral and cognitive functions, as well as central nervous system processes involved in stress reactivity (Chu et al., 2015; Erickson et al., 2011; Nishijima et al., 2013). For example, lifetime stress level appears to negatively influence the hippocampus, but exercise moderates this effect (Head et al., 2012). These seemingly opposing effects of chronic stress and exercise on psychological health might be predicted to have opposing effects on the hypothalamic-pituitary-adrenal (HPA) axis, the neuroendocrine regulatory system that responds to both energetic demands and perception of stressors. However, both chronic exercise (Gerber et al., 2013; Hill et al., 2008; Skoluda et al., 2012; Tremblay et al., 2004) and chronic stress (Allen et al., 2014; Dickerson and Kemeny, 2004; Ulrich-Lai and Herman, 2009) are associated with activation of the HPA axis in humans. This “exercise-glucocorticoid paradox” has been discussed in detail (Chen et al., 2017), with possible explanations. One explanation is that while exercise increases glucocorticoid release acutely, it also facilitates recovery following a stressor. This is illustrated in rats that either engaged in voluntary wheel running prior to 30-min. restraint stress or not. Those that exercised prior to restraint displayed an earlier peak and earlier recovery of plasma glucocorticoids following restraint stress as compared to controls (Hare et al., 2014). Further, quantification of overall glucocorticoid release was lower in exercised rats, a result of a shortened, and perhaps better regulated, HPA response (Chen et al., 2016).

Stress and exercise also influence paraventricular nucleus (PVN) functions. The PVN integrates information from multiple cortical and limbic structures in the context of stress, ultimately resulting in the release of glucocorticoids (i.e., cortisol, corticosterone) from the adrenal cortex (Herman et al., 2016). While this is an adaptive response to acute stressors, prolonged exposure to stress can result in dysregulation of the HPA axis (e.g., elevation of resting cortisol levels), which may have damaging effects centrally and peripherally. Therefore, exercise may have positive effects on HPA functions to promote more adaptive responses to stress (Hare et al., 2014).

Specific effects of exercise on HPA axis functions are not well elucidated, particularly with respect to exercise potentially mitigating HPA axis effects of psychosocial stressors. One study found that acute treadmill running at a high speed increased cFos expression in corticotropin releasing hormone (CRH) neurons in the PVN in rats (Otsuka et al., 2016). However, no difference was found in oxytocin- and vasopressin-immunoreactive cells in the PVN between sedentary and physically active prairie voles (Kenkel and Carter, 2016). Furthermore, the mechanism underlying the stress-buffering effects of exercise within the PVN remain unclear. On one hand, 6 weeks of wheel running buffered against adrenocortical responses and PVN reactivity to white noise in rats (Campeau et al., 2010), whereas 4 weeks of voluntary exercise buffered against corticosterone response but not PVN reactivity to a forced swim stressor in prairie voles (Watanasriyakul et al., 2018). This complication may be due to the fact that the PVN both initiates HPA stress responses and also integrates autonomic signals triggered by exercise (Evanson and Herman, 2015; Michelini and Stern, 2009).

Exercise has been shown to buffer against some psychological stressors and relieve depressive symptoms (Heaney et al., 2014; Zschucke et al., 2015), providing further evidence that it may have benefits at the level of the HPA axis. For example, an 8-week exercise program significantly improved depressive scores and reduced urinary cortisol levels in depressed female adolescents compared to patients who did not participate in the exercise program (Nabkasorn et al., 2005). Depression also is associated with loneliness and social isolation, and many socially isolated individuals live a sedentary lifestyle (Zhai et al., 2015), suggesting a strong association between social isolation and a lack of physical activity. One study found a negative correlation between loneliness and exercise frequency in college students (Page and Hammermeister, 1995). In addition to the psychological impact of social isolation, lonely individuals may also experience damaging physiological changes such as high blood pressure, elevated plasma cortisol, and weakened immune functions (Cacioppo et al., 2003; Choukèr et al., 2002; Cruces et al., 2014; Doane and Adam, 2010). In prairie voles, 4 weeks of voluntary exercise protected against behavioral and endocrine consequences of social isolation. Specifically, physically active animals displayed significantly less depressive- and anxious-like behaviors compared to animals that remained sedentary (Grippo et al., 2014).

The current study was designed to further characterize the protective effects of exercise against potentially damaging chronic isolation using the prairie vole model. In this case, the chronic stress came in the form of social isolation, given previous evidence demonstrating the value of the prairie vole model for investigating physiological consequences of social experiences (Grippo, 2011; Sun et al., 2014; Young et al., 2011). The prairie vole model is particularly appropriate for investigating the effects of chronic social stress because this species typically engages in monogamous socioemotional bonds similar to those seen in humans. Because of these unique behavioral characteristics, the prairie vole provides a useful model for studying behavioral and physiological aspects of social partnerships and social stressors as they relate to stress-related disorders (Ahern et al., 2011; Carter, 1998; McNeal et al., 2014). When monogamous pairs or family members are separated, negative effects on behavior, physiology, and the brain are observed (Lieberwirth et al., 2012; McNeal et al., 2014). Specifically, separating two bonded, opposite-sex prairie voles, or two

sibling prairie voles increases depression- and anxiety-relevant behaviors (McNeal et al., 2014; Grippo et al., 2007). Further, these animals exhibit increased corticosterone and adrenocorticotropic hormone (ACTH) following acute stress when compared to paired animals (McNeal et al., 2014). In addition, the presence of an opposite-sex partner buffers against the negative consequences of chronic mild stress (McNeal et al., 2017). Together, these findings support the utility of this species as a model for the behavioral and physiological consequences of social stress in humans.

In the present study, stress effects were measured by quantifying corticosterone concentrations in both hair and plasma samples, and protective effects of exercise were measured by comparing corticosterone concentrations across experimental groups that either had or did not have access to an exercise wheel. Generally, we predicted that isolation would be associated with several markers of increased HPA activity, and that exercise would mitigate that increase. Based on previous work in rodents (Ferland and Schrader, 2011), including prairie voles (Bosch et al., 2009; McNeal et al., 2014; Pournajafi-Nazarloo et al., 2011), we predicted that animals experiencing social isolation would exhibit elevated corticosterone concentrations in plasma samples. Based on negative effects of social instability in female mice (Jarcho et al., 2016), we also predicted that social isolation would be associated with increases in hair corticosterone concentrations. We further predicted that effects of isolation would be blunted or eliminated in animals that had access to an exercise wheel (Starzec et al., 1983; Watanasriyakul et al., 2018). Lastly, we predicted that certain physiological measures would be systematically correlated to one another. For example, increases in both hair corticosterone and adrenal weight are indicators of long-term HPA axis hyperactivity (Brain and Nowell, 1971; Weiss et al., 2004), and were expected to be affected in a similar pattern in this study.

2.0 METHODS

2.1 Animals

Sixty-two adult female prairie voles, descendants of a wild stock caught near Champaign, Illinois, were used as experimental subjects in this protocol (each first housed with an unstudied female sibling; n = 62 siblings). Females were chosen for the current investigation for several reasons. Previous work with females of this model species has demonstrated behavioral and physiological consequences of depression, anxiety, and increased stress following chronic social isolation (Grippo et al., 2007; Grippo et al., 2008). Further, in humans, loneliness (i.e., the perception of being alone) is reported more frequently by women than men (Prince et al., 1997), and that loneliness is a significant predictor of depression (Cacioppo et al., 2006; Prince et al., 1997). Finally, females are an understudied population (both in human and animal studies; Beery and Zucker, 2011; Klein et al., 2015; Prendergast et al., 2014).

Experimental animals had a mean (± standard error of the mean; SEM) age of 111± 3.4 days, and a body weight of 33.82 ± 0.55 grams. All animals were maintained on a 14/10 h light/dark cycle (lights on at 0630h), with a mean ± SEM ambient temperature of 25 ± 2°C and relative humidity of 40 ± 5%. Animals were allowed food (Purina rabbit chow) and water ad libitum. Offspring were removed from breeding pairs at 21 days of age and housed

in same-sex sibling pairs until the commencement of the experimental procedures. For all procedures described here, only one animal from each sibling pair was studied for physiological responses to social isolation and/or exercise (the other animal in the cage was defined as the "unstudied" sibling). 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 Committees.

2.2 Study outline

All experimental subjects received an ear punch (in both ears) to for identification purposes, to denote that this was the animal in the cage that would be studied for physiological responses to social isolation and/or exercise (the unstudied sibling of each animal was not marked). Six weeks prior to any experimental manipulation, experimental animals were shaved in order to ensure that hair samples collected later reflected only the time period relevant to the study. During the initial six weeks (baseline period), all experimental animals remained housed with their unstudied siblings. At the end of the baseline period, experimental animals were again shaved and hair samples were collected. Experimental animals were then randomly assigned to one of the following experimental groups for five weeks: 1) remained paired with the respective unstudied sibling, with access to an exercise wheel (n=16), 2) remained paired with the respective unstudied sibling, without a wheel (n=15), 3) isolated from the unstudied sibling, with access to a wheel (n=16), and 4) isolated from the unstudied sibling, without a wheel (n=15) (stressor period). These conditions resulted in all groups receiving a change in environmental conditions (either social isolation, addition of a running wheel, or both), with the exception of the paired/no wheel group, which represented the continuous, basal control condition. Experimental animals in the isolated groups were housed individually, without olfactory, auditory, or visual cues from the previous sibling. Previous work with this species has demonstrated that this duration of isolation is sufficient to induce behavioral and physiological consequences (Grippo et al., 2007; Grippo et al., 2008; McNeal et al., 2014; Peuler et al., 2012), and that access to exercise can mitigate these effects (Watanasriyakul et al., 2018). At the end of the five-week stressor period, a second hair sample was collected from each experimental animal, plasma samples were collected, and adrenal and body weights were recorded.

2.3 Experimental conditions

Experimental conditions in which an exercise wheel was made available included continuous access to a running wheel (4.5 in diameter; Super Pet Mouse Silent Spinner Mini Exercise Wheel, Model #100079369, Elk Grove Village, IL) for the five-week stressor period, to allow for voluntary physical activity. Only one wheel was available, regardless of whether there was a single isolated or two paired animals in the cage. Daily distance traveled and daily maximum speed were monitored via an odometer adapted for use with the running wheel (Bell F12 Cyclocomputer, Model # 7001115, Van Nuys, CA). Sedentary paired and isolated animals were housed in a standard cage without a running wheel for the five-week period.

2.4 Hair collection and analysis

To ensure that hair corticosterone samples represented HPA activity during the study, all experimental subjects (but not their unstudied siblings) were shaved at the start of the baseline period, during the light period (between 10am and 12pm). This hair was not collected or analyzed for corticosterone. All subjects remained in standard housing for 6 weeks (with the unstudied sibling in the same cage), at which point another fur sample (baseline) was collected during the light period (between 10am and 12pm) and stored for corticosterone assay. Fur samples were collected by shaving 4cm x 4cm section of fur on the subjects' dorsal rear surfaces. The razor (Andis Pivot Pro PMT-1, Model 23475; Andis Co., Sturtevant, WI) was cleaned with 100% ethanol (and allowed to completely dry) before and after hair sample collection from each subject. Samples were then placed via forceps (also cleaned with 100% ethanol) into an Eppendorf tube. Fur samples were stored at −80° C until assayed for corticosterone. Immediately following baseline hair sample collection, experimental subjects were moved to the assigned experimental housing condition (described above). At the end of the 5-week manipulation period a final hair sample (post-stress) was collected during the light period (between 10am and 12pm).

Hair samples were prepared following a modified previously published protocol (Davenport et al., 2006; Jarcho et al., 2016). Briefly, weighed samples were washed with isopropanol to remove debris. Samples were then chopped into fine pieces with a razor blade to facilitate steroid extraction (Yu et al., 2015). Steroids were then extracted from the hair by incubating the samples in methanol for 48 hours. Finally, the steroid-containing methanol solution was purified by passing the solution through Supelco-select HLB SPE tubes (Sigma-Aldrich). Purified extracts were reconstituted with assay buffer (Arbor Assays, Ann Arbor, MI). Reconstituted samples were assayed in duplicate for corticosterone via commercially available enzyme immunoassay kits (Arbor Assays, Ann Arbor, MI). The detectable range of corticosterone for these kits was 78.125–10,000 pg/ml, and the intra-assay and inter-assay coefficients of variance were 6.36 and 7.75, respectively. Corticosterone concentrations as detected by enzyme immunoassay were then matched with the original weight of the hair collected in order to account for minor variations in hair quantity collected. Corticosterone concentrations are, therefore, expressed in pg/mg of hair.

2.5 Blood collection and analysis

All experimental subjects were anesthetized with a mixture of ketamine (67 mg/kg, sc; NLS Animal Health, Owings Mills, MD) and xylazine (13.33 mg/kg, sc; NLS Animal Health), during the light period (between 10am and 12pm). Blood was sampled within two minutes of the anesthetic injection, from the periorbital sinus via a heparanized capillary tube, and was collected during a period not exceeding 1.5 minutes. The blood was placed immediately on ice, and then centrifuged at 4°C at 3500 rpm for 15min to obtain plasma. Plasma aliquots were stored at −80°C until assayed for circulating corticosterone. Plasma concentrations of corticosterone were measured using a commercial enzyme-linked immunosorbent assay kit, according to the kit instructions (Enzo Life Sciences, ADI-900–097, Farmingdale, NY). Plasma was diluted in assay buffer as necessary (1:500) to yield results reliably within the linear portion of the standard curve. The minimum detection limit of this kit is 0.027 ng/ml. Inter- and intra-assay coefficients of variation are <5% (according to both manufacturer

specifications and confirmed by multiple in-house assays). Cross-reactivity with other steroids or peptides is <1.7%.

2.6 Adrenal gland collection and analysis

Immediately after the collection of blood, each animal was euthanized under anesthesia. Adrenal glands were immediately dissected and weighed. Adrenal weight is expressed both as an absolute measure (g) and as a relative weight to animal body mass.

2.7 Statistical analyses

Data are presented as means ± SEM for all analyses and figures. A value of $p < 0.05$ was considered to be statistically significant. When comparing groups within a given data set (e.g., paired v. isolated on hair corticosterone) the data were analyzed with single-factor or two-factor independent-groups analyses of variance (ANOVA) to compare group (i.e., paired or isolated) and manipulation (i.e., wheel or no wheel) effects, followed by a priori Student's t tests with Bonferroni correction for multiple comparisons. In order to assess synchronicity across physiological responses to chronic social isolation (e.g., association between hair and plasma corticosterone), we calculated Pearson product moment correlations across all biomarkers (i.e., both measures of adrenal weight, plasma corticosterone, and hair corticosterone), and Bonferroni correction was used for multiple comparisons (n=4), resulting in an alpha level of 0.0125 (i.e., 0.05/4).

3.0 RESULTS

3.1 Body weight

Body weight did not differ between experimental groups at either the start or end of the study (Table 1). A two-factor ANOVA yielded no significant main effect of wheel or pairing on body weight at either the start (wheel: $F_{1, 58}$=0.13, p=0.72; pairing: $F_{1, 58}$=0.90, p=0.35) or end (wheel: $F_{1, 58}$=0.11, p=0.74; pairing: $F_{1, 58}$=0.63, p=0.43) of the study. Nor was there a significant wheel by pairing interaction at either the start ($F_{1, 58}$=0.56, p=0.46) or the end ($F_{1, 58}$=0.52, p=0.47) of the study. No follow-up tests were conducted.

3.2 Physical activity

Animals with access to running wheels (both paired and isolated) ran a mean (±SEM) distance of 3.22 ± 0.39 km/day with a mean (±SEM) maximum speed of 1.65 ± 0.27 km/hr. No difference was detected between paired and isolated groups in the daily distance traveled (paired mean ± SEM: 2.67 ± 0.60 km/day isolated mean ± SEM: 3.77 ± 0.47 km/day; t_{30} = 1.45, p = 0.16; Table 1), but paired animals reached a faster maximum speed (paired mean ± SEM: 2.23 ± 0.49 km/hr; isolated mean ± SEM: 1.07 ± 0.14 km/hr; t_{30} = 2.29, p = 0.03).

3.3 Hair corticosterone

Hair samples were collected at the beginning (baseline) and end (post-stressor) of the study to quantify corticosterone as a global measure of HPA axis activity (Table 2). Two-factor ANOVA was conducted to assess hair corticosterone at baseline, and found that neither wheel availability, nor housing status predicted corticosterone concentrations (wheel: $F_{1, 58}$

= 2.76, $p = 0.10$; pairing: $F_{1, 58} = 0.04$, $p = 0.84$). However, the interaction between these factors was significant ($F_{1, 58} = 18.04$, $p < 0.01$), and post-hoc t tests revealed that within isolated animals, those with later access to a wheel had higher corticosterone concentrations at baseline ($t_{31} = 4.05$, $p < 0.01$). The same analyses were used to assess hair corticosterone at the end of the study, and found that neither wheel availability, nor pairing status, nor the interaction term predicted corticosterone concentration (wheel: $F_{1, 58} = 0.47$, $p = 0.50$; pairing: $F_{1, 58} = 0.02$, $p = 0.88$; interaction: $F_{1, 58} = 0.03$, $p = 0.86$).

To evaluate the effect of the manipulation, difference scores in hair corticosterone were calculated by subtracting baseline from post-stress, and these scores were analyzed with two-factor ANOVA. Neither main effect predicted corticosterone concentration (wheel: $F_{1, 58} = 0.06$, $p = 0.81$; pairing: $F_{1, 58} = 0.03$, $p = 0.86$), but the interaction between these factors significantly predicted hair corticosterone concentration ($F_{1, 58} = 4.52$, $p = 0.04$; Fig. 1). Post-hoc t tests comparing means across either pairing status or wheel availability did not detect significant differences (all ps > 0.10).

3.4 Plasma corticosterone

Plasma samples were collected at the end of the study and corticosterone concentrations were analyzed with two-factor ANOVA. Analyses revealed that both main effects predicted plasma corticosterone (wheel: $F_{1, 58} = 4.56$, $p = 0.04$; pairing: $F_{1, 58} = 7.22$, $p < 0.01$; Fig. 2), but the interaction term did not ($F_{1, 58} = 3.44$, $p = 0.07$). Post-hoc t tests revealed isolated animals without wheel access had significantly higher plasma corticosterone than any other experimental group (isolated v. isolated with wheel: $t_{29} = 2.68$, $p = 0.01$; isolated v. pooled paired: $t_{44} = 3.91$, $p < 0.01$), and that no differences existed among the other three groups (all ps > 0.5).

3.5 Adrenal weight

Adrenal glands were collected and weighed at the end of the study. Two-factor ANOVA was used to evaluate both absolute adrenal weight and adrenal:body weight ratio. For absolute adrenal weight, there was a significant interaction ($F_{1, 55} = 4.25$, $p = 0.04$; Fig. 3a), but neither main effect predicted adrenal weight (wheel: $F_{1, 55} = 0.59$, $p = 0.48$; pairing: $F_{1, 55} = 0.14$, $p = 0.71$). For adrenal:body weight ratio, a similar pattern was observed, with a significant interaction ($F_{1, 56} = 4.89$, $p = 0.03$; Fig. 3b), and no significant main effects (wheel: $F_{1, 56} = 1.78$, $p = 0.19$; pairing: $F_{1, 56} = 0.06$, $p = 0.81$). For both measures, no post-hoc t tests between experimental groups revealed significant differences (all ps > 0.05).

3.6 Correlations between physiological measures

Pearson product moment correlation analyses were conducted to evaluate associations across physiological measures. Associations were found between the change in hair corticosterone and both adrenal measures (absolute adrenal weight: $r_{59} = 0.43$, $p = 0.001$; adrenal:body weight ratio: $r_{59} = 0.42$, $p = 0.001$; Fig. 4). Plasma corticosterone was not associated with either measure of adrenal mass (both ps > 0.15).

Associations were further investigated by evaluating the above correlations within experimental groups. In paired animals with wheel access, significant associations were

detected between both measures of adrenal mass and hair corticosterone (absolute adrenal weight: $r_{16} = 0.65$, $p = 0.007$; adrenal:body weight ratio: $r_{16} = 0.70$, $p = 0.003$). No relationship was found between either adrenal measure and plasma corticosterone (both ps > 0.3) for paired animals with wheel access. When assessing within all other experimental groups, no significant associations were found between either measure of adrenal mass and either measure of corticosterone (all ps > 0.06).

4.0 DISCUSSION

Using the socially monogamous prairie vole model, this study investigated the interaction between two factors known to affect HPA axis activity: chronic social isolation and exercise. Our general prediction was that isolated animals would exhibit physiological markers of being chronically stressed, given the disruption of an established social bond, and that access to an exercise wheel would mitigate those physiological markers. Specifically, we predicted elevated corticosterone concentrations in both hair and plasma and increased adrenal weight in isolated animals; and among isolated animals, those with access to an exercise wheel would show lower hair and plasma corticosterone and lower adrenal weight, with these responses being comparable to paired animals.

Social isolation is known to activate the HPA axis [reviewed in (Cacioppo et al., 2015; Sandi and Haller, 2015)] and result in elevated glucocorticoid concentrations (Hawkley et al., 2012). This response tends to be particularly pronounced among species that typically engage in robust social bonds (Hawkley et al., 2012) and in female individuals as compared to males (Dadomo et al., 2018; Haller et al., 1999; Herzog et al., 2009; Iñiguez et al., 2018). Exercise is also known to activate the HPA axis and increase circulating glucocorticoid levels (Gerber et al., 2013; Hill et al., 2008; Skoluda et al., 2012; Tremblay et al., 2004). However, under conditions of chronic stress, exercise mitigates glucocorticoid levels and reduces depression- and anxiety-like behaviors (Campeau et al., 2010; Grippo et al., 2014; Sasse et al., 2008; Watanasriyakul et al., 2018). Parts of the current study align well with these previously observed patterns. First, we observed elevated plasma corticosterone in animals that were isolated and without access to an exercise wheel, as compared to either animals who were paired or those who had access to a wheel, or both. Further, among isolated animals, we observed elevated hair corticosterone concentrations and elevated adrenal size in animals without wheel access. However, our results do not align with the previous patterns entirely. For example, although plasma corticosterone levels were lower in paired animals (versus isolated animals), both hair corticosterone concentration and adrenal size were higher in paired animals with wheel access (versus paired sedentary animals). This pattern suggests that stressors might not affect long-term HPA activity in an additive way, but rather, that HPA activity is quite sensitive to specific social and environmental stimuli. That is, exercise might be effective at mitigating some negative HPA consequences associated with social isolation, but likely does not simply down-regulate all HPA axis responses.

We observed differing patterns between our various measures of physiological responses to stress as a function of social housing and access to a running wheel. Namely, increased concentrations of plasma corticosterone were observed only in animals that were isolated

without access to an exercise wheel. For hair corticosterone and adrenal size the pattern was different, with isolated animals expressing indicators of chronic stress (i.e., elevated hair corticosterone and increased adrenal size) when wheel access was denied, whereas paired animals expressed those same indicators when they had wheel access. These differences across physiological measures are likely due to differences in what exactly is being measured. That is, plasma corticosterone is a point measure, and is useful for quantifying corticosterone at the exact time that the blood is collected. It is an indicator of HPA activity at the time of sampling. Hair corticosterone and adrenal size are indicators of more long term HPA activity. In the case of hair corticosterone, the concentration reflects the amount of hormone deposited along the hair shaft for the entire period that the hair has been growing (i.e., 5 or 6 weeks, in this study), and adrenal size, presumably, also reflects HPA activity during the entire period that a stressor is present. Previous work in rats has demonstrated the utility of quantifying plasma corticosterone for assessing stress over short time periods (e.g., minutes to hours; Stalder and Kirschbaum, 2012), while acknowledging the sensitivity of this measure to various environmental factors (e.g., time of day, time since exercise, time since ingestion of food, etc.; D'Agostino et al., 1982; Girard and Garland, 2002; Heiderstadt et al., 2000; Starzec et al., 1983; Stupnicki and Obminski, 1992). Measuring corticosterone in hair provides a noninvasive method for assessing HPA activity over a longer period of time, and has been shown to effectively detect exposure to chronic stressors (Heiderstadt et al., 2000; Meyer and Novak, 2012; Russell et al., 2012; Scorrano et al., 2015). Our data indicate that hair corticosterone reflected the expected pattern in isolated animals (i.e., elevated when wheel access was denied), but not in paired animals. Interestingly, a similar pattern was observed in adrenal size, another physiological indicator of chronic stress (Gamallo et al., 1986). Unfortunately, the pattern observed in hair corticosterone is limited by the fact that our experimental groups differed at baseline. Prior to manipulation, hair corticosterone was lower in animals that were randomly assigned to the isolation without wheel access experimental group. Additionally, animals that would be isolated with wheel access had higher hair corticosterone concentrations than animals that would remain paired with wheel access. Although change scores were evaluated in an attempt to statistically account for baseline differences, these pre-existing differences may limit the interpretation of hair corticosterone data.

Additional evidence supporting hair corticosterone and adrenal mass as indicators of long-term HPA axis activity is the highly correlated nature of these measures, both when adrenal size was expressed in absolute mass or when expressed as a percent of body mass. Importantly, these associations were strongest when animals experienced environments (i.e., either social or environmental) typically thought of as beneficial to health. Specifically, when animals were with their sibling and had access to the exercise wheel, the correlations between change in hair corticosterone and adrenal mass were strong. When animals were either isolated or without wheel access, the correlations were weak. These findings support a hypothesis that under favorable conditions, the HPA axis is well regulated and physiological sequelae are in line with one another. However, under chronically stressful conditions, the HPA axis may become dysregulated, and the consequences on various organs and tissues may differ. Neither hair corticosterone nor adrenal mass were associated with plasma corticosterone. These patterns indicate that hair corticosterone and adrenal mass, but

possibly not plasma corticosterone, are closely related, and that both hair corticosterone and adrenal mass are reliable measures of chronic social isolation. Plasma corticosterone, on the other hand, while highly effective for detecting acute physiological responses to stress, may be less reliable for detecting accumulated changes associated with chronic stressors, unless multiple samples are collected (Meyer and Novak, 2012). Although we attempted to minimize the influence of short-term influences on plasma corticosterone levels in the current design, it is possible that environmental confounds unsystematically influenced these levels. Further detailed investigations directly comparing repeated measures of hair corticosterone, adrenal function, and plasma corticosterone will provide additional insight into these relationships.

An alternative explanation for the differential pattern of hair corticosterone and adrenal mass in the present study is that increased glucocorticoid production in paired animals with wheel access was a result of increased energy expenditure. That is, elevated corticosterone concentrations in the hair may have represented increased metabolic demands, and not increased stress. Indeed, previous work in rats and mice has demonstrated a strong association between exercise and plasma corticosterone (Coleman et al., 1998; Girard and Garland, 2002; Sipp et al., 1993), a pattern that is also well documented in humans (Tharp, 1975). It may be the case that exercise only has a mitigating effect on HPA activity when individuals are already experiencing some form of psychological stress. In the current study, among paired animals we observed a greater increase in hair corticosterone within animals with wheel access. Further, the only experimental group that did not display a significant increase in hair corticosterone was isolated animals with wheel access. To further support this hypothesis, data from exercise studies in rats housed under different social conditions suggest that the effects of exercise are independent from those of social housing, and that voluntary exercise indeed has stress-buffering effects (Greenwood and Fleshner, 2011).

One additional explanation for increased hair corticosterone and adrenal mass in paired animals with wheel access is competition over wheel access. That is, the exercise wheel may be thought of as a limited resource that the subject animal competed over with their sibling. Indeed, only one wheel was placed in each cage, whether the cage had one (isolated) or two (paired) animals. It is also notable the mean distance traveled in the paired group was similar to that of the isolated group, despite the fact that paired animals shared a wheel with a sibling in the same cage. A reasonable prediction might be that the paired group should have traveled approximately twice the distance than that of the isolated group, similar to what is observed in single- vs. pair-housed rats (Greenwood and Fleshner, 2011). Therefore, among paired animals, the limited availability of an exercise wheel might have represented a stressor (Liesenjohann et al., 2013). On the other hand, for isolated animals that did not have a sibling to compete with, the exercise wheel would not represent a stressor. On the contrary, among these isolated animals, the opportunity to exercise might be a coping mechanism to mitigate the stress of being isolated (Campeau et al., 2010; Greenwood and Fleshner, 2011; McNeal et al., 2017; Sasse et al., 2008; Watanasriyakul et al., 2018).

Taken together, the results from the present study indicate that isolation is a potent psychosocial stressor in the monogamous prairie vole, activating the HPA axis. Further, these results indicate that exercise mitigates the physiological reactivity to social isolation.

These results have important implications for treatment strategies for patients suffering from depressive symptoms or other consequences of social stress, especially those patients whose symptoms are not entirely mitigated through pharmacological means. This study provides a foundation for additional investigation of the benefits of exercise and other environmental factors in mediating behavioral and neurobiological consequences of social stressors. Lastly, the differing physiological patterns from this study support the use of hair corticosterone and adrenal size as indicators of long-term HPA hyperactivity.

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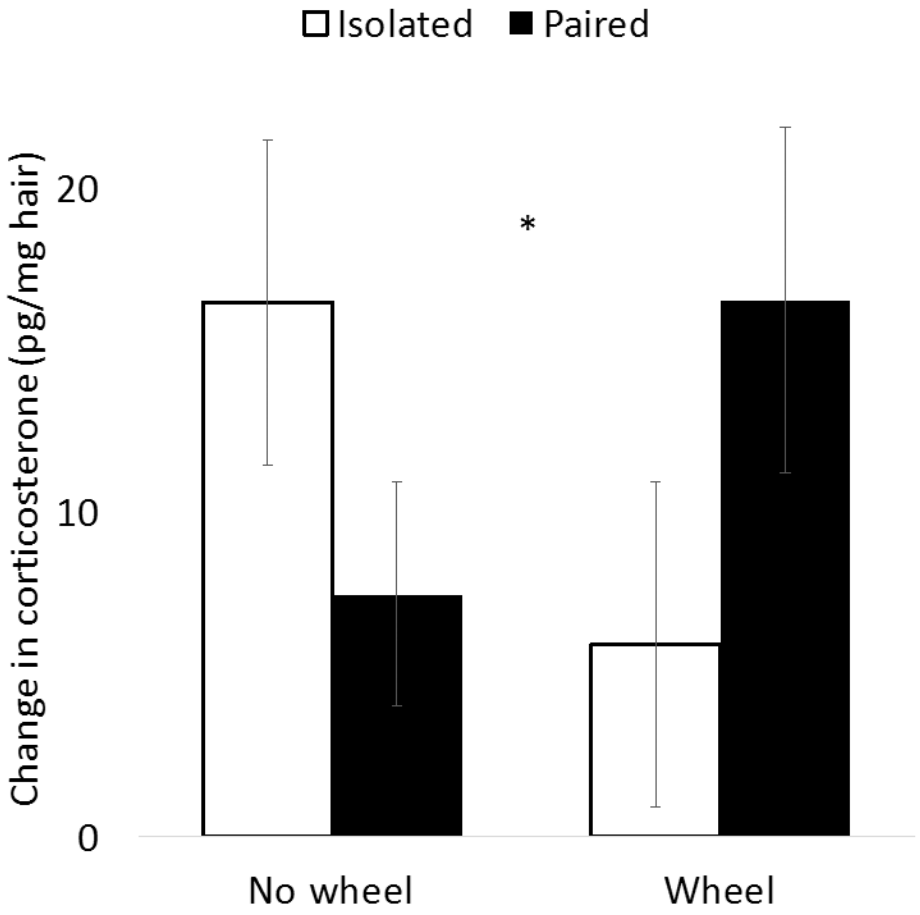


Figure 1.

Change in hair corticosterone concentrations (post-manipulation – baseline) as a function of social isolation and wheel access. Social isolation and wheel access interact such that among isolated animals wheel access results in a mitigated increase in hair corticosterone, whereas among paired animals the opposite pattern was observed. * indicates ANOVA interaction $p < 0.05$.

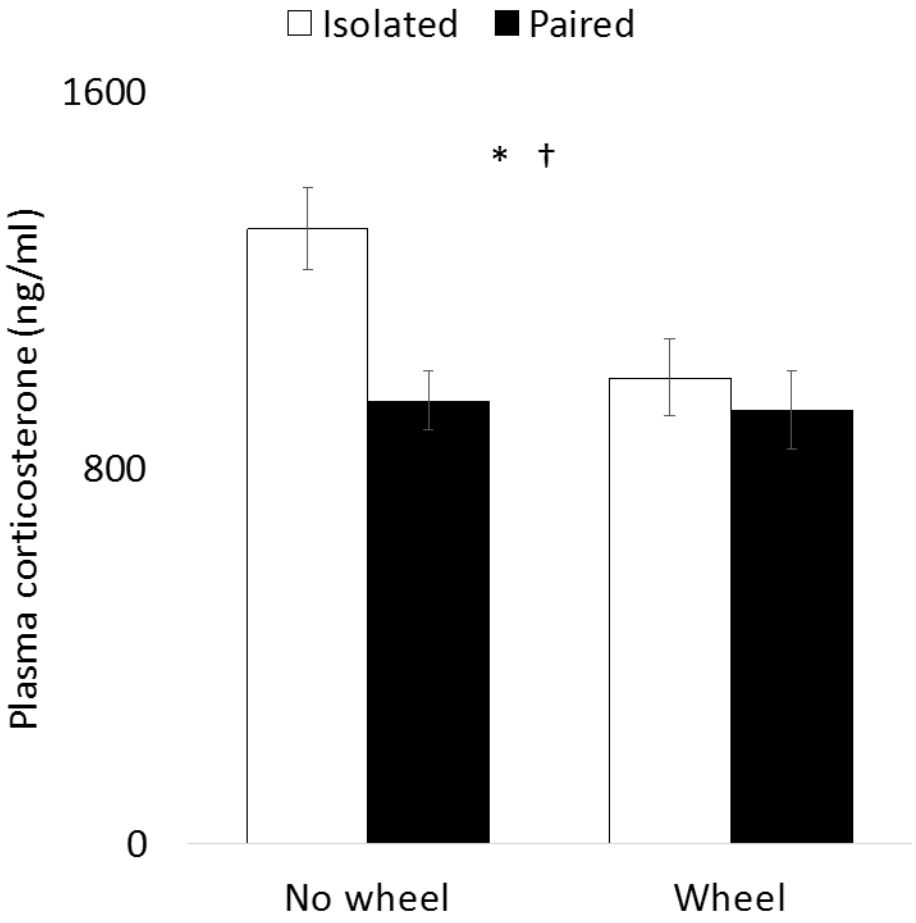
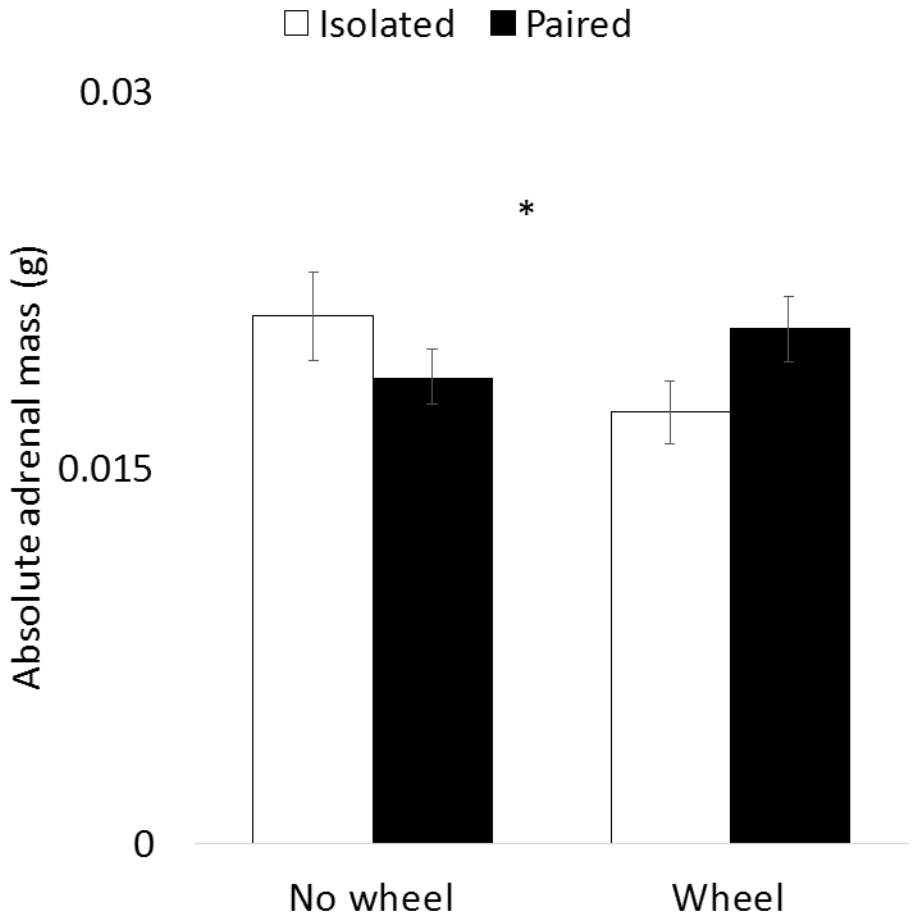


Figure 2.

Plasma corticosterone concentrations as a function of social isolation and wheel access. Elevated plasma corticosterone was observed only in animals that experienced social isolation and did not have access to an exercise wheel. * indicates condition (wheel access or sedentary) $p < 0.05$; † indicates group (paired or isolated) $p < 0.01$.



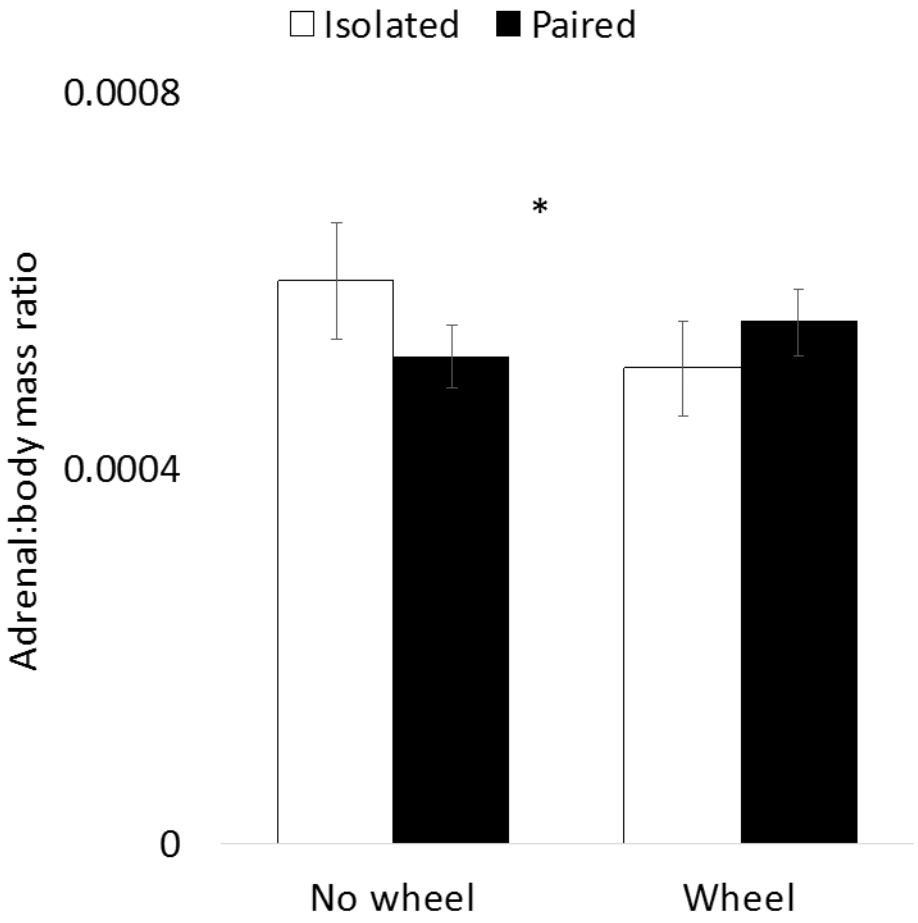


Figure 3.

Adrenal gland mass as a function of social isolation and wheel access. Absolute adrenal mass (a) and adrenal mass relative to body mass (b) are predicted by the interaction between social isolation and wheel access. In both adrenal measures, among isolated animals, wheel access is associated with lower adrenal mass than no wheel access. However, among paired animals the opposite pattern is observed. * indicates ANOVA interaction $p < 0.05$.

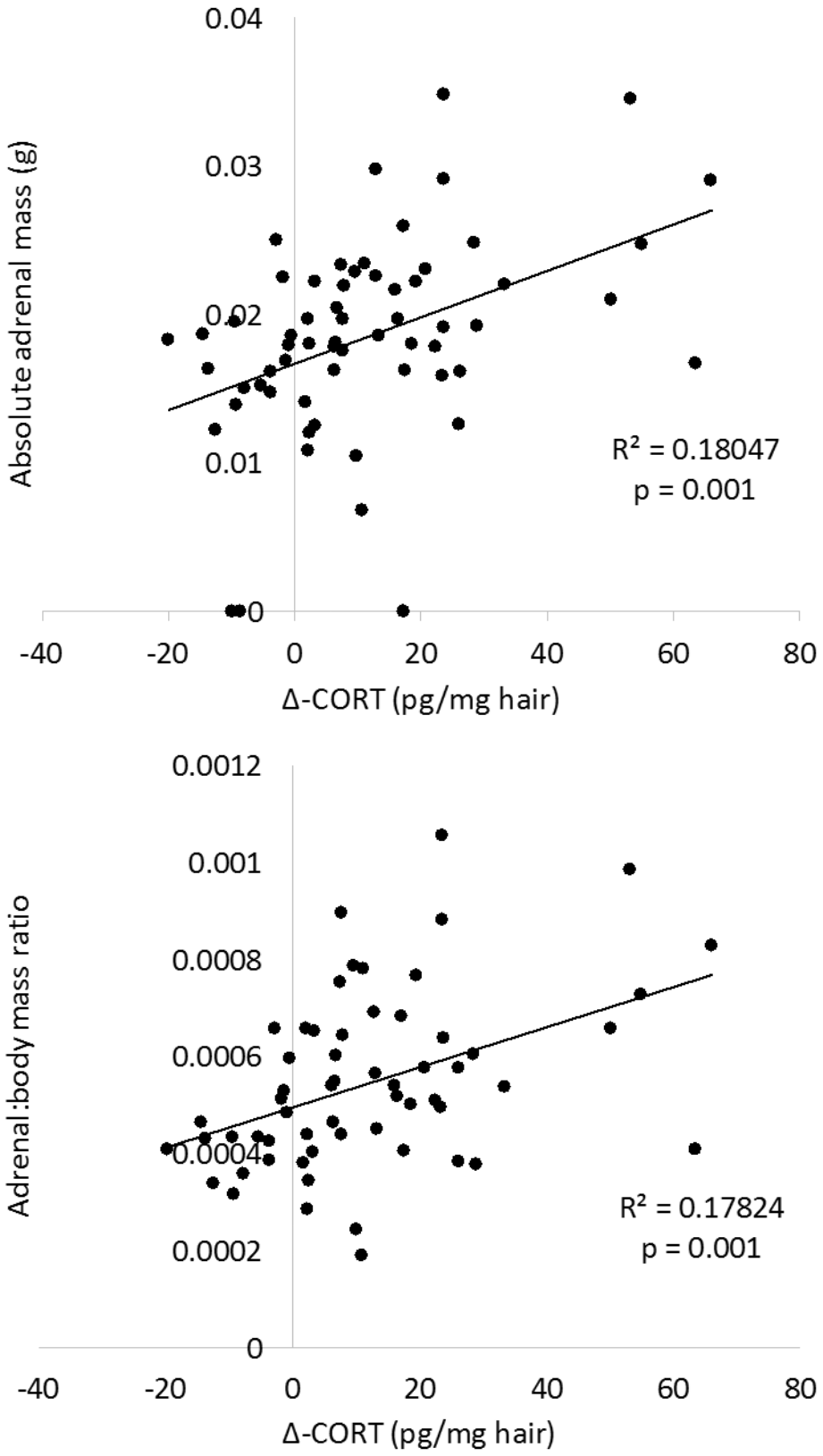


Figure 4.

Association between change in hair corticosterone and adrenal mass. Absolute adrenal mass (a) and adrenal mass relative to body mass (b) are positively associated with change in hair corticosterone from baseline to post-manipulation.

Table 1.

Animal weights and physical activity characteristics (mean ± SEM).

Group	Body mass (g)			Running (km)	
	Start	End	Change	Distance (km/day)	Max speed (km/hr)
Isolated	33.9±1.3	36.5±1.6	2.6±0.9	—	—
Isolated, wheel	32.7±1.1	35.1±1.6	2.4±0.8	2.7±0.6	1.07±0.1
Paired	34.1±1.1	36.6±1.1	2.5±1.1	—	—
Paired, wheel	34.6±1.3	37.1±1.1	2.6±0.7	3.8±0.5	2.2±0.5

Table 2.

Hair corticosterone concentrations at baseline and post-stressor (mean ± SEM).

Group	Corticosterone (pg/mg hair)			
	Baseline	Post-stressor	Change[a]	p[b]
Isolated	14.7±1.2	30.4±5.2	16.5±5.3	0.008
Isolated, wheel	30.5±3.5	36.1±5.5	5.9±4.2	0.182
Paired	23.8±3.0	30.2±4.6	7.5±3.5	0.049
Paired, wheel	19.4±2.4	35.7±5.7	16.5±5.3	0.006

[a] Change calculated as average of difference scores (i.e., post-stressor — baseline) for all individuals in an experimental group.

[b] *p* value corresponds to *t* test comparison of baseline and post-stressor within an experimental group.