1   **Title:** Stress impairs new but not established relationships in seasonally social voles

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Abstract

Affiliative social relationships are impacted by stressors and can shape responses to stress. However, the effects of stress on social relationships in different contexts are not well understood. Meadow voles provide an opportunity to study these effects on peer relationships outside of a reproductive context. In winter months, female meadow voles cohabit with peers of both sexes, and social huddling is facilitated by exposure to short, winter-like day lengths in the lab. We investigated the role of stress and corticosterone (cort) levels in social behavior in short day-housed female meadow voles. A brief forced swim elevated cort levels, and we assessed the effects of this stressor on new and established relationships between females. In pairs formed following exposure to swim stress, the stressor significantly reduced the fraction of huddling time subjects spent with a familiar partner. Swim stress did not affect partner preferences in pairs established prior to the stressor. Finally, we examined fecal glucocorticoid metabolite levels via immunoassay in voles housed under short day (10h light) versus long day (14h light) conditions and detected higher glucocorticoid levels in long day-housed voles. These findings support a role for stress regulation in the formation of social relationships in female meadow voles, and support a potential role for seasonal variation in cort in the behavioral transition from solitary to social. Together they highlight the importance of stress and possibly glucocorticoid signaling for social behavior.

Keywords: Stress, corticosterone, social behavior, partner preference, affiliation, *Microtus pennsylvanicus*, meadow vole, seasonality

Introduction

Social relationships are important for many aspects of life, from reproduction to the provision of emotional support and more direct physical survival benefits. The need for — and targets of — social behaviors also change across the lifespan (Anacker and Beery, 2013). In the wild, animals alter many behaviors with changing seasons (Prendergast et al., 2002). Female meadow voles (*Microtus pennsylvanicus*) transition from solitary and aggressive during the summer reproductive season to communally nesting and affiliative towards other specific individuals. These changes coincide with changes in the regulation of corticosterone (cort) — a glucocorticoid hormone important in the stress response — providing an opportunity to examine the role of stress and corticosterone exposure in social relationships.

Stress and social behaviors affect each other in a variety of ways, which are dependent on the type of stressor, context, and sex of the individual, among other factors (reviewed in Beery and Kaufer, 2015; Hostetler and Ryabinin, 2013). In rats, social defeat stress decreased subsequent social affiliation (Meerlo et al., 1996), while a non-social stressor increased huddling in groups, perhaps as a defense mechanism (Bowen et al., 2012). Affiliation can also moderate the response to stress. Female prairie voles (*Microtus ochrogaster*) exposed to restraint stress showed elevated levels of cort and anxiety-like behavior when isolated following stress, but showed no rise in signs of stress when recovering with a male partner. Active social behavior from the partner, such as allogrooming, is presumed to be an important aspect of these social buffering effects of social partner presence following stress, and is mediated through hypothalamic oxytocin release (Smith and Wang, 2014).

Studies of the development of male-female bonds in socially monogamous prairie voles have revealed declines in cort concentration in both females and males upon pairing with an unfamiliar individual of the opposite sex (DeVries et al., 1995; DeVries et al., 1997). However, the consequences of stress and cort exposure for pair-bonding are sexually dimorphic: development of male partner preferences can be enhanced by exposure to a stressor (forced swim) or an injection of cort, and is inhibited by prevention of the physiological stress response via adrenalectomy (DeVries et al., 1996). In females, in striking contrast, development of a partner preference for a mate is *reduced* following the rise in cort due to stress or cort injection, while adrenalectomy enhances the preference (DeVries et al., 1995; DeVries et al., 1996). The

77 timing of the changes in cort levels may be crucial to the development of the pair bond, as the
78 decrease in cort early in cohabitation appears to be important for females to develop a bond,
79 while the relative increase and return to baseline levels of cort later in cohabitation may be key
80 for males to develop a bond (DeVries et al., 2007). Both meadow and prairie voles have
81 unusually high cort levels — about 3-6 times higher than lab mice, rats, and montane voles
82 (Boonstra and Boag, 1992; Seabloom, 1985; Taymans et al., 1997). The higher basal levels of
83 cort are likely due to target tissue glucocorticoid resistance as demonstrated in prairie voles
84 (Taymans et al., 1997), but changes in cort levels are nonetheless capable of affecting behavior
85 in these voles, as described above.

86 While the role of stress in the development of mate-pair bonds has been well studied in
87 socially monogamous prairie voles, the potential roles of stress and glucocorticoids in same-sex
88 social affiliation remains largely uninvestigated. Same-sex relationships in female meadow voles
89 allow for the study of neurobiological factors supporting the winter transition to social tolerance
90 and affiliation. At an evolutionary level, communal nesting is likely driven by the physical
91 benefits of energy conservation in group huddles; meadow voles do not hibernate, and social
92 huddling enhances survival through the winter (Madison, 1984; West and Dublin, 1984).
93 Nonetheless, day length in the absence of temperature change is a sufficient cue to promote the
94 transition to social huddling in the laboratory, although temperature, food availability, and
95 cohabitation timing and duration also shape social behavior of meadow voles (Beery et al., 2008;
96 Beery et al., 2009; Ondrasek et al., 2015; Parker and Lee, 2003). After a period of cohabitation,
97 females express a preference for huddling more with the partner they have cohabited with than a
98 stranger female, regardless of whether they are siblings or unrelated (Beery et al., 2009).
99 Additional factors that change seasonally and may contribute to this transition include levels of
100 steroid hormones such as estradiol, and oxytocin receptor and corticotropin releasing factor
101 receptor densities and distributions (Beery et al., 2008; Beery et al., 2014; Beery and Zucker,
102 2010; Ferkin and Gorman, 1992; Ferkin and Zucker, 1991).

103 Glucocorticoids are known to change seasonally in many species including meadow
104 voles (Galea and McEwen, 1999; Pyter et al., 2005; Seabloom, 1985), and may contribute to
105 seasonal changes in social behavior. Evidence for the magnitude and direction of seasonal
106 changes in corticosterone is mixed, in part because changes are likely related to the sex and

reproductive status of the individual; pregnant and lactating female meadow voles have the highest corticosterone levels (Boonstra and Boag, 1992). We hypothesized that higher cort levels, such as those seen during reproduction, are not conducive to affiliative behavior, and that lower cort levels in the winter are permissive of more affiliative behavior. We examined the effects of stress on affiliation in female meadow voles housed under short day lengths, as well as effects of stress on cort. We hypothesized that female meadow voles would show reduced formation of same-sex partner preferences following a stressor that raised cort levels, as with opposite-sex partner preferences in female prairie voles. We also examined whether transient stress would affect the expression of partner preferences in pre-existing pairs. We further predicted that voles housed under long day lengths comparable to the breeding season would display increased cort levels compared to those housed under short day lengths, consistent with seasonal variation in behavior. These studies provide insight into the biological mechanisms and environmental factors that impact changes in social behavior.

Methods

Meadow voles were bred locally, with periodic outbreeding to wild-caught individuals trapped within 10 miles of Smith College in Hampshire County, MA. Breeding pairs were housed under a 14:10 light:dark cycle (long day; LD) with lights off at 5pm EST. Offspring were weaned at postnatal day 19 ± 1 d. Females used for these experiments were housed either under LD conditions or a 10:14 light:dark cycle (short day; SD) from weaning. All procedures were approved by the Smith College Institutional Animal Care and Use Committee and conformed to national guidelines.

Experiment 1: Effect of acute stress on corticosterone levels

To test whether acute swim stress affects corticosterone levels, subjects were exposed to a forced swim test (described below) and rapidly decapitated for collection of plasma at one of six times after the stressor. Adult female meadow voles 103 ± 18 days old that had previously been used as stimulus animals in behavioral tests were used in this study, at least three days following any prior testing. Blood was collected from subjects at: 30 minutes (n=9 swim, 8 control), 1h (n=7 swim, 6 control), 3h (n=6 swim, 5 control), 6h (n=6 swim, 6 control), 12h (n=5

swim, 5 control), or 24h (n=5 swim, 5 control) following the end of the swim. Subjects experienced the 3-minute swim stress three hours after the start of the light phase of the day, within a 15-minute range. Subjects were returned to their home cages following the swim and continued to be housed alone until the time of blood collection. “No swim” control subjects were not handled in this time period.

Experiment 2: Effect of acute stress on partner preference formation

To test the effects of acute stress on partner preference formation, adult female voles (n=16 per group, 72 ± 9 days old, housed under SD conditions) were exposed to the forced swim test (swim stress), or received no stress (no swim control). All subjects were then moved into a new cage for pairing with a novel female partner. Partners in the swim group were exposed to swim stress immediately prior to pairing. Focal voles were distinguished from partners by a small patch of shaved fur. All pairs cohabitated for 24 hours following swim or control treatment, after which they were tested in the partner preference (PP) test (PPT).

Experiment 3: Effect of acute stress on partner preference in established relationships

Female subjects were paired with same-sex littermates from weaning until the time of the experiment (n=10 per group, 75 ± 5 days old, housed under SD conditions), when they were given the forced swim stress and returned to their home cage. The PPT was conducted following two hours of cohabitation.

Experiment 4: Effect of day length on corticosterone levels

To test the effect of day length on corticosterone, glucocorticoid metabolite levels were analyzed in feces of LD and SD females (83 ± 55 days old). Approximately 20 fecal boli were collected from the home cage 24 hours following a cage change, 6-8 hours following the start of the light cycle, while the voles were kept in a separate holding cage. The boli were frozen in foil on dry ice and stored at -80°C . Hormone levels were analyzed by an enzyme-linked immunosorbent assay (ELISA, see below for details).

Forced swim stressor

The swim stress was induced using a Porsolt Forced Swim Test Apparatus (#60160, Stoelting Co., Wood Dale, IL, USA), a clear cylinder with a diameter of 20cm and height of 45cm, with room temperature tap water filled to a height of 18cm. Each vole was moved from its home cage directly into the water where it remained for 3 minutes, until it was removed from the water using a mesh strainer, and dried briefly with a towel prior to being returned to the home cage (experiments 1 and 3) or placed in a clean cage (experiment 2). Voles exhibited active swimming, pawing at the sides of the apparatus, as well as floating when immobile. The apparatus was rinsed thoroughly and water was replaced between each subject tested. The 3-minute swim stress stressor has been shown to elevate cort in prairie voles, peaking at 15-30 minutes after the stressor (DeVries et al., 1995; Taymans et al., 1997).

Partner Preference Test

This test and apparatus have been described in previous work (Ahern et al., 2009; Anacker et al., 2014). The PP apparatus was 75 x 20 x 30cm made of plexiglass painted white on the outside, and consisted of three equal-sized chambers arranged linearly, separated by walls with openings 8cm wide through which the test subject could move freely. The partner was tethered to one end of the apparatus by a collar attached to a leash that swiveled and allowed free range of movement within a restricted range of the chamber. A stranger vole, an age-matched female, was tethered in the opposite chamber. The subject was placed in the center chamber and allowed to move freely in the apparatus throughout the 3-hour test. Following the test the subjects were returned to their home cages.

The PPT was recorded by a video camera mounted to the ceiling. Behavior was scored by a trained observer blind to the treatment of the subjects, watching up to 8X speed. Huddling behavior (side by side motionless contact) and time spent in each chamber were scored for the focal animal in each test and converted to real time using custom software (InterVole Timer version 1.6, A. Beery, available upon request).

Fecal corticosterone assay

Pellets were dried at 95°C for 5.5 hours, then crushed. Sample mass of 0.1g was added to 1.5mL 80% methanol (modified from Mateo and Cavigelli, 2005; Touma et al., 2003). Samples from paired female cagemates were indistinguishable and combined for the assay, resulting in 4

SD samples from 8 voles and 7 LD samples from 12 voles. The samples were then vortexed and centrifuged at 2500 RCF for 20 minutes, and the supernatant was extracted and stored at -20°C until analysis.

A Corticosterone EIA Kit (#500655, Cayman Chemical, Ann Arbor, MI, USA) was used to measure levels of glucocorticoid metabolites in feces. Kit reactivity and cross-reactivity was as follows: corticosterone 100% reactivity; 11-dehydrocorticosterone 11% cross-reactivity; 11-deoxycorticosterone 7%; progesterone 0.31%; cortisol 0.17%; aldosterone 0.06%; testosterone 0.03%; pregnenolone 0.02%; 5 α -DHT 0.01%; androstenedione, cortisone, DHEA, DHEA-S <0.01%). Kit instructions were followed and samples were diluted 1:100 based on pilot assays. The intra-assay coefficient of variation was 19.1.

Statistical Analyses

The effect of swim stress on cort level was analyzed by a two-way analysis of variance (ANOVA). Post-hoc pairwise comparisons, supported by the significant interaction of the effects of treatment and time, were used to determine the timeline of the cort response and corrected for multiple comparisons using the False Discovery Rate method of Benjamini and Hochberg (1995).

Behavior in the PPT was analyzed by a two-way ANOVA with familiarity with the stimulus animal (partner or stranger) as a within-subjects factor and treatment (swim stress or no swim control) as a between-subjects factor. *A priori* *t*-tests were used to determine whether there was a significant PP for each treatment group and whether partner huddling time differed between groups. ‘Partner huddling fraction’ was calculated as the fraction of time the subject spent huddling with the partner out of total huddling time (time with partner plus time with stranger); this single variable avoids violation of the assumption of independence between partner and stranger variables. This was used to make comparisons between stress and control groups using a *t*-test, or Welch’s *t*-test when data were not normally distributed. Subjects were not included in the partner huddling fraction analysis if they huddled less than 5 minutes total.

All other comparisons were performed as *t*-tests, described in the text, and all tests were performed 2-tailed. Statistical analyses were performed in Prism version 5.02 and JMP version 8 (SAS, Inc.). Correction for multiple comparisons via False Discovery Rate was conducted in R

version 3.2.1 (the R project for statistical computing). Effect sizes were calculated and reported for ANOVA using η^2 and for *t*-tests using Cohen's *d*. The Practical Meta-Analysis Effect Size Calculator (<http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php>) was used to estimate Cohen's *d* for *t*-tests.

Results

Experiment 1: Effect of swim stress on corticosterone levels

Swim stress significantly raised plasma cort levels ($F(1,60)=10.8$, $p=0.002$, $\eta^2=0.095$), and there was a significant interaction between the stress treatment and time ($F(5,60)=5.55$, $p<0.001$, $\eta^2=0.243$), indicating that the stress treatment affected cort levels at specific time points. There was also a significant effect of time on cort levels ($F(5,60)=3.16$, $p=0.013$, $\eta^2=0.138$). Cort levels exhibited the highest observed elevation 30 minutes following the stressor and then declined (Fig 1). False Discovery Rate corrected pairwise comparisons showed that at 30 minutes post-swim, cort levels were significantly higher in stressed animals compared to controls ($p<0.001$, $d=2.99$). Differences at 1 and 3h did not reach significance ($p=0.059$, $p=0.088$), and later time points were statistically indistinguishable.

Experiment 2: Effect of acute stress on partner preference formation

Relative preference for the partner was assessed as the fraction of time spent huddling with the partner out of total huddling in the PPT, and was significantly lower in stressed subjects compared to controls (Welch's $t(17.8)=2.48$, $p=0.023$, $d=1.01$; Fig 2A). The effects of familiarity and stress on huddling times were also assessed by 2-way ANOVA (Fig 2B). There was a main effect of familiarity on huddling behavior in the PPT, such that subjects in both stress and control groups spent more time huddling with the partner than the stranger ($F(1,56)=20.8$, $p<0.0001$, $\eta^2=0.266$). Stressed subjects spent less overall time huddling with stimulus animals than control subjects did, but this effect did not reach statistical significance ($F(1,56)=3.57$, $p=0.064$, $\eta^2=0.023$). Similarly, the predicted interaction between stress and familiarity with the stimulus animal neared, but did not reach statistical significance ($F(1,56)=3.84$, $p=0.055$, $\eta^2=0.030$). *A priori t*-tests revealed that there was a significant preference for huddling with the

partner over a stranger female in the PPT for control subjects ($t(30)=4.03$, $p<0.001$, $d=1.42$), and also in stressed subjects ($t(30)=2.68$, $p=0.012$, $d=0.948$).

Experiment 3: Effect of acute stress on partner preference in established relationships

There was no difference in the partner huddling fractions of stressed and control subjects ($t(13)=0.280$, $p=0.784$, $d=0.585$; Fig 2C). There was a main effect of familiarity on huddling time ($F(1,36)=22.8$, $p<0.0001$, $\eta^2=0.039$) and no effect of stress ($F(1,36)=0.0141$, $p=0.906$, $\eta^2<0.001$) or interaction between variables ($F(1,36)=0.0138$, $p=0.907$, $\eta^2<0.001$), revealing that the partner was preferred over the stranger regardless of stress condition in the pairs that had been cohoused since weaning (Fig 2D). Accordingly, *a priori* *t*-tests revealed a significant preference for huddling with the partner over the stranger in controls ($t(18)=3.19$, $p=0.005$, $d=1.43$), and in stressed subjects ($t(18)=3.57$, $p=0.002$, $d=1.60$).

Experiment 4: Effect of day length on corticosterone levels

The level of fecal glucocorticoid metabolites was significantly higher in LD than SD subjects ($t(9)=2.43$, $p=0.038$, $d=1.52$; Fig. 3).

Discussion

The present studies demonstrate that the ability of female meadow voles to form new preferences for specific same-sex individuals is impaired by exposure to acute stress that raises corticosterone levels. Importantly, such stress did not affect the partner preference in established relationships. Impairment of partner preference formation by acute stress in meadow voles parallels the effects of stress on formation of opposite sex partner preferences in female prairie voles (DeVries et al., 1995; DeVries et al., 1996). In both species, stress reduced the relative amount of huddling with a new partner, whether for a male partner in prairie voles or a same-sex partner in meadow voles.

There are some important differences in the molecular mechanisms supporting mate affiliation in prairie voles and peer affiliation in meadow voles; for example, interactions between dopamine and oxytocin receptor signaling in the nucleus accumbens are important for

prairie vole male-female bond formation (Liu and Wang, 2003; Young et al., 2014), but dopamine receptor blockade does not impair same-sex partner preferences in meadow voles (Beery and Zucker, 2010). There are also similarities in mechanisms; for example, oxytocin enhances each type of affiliation in females (Beery and Zucker, 2010; Williams et al., 1994), and it may be that stress acts upon these similar pathways to produce the same behavioral effect. One report in prairie voles suggests that same-sex affiliation may be enhanced by stress in this species (DeVries and Carter, unpublished data referenced in Carter, 1998), however it was not specified whether this was tested in new or established pairs of females.

In contrast to the observed effects of stress on new pairs, there was no evidence of an effect of stress on partner preferences in female meadow voles in established pairs that had been cohoused from weaning. Both stressed and control groups showed a strong PP after the long cohabitation, with virtually no time spent huddling with the stranger (Fig. 2C-D), in accordance with results from unrelated female voles cohoused for 6 weeks (Beery et al., 2009). Because of this ceiling effect of PP, it was not possible for us to detect whether there may have been an enhancing effect of stress on the established bond, but it clearly did not impair expression of the PP. Partner and stranger huddling times differed in experiments two and three (Fig. 2B,D), which is likely due to the different cohabitation durations. 24 hours represents the minimal cohousing window to exhibit a significant preference for the partner, while animals cohoused for two weeks show stable preferences with less apparatus exploration (Beery et al., 2009). It is unlikely that the difference in kinship between the two experiments played a role in the stability of the long-term partnerships, as equivalent levels of huddling are seen in female siblings and unfamiliar voles cohoused from weaning (Beery et al., 2009). Instead, the present data suggest that after the shorter cohabitation period some subjects have not yet formed a “complete” PP and do spend time huddling with the stranger as well, and possibly that these conditions lead to greater levels of total huddling. The difference in individual variation between shorter and longer cohabitations suggests that with more cohabitation time, virtually all subjects would show a complete PP.

The mechanisms that sustain social relationships are distinct from those that form the bonds initially. In prairie voles, specific subtypes of dopamine and opioid receptors mediate either bond formation or maintenance, even within a single brain region: the nucleus accumbens (Aragona et al., 2006; Resendez et al., 2012). Although the mechanism by which stress affects

female social bond formation is unknown, evidence from the present studies suggests that this mechanism does not influence the preference for an established partner.

While the present study showed a significant reduction in the partner huddling fraction in new pairs in the stress condition, this effect was more subtle than with opposite sex pairings in prairie voles. It is worth noting that the duration of cohabitation with the partner differed between the studies conducted in female prairie voles (DeVries et al., 1995; DeVries et al., 1996) and the present study in meadow voles, which may account for the difference in the magnitude of the effect of stress on PP. As was common in early work with prairie voles, females were only exposed to their social partner for 6 or fewer hours before the PP was assessed. In meadow voles 24 hours are needed for the development of a strong same-sex PP (Beery et al., 2009), but the effects of stress and stress-induced corticosterone may not be as pronounced with this longer time period.

It would be of interest in future studies to assess whether the nature of the stressor shapes its effect on affiliative behavior: for example, social stressors such isolation, crowding, and social defeat might impact partner preference formation in different ways from each other and from physical stressors. Physical stressors might also vary in their effects based on their nature and magnitude.

It is possible that the rise in cort level following swim stress is not the direct cause of the alteration in partner preference. Future studies could also address whether cort administration would affect the partner preference in the same way as the stressor, as in prairie voles (DeVries et al., 1995). Further, it is important to understand longer-lasting alterations in glucocorticoid levels such as variation across seasons. Seasonal changes in fecal glucocorticoid metabolites are observed in many species of animals and may relate to altered metabolism, and/or to changes in circulating cort levels, as opposed to changes in the stress response itself. The sampling method employed in the present study — examining the integral levels of fecal glucocorticoid metabolites over a 24-hour period — is particularly useful for capturing baseline hormone levels when long-term effects are anticipated, such as those due to season or day-length (Goymann, 2005; Touma and Palme, 2005). In the present study, fecal glucocorticoid metabolite levels differ with day length in a way that parallels the seasonal changes in social behavior. We found higher levels of fecal glucocorticoid metabolites in LD than SD voles not engaged in reproductive

activities; fecal glucocorticoid metabolites reflect the circulating concentration of free cort that is available to act on tissues. This supports the idea that the production of glucocorticoids is highest in the breeding season, when females are less likely to be social with each other, although a direct effect of cort was not tested in the present studies. These results are consistent with the hypothesis that high glucocorticoid levels are not supportive of formation of social bonds in female meadow voles, and thus these relationships are more likely to form under short day, low cort conditions, as experienced in the winter. Further experiments will be needed to test this hypothesis.

Several other studies have found preliminary evidence of changes in circulating cort in meadow voles across seasons. Adrenal weights and serum corticosterone production in response to ACTH peaked in the breeding season in wild-caught meadow voles of both sexes (Seabloom, 1985). In addition, basal cort levels were highest in females captured in the breeding season relative to the non-breeding season (Galea and McEwen, 1999), and pregnant and lactating females had the highest cort levels of all (Boonstra and Boag, 1992). While these studies are consistent with our findings, one study of plasma cort in male meadow voles found an effect in the opposite direction: in wild-caught males housed under 16 hours of light or 8 hours of light per day for 12 weeks, free cort was significantly higher under SD conditions (Pyter et al., 2005). While the subjects and conditions of this study were different from ours in a number of ways, the difference in the direction of effect is notable and may represent a sex difference in seasonal cort regulation. Other studies of male meadow voles show minimal changes in cort across seasons, and sometimes indicate higher levels in winter than in summer or the breeding season (Galea and McEwen, 1999; Seabloom, 1985). As males do not exhibit day-length dependent changes in their ability to form same-sex attachments (Beery et al., 2009), this further supports the idea that fluctuations in glucocorticoids may be important for the changes in female social behavior.

Further evidence from analyses at different levels of the stress system of meadow voles support seasonal and/or day-length dependent changes in stress hormone signaling. Corticotropin releasing factor (CRF) receptor (CRF₁ and CRF₂) levels differed with day length exposure; overall, CRF₁ receptor levels were higher in long day conditions than short day, particularly in the CA1 and CA3 regions of the hippocampus, while CRF₂ receptor levels were higher in the CA3 region of the hippocampus under short day conditions (Beery et al., 2014). Taken together

with evidence that CRF expression levels increase in long days in bank voles (Peacock et al., 2004), and the present finding that LD females had higher levels of glucocorticoid metabolites in their fecal samples compared to females housed in SDs, we propose that the stress signaling and feedback pathway may be more active during long days than short days. While higher glucocorticoid signaling is not the same as higher stress reactivity, some studies provide support for the idea that long day or summer phenotype meadow voles are more anxious. Meadow voles (particularly females) housed in long days spent less time in the light portion of a light/dark box test, consistent with greater anxiety behavior (Ossenkopp et al., 2005). A field study of wild male meadow voles also found differences in open field exploration (Turner et al., 1983). This adds further support to the hypothesis that stress levels may be higher in summer and prevent development of partner preferences.

Conclusions

The present study indicates that stress increases glucocorticoid concentrations and alters the propensity of female meadow voles to form social preferences for new partners. Higher sociability is observed in this species in winter months; one possible explanation is that as glucocorticoid levels decrease in the winter — corresponding to the lower fecal glucocorticoid metabolite levels measured in voles housed in short day conditions in this study — a state more permissive of affiliation may arise. Stress did not detectably impact established relationships between female partners. These findings indicate that stress and hormone levels are important to consider in the development of novel social relationships outside a reproductive context.

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Figure Captions

Figure 1. Corticosterone levels following swim stress and in time-matched controls over 24 hours. Corticosterone levels were significantly higher in subjects exposed to swim stress 30 minutes following the stressor, compared to controls. At later time points following stress, there were no significant differences between the stress group and controls. Variations in corticosterone due to circadian cycles can be seen in both groups. The dark bar on the x-axis indicates the dark portion of the light cycle. *** $p < 0.001$

Figure 2. Effects of swim stress on formation of partner preference (A-B) or on established partner preferences (C-D). A) The fraction of time spent huddling with the partner out of total time huddling was significantly higher in control subjects than in stressed voles. B) Both stressed and control subjects huddled significantly longer with their partner than with a stranger. The effect of stress treatment on huddling time approached but did not reach significance ($p = 0.06$) C) There was no significant effect of stress on the fraction of huddling time spent with the partner in the established pairs. D) Both stressed and control subjects in an established partnership huddled significantly more with their partner than with a stranger. Points represent individual subjects that met criterion of spending at least 5 minutes huddling. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Figure 3. Fecal glucocorticoid metabolite levels were higher in long day-housed females than in short day-housed females. Samples were collected 24 hours after cage change. * $p < 0.05$

Fig 1

Fig 2

Fig 3