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Sex differences in stress-induced social withdrawal: independence from adult gonadal hormones and inhibition of female phenotype by corn cob bedding

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Abstract

There is compelling evidence for important sex differences in behavioral and hormonal responses to psychosocial stress. Here we examined the effects of gonadal hormones on behavioral responses to social defeat stress in monogamous California mice (*Peromyscus californicus*). Three episodes of social defeat induced social withdrawal in intact females but not males. Gonadectomy blocked corticosterone responses to defeat in females and sensitized male corticosterone responses. However, gonadectomy had no effects on social interaction behavior, suggesting that social withdrawal is not dependent on gonadal hormones in the adult California mouse. In contrast, defeat reduced exploratory behavior in the open field test for intact but not castrated males. We also examined the effects of social defeat on social interaction behavior when California mice were raised on corn cob bedding, which has estrogenic properties. In this dataset of over 300 mice, we observed that social defeat did not induce social withdrawal when females were raised on corn cob bedding. This finding suggests that the use of corn cob in rodent studies could mask important sex differences in the effects of stress on brain and behavior. Although gonadal hormones do not affect social withdrawal behavior in adults, our data suggest that hormones may act earlier in development to induce a more resilient social phenotype.

Introduction

Social defeat stress has emerged as a robust and ethologically relevant approach to understanding neurobiological and behavioral responses to psychosocial stress (Golden et al., 2011; Huhman, 2006; Miczek et al., 2008). The procedure typically consists of repeated

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exposure to about 10 minutes of physical confrontation with a larger aggressive male (Berton et al., 2006; Blanchard et al., 1993; Huhman et al., 2003; Kudryavtseva et al., 2004; Martinez et al., 1998). One of the most consistent behavioral responses to defeat stress is withdrawal from social situations, which has been observed in a diverse group of birds (Carere et al., 2001), rodents (Haller and Bakos, 2002; Krishnan et al., 2007; Kudryavtseva et al., 2000; Schimozuru et al., 2006; Trainor, 2011; Warren et al., 2012) and primates (Kramer et al., 1999; Willard and Shively, 2012). It has been difficult to apply the social defeat model in *M. musculus* and *Rattus norvegicus* because female aggression levels in these species is relatively low (Ter Horst et al., 2009), but see (Holly et al., 2012; Solomon et al., 2007). This has impeded our ability to contrast behavioral responses to defeat stress in males and females.

Gonadal hormones have important effects on the function of the hypothalamic-pituitary-adrenal axis (HPA), and it has been hypothesized that these effects may contribute to sex differences in susceptibility to stress (Goel and Bale, 2009). In the adult, estrogens generally exaggerate the effects of stress on glucocorticoid secretion (Lund et al., 2006; Viau and Meaney, 1991; Weiser and Handa, 2009). In contrast, exposure to estrogens during postnatal development appears to inhibit HPA activation in response to psychosocial stress (Evuarherhe et al., 2009; Patchev et al., 1995). Overall, these data suggest that developmental exposure to estrogens or estrogen-like compounds could have important effects on the development of sex differences in hormonal and behavioral responses to stress.

The California mouse (*Peromyscus californicus*) is a monogamous species in which both males (Bester-Meredith et al., 1999) and females (Silva et al., 2010) have high aggression levels. The reactivity of the HPA axis has been well characterized in this species (Harris et al., 2012), and females but not males have elevated corticosterone levels following resident-intruder testing (Trainor et al., 2010). We previously demonstrated that females but not males display a social withdrawal phenotype following three episodes of social defeat stress (Trainor et al., 2011). We tested whether sex differences in behavioral and corticosterone responses to social defeat were mediated by gonadal hormones. Although gonadectomy had major effects on corticosterone responses, the effects on social behavior were surprisingly modest. In contrast, we show that defeat-induced social withdrawal is blunted in females raised on corncob bedding. Corncob bedding is becoming more prominent in animal facilities due to its lower cost and ability to absorb moisture, and we recently discovered that corncob bedding alters the behavioral actions of estrogens in male California mice (Villalon Landeros et al., 2012). These data suggest the use of corncob bedding may mask sex differences in behavioral responses to stress.

Materials and Methods

Animals

California mice were obtained from our laboratory colony and housed in clear polypropylene cages. Harlan Teklad 2016 food, which is phytoestrogen free, and water were provided *ad libitum*. Cage bedding is described for each experiment. Mice were maintained on 16h light/8h dark cycle (lights off 1400 PST). All mice were 3 month old adults and housed 2–3 per cage in same sex groups. All testing procedures were approved by the UC Davis Institutional Animal Care and Use Committee. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Experiment 1: Effects of gonadal hormones on sensitivity to social defeat

This study was conducted between Jan 2010–Dec 2010 during which all mice were housed on recycled cardboard bedding (Carefresh, Absorption Corp). Male and female California mice were randomly assigned to be gonadectomized or undergo sham surgery under isoflurane anesthesia. Male castration or sham surgeries were completed under as previously described (Trainor and Marler, 2001). Female ovariectomy or sham surgeries were made via a single midline dorsal skin incision. All mice were housed individually for one week during recovery and then group housed with their original cagemates. An additional 3 week recovery period ensured the absence of any residual effects of gonadal hormones during behavioral testing. Mice were then randomly assigned to undergo 3 episodes of social defeat or handling on consecutive days, which were conducted during the first 3 hours of the dark phase (1430–1730) under dim red light. Mice assigned to social defeat were placed into the home cage of a same sex aggressive animal for 7 minutes or until the focal mouse was exposed to 10 offensive attacks (note small modification in Exp. 2). Control mice were introduced into an empty cage for 7 min. Immediately after the third episode of defeat or handling, each mouse was anesthetized with isoflurane and a retro-orbital blood sample was collected for corticosterone measurements. All mice were then returned to their homecage and undisturbed for 4 weeks until social interaction testing. Episodes of social defeat were video recorded. The number of times the resident attacked the intruder (by biting or boxing) was summated in each episode. The number of times the intruder engaged in defensive behavior (standing on hind legs or aggression), freezing, and escape behavior was also quantified.

Social interaction testing consisted of 3 phases (open field, acclimation, and interaction) and was conducted in the dark phase (1430–1730) under dim red light. Testing was conducted in a large Plexiglas open field that contained no bedding (89×63×60 cm). During the open field phase of testing each mouse was introduced into the arena for 3 min. Time spent in the center of the arena and total distance traveled was recorded (Any-Maze, Stoelting). During the 3 min acclimation phase of testing, a small wire cage was introduced against one wall of the arena (without removing the focal mouse from the arena) and time spent within 8 cm of the cage (interaction time) or within the 2 corners (8 × 8 cm each) opposite the wire cage (corners time) was recorded. Finally, an unfamiliar same-sex stimulus mouse was placed into the wire cage for 3 min. Sample sizes were 9–16 mice per group.

Corticosterone was assayed using an I¹²⁵ labeled radioimmunoassay kit (MP Biomedicals, Solon, OH) that has been used previously with California mice (Glasper and DeVries, 2004; Trainor et al., 2010). California mice have very high baseline corticosterone levels, so samples were diluted 1:2000. The sensitivity of this assay is 25 ng/mL. The intrassay coefficient of variation was 3.5%.

Behavioral data during bouts of social defeat were analyzed using repeated measures ANOVA testing for effects of sex and gonadectomy. Based on Q-Q plots, corticosterone data and time spent in corner zones were log transformed to normalize the distributions. All data were analyzed using SPSS with three-way ANOVA testing for effects of sex, gonadectomy and stress. Behavioral data from social interaction tests used three-way ANOVA and planned comparisons testing for effects of defeat stress within sex/hormone treatment groups (e.g. control vs. stress for intact females).

Experiment 2: Effects of cage bedding and male siblings on sensitivity to social defeat

We recently discovered that the use of corncob bedding has important effects on estrogen signaling in male California mice (Villalon Landeros et al., 2012). In the process of conducting this study, our breeding colony was kept on corncob bedding (1/8 inch,

Andersons, #88) for about one year (Sept 2010–Oct. 2011). At the end of this period the colony was switched to aspen sanichips (Harlan Teklad, #7090). We hypothesized that exposure to the estrogenic components of corncob bedding during development would affect female behavioral responses to defeat. We combined behavioral data from 9 social defeat studies conducted between Apr. 2009 and Oct. 2012 (Table 1). All studies used a standard experimental design and there were no changes in husbandry practices besides the bedding type over this time frame. This allowed us to test whether the effects of social defeat on social interaction behavior varied across different bedding types. Specifically, we expected that exposure to corncob bedding (and its estrogenic components) would reduce the social withdrawal response in females exposed to defeat stress.

A total of 353 mice were included for this analysis which includes published (Greenberg et al., in review; Trainor et al., 2011) and unpublished data. All studies used for this analysis used the same design. Males and females were randomly assigned to social defeat or control handling as described for experiment 1. Stimulus mice were housed on the same bedding type as focal mice. One small modification was made for experiments started March 2012 and after. Bouts of defeat were halted after 7 offensive attacks instead of 10. This change was made to equalize the intensity of aggression for males and females (Table 1). Two to 4 weeks later, mice were tested in social interaction tests as described in experiment 1. Time spent in the interaction zone was analyzed using SPSS with three-way ANOVA (sex, stress, bedding). Because of the large number of experimental groups (12), we used Student-Newman-Kuels post-hoc tests to control for family-wise error rates.

Results

Experiment 1: Effects of gonadal hormones on sensitivity to social defeat

The effects of social defeat on acute corticosterone responses depended on sex and gonadal status (Fig. 1A, $F_{1,37}=4.36$, $p < 0.05$). Intact females exposed to defeat had significantly higher corticosterone levels compared to intact control females, whereas there was no difference among ovariectomized females. Intact males exposed to defeat did not differ from intact control males whereas castrated males exposed to defeat had significantly higher corticosterone levels than castrated control males (Fig. 1A). These differences in corticosterone were not due to differences in how the residents behaved towards focal animals, as gonadectomized males and females were exposed to equivalent levels of aggression (Fig. 1B). Gonadectomy also did not affect the frequency of escapes (Fig. 1C), freezing (Fig. 1D), or the frequency of defensive behavior (all p 's > 0.3). However, females showed more escape attempts from residents than males (Fig. 1C, $F_{1,44}=6.27$, $p < 0.05$).

Defeat reduced social interaction time for females but not males (Fig. 2A, $F_{1,85}=4.05$, $p < 0.05$). Although the overall sex $\times$ stress interaction term was not significant, two-way ANOVA of males and females demonstrated clear sex differences. Defeat reduced social interaction time in females (Fig. 2A, $F_{1,54}=6.4$, $p < 0.01$) but not males ($p > 0.5$). Planned comparisons indicated social defeat reduced social interaction time in both intact and ovariectomized females compared to control females ($p < 0.01$). There were no differences in time spent interacting with an empty cage during the acclimation phase (Fig. 2B). Similar results were seen for time spent in the corner zones. Defeat increased time spent in the corner zones during the social interaction phase in females (Fig. 2C, $F_{1,54}=4.0$, $p < 0.05$) but not males ($p > 0.4$). There were no effects of gonadectomy. There were no differences in time spent in the corners during the acclimation phase, in the absence of a social stimulus (Fig. 2D).

In the open field phase, there was a nonsignificant trend for a sex $\times$ stress interaction in time spent in the center of the open field ($F_{1,85}=3.7$, $p = 0.056$). For males, defeat reduced time

spent in the center of the open field for intact males but not castrated males (Fig. 3A). For females, defeat increased time spent in the center of the open field of gonadectomized females but not intact females (Fig. 3A). There were also no significant differences in activity or time spent in the center of the arena during the open field phase, when the arena was empty (Fig. 3B).

Experiment 2: Effects of cage bedding on sensitivity to social defeat

This very large data set confirmed that the effect of social defeat on social interaction behavior was different in males and females (Fig. 4, sex $\times$ stress $F_{1,341} = 8.48$, $p < 0.01$). In addition, this interaction was clearly modulated by the type of cage bedding used (stress $\times$ bedding $F_{2,341} = 3.34$, $p < 0.05$). Post-hoc tests revealed that social defeat reduced female social interaction time in mice raised on carefresh or sanichip bedding (indicated with * in Fig. 4A). There was no significant effect of stress on social interaction behavior in females raised on corncob bedding. In females raised on non-corn-cob bedding, social defeat significantly reduced the time spent interacting with a social stimulus by 30% compared to the controls. In contrast stressed females raised on corncob bedding spent 17% less time interacting with the social stimulus than controls, a difference that was not statistically significant when controlling for experiment-wise Type-I error. There was no effect of defeat on social interaction behavior in males raised on carefresh or corncob bedding. For mice raised on sanichip bedding, social interaction was lower in males exposed to defeat compared to controls, but this difference was not significant. It is important to note that for mice raised on non-corn-cob bedding (sanichips and carefresh) stressed males spent significantly more time interacting with a social stimulus than stressed females (indicated with † in Fig. 4A), a difference that was absent when corncob bedding was used. There were also no differences in time spent in the interaction zone among control animals (male and female) across any of the bedding types. These findings emphasize that the use of corncob bedding reduces sex differences in behavioral responses to social defeat stress. Similar to experiment 1, there were no significant differences in time spent interacting with an empty cage during the acclimation phase (Fig. 4B, all p 's > 0.3).

In general the amount of time spent in the corners during the social interaction phase followed a pattern opposite to time spent in the interaction zone, although there were subtle differences. On average stressed mice spent more time in the corner zone than control mice (Fig. 4C, $F_{1,341} = 6.1$, $p < 0.02$). This effect was driven primarily by a strong effect of stress in females ($F_{1,224} = 8.22$, $p < 0.01$) as there was no significant effect of stress in males ($p > 0.3$). Post-hoc tests only detected a significant effect of stress in females raised on the carefresh bedding (Fig. 4C).

During the open field phase there was a significant main effect of bedding on total distance travelled (Fig. 5A, $F_{1,289} = 14.8$, $p < 0.01$), with mice raised on corncob bedding showing significantly less activity. The effect of defeat stress on time spent in the center of the open field was different in males and females (Fig. 5B, Table 2, $F_{1,289} = 3.9$, $p < 0.05$). Stressed males spent significantly less time in the center of the arena than control males ($F_{1,98} = 8.43$, $p < 0.01$) whereas there were no differences between stressed females and controls. This effect was primarily driven by control males housed on carefresh bedding, which spent more time in the center than control males housed on other bedding types.

Repeated measures ANOVA showed that stressed mice raised on sanichips were exposed to fewer offensive attacks than mice raised on the other bedding types (Table 1, $F_{1,142} = 3.7$, $p < 0.03$). However, this effect was driven by a change in our protocols to equalize the levels of aggression males and females are exposed to during defeat. If mice tested after this modification are excluded from the analyses, males are exposed to more offensive attacks than females ($F_{1,134} = 7.6$, $p < 0.01$) and there is no effect of bedding on the number of

offensive attacks ($p > 0.29$). There were no correlations between numbers of offensive attacks and social interaction behavior.

Discussion

Gonadectomy had major effects on corticosterone levels following social defeat, consistent with previous studies that gonadal steroids contribute to sex differences in HPA activation. However, despite these strong effects on corticosterone secretion, ovariectomy did not block social withdrawal in stressed females nor did castration induce social withdrawal in stressed males. These results suggest that sex differences in social withdrawal responses to defeat stress in California mice are not mediated by activational effects of gonadal hormones. Furthermore, it also appears unlikely that social withdrawal is dependent on increased corticosterone secretion following defeat. In contrast, our data implicate developmental factors as a key component governing sex differences in behavioral responses to stress. Females raised on corn cob bedding had behavioral responses to defeat that more closely resemble stressed males. This raises the possibility that steroid hormones may act during development to masculinize brain circuits controlling behavioral responses to defeat.

Many rodent studies have demonstrated that female glucocorticoid responses to various stressors have a greater magnitude or longer duration than males (Grippe et al., 2007; Trainor et al., 2010). Furthermore, testosterone generally inhibits HPA activation in adults (Viau, 2002; Viau et al., 1999) whereas estradiol facilitates HPA activation (Carey et al., 1995; Weiser and Handa, 2009). Consistent with these findings we observed that castration, which we previously showed reduced California mouse testosterone levels (Trainor and Marler, 2001, 2002), facilitated corticosterone release following defeat. Although it appears that ovariectomy blocked corticosterone responses to defeat, the absence of a significant increase in corticosterone may be due in part to the fact that corticosterone levels were slightly higher in ovariectomized females not exposed to defeat. Several interventions that block glucocorticoid action during defeat stress also block behavioral changes induced by defeat (Calfa et al., 2006; Hartmann et al., 2012). For example, deletion of the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) gene in mice reduces corticosterone levels following social defeat and eliminates defeat induced social withdrawal (Lehmann et al., 2012). Our results suggest that social withdrawal and defeat-induced corticosterone responses can be uncoupled, because behavior of California mice in the social interaction test was not linked to corticosterone responses following social defeat. Even though castrated males had increased corticosterone levels immediately following defeat, we saw little evidence for a social withdrawal phenotype. Similarly, both intact and ovariectomized females exposed to defeat showed reduced social interaction behavior. Overall, these data suggest that the social withdrawal phenotype is not dependent on acute changes in corticosterone following defeat. This finding is consistent with the idea that glucocorticoids do not signal the valence of a social situation but more closely reflect energetic demands (Buwalda et al., 2012). While gonadal hormones do not appear to act in adults to mediate sex differences in behavioral responses to social stress, we discovered that these responses are inhibited by exposure to corn cob bedding.

Experiment 2 has an extremely large sample size for a rodent behavioral study, and used a consistent experimental procedure over a three year period. Social interaction data in the acclimation and interaction phase, especially for control animals, is remarkably consistent over this time. This consistency suggests that any effects of bedding on locomotor behavior have a minimal impact social interaction behavior. A key finding is that differences in social behavior only emerged in animals exposed to defeat stress. One weakness however is that mice were raised and tested on the same bedding type, so it is not possible to separate out organizational versus activational effects of the bedding on behavior. Previous studies of

corncob demonstrated that the effects of this bedding on behavior are mediated by THF-diols, which have estrogenic properties and upregulate COX2 activity (Markaverich et al., 2007). During postnatal development, estrogens stimulate COX2 enzyme activity, increasing the production of prostaglandins that masculinize the brain (McCarthy et al., 2009). If the estrogenic properties of corncob are more important for inducing a resilient social phenotype, this effect is more likely to occur during development. Gonadectomy of mature adults had no effect on social withdrawal phenotypes, suggesting that any effects of steroid hormones on defeat induced social withdrawal are more likely to occur during prenatal (Hines and Goy, 1985; Tobet et al., 1986) or postnatal (Bangasser and Shors, 2008; Han and De Vries, 2003; Lansing and Lonstein, 2006) development. These data provide a strong rationale for future work examining the effects of hormones on the development of sex differences in behavioral and neurobiological responses to stress.

The fact that California mice raised on corncob bedding had blunted behavioral responses to social defeat stress should be of concern to researchers using rodent models. We previously demonstrated that male California mice raised on corncob bedding had fewer estrogen receptor α immunoreactive cells in the bed nucleus of the stria terminalis and ventromedial hypothalamus, and that the number of phospho-extracellular signal regulated kinase positive cells was reduced across several nuclei in the social behavior network (Villalon Landeros et al., 2012). The effects of corncob bedding on these pathways in females are currently unknown. The use of corncob bedding inhibits sexual behavior in both males and females (Markaverich et al., 2002b), an effect that is mediated by THF-diols (Mani et al., 2005; Markaverich et al., 2002a). Effects of corncob bedding extend beyond social contexts, as both males and females raised on corncob had lower levels of activity in the open field phase of testing. Our results as well as others suggest that the increased adoption of corncob bedding in animal facilities could mask important sex differences in the effects of stress or early life experience on behavior. Our data also showed more subtle differences between carefresh (paper-based) and sanichips (wood chip based). Recent studies have suggested that wood-chip based bedding has higher lipopolysaccharide (LPS) content than paper-based bedding (Whiteside et al., 2010), which could result in differences in inflammatory responses (Ewaldsson et al., 2002). A recent study showed that an LPS challenge enhanced social withdrawal behavior in C57Bl6/J mice exposed to defeat (Wohleb et al., 2012). It is interesting to note that among California mice exposed to defeat, the males and females showing the lowest levels of social interaction data were housed on sanichip bedding.

There was evidence in both studies for a weak effect of social defeat on male exploratory behavior. In experiment 2 social defeat decreased time spent in the center of an open field for males but not females. In experiment 1, gonadectomy blocked this effect in males. Several studies have demonstrated that social defeat reduces time exploring the open arms of the elevated plus maze (Calfa et al., 2006; Heinrichs et al., 1992; Reul et al., 1997). This effect is thought to be mediated by elevated levels of glucocorticoids because exogenous corticosterone treatment has anxiogenic effects (Mikics et al., 2005) and infusion of RU-486 (an antagonist to both glucocorticoid and progesterone receptors) in to the lateral septum reverses the anxiogenic effects of defeat in the elevated plus maze (Calfa et al., 2006). Interestingly, male California mice resemble the “unsusceptible” phenotype following social defeat in C57Bl6 mice, characterized by the absence of social withdrawal and increased anxiety-like behavior in the elevated plus maze (Krishnan et al., 2007). It’s possible that an open field phase longer than 3 min would detect a stronger anxiogenic effect in male California mice. However, in a previous study a defeat induced only a very weak anxiogenic effect in male California mice in light-dark box testing (30 min).

Social defeat has become an important method for studying behavioral and neurobiological changes associated with mental disorders. Defeat reduces social approach behavior across a

wide spectrum of species, and social withdrawal is an important component of anxiety and mood disorders (Krishnan and Nestler, 2011). One weakness of this model has been that only a few studies have considered the effects of social stress on females, largely because aggression levels in female domesticated rats and mice are relatively low. Further study of species with high female aggression levels such as Syrian hamsters (Farruzzi et al., 2005; Huhman et al., 2003) and California mice (Davis and Marler, 2003; Silva et al., 2010) provides an important opportunity to address this deficiency. Our results also illustrate how “routine” aspects of animal studies such as cage bedding can have a major impact on experimental results.

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Highlights

Gonadectomy exaggerates corticosterone responses to social defeat in males and blocks increases in corticosterone in females

Defeat-induced social withdrawal in females is independent of the activational effects of gonadal hormones

Corncob bedding inhibits female social withdrawal responses to defeat but has no effect on control animals

Figure 1.

Hormone (A) and behavioral (B–D) data during social defeat stress. Gonadectomy had opposite effects on corticosterone following a third episode of defeat stress in males and females (A). Gonadectomy in focal animals did not affect the aggressive behavior of residents (B) or coping behavior of focal animals (C, D). * $p < 0.05$, *** $p < 0.001$ for planned comparisons following 3-way ANOVA. † $p < 0.05$, effect of sex in repeated measures ANOVA.

Figure 2.

Behavioral responses during social interaction testing in mice assigned to gonadectomy (gdx) or sham surgery. Social defeat stress reduced the amount of time females spent in the interaction zone in the presence of an unfamiliar same-sex stimulus mouse (A) and increased the amount of time spent in the corners opposite the interaction zone (B). There were no differences in time spent in the interaction zone or corner zones in the presence of an empty cage during the acclimation phase (B, D). Three-way ANOVA was followed by separate two-way ANOVA's for males and females testing for effects of gonadectomy and stress. ** $p < 0.01$, effect of stress, * $p < 0.05$, effect of stress. n 's = 9–16 per group.

Figure 3.

Behavioral responses during the open field phase in mice assigned to gonadectomy (gdx) or sham surgery. Social defeat reduced time spent in the center of the open field for intact males but increased time spent in the center for gdx females (A). There were no differences in total distance traveled. * $p < 0.05$, ** $p < 0.01$ for planned comparisons following 3-way ANOVA.

Figure 4.

Behavioral responses during social interaction tests in mice raised on different forms of cage bedding. Social defeat reduced time spent in the interaction zone (A) in females raised on cardboard based bedding (carefresh, cf) or woodchip based bedding (sanichip, sc) but had no significant effect on females raised on corncob (cc) bedding. Social defeat increased time spent in the corner zones during the social interaction phase (C) in females but not males. There were no differences in time spent in the interaction zone or corner zones in the presence of an empty cage during the acclimation phase (B, D). Three-way ANOVA was followed by Student-Newman-Keuls post-hoc tests (* $p < 0.05$ control vs. stress, † $p < 0.05$ male vs. female). See table 1 for sample sizes.

Figure 5.

Behavioral responses during the open field phase in mice raised on different forms of cage bedding (carefresh, cf; sanichip, sc; corncob, cc). Social defeat reduced time spent in the center of the open field for males but not females (A). Males and females raised on corncob bedding showed lower activity levels (B). Three-way ANOVA was followed by Student-Newman-Keuls post-hoc tests. See table 1 for sample sizes. (* $p < 0.05$ control vs. stress; † $p < 0.05$, significantly different from control male, cf; ** $p < 0.01$, main effect of bedding).

Table 1

Sample information and dates for Experiment 2

Author	Start	End	Bedding	control males	stressed males	control females	stressed females
BCT	Apr 2009	Jul 2009	carefresh	9	10	11	16
EYT	Jan 2010	Dec 2010	carefresh	9	8	16	15
GDG	Apr 2011	Sept 2011	corncob	8	9	19	20
KLC	Aug 2011	Jan 2012	corncob	0	0	18	30
MQS	Oct 2011	Dec 2011	corncob	11	11	10	12
SAF	Sept 2011	Oct 2011	corncob	4	4	3	3
SAL	Feb 2012	Sept 2012	sanichips	9	5	8	10
BCT	Mar 2012	Mar 2012	sanichips	6	7	11	14
ALM	Sept 2012	Oct 2012	sanichips	7	6	7	7
<i>Total n's</i>							
			carefresh	18	18	27	31
			corncob	23	24	50	65
			sanichip	22	18	26	31
<i>Number of offensive attacks per episode of defeat</i>							
			carefresh	N/A	7.2±0.4	N/A	6.6±0.4
			corncob	N/A	7.7±0.4	N/A	6.5±0.2
			sanichip	N/A	6.1±0.4	N/A	5.8±0.3

Table 2Percent time (mean $\pm$ s.e.) in center during open field phase

<i>Experiment 1</i>				
Gonad status	control males	stressed males	control females	stressed females
intact	25.2 $\pm$ 3.9	16.3 $\pm$ 2.7 [*]	16.7 $\pm$ 3.4	16.3 $\pm$ 2.4
gonadectomized	22.2 $\pm$ 4.2	21.0 $\pm$ 2.2	12.8 $\pm$ 2.5	19.4 $\pm$ 2.0 [*]
<i>Experiment 2</i>				
Bedding	control males	stressed males	control females	stressed females
carefresh	25.2 $\pm$ 3.9	16.3 $\pm$ 2.7 [*]	16.7 $\pm$ 3.4	16.3 $\pm$ 2.4
comcob	18.0 $\pm$ 1.6 [†]	14.7 $\pm$ 1.3	18.4 $\pm$ 1.2	17.6 $\pm$ 1.0
sanichips	17.0 $\pm$ 1.8 [†]	13.7 $\pm$ 1.7	15.5 $\pm$ 1.1	16.7 $\pm$ 1.3

^{*}
p < 0.05, vs control,

[†]
p < 0.05 vs. carefresh