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Exposure to chronic variable social stress during adolescence alters affect-related behaviors and adrenocortical activity in adult male and female inbred mice

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Abstract

Rodent models provide valuable insight into mechanisms that underlie vulnerability to adverse effects of early-life challenges. Few studies have evaluated sex differences in anxiogenic or depressogenic effects of adolescent social stress in a rodent model. Further, adolescent stress studies often use genetically heterogeneous outbred rodents which can lead to variable results. The current study evaluated the effects of adolescent social stress in male and female inbred (BALB/cJ) mice. Adolescent mice were exposed to repeat cycles of alternating social isolation and social novelty for 4 weeks. Adolescent social stress increased anxiety-related behaviors in both sexes and depression-related behavior in females. Locomotion/exploratory behavior was also decreased in both sexes by stress. Previously stressed adult mice produced less basal fecal corticosteroids than controls. Overall, the novel protocol induced sex-specific changes in anxiety- and depression-related behaviors and corticoid production in inbred mice. The chronic variable social stress protocol used here may be beneficial to systematically investigate sex-specific neurobiological mechanisms underlying adolescent stress vulnerability where genetic background can be controlled.

Keywords

adolescent; social stress; depression; anxiety; BALB/cJ

Adolescence is a developmental period characterized by profound behavioral and neurobiological changes associated with increased novelty seeking and emotionality (Brenhouse & Andersen, 2011; Spear, 2000). Peer-directed social interactions are particularly salient to adolescents as they fine-tune complex social skills (Sachser, Kaiser, & Hennessy, 2013). Human studies suggest that the adolescent brain may be uniquely susceptible to long-lasting stress effects that can manifest as affective disorders into

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adulthood (Heim & Nemeroff, 2001). Preclinical studies also suggest that adolescents are vulnerable to social stress; social isolation, social defeat, and social instability all increase adult anxiety- and depression-related behaviors in rats and mice (Bourke & Neigh, 2011; Hong et al., 2012; Lukkes, Mokin, Scholl, & Forster, 2009; Ros-Simó & Valverde, 2012; Scharf, Sterlemann, Liebl, Müller, & Schmidt, 2013; Schmidt, Scharf, et al., 2010; Schmidt, Trümbach, et al., 2010; Vidal et al., 2007). These social stress paradigms have primarily been implemented in outbred male rodents. This presents a limitation because genetic manipulations – powerful tools to elucidate biological mechanisms of complex phenotypes – are normally generated in inbred mouse strains and results are highly dependent on genetic background (Sittig et al., 2016). Furthermore, few studies have specifically compared the effects of adolescent social stress in both sexes and on both anxiety- and depression-related behavior (Table 1). Given these limitations, the current study measured adult anxiety- and depression-related behavior in male and female inbred mice exposed to a novel adolescent social stress protocol. Our protocol included stressors that are known to affect both female and male mice (i.e. social isolation and novelty, respectively) (Arndt et al., 2009; Bartolomucci et al., 2004; Brown & Grunberg, 1995).

In rodents, adolescent development is typically considered to occur from postnatal day (PND) 21–59 (McCormick & Mathews, 2010). During this time, the hypothalamic-pituitary-adrenal axis (HPA) undergoes significant maturation, which can be modified by chronic stress. For example, adolescent social stress leads to blunted basal corticosterone levels in adult mice and rats which may follow an initial period of HPA hyperactivity during stress exposure (Caruso, McClintock, & Cavigelli, 2014; Ros-Simó & Valverde, 2012; Scharf et al., 2013; Schmidt, Scharf, et al., 2010). Stress-induced alterations in HPA regulation are one way in which adolescent stress may increase adult affective disorders (McCormick & Green, 2013). Prolonged glucocorticoid (GC) exposure due to repeated stress may alter adolescent hippocampus, amygdala, and prefrontal cortex development resulting in sensitization of stress-responsive neural circuits that regulate emotion-related behavior (Heim & Nemeroff, 2001; Isgor, Kabbaj, Akil, & Watson, 2004; Leussis & Andersen, 2008; McCormick, Hodges, & Simone, 2015). Adolescent social stress leads to reductions in myelination, synaptic density, and dendritic complexity in these regions (Leussis & Andersen, 2008; McCormick & Green, 2013; Tsai et al., 2014), although the precise mechanisms by which chronic adolescent social stress leads to anxiety and depression symptoms remain unclear. Rodent models are important tools for investigating these processes. However, the results of adolescent social stress studies in rodents are quite variable (Table 1). Several studies report significant sex-differences in vulnerability, while others report no enduring neurobiological or behavioral changes in either sex (Bourke & Neigh, 2011; Butler, Carter, & Weiner, 2014; Hong et al., 2012; Isgor et al., 2004). Different outcomes among studies may be compounded by genetic variability in commonly-studied outbred animals (e.g. Sprague-Dawley rats, Long-Evans rats, and CD1 mice) (Caruso et al., 2014; Scharf et al., 2013; Schmidt, Trümbach, et al., 2010).

The early adolescent stage is a sensitive period in rodent development during which social stress (e.g. isolation or social instability) can cause persistent alterations in adult behavior (McCormick et al., 2015; Sachser, Durschlag, & Hirzel, 1998). Research with inbred mouse strains can facilitate the discovery of genetic and environmental factors mediating the

adverse effects of adolescent social stress (Jacobson & Cryan, 2007). However, limitations of established social stress models should be taken into account for research involving inbred mouse strains. Paradigms utilizing social instability (e.g., repeat exposure to novel social partners) are advantageous because large cohorts of mice can be exposed to uncontrollable and continuously stressful situations. Social instability disrupts social group composition and increases inter-male aggression during a time when social skills are acquired (Sachser et al., 2013; Schmidt et al., 2007; Schmidt, Scharf, et al., 2010). In addition, different inbred mouse strains show different levels of baseline sociability (e.g. Brodtkin 2007), and thus strain selection may influence outcomes. Isolation is also easily applied, but may be more stressful for females than males or for high- vs. low-sociability inbred mice (e.g. C57BL/6J vs. BALB/cJ) (Arndt et al., 2009; Bartolomucci et al., 2004; Brown & Grunberg, 1995). For example, isolation increased basal corticosterone levels in adult female C57BL/6NCrl mice but had no effect on female BALB/cAnNCrl mice or males of either strain (Arndt et al., 2009). Finally, adolescent social stress exposure should be limited to developmental periods within an age-range that is commonly-accepted as rodent adolescence (PND 21–59), but some researchers utilize stress protocols that continue into adulthood (Table 1). Thus, the chronic variable social stress (CVSS) protocol was designed to be ethologically-appropriate, age-specific, and to include multiple adolescent social stressors that may affect both males and females across multiple inbred mouse strains.

The current study tested the effects of CVSS, administered during adolescent development, on adult behavior and HPA function in inbred BALB/cJ male and female mice. BALB/cJ mice were chosen for this initial experiment because they are typically considered to be a high-anxiety strain and a number of studies have demonstrated that they are more stress-susceptible than other inbred strains (reviewed in Belzung & Griebel, 2001; Jacobson & Cryan, 2007). The combined effect of repeated adolescent social isolation and novelty exposure on adult HPA activity and behavior in both male and female inbred mice remains uncharacterized. We hypothesized that adolescent CVSS would increase adult anxiety- and depression-related behaviors and alter basal HPA activity in both sexes.

METHODS

Animals

Male and female BALB/cJ mice (The Jackson Laboratory, Bar Harbor, ME) were bred and pups remained with the dam until weaning (PND 22). Mice were housed in polycarbonate cages (28 cm × 17 cm × 12 cm) with corn-cob bedding and maintained on a reverse 12:12 h light:dark schedule (lights off 06:00 h) with *ad libitum* food and water. All procedures were approved by the Pennsylvania State University IACUC committee.

Chronic Variable Social Stress Procedure

At weaning, mice from 8 litters were assigned to either CVSS or control (CON) groups ($n = 7\text{--}9/\text{sex}/\text{group}$), with littermates evenly distributed among groups. CVSS was administered from PND 22–50. This age-range was chosen to limit stress exposure to a developmental period commonly accepted as the early- to late-adolescent stages of development, based on age-specific behavioral, neurobiological, and pubertal changes (McCormick & Mathews,

2010). Note that early adolescence has also been designated as the juvenile period (Andersen, 2003; Spear, 2000). Timing and duration of CVSS were consistent with previous adolescent social stress protocols used with outbred mice, with the exception that CVSS concluded during late-adolescence, whereas some prior stress protocols have continued into adulthood (Scharf et al., 2013; Schmidt, Scharf, et al., 2010; Schmidt, Trümbach, et al., 2010). CVSS consisted of repeated cycles of individual-housing (3 days) and re-socialization with 2 unfamiliar same-sex cagemates (4 days). CON mice were housed with same-sex cagemates (3/cage) where they remained throughout adolescence.

Glucocorticoid Measurements

CVSS effects on HPA activity were assessed with non-invasive measures of fecal corticoid metabolites (fCORT) to estimate diurnal peak and trough corticosterone production (Cavigelli et al., 2005). Measures of plasma corticosterone more precisely reflect stress-induced changes in HPA activity, but repeated blood sampling can cause additional stress and elevate corticosterone levels within minutes (Touma, Sachser, Möstl, & Palme, 2003; Tuli, Smith, & Morton, 1995). Fecal measures limit the impact of repeated sampling procedures on subsequent behavior and circadian corticosterone rhythms to provide an integrated measure of unbound (i.e., biologically active) corticosterone (Cavigelli et al., 2005). On PND 51, all mice were moved from group- to individual-housing in wire-bottom cages for 1-week of acclimation prior to fecal collection. We have previously found that basal fCORT levels return to pre-isolation levels after 4 days (Caruso et al., 2014). On PND 59, feces were collected every 4h, with two time points during the diurnal fCORT peak (08:00 and 12:00 h) and two times during the trough (20:00 and 24:00 h). fCORT was extracted then quantified using radioimmunoassay [¹²⁵I] kits (MP Biomedicals, Solon, OH) as previously described (Caruso et al., 2014). Briefly, fCORT was extracted with ethanol, evaporated with air, and reconstituted in methanol. Reconstituted samples were diluted with kit-provided diluent (1:5) to ensure primary binding along the linear portion of the standard curve (20–80% binding). Samples were assayed in duplicate and those with a coefficient of variation greater than 15% were re-analyzed. Inter-assay and intra-assay coefficients of variance were 10.3 and 9.7%, respectively. We analyzed mean peak and trough fCORT concentrations (ng/g) and total dried fecal mass (g). Following fecal collection, mice were re-housed with their original same-sex cagemates (3/cage) on regular bedding.

Elevated Plus-Maze

On PND 60, anxiety-related behavior was measured on the elevated plus-maze (EPM) under dim red lights (~30–50 lux) during the active period (13:00 – 17:00 h). Consistent with prior studies in the adolescent social stress literature (Scharf et al., 2013; Sterlemann et al., 2008), mice were first tested on the EPM because performance in this test is highly sensitive to prior experiences (Crawley, 2008). The EPM consisted of two open (30 × 5 cm) and two closed arms (30 × 14.5 × 5 cm) elevated 42 cm off the ground. During the 5 min test, percent time on open arms, percent open arm entries, number of closed arm entries, and number of head dips were measured. Reduced percent time on/entries into open arms represent unconditioned anxiety which is independent of locomotion, whereas a reduction in closed arm entries signifies reduced locomotor activity (File, 2001). Arm entry was defined as two limbs passing the threshold of an arm.

Sucrose Preference Test

Depression-related behavior was assessed with the sucrose preference test (SPT) 6 days after EPM testing. Reduced sucrose preference is widely considered a measure of anhedonia-related behavior (McCormick & Green, 2013). For SPT, mice at PND 66 were given 24-hr access to two bottles: one contained tap water and the other contained 1% sucrose. This concentration was previously shown to produce a robust sucrose preference (~85%) in BALB/cJ mice (Lewis et al., 2005). Bottle positions were switched after 12 h to control for side bias. Sucrose preference was quantified as: mass of sucrose solution intake (g)/total fluid intake (g)*100.

Forced Swim Test

Depression-related behavior was also measured in the forced swim test (FST) under dim red lights (~30–50 lux) during the active period (13:00 – 15:00 h). FST behavior was assessed 3 days after the SPT, on PND 69. Previous studies have found little difference in performance when behavioral tests were administered at inter-test intervals of 1–2 days vs. 1 week (Paylor, Spencer, Yuva-Paylor, & Pieke-Dahl, 2006). The FST was conducted by placing mice into an inescapable glass beaker (18.5 wide × 24.5 cm high) filled with 25–27°C water for 6 min. The time spent immobile (s), defined as floating in an upright position and making only those movements necessary to keep its head above water, was measured during the last 4 min of the test period (Porsolt, Bertin, & Jalfre, 1977). Increased immobility in the FST is considered passive behavioral coping in rodents (McCormick & Green, 2013).

Statistics

Data were analyzed using two-way ANOVAs (sex × stress) or repeated measures ANOVA (time of day × sex × stress condition) where appropriate. Tukey's HSD tests were used for post-hoc analyses and p -values < 0.05 were considered significant. Distributions of all variables were checked for normality. Outliers were detected using a Grubb's outlier test (Grubbs, 1969) and excluded from analyses (sucrose preference test: 3/31 data points excluded; fCORT concentration: 1/64 data points excluded). Pearson's correlation analysis was used to evaluate the relationship between fCORT concentrations and anxiety/depression-related behavior for males and females and females separately. Figures depict untransformed values. All analyses were performed and figures made using R v3.3.2.

RESULTS

Fecal Corticoids

Analyses of peak and trough fCORT concentrations were performed separately for males and females due to a significant time × sex × stress condition interaction ($F_{1,27} = 10.09$, $p < 0.01$). In males, CVSS mice had lower fCORT concentrations than CON males during both the diurnal peak and trough (Fig. 1A; $F_{1,14} = 6.81$, $p < 0.05$). In females, CVSS mice had lower fCORT concentrations than CON mice during the diurnal peak, but no difference was observed during the diurnal trough (Fig. 1B; $F_{1,13} = 10.61$, $p < 0.01$). During the fCORT peak, male and female CVSS mice produced less fecal mass than CON mice ($F_{1,23} = 4.81$, p

< 0.05; Mean $\pm$ S.E.M. for CON vs. CVSS: 0.57 ± 0.03 vs. 0.49 ± 0.02 g dry weight). No other significant differences were observed for fecal mass produced.

Elevated Plus-Maze

Relative to CON mice, male and female CVSS mice had decreased percent time on open arms (Fig. 1C; $F_{1,28} = 7.29$, $p < 0.05$), decreased percent open arm entries (Fig. 1D; $F_{1,28} = 19.31$, $p < 0.001$), and fewer head dips ($F_{1,28} = 27.37$, $p < 0.001$; Mean $\pm$ S.E.M. for CON vs. CVSS 11.07 ± 0.93 vs. 4.56 ± 0.82). CVSS mice also exhibited decreased locomotion relative to CON mice, as indicated by fewer closed arm entries (Fig. 1E; $F_{1,28} = 21.03$, $p < 0.001$). There were no main effects of sex or interaction effects for percent time on open arms, percent open arm entries, closed arm entries, or head dips.

Sucrose Preference Test

CVSS females exhibited decreased sucrose preference relative to CON females; this was not observed in males (Fig. 1F; $F_{1,24} = 9.11$, $p < 0.01$). There were no other significant effects for sucrose preference.

Forced Swim Test

In the FST, there was no effect of CVSS on depression-related behavior, but overall females had increased immobility compared to males ($F_{1,28} = 9.79$, $p < 0.001$; Mean $\pm$ S.E.M. for Female vs. Male 148.11 ± 13.50 88.42 ± 13.50). There were no other significant effects on FST behavior.

Correlations

There was a significant positive correlation between peak fCORT concentrations and behavior in the EPM and SPT (Table 2). Peak fCORT concentrations were positively correlated with EPM closed arm entries for both females ($R^2 = 0.28$, $p < 0.05$) and males ($R^2 = 0.38$, $p < 0.05$). Females also exhibited a significant positive correlation between peak fCORT concentrations and sucrose preference ($R^2 = 0.47$, $p < 0.05$). There were no other significant correlations between fCORT concentrations and anxiety- or depression-related behaviors.

DISCUSSION

Overall, there are several advantages to the adolescent CVSS model of stress vulnerability. CVSS induces sex-specific effects on anxiety- and depression-related behaviors in inbred adult mice, which allows for future studies on mechanisms susceptibility in both sexes. CVSS includes two different adolescent social stressors (i.e. isolation and novel social partners) in order to target individual differences in behavioral and HPA responses to different kinds of social stressors (Caruso et al., 2014; Scharf et al., 2013; Schmidt, Trümbach, et al., 2010). Finally, CVSS successfully induced adult stress effects in an inbred mouse. This complements the majority of prior studies that have been conducted with outbred rats and mice, where genetic variability may influence adolescent stress susceptibility (Scharf et al., 2013; Schmidt, Scharf, et al., 2010; Schmidt, Trümbach, et al., 2010). We are unaware of any other study that has compared sex-specific impacts of chronic

adolescent social stress on adult behavior and physiology in inbred mice (i.e., in the absence of genetic variability). CVSS provides a useful model for future comparisons of strain- and sex-specific effects of stress on anxiety- and depression-related behaviors to best elucidate genetic and environmental influences on stress-related affective disorders.

The current study extends prior findings by showing that combined exposure to social isolation and novelty during adolescent development has some similar effects on adult male and female anxiety-related behavior in inbred BALB/cJ mice. Mice that experienced adolescent chronic variable social stress (CVSS) displayed altered affect-related behaviors during adulthood. When tested in the EPM, both male and female adult CVSS-exposed mice exhibited a 35–40% reduction in open arm time and entries relative to CON mice. CVSS mice also exhibited reduced locomotor activity on the EPM which was recently acknowledged to be an important component of rodent anxiety-related phenotypes (Thompson, Grabowski-Boase, & Tarantino, 2015). C57BL/6J, BALB/cJ, and DBA/2J mice treated with prototypical anxiolytics exhibited increased locomotor activity in several anxiety-related behavior tests with no effect on standard anxiety-related behaviors (e.g., time in center of open field or open arm time in EPM) (Thompson et al., 2015). Thus, CVSS-induced changes in locomotor activity in a novel environment are difficult to decouple from typical anxiety-related behaviors and may reflect an anxiety-related state. The magnitude of the increase in anxiety-related behavior in the current study is comparable to prior studies that used different adolescent stress paradigms with outbred rodents (Table 1). Previous studies have shown that: (1) adolescent social isolation leads to ~25–30% increase in adult anxiety-related behavior in genetically diverse outbred male rodents (Lukkes et al., 2009; Ros-Simó & Valverde, 2012), (2) adolescent social instability increases adult anxiety-related behavior by ~25–60% in both male and female outbred animals (McCormick, Smith, & Mathews, 2008; Scharf et al., 2013; Schmidt, Scharf, et al., 2010; Schmidt, Trümbach, et al., 2010), and (3) social defeat, and combined isolation, restraint, and social defeat (mixed modality stress) increase adult anxiety-related behavior by ~30–40% in adult male and female outbred rats (Bourke & Neigh, 2011; Vidal et al., 2007). Taken together, adult rodents exposed to stressful adolescent social experiences exhibit behavioral phenotypes indicative of heightened fear and anxiety.

Adolescent CVSS increased adult depression-related behavior in a sex- and test-dependent manner. We found a 20% reduction in sucrose preference in CVSS females which represents an anhedonia-related behavior (McCormick & Green, 2013). Control males failed to exhibit a preference for 1% sucrose, whereas prior studies have indicated that BALB/cJ males show a strong preference at this concentration (Lewis et al., 2005). It is unclear why our results differ from this prior study, but because of the lack of preference in control males, we are unable to determine whether stress influenced male anhedonia-related behavior. There was no effect of adolescent social stress on FST behavior, but overall females exhibited more immobility than males. Similar results were previously reported in adolescent rats (Leussis & Andersen, 2008). The current SPT and FST results suggest that female mice are more vulnerable to depression-related effects of adolescent social stress than males. The increased anhedonia-like response in females may have resulted from the fact that SPT requires isolation, which may have exacerbated decreased preference effects in females vs. males. Sex differences have been observed for a number of different rodent models of depression-

related behavior, but the direction of these sex differences are highly dependent on the behavioral parameters that were investigated and the type/duration of stressors used (Dalla, Pitychoutis, Kokras, & Papadopoulou-Daifoti, 2012).

Few studies have assessed adult depression-related behavior following adolescent social stress. Similar to our findings, mixed modality adolescent stress reduced sucrose preference by ~25% and increased FST immobility by ~35% in adult outbred female rats, whereas males were unaffected (Bourke & Neigh, 2011). Social instability also increased immobility in the tail-suspension test in outbred male mice, although females have not been assessed (Schmidt, Scharf, et al., 2010). However, 20% of these mice were resilient to adolescent social stress and did not exhibit increased anxiety- or depression-related behavior (Scharf et al., 2013), and resiliency was associated with a heterogeneous genetic background (Schmidt, Trümbach, et al., 2010). These results accentuate the need to model adolescent social stress in both male and female inbred mice to control genetic variability. The current study suggests that, under conditions of limited genetic variability, adolescent social stress may lead to anxiety-related processes in both males and females and to depression-related processes only in females.

Importantly, CVSS may have sensitized behavioral responses to subsequent social stressors. All mice were isolated for fCORT measures and then rehoused 24 hrs prior to EPM testing. Previous studies have found no effect of social isolation on anxiety-related behavior in adult BALB/cAnNCrl mice (Arndt et al., 2009), but we are unaware of any studies that have assessed the impact of a short 24-hr resocialization period on anxiety-related behavior. The magnitude of the CVSS-induced anxiety-related behavior in the current study may have been modulated by isolation and 24-hr resocialization prior to EPM. Previous studies have found that anxiety-related effects of adolescent stress are potentiated in adult mice re-exposed to a social stressor during adulthood (Jacobson-Pick, Audet, Nathoo, & Anisman, 2011). Future studies are necessary to evaluate this “two-hit” hypothesis.

Social experiences during adolescence shape adult HPA regulation (Bourke & Neigh, 2011; Caruso et al., 2014; Schmidt, Scharf, et al., 2010). Prolonged GC exposure is one mechanism by which adolescent social stress may reprogram limbic brain development conferring vulnerability to anxiety and depression (McCormick et al., 2015). CVSS was associated with reduced HPA activity in males and females, as indicated by lower fCORT concentrations at the end of adolescence. This effect was predominately due to reduced diurnal peak fCORT concentrations. CVSS mice also produced less fecal mass than CON mice during the diurnal peak, which could have artificially elevated fCORT concentrations (Cavigelli et al., 2005). Therefore, actual peak GC production in CVSS mice may have been even more diminished than we were able to document with the fCORT measure. We also found dramatic sex differences in fCORT concentrations which were surprising because in other rodents, males typically excrete more fecal GC metabolites than females (Cavigelli et al., 2005; Touma et al., 2003). Results may reflect sex differences in HPA responses to isolation which has no effect, or even reduces, GC production in males, but causes a substantial increase in females (Arndt et al., 2009; Kamakura, Kovalainen, Leppäluoto, Herzig, & Mäkelä, 2016; Palanza, Gioiosa, & Parmigiani, 2001).

Given that adolescent social experiences prepare an organism for future social environments, hypoactive adult HPA activity following adolescent social stress may limit long-term deleterious effects of prolonged GC exposure during stressful adult conditions. While reduced fCORT levels in CVSS mice could indicate less adolescent adversity, we do not believe this to be the case because increased anxiety-related behavior was also observed in these mice. Several studies have reported HPA hypoactivity in rats and mice exposed to adolescent social/non-social stress coupled with increased anxiety- and depression-related behavior (Jacobson-Pick et al., 2011; Ros-Simó & Valverde, 2012; Scharf et al., 2013). Moreover, adult male C57BL/6N mice with high basal CORT levels following chronic restraint stress exhibited reduced anxiety-like behavior and increased locomotion in the EPM and OFT relative to stressed mice with low basal CORT (Kim, Jung, Kim, Min, & Yoon, 2013). Significant correlations in peak fCORT concentrations with closed arm entries on the EPM and with sucrose preference further suggests that stress-induced HPA dysregulation is associated with individual differences in EPM and SPT behavior. Alternatively, prior experience with isolation may have led to HPA habituation in CVSS mice and reduced CORT production during the week of isolation before fecal sampling. Finally, adolescent social instability has been shown to reduce basal corticosteroid-binding globulin levels, resulting in increased free (i.e., bioactive) CORT in rats (McCormick, Merrick, Secen, & Helmreich, 2007). Whereas plasma CORT measurements reflect total CORT (i.e., free and bound CORT), fCORT measurements provide an index of only free, biologically-active, CORT levels (Cavigelli et al., 2005). As a result, we cannot rule out the possibility CVSS influenced the proportion of biologically active to inactive CORT levels. Further work is necessary to disentangle this relationship.

Limitations

There are some limitations to consider when interpreting the current results. The use of a single inbred strain may limit the generalizability of our results, particularly because these effects were found in BALB/cJ mice that normally exhibit relatively high levels of anxiety/depression-related behavior (Belzung & Griebel, 2001; Jacobson & Cryan, 2007). The effects of adolescent stress may be potentiated in mice that display an anxious phenotype under basal conditions. For example, BALB/cOlaHSD and C57BL/6JolaHsd adult males both exhibit depression-related behavior following exposure to chronic social defeat stress. However, exposure to a less severe social defeat paradigm only caused these behavioral effects in BALB/cOlaHSD males suggesting increased sensitivity to stress in this strain (Savignac et al., 2011). Furthermore, strain differences in anxiety/depression-related behavior have been associated with genetic polymorphisms known to modulate stress-susceptibility (Jacobson & Cryan, 2007). Future studies that incorporate multiple inbred and/or genetically modified mice will strengthen the translational value of our findings and provide valuable insight into the genetic and environmental factors that mediate CVSS-induced phenotypes.

A second limitation concerns the use of behavioral test batteries. Test batteries are an indispensable resource for characterizing behavioral phenotypes among a limited number of animals (Crawley, 2008). In the current study, behavioral tests were presented in the same order for all mice. It is therefore conceivable that prior experiences with novelty (i.e., EPM

testing) could have impacted performance in subsequent tests. Few studies have systematically evaluated test order effects within a test battery. In one study, few differences were found when performance was compared between 4 different behavioral test orders (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001). In lieu of a more comprehensive understanding of test order effects, future studies that vary test order within experimental conditions will minimize results that are idiosyncratic to a particular test order.

Conclusion

The current study indicates that CVSS, a novel adolescent social stress protocol, induces sex-specific changes in depression-related behavior, but not HPA activity or anxiety-related behavior, in an inbred mouse strain. Results are consistent with findings in outbred rats and mice exposed to similar stress paradigms. The CVSS protocol may be beneficial for studies on neurobiological mechanisms involved in sex-specific vulnerabilities to adolescent social stress.

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Figure 1.

Exposure to adolescent CVSS alters HPA function and affect-related behavior in adult mice.

(A) Fecal corticoid metabolite (fCORT) concentrations were lower in CVSS males than CON males. (B) Diurnal peak fCORT concentrations were lower in CVSS than CON females. (Note differences in Y axis scales between males and females). (C) In the EPM, CVSS mice spent less time on the open arms, (D) made fewer open arm entries, and (E) fewer closed arm entries than CON mice. In the SPT, CVSS females exhibited reduced

sucrose preference relative to CON females (F). Bars represent mean $\pm$ SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

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Table 1

Experiments investigating the impact of adolescent social stress on adult affect-related behavior.

Author:	Sex:	Strain + Species:	Age at stress:	Stress type:	Age at testing:	Test:	^c Anxiety and depression:
<i>Schmidt et al. (2010a)</i>	Female	CD1 Mice	P31 – P80	^a Social Instability	P85	NSFT	↑
						EPM	=
<i>Schmidt et al. (2010b)</i>	Male	CD1 Mice - Resilient	P31 – P80	^a Social Instability	P108	OFT	=
						NSFT	=
		CD1 Mice - Vulnerable				OFT	↑
						NSFT	↑
<i>Scharf et al. (2013)</i>	Male	CD1 Mice	P28 – P77	^a Social Instability	P78	OFT	↑
						EPM	=
						SI	=
						TST	=
					P450	EPM	=
<i>McCormick et al. (2008)</i>	Male	Long-Evans Rats	P30 – P45	^b Social Instability	P70	EPM	↑
	Female					EPM	↑
<i>Bourke & Neigh (2011)</i>	Male	Wistar Rats	P37 – P49	Mixed Modality	P96	EPM	=
						FST	=
						SPT	=
	Female				P96	EPM	↑
						FST	↑
						SPT	↑
<i>Vidal et al. (2007)</i>	Male	Wistar Rats	P45 – P47	Social Defeat	P78	SA	↑
<i>Ros-Simó & Valverde (2012)</i>	Male	CD1 Mice	P21 – P70	Social Isolation	P71	OFT	↑
						EPM	↑
						LDB	↑

Author:	Sex:	Strain + Species:	Age at stress:	Stress type:	Age at testing:	Test:	c Anxiety and depression:
						TST	↑
Lukkes et al. (2009)	Male	Sprague-Dawley Rats	P21 – P42	Social Isolation	P56	OFT	↑
						SI	↑
Butler et al. (2014)	Female	Long-Evans Rats	P31–P73	Social Isolation	P85	EPM	=
						NEL	=
						LDB	=
Hong et al. (2012)	Male	Sprague-Dawley Rats	P30 – P50	Social Isolation	P70	SPT	=
						FST	=
	Female					SPT	↑
						FST	↑

Note: NSFT = Novelty suppressed feeding test; OFT = Open field test; SI = Social interaction test; EPM = Elevated plus-maze; TST = Tail suspension test; SPT = Sucrose preference test; SA = Social Avoidance; LDB = Light-Dark Box; NEL = Novelty-evoked locomotion;

^aCage composition changed 2x/week for 7 weeks;

^b1 h daily social isolation + re-housing with a novel cagemates;

^c(↑) Increased depression-/anxiety-related behavior, (=) No change in behavior;

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Table 2

Correlations between fCORT concentrations and affect-related behaviors

		Females	Males
Diurnal Peak fCORT vs.			
EPM: Open Arm Time (%)	<i>r</i>	0.25	−0.01
	<i>R</i> ²	0.06	0.0001
	<i>p-value</i>	0.37	0.96
EPM: Open Arm Entries (%)	<i>r</i>	0.41	0.29
	<i>R</i> ²	0.17	0.08
	<i>p-value</i>	0.13	0.26
EPM: Head Dips	<i>r</i>	0.42	0.41
	<i>R</i> ²	0.17	0.18
	<i>p-value</i>	0.12	0.11
EPM: Closed Arm Entries	<i>r</i>	0.53	0.62
	<i>R</i> ²	0.28	0.38
	<i>p-value</i>	0.04 *	0.01 *
SPT: Sucrose Preference (%)	<i>r</i>	0.69	−0.20
	<i>R</i> ²	0.47	0.04
	<i>p-value</i>	0.01 *	0.47
FST: Time Spent Immobile (sec)	<i>r</i>	−0.20	−0.47
	<i>R</i> ²	0.04	0.22
	<i>p-value</i>	0.46	0.07
Diurnal Trough fCORT vs.			
EPM: Open Arm Time (%)	<i>r</i>	−0.08	−0.24
	<i>R</i> ²	0.007	0.06
	<i>p-value</i>	0.77	0.36
EPM: Open Arm Entries (%)	<i>r</i>	−0.29	0.34
	<i>R</i> ²	0.08	0.12
	<i>p-value</i>	0.27	0.20
EPM: Head Dips	<i>r</i>	−0.17	0.04
	<i>R</i> ²	0.03	0.002
	<i>p-value</i>	0.53	0.86
EPM: Closed Arm Entries	<i>r</i>	−0.004	0.44
	<i>R</i> ²	0.000	0.19
	<i>p-value</i>	0.99	0.09

		Females	Males
SPT: Sucrose Preference (%)	<i>r</i>	0.003	−0.08
	<i>R</i> ²	0.000	0.007
	<i>p-value</i>	0.99	0.76
FST: Time Spent Immobile (sec)	<i>r</i>	−0.22	−0.29
	<i>R</i> ²	0.05	0.09
	<i>p-value</i>	0.41	0.26

Pearson correlation coefficients and regression *R*² statistic comparing fCORT concentrations at the diurnal peak and trough to behavior in the EPM, SPT, and FST. Correlation coefficients were calculated for females and males separately. *n* = 13–16 pairs in each analysis.

* indicates *p* < 0.05.