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Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice

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Abstract

Exposure to stress is highly correlated with the emergence of mood-related illnesses. Because major depressive disorder often emerges in adolescence, we assessed the effects of social defeat stress on responses to depressive-like behaviors in juvenile mice. To do this, postnatal day (PD) 35 male c57BL/6 mice were exposed to 10 days of social defeat stress (PD35-44), while control mice were handled daily. Twenty-four hours after the last episode of defeat (PD45), separate groups of mice were tested in the social interaction, forced swimming, sucrose preference, and elevated plus-maze behavioral assays ($n = 7$–12 per group). Also, we examined body weight gain across days of social defeat and levels of blood serum corticosterone 40 min after the last episode of defeat stress. Our data indicates that defeated mice exhibited a depressive-like phenotype as inferred from increased social avoidance, increased immobility in the forced swim test, and reduced sucrose preference (a measure of anhedonia), when compared to non-defeated controls. Defeated mice also displayed an anxiogenic-like phenotype when tested on the elevated plus-maze. Lastly, stressed mice displayed lower body weight gain, along with increased blood serum corticosterone levels, when compared to non-stressed controls. Overall, we show that in adolescent male c57BL/6 mice, social defeat stress induces a depression- and anxiety-like phenotype 24 h after the last episode of stress. These data suggest that the social defeat paradigm may be used to examine the etiology of stress-induced mood-related disorders during adolescence.

Introduction

Exposure to stress has been linked to the etiology of mood-related disorders (Krishnan & Nestler, 2008). In particular, stressful events have been causally linked to anxiety-related and major depression syndromes (Tsoory et al., 2007). Throughout the literature, preclinical, clinical, and postmortem investigations have primarily examined how both stress and antidepressant pharmacotherapy influence neurobiological homeostatic mechanisms that in turn may underlie the neurobiology of major depressive disorder (Krishnan et al., 2007; Kupfer et al., 2012). To date, most of these studies have examined the underpinnings of mood-related disorders using adult populations. This is surprising given that epidemiological reports suggest that the first incidence/episode of depression is most often reported prior to adulthood (Lewinsohn et al., 1993; Paus et al., 2008).

Adolescence, the transitional stage between childhood and adulthood, is characterized by distinct neurobiological changes that underlie the emergence of sex differences (Eiland & Romeo, 2013; Spear, 2000), as well as other age-specific behavioral phenotypes, including increased social activity, playfulness and risk-seeking behavior (Doremus-Fitzwater et al., 2010; Laviola et al., 2002; Richards et al., 2012). As such, juveniles are more likely to experience stressful life events (Charney & Manji, 2004), and are simultaneously more sensitive to the deleterious effects of stress (Stone & Quartermain, 1997; McCormick et al., 2010). This makes adolescence a unique developmental stage to examine how specific stressors, such as social stress, influence and/or precipitate the development of mood-related disorders. At the preclinical level, the social defeat paradigm is considered one of the most robust models of stress-induced mood-related illnesses (Berton et al., 2006). When compared to other animal models of depression, such as chronic unpredictable mild stress (Willner et al., 1987) or the forced swim test (Porsolt et al., 1977), the social defeat paradigm possesses higher face, predictive, and ethological validity that results in enduring behavioral and neurobiological changes that mimic several symptoms of the human condition (Berton et al., 2006; Krishnan & Nestler, 2008). For example, in adult rodents, social defeat stress results in increased activation of

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the hypothalamic-pituitary-adrenal (HPA) axis, as inferred by elevated levels of blood serum corticosterone (Buwalda et al., 1999), in addition to decreased preference for sucrose (a measure of anhedonia; Willner et al., 1987), increased sensitivity to helplessness measures (Warren et al., 2013), and increased social avoidance – behaviors collectively described as a depressive-like phenotype (Krishnan & Nestler, 2008).

Although few investigations have examined the effects of early-life social defeat stress on mood-eliciting behavioral tasks in adulthood (Buwalda et al., 2013; Ver Hoeve et al., 2013; Watt et al., 2009), the social defeat model has not been thoroughly examined as a potential model of stress-induced depression within the juvenile period of development. Thus, the current investigation was designed to assess behavioral responsivity to a range of emotion-eliciting stimuli shortly (24 h) after chronic social defeat stress in adolescent (postnatal day [PD] 35) male c57BL/6 mice.

Materials and methods

Animals

Five week-old male c57BL/6 mice, and adult (retired) male CD1 breeders, were used in this study. The c57BL/6 mice were obtained from the Department of Psychology Mouse-Breeding Colony at California State University San Bernardino (CSUSB), while the CD1 retired breeders were purchased from Charles River Laboratories. Mice were housed in standard polypropylene cages containing wood shavings (c57BL/6, four per cage; CD1, one per cage) and placed on a 12-h light/dark cycle (lights on at 7:00 A.M.) with unrestricted access to food and water. Experiments were conducted in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and with approval of the Institutional Animal Care and Use Committee at CSUSB.

Social defeat stress and experimental design

Because social defeat stress follows the resident/intruder paradigm (Kudryavtseva et al., 1991; Miczek, 1979), where conflict stress involves the threat from a more dominant resident counterpart, we selected to use the inbred CD1 strain of mice as aggressors for this investigation (Parmigiani et al., 1999). Briefly, CD1 male mice with consistent attack latencies (≤30 s on three consecutive screening tests) were housed in cages fitted with perforated Plexiglas separators, which allow sensory contact without physical contact, and used to stress/defeat the experimental c57BL/6 mice. Specifically, adolescent (PD35) c57BL/6 mice were exposed to a 10 min long defeat episode, and then housed for the remainder of the day in the compartment next to the aggressor. In the event that the CD1 mouse was exhibiting overly aggressive attacks (continuous biting even after the experimental mouse displayed submissive posturing), the defeat bout was immediately terminated (Golden et al., 2011; Iñiguez et al., 2014). This procedure was repeated for 10 consecutive days (PD35-44) with different CD1 aggressors each day. The age at the start of social defeat stress (PD35) was selected because it roughly approximates mid-adolescence (Eiland & Romeo, 2013; Tirelli et al., 2003), a

Table 1. Experimental groups.

| Group	Subjects		Age	Interval	Procedure
1	Control	$n = 10$	PD 35–44	24 h	Social interaction
	Defeated	$n = 10$			
2	Control	$n = 8$	PD 35–44	24 h	Forced swim test
	Defeated	$n = 10$			
3	Control	$n = 10$	PD 35–44	24 h	Sucrose preference
	Defeated	$n = 12$			
4	Control	$n = 9$	PD 35–44	24 h	Elevated plus-maze
	Defeated	$n = 7$			
5	Control	$n = 10$	PD 35–44	40 min	Corticosterone assay
	Defeated	$n = 9$			

PD, postnatal day.

developmental stage in which the onset of major depressive disorder is most often reported in humans (Burke et al., 1990). Non-defeated (control) adolescent mice were handled daily and housed in similar cages, one on each side of a perforated Plexiglas partition. Immediately after the last defeat episode (i.e. PD44), both stressed (defeated) and non-stressed (control) mice were single housed. Twenty-four hours later (PD45), separate groups of c57BL/6 mice were randomly assigned to the different behavioral tasks described below. Separate groups of adolescent c57BL/6 mice were used for each experiment in order to avoid possible carry-over effects (Table 1). All behaviors were recorded via an automated video tracking system (Noldus®), except for the forced swim test, which was scored by observers blind to stress conditions. Lastly, for the corticosterone immunoassay experiment, a separate group of mice was killed 40 min after the 10th defeat episode (PD44) in order to examine the activation of the HPA axis as a function of social defeat stress (Krishnan et al., 2007; Warren et al., 2013).

Social interaction test

The social interaction test is used to assess social avoidance behavior (Berton et al., 2006). This is a two-step test (Krishnan et al., 2008), conducted under red light conditions. In the first 2.5 min session, a c57BL/6 mouse is allowed to freely explore an open field arena (40 cm length × 40 cm width × 40 cm height; Figure 1a shows the schematic). Along one side of the arena is a circular (7 cm diameter) wire cage (Stoelting Co., Wood Dale, IL) that remains empty during the first trial (target absent). The experimental c57BL/6 mouse is then removed from the open field arena and a novel CD1 male mouse is placed into the wire cage. In the second 2.5 min trial (target present), the experimental c57BL/6 mouse is reintroduced into this arena now containing a social target (unfamiliar CD1 mouse) within the wire cage. In this investigation, time (s) spent in the interaction zone (8 cm wide corridor surrounding the wire cage) and the corner zones (10 × 10 cm) were the dependent variables (Iñiguez et al., 2014). Additionally, we recorded the distance traveled (cm) during the first 2.5 min of the social interaction test to examine whether basal locomotor activity could be influenced by social defeat stress.

Figure 1. Social defeat stress induces avoidance behaviors in adolescent c57BL/6 male mice. (a) Schematic of the social interaction/avoidance arena illustrating the geographic location and size of the interaction zone and corner zones with respect to the enclosure (circular wire cage) in which a social target (CD1 mouse) is positioned. (b) Control (non-stressed; $n = 10$) mice showed significantly higher levels of interaction time ($p < 0.05$), whereas defeated mice ($n = 10$) spent significantly less time interacting ($p < 0.05$), in the presence of a social target. (c) This avoidance behavior as a result of social defeat stress was also evident when assessing time in the corner zones, in which defeated (stressed) mice spent significantly more time in the corner zones in the presence of the social target ($p < 0.05$). (d) No differences in locomotor activity (distance traveled in cm) during the first 2.5 min of the social interaction test was observed between the groups ($p > 0.05$). *$p < 0.05$, within group comparison (presence versus absence of social target). $\Psi p < 0.05$, between group comparison (control versus defeated). Data are presented as mean + SEM.
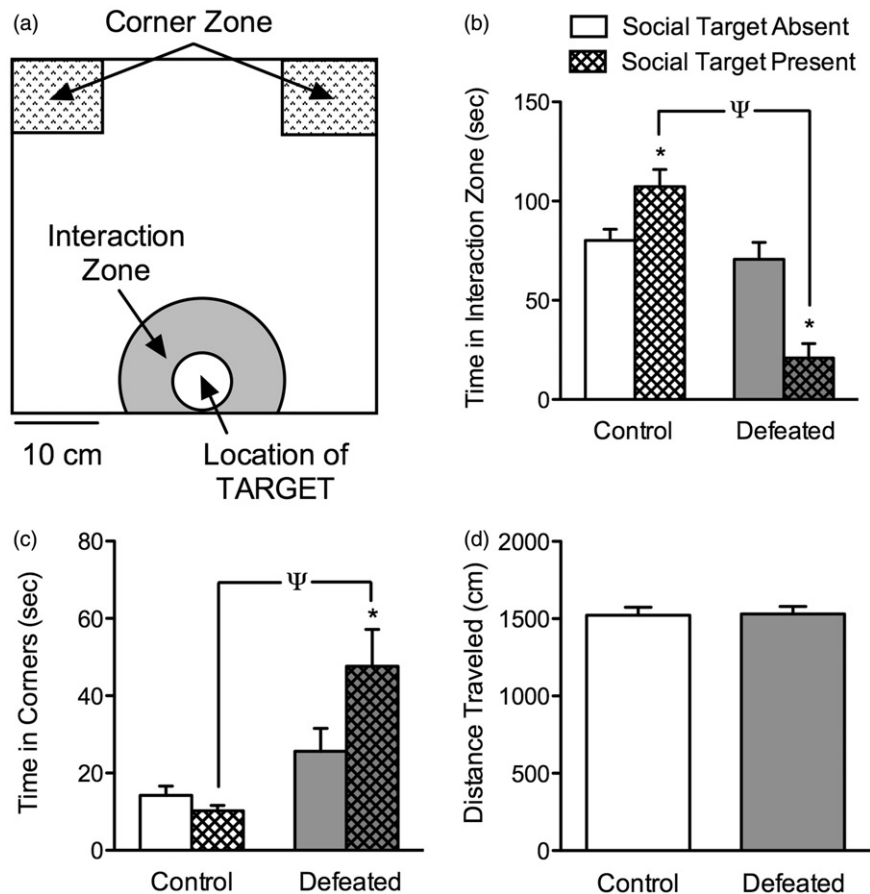
Forced swim test

The forced swim test is a behavioral procedure in which rodents are forced to swim under inescapable conditions. Initially, rodents engage in escape-like behaviors but eventually adopt a posture in which they make only the movements necessary to maintain their head above water; however, antidepressant treatment can significantly increase their escape-directed behaviors (Iñiguez et al., 2010a), an effect that has been correlated with antidepressant efficacy in humans (Porsolt et al., 1977). Conversely, an animal that spends more time immobile is considered to be more sensitive to the effects of inescapable stress (Iñiguez et al., 2010b). This task was carried out according to published protocols (Iñiguez et al., 2014). Specifically, mice were forced to swim once in a 4 L Pyrex glass beaker containing 3 L of water ($24 \pm 1\,^{\circ}C$) for 6 min. All cylinders were emptied and cleaned between mice. The time (s) to initially adopt a posture of immobility (latency to immobility), as well as the total time (s) spent immobile, during the last 5 min of the test, were the dependent variables.

Sucrose preference

The sucrose preference test was assayed using published protocols (Iñiguez et al., 2009). This test consisted of a 2-bottle procedure in which mice were given the choice between consuming water or a 1% sucrose solution. This test has been widely used across the literature to examine the effects of stress-induced anhedonia (Willner et al., 1987), a reduced ability to experience pleasure. Adolescent

mice were habituated to drink water from two bottles during the last 5 days of social defeat (PD40-44). On PD45, 24 h after the last social defeat episode, animals were single housed in a cage that had two drinking bottles. One of the bottles had water, while the other bottle had a 1% sucrose solution. Water and sucrose consumption was measured the following day (8:00 A.M.). The position of the sucrose bottle was counterbalanced (left versus right) across the different cages to control for potential side-preference bias. Preference for sucrose over water (sucrose/[sucrose + water]) was used as a measure for sensitivity to reward (Warren et al., 2011).

Elevated plus-maze

The elevated plus-maze is a classic test of anxiety-like behavior (Montgomery, 1955), that uses the natural reluctance of rodents to explore open spaces (Pellow et al., 1985). The maze (Stoelting Co., Wood Dale, IL) was made of gray plastic and consisted of two perpendicular intersecting runways (5 cm wide × 35 cm long). One runway had tall walls (closed arms; 15 cm in height), and the other one had no walls (open arms). The arms were connected together by a central area (5 × 5 cm), and the maze was elevated 40 cm from the floor. At the beginning of the test, under controlled light conditions (~90 lux), rodents were placed in the central area, facing one of the open arms, and the cumulative time (s) spent in the open- and closed-arms was recorded (Iñiguez et al., 2014). In addition, the total distance (cm) traveled was recorded throughout the 5 min test.

Corticosterone immunoassay

A separate set of adolescent c57BL/6 mice was used to examine how repeated episodes of social defeat would influence the activation of the HPA axis, as inferred by levels of trunk blood serum corticosterone (Backström & Winberg, 2013). Forty minutes after the 10th episode of social defeat (PD44), mice were decapitated, and trunk blood was collected into standard Heparin-coated collection tubes and placed on ice (Krishnan et al., 2007). Blood was centrifuged (1500g) for 15 min at 4 °C. Serum supernatant was collected, and stored at −20 °C, until corticosterone levels were assayed as previously described (Warren et al., 2013), per manufacturer's instructions (Assay Designs, Ann Arbor, MI).

Statistical analysis

Assignment of adolescent mice to the different experimental conditions was random. Data was analyzed using ANOVA techniques, with stress (control versus defeat), presence of social target (absent versus present; repeated measure), and body weight (across days of defeat; repeated measure) as sources of variance. Two-tailed Student's t-tests were used for analyses implicating two-group comparisons. Data are presented as the mean + SEM. Statistical significance was defined as $p < 0.05$.

Results

Social interaction

The effects of 10 days of social defeat stress during adolescence on social behaviors, 24 h after the last defeat (PD45), are shown in Figure 1(b–d). A two-way ANOVA, with stress and presence of social target as independent variables, indicated that the time spent in the interaction zone (Figure 1b) was dependent on a stress main effect ($F_{(1,36)} = 39.9$, $p < 0.0001$), and a stress by presence of target interaction ($F_{(1,36)} = 25.76$, $p < 0.001$). Post hoc analyses indicated that control (non-stressed) adolescent mice ($n = 10$) showed significantly higher levels of social interaction when the target was present (target absent versus present, $p < 0.05$). In contrast, social interaction levels were significantly reduced in mice exposed to social defeat ($n = 10$) in the presence of a social target (target absent versus present, $p < 0.05$), or when compared to non-stressed controls (between group comparison, $p < 0.05$).

The time spent in the corner zones (Figure 1c), another measure of social avoidance (Krishnan et al., 2008), also indicated that the total time spent in the corners was dependent on a stress main effect ($F_{(1,36)} = 17.77$, $p < 0.001$), as well as a stress by presence of target interaction ($F_{(1,36)} = 5.05$, $p < 0.03$). Specifically, socially defeated c57BL/6 mice spent significantly more time in the corner zones in the presence of the social target (target absent versus present, $p < 0.05$), or when compared to controls (target present, $p < 0.05$). Importantly, no differences in total distance (cm) traveled were observed between the groups during the target absent (2.5 min) condition of the social interaction test (Figure 1d; $p > 0.05$).

Forced swim test

Adolescent social defeat stress increases sensitivity to behavioral despair in the forced swim test (Figure 2). Twenty-four hours after the last exposure to social defeat stress (PD45), adolescent mice were exposed to a 6-min episode of inescapable swimming stress. Here, the defeated mice ($n = 10$) displayed shorter latencies (s) to adopt a posture of immobility ($t_{(16)} = 2.76$, $p < 0.01$; Figure 2a), and spent significantly more time (s) in the immobile position ($t_{(16)} = 2.32$, $p < 0.02$; Figure 2b), when compared to non-stressed control mice ($n = 8$).

Sucrose preference

Adolescent social defeat stress decreases preference for sucrose in adolescent c57BL/6 mice (Figure 3). Twenty-four hours after the last episode of social defeat stress, the defeated mice ($n = 12$) showed a significant decrease in preference for a 1% sucrose solution (Figure 3a), when compared to non-stressed controls ($n = 10$); $t_{(20)} = 3.73$, $p < 0.01$. No differences in total liquid (mL) intake (sucrose + water) were observed between the groups ($p > 0.05$; Figure 3b).

Elevated plus-maze

Figure 4 shows the effects of adolescent social defeat stress on the anxiogenic environment of the elevated plus-maze. When compared to non-stressed controls, ($n = 9$), socially defeated adolescent mice ($n = 7$) spent significantly more time in the closed arms ($t_{(14)} = 4.87$, $p < 0.001$; Figure 4a), while spending less time in the open arms ($t_{(14)} = 2.06$, $p < 0.02$; Figure 4b) of the maze. Importantly, no differences in total locomotor activity between defeated (stressed) and control mice were evident during the 5 min test ($p > 0.05$; Figure 4c).

Figure 2. Effects of adolescent social defeat stress on forced swimming behavior, 24 h after the last defeat episode (postnatal day 45). Defeated (stressed; $n = 10$) adolescent mice exhibited a depressive-like behavior as inferred by (a) lower time to adopt an initial posture of immobility and conversely (b) spending significantly more time in the immobile position, when compared to control (non-stressed; $n = 8$) mice. *Significantly different from controls ($p < 0.05$). Data are presented as mean time (s) + SEM.
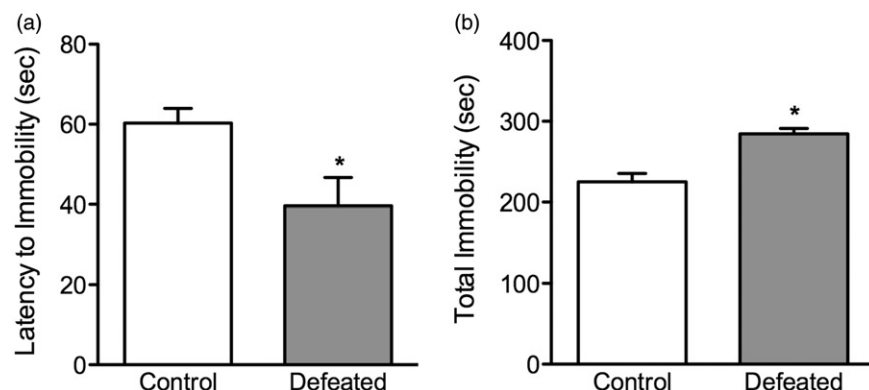
Figure 3. Effects of social defeat stress on sucrose preference in adolescent male c57BL/6 mice. (a) Twenty-four hours after social defeat (postnatal day 45), stressed (defeated; $n = 12$) adolescent mice displayed a decreased preference for a 1% sucrose solution, when compared to control (non-stressed; $n = 10$) mice. (b) No differences in total liquid intake (sucrose + water) were detected between the groups. *Significantly different from controls ($p < 0.05$). Data are presented as percentage or total mL consumed (mean + SEM).
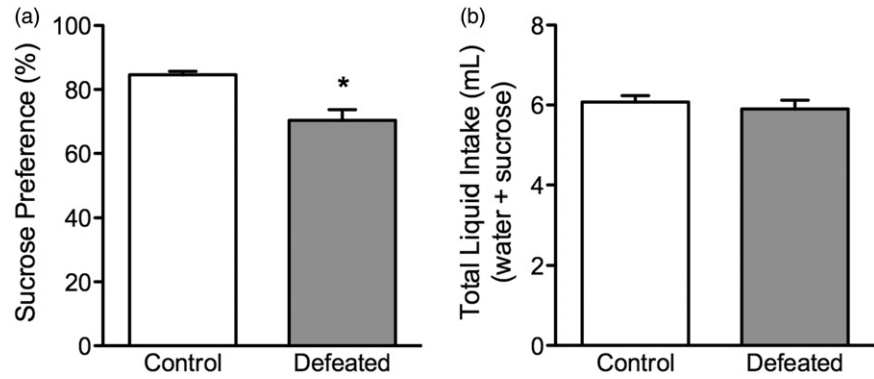
Figure 4. Effects of 10 days of adolescent social defeat stress on anxiety-like behavior in the elevated plus-maze. Twenty-four hours after the last social defeat episode, the stressed (defeated; $n = 7$) mice spent (a) significantly more time (s) in the closed arms, (b) while spending less time in the open arms of the maze, when compared to control (non-stressed; $n = 9$) mice. (c) No differences in the total distance (cm) traveled within the 5 min test were observed between the groups ($p > 0.05$). *Significantly different from controls ($p < 0.05$). Data are presented as mean time (s) and distance traveled (cm) + SEM.
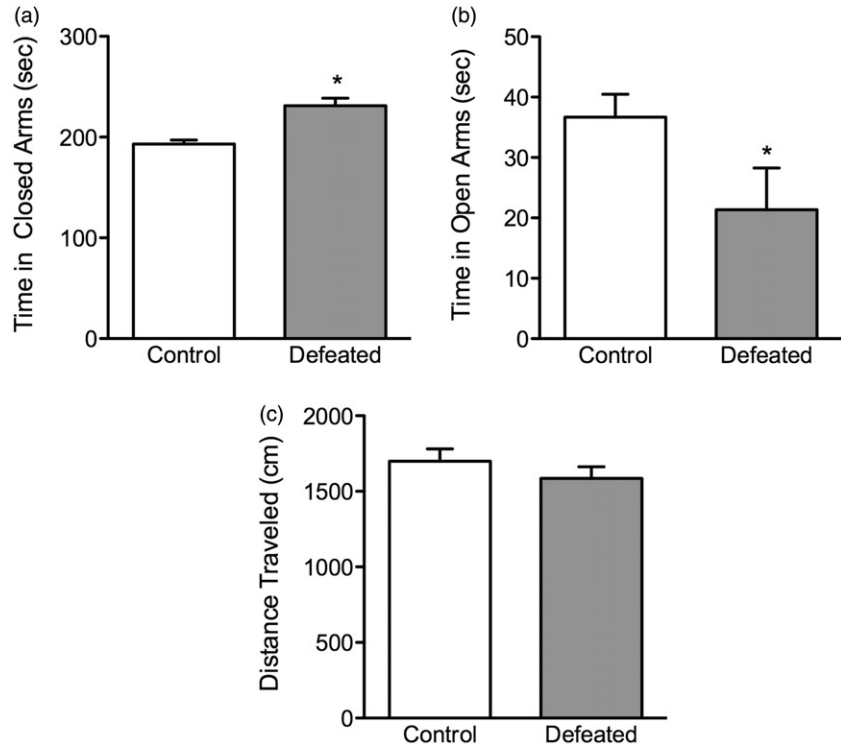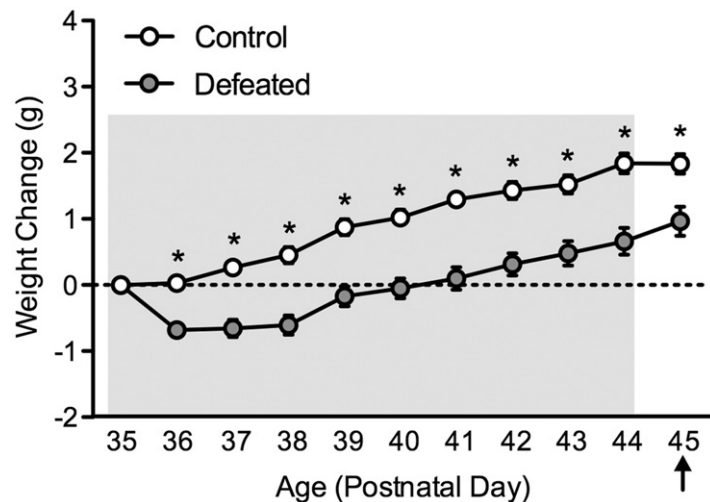
Figure 5. Effects of social defeat stress on body weight gain in adolescent male c57BL/6 mice. Social stress (postnatal day 35–44) resulted in significantly lower body weight gain across days of social defeat, starting on day 2 of stress exposure, when compared to non-stressed (control) mice ($n = 10$ per group). Body weight remained significantly decreased in the defeated group 24 h after the last day of stress exposure (postnatal day 45). Arrow indicates day of behavioral testing. *Significantly different when compared to controls ($p < 0.05$). Data are presented as weight change in grams (mean + SEM).

Body weight

Figure 5 shows the effects of 10 days of social defeat stress on body weight change in adolescent male c57BL/6 mice. Body weight was recorded throughout the 10 days of social defeat (PD35-44). Weight change was calculated by subtracting the body weight of the animal from the initial weight on PD35; thus a positive number would suggest weight increase, while a negative number would indicate body weight decrease (Warren et al., 2013). A mixed-design repeated measures ANOVA revealed that body weight changed as a function of stress (between group main effect: $F_{(1,18)} = 7.41$, $p < 0.001$), day of social defeat episode (repeated measure main effect: $F_{(9,162)} = 7.45$, $p < 0.01$), and a stress by day of defeat interaction ($F_{(9,162)} = 2.51$, $p < 0.01$). *Post hoc* analyses revealed that when compared to control mice ($n = 10$), defeated mice ($n = 10$) displayed lower weights as of the second day (i.e. PD36) of stress ($p < 0.05$, respectively). Twenty-four hours after the last episode of stress (PD45), adolescent mice exposed to social defeat weighed significantly less than the control mice ($t_{(18)} = 2.14$, $p < 0.05$).

Corticosterone immunoassay

Figure 6 shows the effects of social defeat stress on blood serum corticosterone levels in adolescent c57BL/6 mice. Forty min after the last episode of social defeat (PD44), the stressed mice (defeated, $n = 9$) exhibited significantly higher levels of corticosterone ($t_{(17)} = 2.59$, $p < 0.001$), when compared to non-stressed controls ($n = 10$).

Discussion

The present investigation was designed to evaluate whether the social defeat stress model (Berton et al., 2006), a common paradigm used to examine the behavioral, physiological, and molecular underpinnings of mood-related disorders (Krishnan & Nestler, 2011; Trainor et al., 2011), would result in a depressive-like phenotype in adolescent c57BL/6 male mice. This approach was taken because stress exposure during the juvenile stage of development increases the vulnerability to develop mood-related disorders, such as depression- and
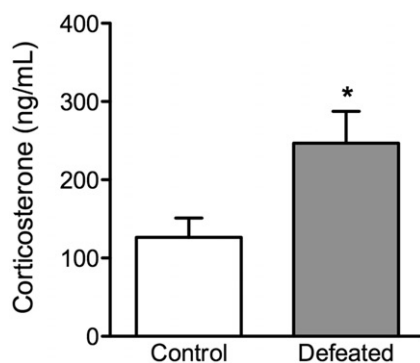
Figure 6. Effects of social defeat stress on adolescent neuroendocrine stress response. (a) Trunk blood of c57BL/6 mice was taken 40 min after the 10th episode of social defeat stress (i.e. postnatal day 44). Adolescent mice exposed to social defeat stress (defeated; $n = 9$) displayed significantly increased serum corticosterone levels when compared to non-stressed mice (control; $n = 10$). Data are presented as concentrations in ng/mL. *Significantly different than control mice ($p < 0.05$). Data are presented as mean + SEM.

anxiety-related syndromes (Spear, 2000; Wals & Verhulst, 2005). The social defeat paradigm was selected because social stressors are of particular relevance to juvenile populations, in the form of bullying and increased child-parent conflict (Gladstone et al., 2006). Our findings indicate that exposure to 10 days of defeat stress during adolescence (PD35-44) results in an overall depression- and anxiogenic-like phenotype as indicated by both behavioral and physiological responses that are commonly used to evaluate mood-related syndromes in murine models of depression (Krishnan & Nestler, 2011; Trainor et al., 2011).

When tested on the social interaction test, defeated adolescent mice displayed an avoidant behavioral response, by spending significantly less time in the interaction zone, while spending more time in the corner zones, when compared to non-defeated controls. This stress-induced avoidance behavior mirrors that of adult c57BL/6 male mice exposed to social defeat stress (Iñiguez et al., 2010b), wherein this response is long-lasting (Krishnan et al., 2007) and reversed by chronic, but not acute, administration of traditional antidepressants (Berton et al., 2006). Furthermore, when assessing sensitivity to a subsequent helplessness-related stressor, namely the forced swim test, we found that defeated adolescent mice displayed increased sensitivity to despair measures, as inferred from decreased time to adopt a posture of immobility and a total increased time spent immobile, when compared to controls (Figure 2). This depression-like behavioral phenotype resembles that of early-adult (∼PD56) c57BL/6 male mice exposed to a similar defeat stress regimen (Huang et al., 2013), yet here, we extend these findings to mid-adolescence (PD45), the developmental stage when the first incidence of depression is most commonly reported (Burke et al., 1990). Because social avoidance and increased helplessness behaviors are considered symptoms of numerous psychiatric illnesses in addition to major depression (APA, 2000; Berton et al., 2006), we assessed whether adolescent social defeat would influence sucrose preference in a two-bottle choice test as a complementary measure of depression-like behavior. Specifically, we selected this additional measure because anhedonia (Papp et al., 1991), the reduced ability to experience pleasure, is one of the core symptoms of clinical depression (APA, 2000), and rodents exposed to unpredictable environmental stressors typically exhibit a reduced preference for sweet solutions (Willner et al., 1987). Here, we found that defeated adolescent mice displayed a reduced preference for the sucrose solution, without changes in total fluid intake, when compared to controls (Figure 3). Collectively, the data from the social interaction, forced swim, and sucrose preference tests suggest that exposure to social defeat stress during adolescence results in the expression of core and common endophenotypes of major depressive disorder, which include avoidance, helplessness, and anhedonia (Krishnan & Nestler, 2008).

The co-occurrence of psychiatric illnesses, such as major depression and generalized anxiety disorder is very common in children and adolescents (Axelson & Birmaher, 2001). Consequently, an ''anxious-depression'' classification has been included in the new fifth edition of the diagnostic and statistical manual of mental disorders (DSM-5; Das-Munshi

et al., 2008). For this reason, when assessing stress-induced mood-related phenotypes at the preclinical level, anxiety-related tests, in addition to behavioral despair measures, are commonly implemented (Beuke et al., 2003). Thus, we examined how adolescent social defeat stress would influence responses to the anxiogenic environment of the elevated plus-maze (Montgomery, 1955). We found that defeated mice spent significantly less time in the open arms, while spending more time in the closed arms of the maze, when compared to controls – a classic anxiogenic-like response (Pellow et al., 1985). This increased sensitivity to anxiety-like behavior in adolescent mice mimics that of adult rodents reported after social defeat (Berton et al., 2006), highlighting that social defeat stress influences sensitivity to anxiety-inducing environments in adolescent mice in a similar manner as adults (Warren et al., 2013).

In addition to mood-related behaviors, physiological changes – such as fluctuations in body weight and levels of blood corticosterone – are commonly used to evaluate depression-related phenotypes. For example, weight disorders are a common characteristic of pediatric major depressive disorder (APA, 2000), and therefore, we monitored weight gain across days of defeat stress. Not surprisingly, we found that defeated mice displayed decreased body weight gain as of the second day of defeat, and remained significantly lower 24 h after the last episode of stress (PD45). This stress-induced weight reduction could potentially suggest a weight disorder that mimics another endophenotype of clinical depression (APA, 2000). It is important to note that although the defeated mice weighed significantly lower than the non-stressed group, we found no differences in basal locomotor activity as a function of stress exposure across the different behavioral tests, indicating that the depressive-like phenotype (i.e. social avoidance, helplessness, and decreased preference for sucrose) was not attributed to stress-induced decreases in body weight. In addition to fluctuations in body weight, another common physiological mechanism by which the brain responds to stress is the activation of the HPA axis (McCormick et al., 2010). Accordingly, we examined levels of blood serum corticosterone 40 min after the last episode of social defeat stress in a separate subset of adolescent mice (Group 5 in Table 1). This approach was taken because unlike most acute stressors, where maximal HPA activation occurs within the first 10 min post stress termination, there is a 20–40 min delay of maximal HPA activation after social conflicts (Heinrichs & Koob, 2006). Here, we found that adolescent mice exposed to social defeat stress displayed significantly higher levels of serum corticosterone when compared to non-defeated mice, reflecting the activation of the HPA axis as a result of social defeat exposure (Krishnan et al., 2007). This finding is likely mediating, at least in part, the depression- and anxiety-like phenotype observed across the different behavioral tests, since previous studies indicate that persistent activation of the HPA axis is associated with mood-related responses in rodents (Warren et al., 2013). While the neurobiological mechanism(s) underlying this defeat-induced depressive- and anxiogenic-output is not well understood, such behavioral responses in adult rodents have been correlated with alterations in post-receptor signaling molecules, namely brain derived neurotropic factor (BDNF)-related signaling (Eisch et al., 2003; Jiang & Salton, 2013), within the dopaminergic reward system (i.e. ventral tegmental area [VTA]–nucleus accumbens circuitry). In particular, stress-induced upregulation of VTA BDNF and several of its downstream effectors, including the extracellular signal-regulated kinase, have been found to mediate depression-like behaviors in both adult and adolescent rodents (Iñiguez et al., 2010b, 2014). Indeed, recent evidence links the stress-induced increases in glucocorticoids and depression-like behavior with dysregulation of dopaminergic neurons within the adolescent VTA (Niwa et al., 2013). Conversely, juvenile administration of the antidepressant fluoxetine (Prozac), the only Food and Drug Administration approved pharmaceutical for pediatric depression (Zito et al., 2006), regulates the activation of VTA BDNF-related molecules in an antagonistic manner (Warren et al., 2011; Iñiguez et al., 2014). However, we must emphasize that clinical diagnosis of depression- and anxiety-related syndromes are based on behavioral abnormalities only, with no known biologically based diagnostic distinctions (Krishnan & Nestler, 2008).

Murine models of depression have proven to be instrumental in the study of affective illnesses (Krishnan & Nestler, 2011). Here, we extend the social defeat paradigm as a valuable model to study adolescent depression- and anxiety-related syndromes. In adult rodents, social defeat stress induces, in most animals, the development of an array of behavioral and physiological changes that are reminiscent of depression- and anxiety-related symptoms. These animals are traditionally referred to as susceptible. However, social defeat also produces a small subgroup of animals that do not develop social avoidance, described as resilient mice (Krishnan et al., 2007). While the present results mirror those of adult rodents, future studies will be needed in order to examine if the resilient-like behavioral phenotype is evident in adolescent male c57BL/6 mice, as it is reported in early-(Huang et al., 2013) and mid-adulthood (Krishnan et al., 2007). Also, a limitation of the present study is that we did not assess the effects of social defeat stress in adolescent female subjects, thus hindering the interpretability of our results to the clinical setting, where twice as many girls (versus boys) are diagnosed with major depression (Hankin et al., 1998). Moreover, further characterization of social defeat stress on adolescent-specific behaviors, such as decision-making, play behavior, and sleep-wake patterns (Malkesman & Weller, 2009) will be required to further validate this paradigm as an adolescent model of affective distress. Meanwhile, it would be interesting to investigate the ability of potential antidepressant pharmaceuticals to acutely reverse the sequelae of juvenile social defeat stress, in a somewhat similar fashion as chronic administration of fluoxetine (Berton et al., 2006).

Conclusions

The use of animal models, though not without limitations (Krishnan & Nestler, 2011), is invaluable to our understanding of stress-induced mental illness (Krishnan & Nestler, 2008). However, until recently, most models of stress-induced

depression have primarily implemented stressors in adult-hood, rather than adolescence, the developmental stage when the first incidence of depression is most often reported (Kessler et al., 2001). Here, we demonstrate that exposure to 10 days of defeat is a potent social stressor in adolescent male c57BL/6 mice capable of inducing depression- and anxiety-like symptomatology (Krishnan & Nestler, 2011), making the social defeat paradigm a potential novel preclinical model to study adolescent stress-induced psychopathologies. Developing a suitable juvenile animal model of mood-related disorders is critical to understand and identify the basic neurobiological mechanisms underlying the etiology of anxious-depression (Das-Munshi et al., 2008), which in turn, will further assist in the development of more effective and safer pharmacological therapies for juvenile populations (Findling et al., 2006).

Acknowledgements

Declaration of interest

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