

Gains and Losses: Resilience to Social Defeat Stress in Adolescent Female Mice

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ABSTRACT

BACKGROUND: Adolescence is a unique period of psychosocial growth during which social adversity can negatively influence mental health trajectories. Understanding how adolescent social stress impacts males and females and why some individuals are particularly affected is becoming increasingly urgent. Social defeat stress models for adolescent male mice have been effective in reproducing some physical/psychological aspects of bullying. Designing a model suitable for females has proven challenging.

METHODS: We report a version of the adolescent male accelerated social defeat stress (AcSD) paradigm adapted for females. Early adolescent C57BL/6J female mice ($N = 107$) were exposed to our modified AcSD procedure twice a day for 4 days and categorized as resilient or susceptible based on a social interaction test 24 hours later. Mice were then assessed for changes in Netrin-1/DCC guidance cue expression in dopamine systems, for inhibitory control in adulthood using the Go/No-Go task, or for alterations in dopamine connectivity organization in the matured prefrontal cortex.

RESULTS: Most adolescent females showed protection against stress-induced social avoidance, but in adulthood, these resilient females developed inhibitory control deficits and showed diminution of prefrontal cortex presynaptic dopamine sites. Female mice classified as susceptible were protected against cognitive and dopaminergic alterations. AcSD did not alter Netrin-1/DCC in early adolescent females, contrary to previous findings with males.

CONCLUSIONS: Preserving prosocial behavior in adolescent females may be important for survival advantage but seems to come at the price of developing persistent cognitive and dopamine deficiencies. The female AcSD paradigm produced findings comparable to those found in males, allowing mechanistic investigation in both sexes.

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Adolescence is a unique and sensitive period during which adversity can greatly impact ongoing brain development and mental health trajectories. The ongoing changes in brain architecture and cognitive function, which are driven by the prolonged maturation of the prefrontal cortex (PFC) and gradual refinement in inhibitory control, render adolescents highly vulnerable to environmental challenges and more prone to developing psychiatric illness later in life (1–6). Adolescence is also an age of psychosocial growth when boys and girls spend more time socializing with peers than at any other age, and peers are their primary and most important source of appraisal (7). Unfortunately, peer victimization and cyberbullying are prevalent during adolescence (8–10) and can drastically increase internalizing disorders and psychiatric risk (10–15). However, there are important individual differences in response to social stress, with some adolescents being unaffected by social adversity and others who are susceptible being affected differently (16,17).

Multiple factors, including age (11), influence individual differences in adolescent resilience and susceptibility to psychiatric consequences of stress (6,18), with biological sex playing a major role. There are noticeable sex differences in the age of

onset and prevalence of psychiatric disorders that emerge in adolescence, including depression and substance use disorder (19,20). Although females exposed to adversity in adolescence show a higher risk of developing mood disorders than males (21–24), sex-specific sensitivity to environmental threats seems to depend on the type of stressor and on the physiological and/or behavioral outcome examined. While females show elevated levels of salivary cortisol in response to social rejection, males react more to achievement challenges (25). Conversely, cyberbullying is associated with depression and anxiety in girls more than in boys, but it is linked to conduct disorders in boys to a greater extent than in girls (26). Understanding why/how social stress in adolescence affects males and females differently, in both the short- and long-terms, is critical and timely, considering the increasing incidence in peer victimization and depression in youth and the need to inform early detection, prevention, and intervention programs.

Modeling social stress in adolescent male and female rodents can provide insights regarding the dimorphic sensitivity to adversity. Social interactions in rodents are particularly important during adolescence, when social behaviors such as play and social exploration emerge for the first time (27–30).

To reproduce certain physical and psychological aspects of the stress that is experienced by victims of bullying, researchers have used the chronic social defeat stress model in adolescent male rodents, in which a mouse (or rat) is subjected to repeated physical attacks and forced into submission by an aggressive mouse (31–42). Mice are then classified as social avoidant (susceptible) or nonsocial avoidant (resilient). We recently adapted the accelerated social defeat (AcSD) version of the chronic social defeat stress model to expose male mice to social stress during specific adolescent chronological periods (43,44) to be able to capture critical windows of vulnerability and assess possible molecular players (43–45). We found that AcSD specifically during early adolescence altered social behavior soon after exposure and had enduring detrimental consequences on impulse control (46,47). Although not all males exposed to AcSD showed impaired social behavior in adolescence, all defeated mice exhibited impulse control deficits in adulthood. This indicates that a socially avoidant phenotype is not a consistent measure of susceptibility to AcSD in adolescence and that there may be a tradeoff between protection against social deficits in adolescence and poor inhibitory control in adulthood (48). Indeed, all mice subjected to AcSD showed reduced expression of the Netrin-1 guidance cue receptor gene *Dcc* (46), which controls the protracted growth of dopamine axons from the nucleus accumbens (NAcc) to the PFC, an event that occurs in parallel to the gradual refinement of impulse control (49–51).

Because dominance hierarchies in C57BL/6J mice involve males fighting against males, but not females, building a model of adolescent social defeat stress in female mice has been challenging for many groups. Here we sought to overcome this limitation by modifying and adapting our adolescent AcSD male model to female mice. Using a combination of behavioral, molecular, anatomical, and cognitive measures, we were able to assess, for the first time, the short- and long-term impact of social defeat stress specifically in early adolescent female mice. We were also able to compare these effects to those that have been reported in male mice exposed to social stress during early adolescence. Previous findings have shown substantial sex differences in response to social stress in adolescent rats (52–57) and in sensitivity to the effects of drugs of abuse in mice (44). Therefore, we hypothesized divergent short and/or long-term outcomes of AcSD in female mice compared with the ones that we previously reported in males (46).

METHODS AND MATERIALS

Animals

Experiments were performed in accordance with the Canadian Council of Animal Care and approved by the McGill University and Douglas Hospital Animal Care Committee.

AcSD Stress Paradigm for Adolescent Female Mice

We used the AcSD stress paradigm (Figure 1A) that we used in our previous studies with male mice (46,47) but made modifications for early adolescent females. This paradigm consists of CD-1 screening and priming for aggressive behavior followed

by the AcSD (see the [Supplemental Methods and Materials in Supplement 1](#) for a full description). Briefly, during phase 1 of the priming and screening of CD-1 aggressor, an adult male C57BL/6J mouse is introduced to a male CD-1 mouse's home for 3 consecutive days. During phase 2, an adult male C57BL/6J mouse is introduced to the CD-1's home cage for only 30 seconds and is then immediately replaced by an early adolescent (postnatal day [PND] 24–25) female C57BL/6J mouse for 5 minutes. This priming is done twice a day (9:00 AM and 2:00 PM) and lasts 3 to 4 days until a subset of CD-1 mice become consistently aggressive toward the adolescent female mice. Adolescent female mice used during the priming phase are not used in the rest of the experiment.

The AcSD consists of 2 defeat sessions per day for a total of 4 consecutive days during which an adult C57BL/6J mouse is introduced into the CD-1 compartment for a period of 30 seconds (to prime the CD-1 for aggression) and is immediately replaced by an experimental female adolescent (PND 25) C57BL/6J mouse, which is left with the CD-1 for 10 minutes or until 10 attacks occur (Table 1). Control C57BL/6J adolescent females are housed in similar 2-compartment rat cages with a conspecific every day, and no physical interaction occurs between conspecific mice. Twenty-four hours after the last AcSD session, C57BL/6J adolescent mice are assessed in a social interaction task (SIT) to measure approach and/or avoidance behavior toward a novel adult male CD-1 social target (58,59). The amount of time spent (seconds) in the interaction zone is estimated when a target is absent and when the target is present. The amount of time between sessions 1 and 2 is approximately 1 minute. The social interaction ratio is calculated as the amount of time spent in the interaction zone with the target divided by the amount of time spent in the interaction zone without the target. Defeated mice with a ratio < 1.00 are classified as susceptible, and those with a ratio > 1.00 are classified as resilient (Figure 1B).

Elevated Plus Maze

Twenty-four hours after the SIT, females were tested in the elevated plus maze to assess risk taking-like behavior.

Go/No-Go Task

The Go/No-Go task was used to measure inhibitory control in adulthood as in (46,47,50,60). Responses to the Go cue were recorded as hits, and responses to the No-Go cue were recorded as commission errors and considered a measure of action impulsivity (61–63).

Molecular and Neuroanatomical Analysis

A description of Western blot, quantitative polymerase chain reaction, and stereology of PFC tyrosine hydroxylase immunofluorescence experiments is in the [Supplemental Methods and Materials in Supplement 1](#).

Experimental Design and Statistical Analyses

Statistical analyses are described in [Supplemental Methods and Materials in Supplement 1](#) and in [Supplement 2](#).

Sensitivity to Social Stress in Adolescent Female Mice

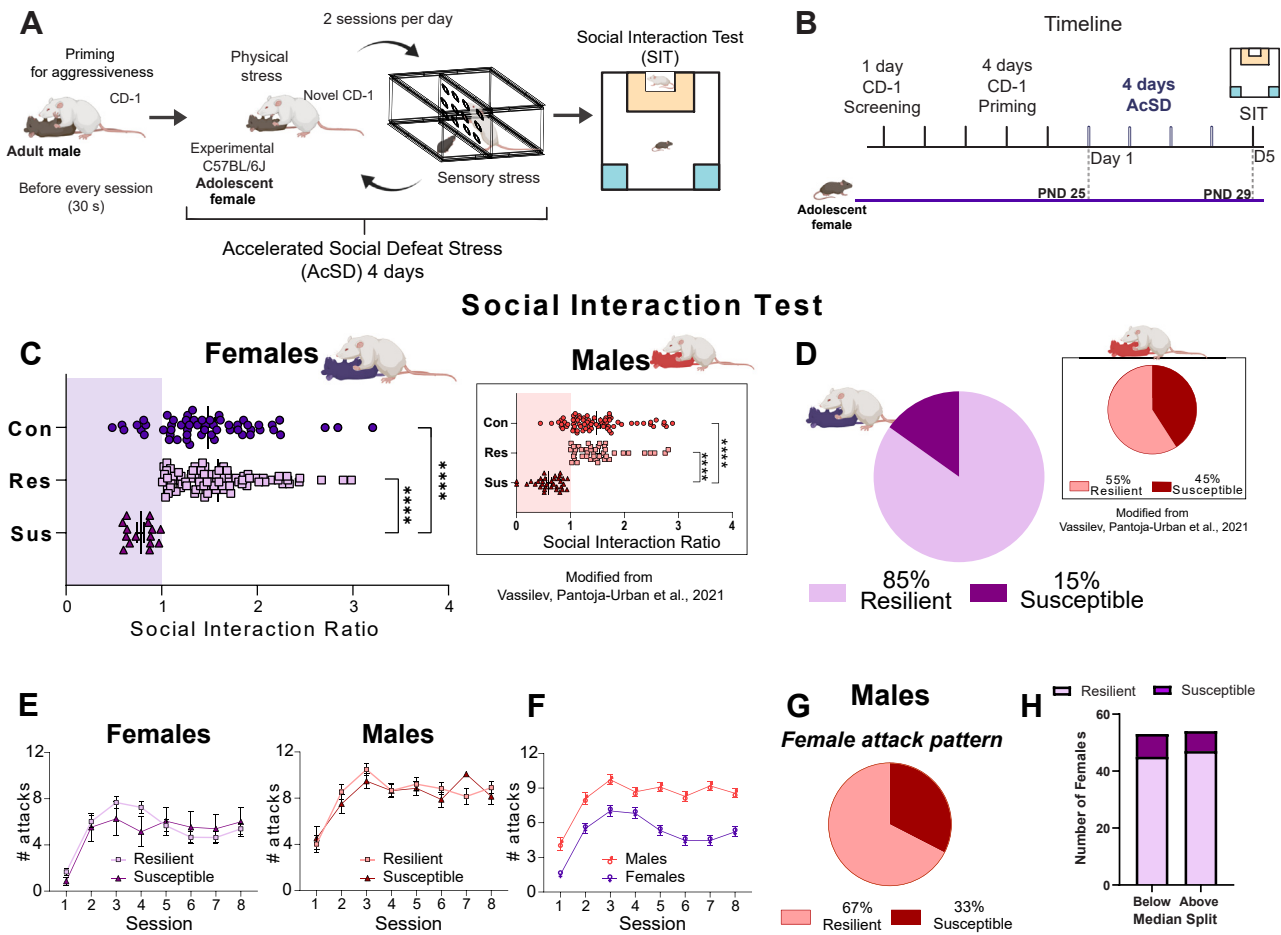


Figure 1. High proportion of resilient female mice after exposure to AcSD stress. **(A)** Graphic representation of AcSD stress paradigm for adolescent mice. **(B)** Timeline of AcSD stress in female mice. **(C)** SIT results after AcSD in female adolescent mice; significantly different at $***p < .0001$. Inset: Data reproduced from Vassilev *et al.* (46) showing SIT after AcSD for adolescent male mice (significantly different at $***p < .001$). **(D)** Proportion of resilient and susceptible female mice. The proportion of the resilient phenotype was higher in females than in males. Inset: Data reproduced from (46) showing that most males exposed to AcSD in adolescence showed resilience. **(E)** The number of attacks between resilient and susceptible mice is not significantly different in either females or males. **(F)** Female mice received fewer attacks than male mice during the AcSD. **(G)** Results from the AcSD experiment with adolescent males exposed to the “female” pattern of attacks. The resilient vs. susceptible proportion does not differ from the one that we found using the typical male AcSD protocol. **(H)** Median split on the cumulative number of received attacks in female mice. The proportion of females that were categorized as susceptible or resilient does not differ between the low and high received attack groups. All data are shown as mean \pm SEM. AcSD, accelerated social defeat; con, control; PND, postnatal day; res, resilient; SIT, social interaction task; sus, susceptible.

Table 1. Definition of an Attack During the Accelerated Social Defeat Paradigm

Attack Conditions	Description
1	An attack is defined as “when the CD1 mouse bites the C57BL/6J mouse, and the C57BL/6J mouse moves away in response to the bite.”
2	A bite is defined as “when the CD1 mouse places its teeth on any part of the C57BL/6J mouse’s body.”
3	Moving away is defined as “when the C57BL/6J mouse moves both of its hind paws from the position they were in before the CD1 engaged it.”
4	There must be ~2 seconds between separate attacks. If more than 1 bite occurs <2 seconds apart, count as 1 attack.
5	If the CD1 mouse bites more than once in succession without a break (<~2 s apart), gently separate animals.
6	If the CD1 mouse bites and does not let go, gently separate animals.
7	If the C57BL/6J mouse becomes trapped in a corner or is pinned down by the CD1 mouse and cannot move away in response to a bite, gently separate animals with a ruler.
8	In attack conditions 5–7, count as 1 attack (up to separation).

RESULTS

The AcSD Model Adapted for Female Mice Reveals That Most Females Do Not Show Stress-Induced Susceptibility in Adolescence

The modified AcSD model (Figure 1A) leads to consistent attacks from the CD1 aggressor to the experimental adolescent female mice—as operationally defined in Table 1—across all 8 sessions. The majority (85%) of female mice that were exposed to AcSD did not display social avoidance in the SIT (i.e., resilient phenotype). This finding was replicated in 6 different cohorts (Figure S1 in Supplement 1) and is shown as pooled data (Extended data Figure 1C in Supplement 2). The proportion of resilience in females is significantly greater than the proportion of resilience that we reported in early adolescent males [55% from (46)] (Extended data Figure 1D in Supplement 2). Importantly, there was no difference in the number of received attacks between resilient and susceptible male and female groups (Extended data Figure 1E in Supplement 2). However, when comparing the number of attacks between females and males [using the male data derived from our published study (46)], we found that females received fewer attacks than males across all defeat sessions (Extended data Figure 1F in Supplement 2).

To test whether reduced physical harm in females could account for their increased resilience, we adopted 2 strategies. First, we reproduced the “female” pattern of attacks in a cohort of adolescent males using the limited-attacks strategy that we have described previously (46). This manipulation does not significantly alter the proportion of resilient (67%) versus susceptible (33%) phenotypes in males (Extended data Figure 1G in Supplement 2). Second, we performed a median split on the cumulative number of received attacks by the females used in all the experiments ($N = 107$). The proportion of females that were categorized as susceptible or resilient did not differ between the low and the high received attack groups (Extended data Figure 1H in Supplement 2). This was also true when we compared the proportion of susceptibility and resilience between the 75th and 25th percentile of the female attack distribution (Extended data Figure 1H in Supplement 2). These results showed that the AcSD model can be used to study the effects of social defeat stress in adolescence in both males and females and that females are less vulnerable to exhibiting social avoidance to an adult aggressor male, regardless of the number of attacks received. Moreover, an awake, behaving social target is necessary for susceptible defeated females to show social avoidance following adolescent AcSD (Figure S2 in Supplement 1).

To assess whether exposure to AcSD in adolescent females alters risk taking-like behavior, we tested them in the elevated plus maze 48 hours after exposure (Figure 2A). The percent of time spent in the open arms was similar across the control, resilient, and susceptible groups (Extended data Figure 2B in Supplement 2), and there was no correlation between the amount of time spent in the interaction zone with the target present in the SIT and the percentage of time spent in the open arms (Extended data Figure 2C in Supplement 2). In our previous studies with males, we found increased risk taking-like behavior in the elevated plus maze test in the resilient group

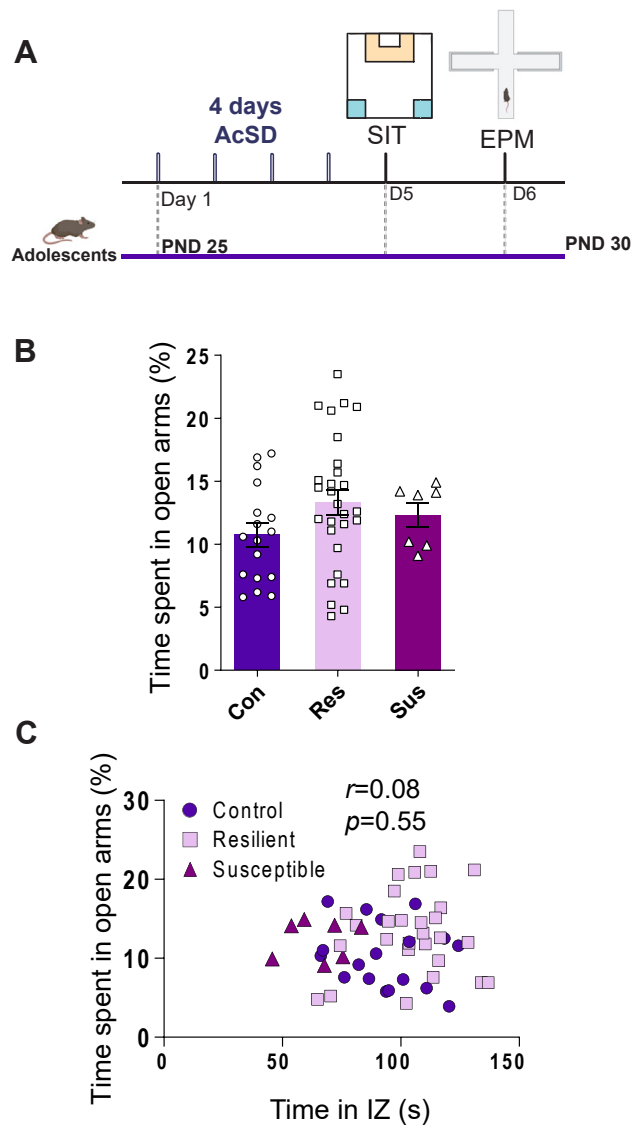


Figure 2. AcSD in adolescent female mice did not lead to changes in risk taking-like behaviors. **(A)** Experimental timeline. **(B)** AcSD in adolescent female mice did not lead to changes in risk taking-like behavior measured by the EPM test, $p = .18$. **(C)** There was no correlation between the amount of time spent in the open arms of the EPM and the amount of time spent in the IZ during the SIT in female mice, Pearson's $r_{53} = 0.08$, $p = .55$. All data are shown as mean \pm SEM. AcSD, accelerated social defeat; con, control; EPM, elevated plus maze; IZ, interaction zone; PND, postnatal day; res, resilient; SIT, social interaction task; sus, susceptible.

compared with both the control and susceptible groups, and this change was positively correlated with social avoidance behavior (46).

Social Stress in Adolescence Does Not Alter the Netrin-1/DCC Pathway in the Mesolimbic Dopamine System in Females

To determine whether AcSD in adolescence dysregulates the Netrin-1/DCC pathway in female mice, we measured *Dcc*

messenger RNA (mRNA) expression in the ventral tegmental area—where 99% of dopamine neurons express *Dcc* (64)—and Netrin-1 protein levels in the NAcc 1 week later (Figure 3A). We found no difference in *Dcc* mRNA or Netrin-1 levels across the control, resilient, and susceptible groups (Extended data Figure 3B in Supplement 2). These findings contrast with the selective dysregulation of the Netrin-1/DCC pathway that we have observed in males exposed to AcSD during adolescence (46). *Dcc* mRNA is highly enriched in ventral tegmental area dopamine neurons, with no difference between the sexes in the percentage of dopamine neurons expressing this transcript (64).

Only Resilient Females Show Inhibitory Control Deficits in Adulthood

We used the Go/No-Go task to assess the effect of AcSD in adolescence on inhibitory control in adulthood in female mice (Figure 4B). Resilient females exhibited a greater proportion of commission errors than controls in adulthood. This effect was evident in the last sessions of the task. Surprisingly, the proportion of commission errors in susceptible mice that showed social avoidance in adolescence was similar to that of control mice (Extended data Figure 4C in Supplement 2).

Next, because of the differences in sample sizes across groups and the large individual differences in action impulsivity in females, we performed an unbiased analysis of the No-Go

performance data to classify them into good versus poor performers. We conducted agglomerative hierarchical clustering analysis using the Ward method on the proportion of commission errors. The dendrogram revealed 2 main clusters (Figure 4D) ($k = 2$) corresponding to good and poor performers in the No-Go trials (Figure 4E). The majority of both control (85%) and susceptible (95%) mice were classified in the good performance cluster. In contrast, defeated mice that showed resilience (80%) were mostly classified in the poor performance cluster (Extended data Figure 4E in Supplement 2). These findings corroborate that female mice that do not show AcSD-induced social avoidance in adolescence are vulnerable to enduring deficits in inhibitory control. Instead, socially avoidant defeated females show protection against developing deficits in action impulsivity in adulthood. These findings raise the intriguing possibility that preserving social behavior in adolescence occurs at the expense of developing cognitive dysfunction in adulthood (48).

AcSD in adolescence did not alter the proportion of hits in females, indicating that defeated and control mice engaged in the task equally (Figure S3 in Supplement 1). To rule out differences in motivation for food reward, we tested adult female mice on a progressive ratio task. We found no difference in progressive ratio performance across groups (Figure S4 in Supplement 1).

In females, stress-induced susceptibility in adolescence predicts vulnerability to cognitive deficits in adulthood, with

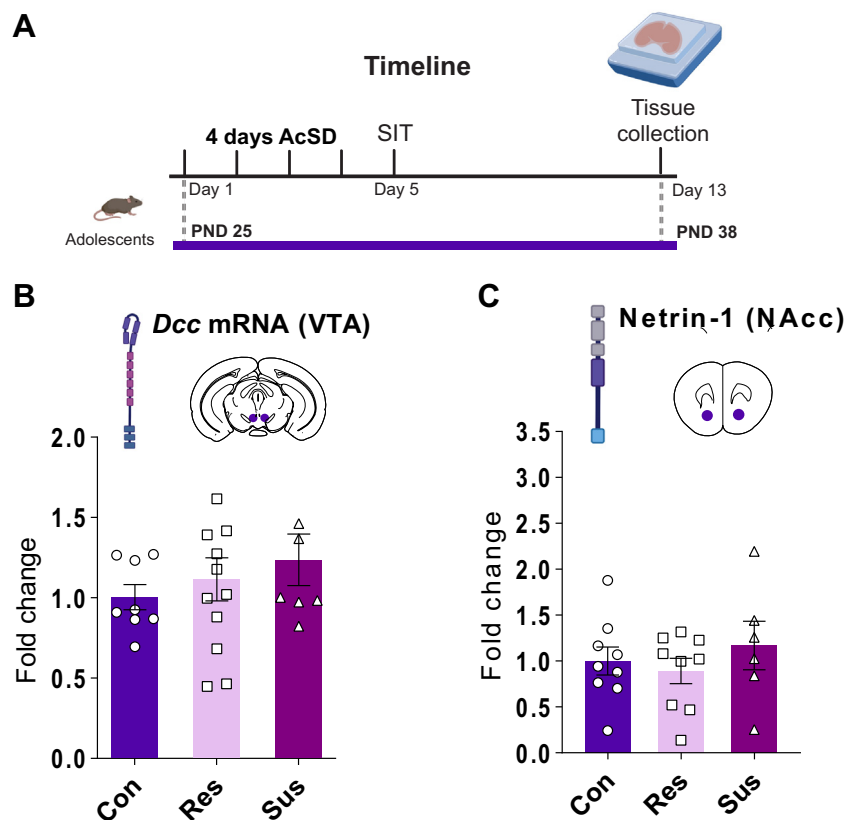


Figure 3. Following accelerated social defeat in adolescence, there were no changes in the Netrin-1/DCC system in female mice. **(A)** Experimental timeline. **(B)** Accelerated social defeat in adolescent female mice did not lead to changes in *Dcc* mRNA expression in the VTA. **(C)** Accelerated social defeat in adolescent female mice did not lead to changes in Netrin-1 protein levels in the NAcc. All data are shown as mean \pm SEM. AcSD, accelerated social defeat; con, control; mRNA, messenger RNA; NAcc, nucleus accumbens; PND, postnatal day; res, resilient; SIT, social interaction task; sus, susceptible; VTA, ventral tegmental area.

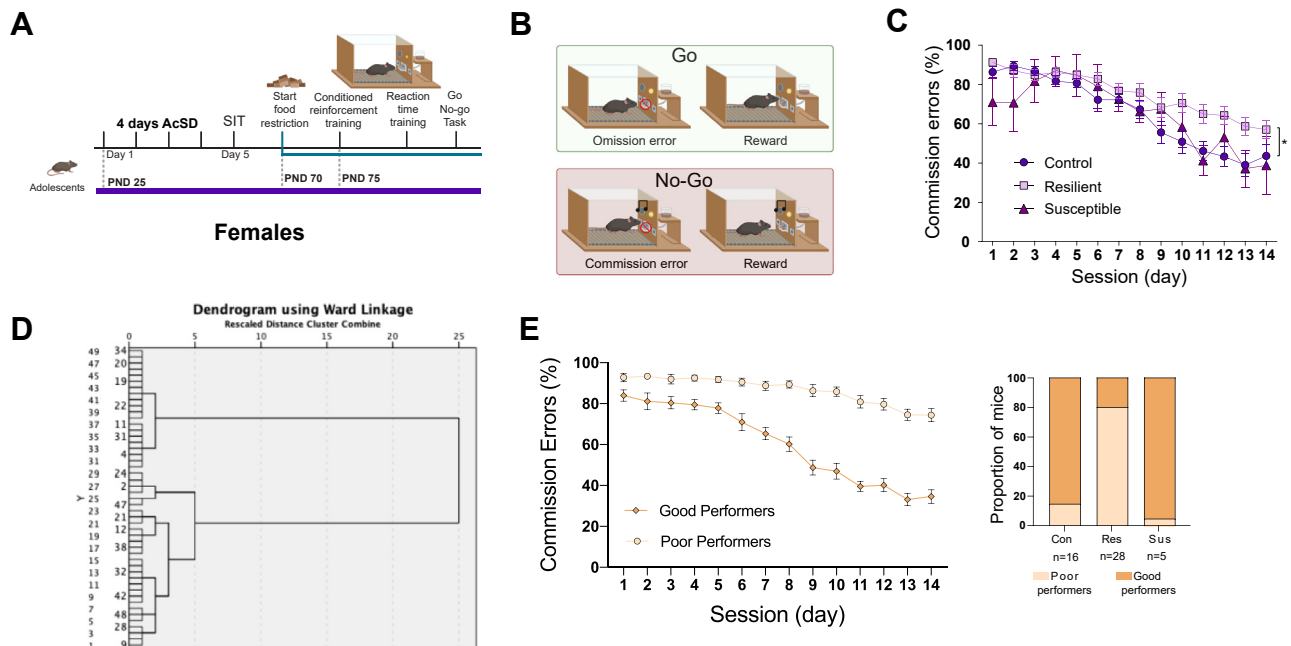


Figure 4. Only resilient female mice showed AcSD-induced deficits in impulse control in adulthood. **(A)** Experimental timeline. **(B)** Graphic representation of the Go/No-Go task. **(C)** Resilient female mice maintained a higher proportion of commission errors in comparison to control and susceptible mice, significantly different at $p < .05$. **(D)** Dendrogram showing hierarchical clustering of subjects based on percentage of commission errors during 14 days of the Go/No-Go task. **(E)** Cluster analysis classified cases into one of two groups, good and poor performers, based on commission error scores. Most resilient females were classified in the poor performance cluster, compared with most control and susceptible females, which were classified in the good performer cluster. All data are shown as mean \pm SEM. AcSD, accelerated social defeat; con, control; PND, postnatal day; res, resilient; SIT, social interaction task; sus, susceptible.

“protection” in adolescence predicting vulnerability in adulthood. In our study with males, we showed that defeated males developed deficits in action impulsivity in adulthood regardless of their behavior in the SIT in adolescence (46). Furthermore, hierarchical clustering analysis of the proportion of commission errors data collected in our previous study with males (46) show that most male control mice were classified in the good performance cluster, but almost all defeated mice were classified in the poor performance cluster (Figure S5 in Supplement 1). Therefore, resilience and susceptibility to short- and long-term effects of AcSD in adolescence are specific to the behavioral outcome and vary as a function of sex.

Resilient Females in Adolescence Show a Depletion of Presynaptic Sites in PFC Dopamine Axons in Adulthood

To assess whether AcSD in adolescent females affects the developing dopamine system, we quantified the expanse of the dopamine projection to the cingulate, prelimbic, and infralimbic subregions of the PFC in adulthood. We also examined the number and density of dopamine varicosities in these regions (Figure 5A, B). There were no significant differences across groups in the volume that dopamine axons occupied in the cingulate, prelimbic, and infralimbic subregions (Extended data Figure 5C in Supplement 2). Alterations in the volume that PFC dopamine axons occupy are a proxy for changes in mesocortical dopamine axon growth in adolescence, which is a process mediated by DCC receptor signaling (50). The lack of AcSD-induced changes in this metric is

consistent with the lack of changes in DCC receptor expression in dopamine neurons observed in adolescence. However, there is a trend suggestive of a reduction in the total number of dopamine varicosities in mice that display resilience in adolescence (Extended data Figure 5D in Supplement 2). In fact, dopamine varicosity density was reduced in adult females that displayed resilience to stress-induced social avoidance in adolescence (Extended data Figure 5E in Supplement 2). These findings contrast with our studies in males, in which defeated mice, regardless of social avoidance phenotype, showed both *Dcc* mRNA downregulation in dopamine neurons in adolescence and increased PFC dopamine input volume in adulthood (46).

Social Stress in Adolescence Leads to Increased Body Weight in Defeated Females

Although body weight during the AcSD did not differ between control and defeated females, in adulthood, all defeated mice showed an increase in body weight compared with controls (Figure 6A). To examine changes in weight gain, we compared body weight on the last day of the social defeat and in adulthood and found a significant increase in all defeated versus control groups (Extended data Figure 6A in Supplement 2). These delayed effects of AcSD on body weight were not identified in our previous study with male mice (43) (see Figure S5 in Supplement 1), although alterations in male body weight have been reported using a different social stress procedure and later timing of exposure than the ones that were applied in our study (32).

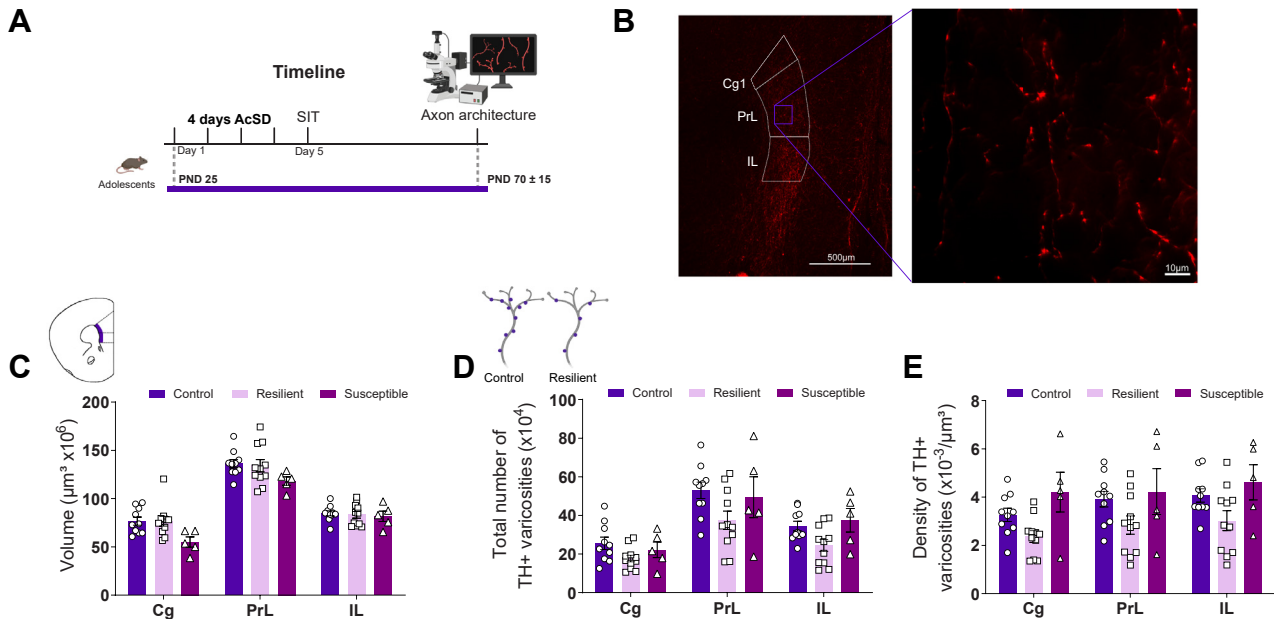


Figure 5. AcSD in adolescence depleted presynaptic sites from dopamine axons in the prefrontal cortex in adulthood, but only in resilient females. **(A)** Timeline of the experiments. **(B)** Photomicrographs show TH-immunolabeled dopamine axons in the Cg, PrL, and IL subregions of the prefrontal cortex (5× and 100× magnification). White arrows indicate examples of TH-positive varicosities. **(C)** There were no differences across groups in estimated dopamine input volume in any of the subregions. **(D, E)** Resilient female mice showed reduced **(D)** total number and **(E)** density of dopamine varicosities compared with control mice. All data are shown as mean ± SEM. AcSD, accelerated social defeat; Cg, cingulate; IL, infralimbic; PND, postnatal day; PrL, prelimbic; SIT, social interaction task; TH, tyrosine hydroxylase.

DISCUSSION

Models of social defeat stress in adolescence have been used in male mice and in male and female rats to reproduce certain physical and psychological aspects of the stress experienced by victims of bullying and domestic violence (31–42,52–55,57,65–67). Here, we were able, for the first time, to assess short- and long-term effects of social defeat stress in early adolescent female mice using a modified adolescent male AcSD paradigm (46,47). Most females showed resilience against stress-induced social avoidance in adolescence, but in adulthood, these resilient females developed inhibitory control deficits and showed a depletion of presynaptic sites in PFC dopamine axons. In contrast, female mice that would typically be classified as susceptible based on their SIT profile were protected against impulse control deficits and reduced PFC dopamine connectivity in adulthood. While AcSD in early adolescence alters *Dcc* mRNA expression in dopamine neurons in males (46), this was not observed in females, suggesting that during this early adolescent developmental period, the Netrin-1/DCC guidance system is not involved in the behavioral changes that are observed in the female resilient group.

Social defeat stress during the same chronological window during adolescence affects males and females differently, with a higher percentage of susceptible males (45%) than females (15%), independent of the number of attacks received. Although the source of this difference is unknown, preserving sex-specific adolescent social behavior trajectories may be important to cope with an organism’s environmental demands (27,30). A study with rats showed that social exploration in

males did not change across adolescence, but in females it peaked at approximately postnatal day 30–38 (29). The higher proportion of resilient females in our study may reflect a protective process aimed at maintaining a behavior that is relevant to females during this developmental stage. Favoring control levels of social behavior in adolescence, regardless of sex, must be important because rodents engage in social play and exploration in adolescence more than at any other age (27,29,56). Significantly more males exposed to the AcSD

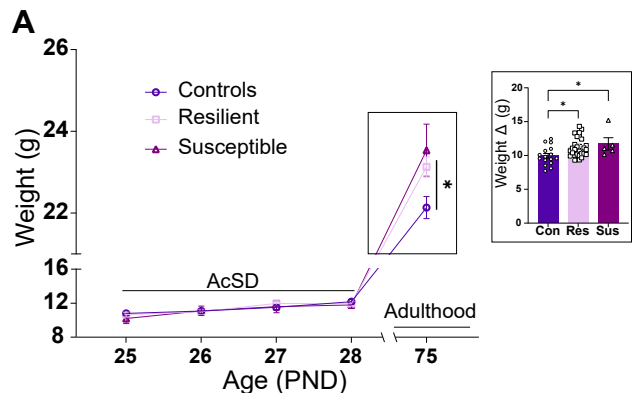


Figure 6. After AcSD, defeated female mice weighed more than the control mice. **(A)** Weights of females during AcSD and in adulthood. Inset: difference in weight between PND 75 and PND 28, showing that defeated mice gained more weight than controls; significantly different at **p* < .05. All data are shown as mean ± SEM. AcSD, accelerated social defeat; con, control; PND, postnatal day; res, resilient; sus, susceptible.

paradigm (or to chronic social defeat stress) in adulthood show susceptibility compared with those exposed in adolescence (46,58). This susceptibility is also greater in C57BL/6 female mice exposed to a modified version of the chronic social defeat stress paradigm in adulthood (68) or in California female mice exposed to defeat (65,66,69) than in female C57BL/6 mice subjected to AcSD in adolescence. Similar age-dependent effects have been described in female rats exposed to social defeat in adolescence versus adulthood (56). Social behaviors early in life seem to be protected whereas preserving cognitive processing is more prevalent at a later time point (46). Therefore, a resilient versus susceptible classification based on measures of performance at a specific age is problematic because protection and risk are domain specific and vary according to age and sex (16,17,70,71). We encourage caution against the use of social avoidance behavior as a main measure of stress vulnerability.

Facilitating social exploration in adolescent females seems to come at the expense of developing enduring cognitive deficits because most defeated resilient females showed poor adult impulse control. In contrast, females that showed susceptibility in adolescence performed at similar levels in the Go/No-Go task in adulthood as controls. In humans, a tradeoff has been shown between resilience to maladaptive behavioral outcomes in adolescence and increased vulnerability in other health domains, including metabolic disorders, in adulthood (72). Coping strategies in adolescence have been found to exert a sex-specific influence on the cognitive effects of adolescent stress (73,74). Why resilience to the detrimental effects of AcSD is specific to the behavioral outcome and why social protection may come at the price of developing adult cognitive impairment are thought-provoking questions that remain to be addressed. PFC dopamine plays a pivotal role in impulse control (75), which improves gradually from adolescence to adulthood, paralleling dopamine maturation (43,76,77). It is notable that in this study, we found that only females that were protected against social avoidance in adolescence and that developed cognitive impairment in adulthood showed impoverished adult PFC dopamine synaptic connectivity. The presence and absence of AcSD-induced social deficits in adolescence may be associated with a specific configuration of changes in mesocortical dopamine development, including structural changes in postsynaptic dopamine targets (35,78), and these alterations may be causally linked to adult cognitive phenotypes.

During adolescence, the PFC is still maturing and remains sensitive to environmental influence. The Netrin-1/DCC guidance cue pathway controls the extent of the mesocortical dopamine input in adolescence by promoting mesolimbic dopamine axon targeting in the NAcc, preventing them from growing ectopically to the PFC (45,50,60). Males and females show an identical target-dependent pattern of DCC receptor expression in mesocorticolimbic dopamine regions (44,79), suggesting that the overall role of the Netrin-1/DCC pathway in segregating mesolimbic and mesocortical projections is the same regardless of sex. AcSD in early adolescence downregulates *Dcc* levels and alters the extent of the dopamine input to the PFC, which is a proxy for ectopic mesolimbic dopamine axon growth to this region (46). However, in early adolescent females, the Netrin-1/DCC system is not altered in

defeated mice, which is consistent with the lack of changes in the expanse of the dopamine input to the PFC (i.e., volume) in adulthood. The impact of social stress during this early adolescent period on the developing dopamine system appears to be sexually dimorphic and to involve divergent molecular mechanisms [see (55)]. Perhaps local expression of guidance cues in postsynaptic partners of PFC dopamine axons is involved, including changes in the SLIT/ROBO signaling, which has been shown to play a role in social stress susceptibility in adult female, but not male, mice using a model of chronic variable stress (80). It is also possible that the Netrin-1/DCC system in females is sensitive to AcSD but at a different adolescent age. Exposure to amphetamine during adolescence induces sex- and age-specific alterations in ventral tegmental area *Dcc* expression and in NAcc Netrin-1 (44). In our studies, chronological age between male and female mice overlaps, but their biological developmental trajectories may be quite different, considering that the timing of dopamine development in adolescence in rodents is highly sex specific (29,81–85). An interplay between stress and the developmental effects of gonadal hormones is also likely to be implicated, including in the female-specific increase in adult body weight [for example, see (83,86–89)]. Changes in stress hormones in defeated females may also be involved in the effects of stress on dopamine and cognitive development (53,68,90). Detailed longitudinal assessment of gonadal and stress hormone profiles elicited by AcSD will shed light on this question and guide future functional/mechanistic experiments.

Understanding how adolescent social stress affects neurodevelopment, both early and enduringly, and why only some individuals are susceptible is urgently needed considering the high prevalence of bullying in North America (91). Adolescent social stress models that capture and quantify individual differences in stress sensitivity can provide insights into this issue. Studies with rats have shown that adolescent females but not males show social avoidance following chronic stress (52). However, females have shown greater sensitivity to developing depression-like abnormal traits and learning and memory deficits (53,54). While chronic adolescent stress in male and female rats enhances adult immune reactivity, the underlying mechanisms and physiological consequences are sex specific (55,57). Our AcSD model could be used with adolescent male and female mice. We showed that exposure to social stress during the same chronological window in adolescence affected the developing mesocortical dopamine system and cognitive control differently in males and females and appears to involve sex-specific molecular processes. We propose that individual differences in the way that social stress affects sociability in adolescence are associated with a unique pattern of developmental dopamine signatures and that these changes may predispose to or protect against cognitive harm later in life. Indeed, social behavior in rats is critical for setting up synaptic connections in the developing PFC and for establishing cognitive processing in adulthood (92). Our study highlights the importance of conducting longitudinal preclinical studies and including both sexes.

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AHP-U, SR, and CF designed the research; AHP-U, SR, AM, and MG performed the research and acquired data; AHP-U, SR, GH, and MG analyzed the data; and AHP-U, SR, and CF wrote the paper.

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Data for male mice presented in Figure 1C–F and Figures S4 and S5 in Supplement 1 have been modified from our previous publication (43). Figure illustrations were created using templates from BioRender.com. Melina Jaramillo Garcia (Molecular and Cellular Microscopy Platform in the Douglas Hospital Research Centre) helped set up the imaging experiments and the analysis.

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ARTICLE INFORMATION

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