A history of chronic adolescent stress attenuates cued fear-potentiated startle in adult female Wistar rats

Amy J. Wegener
Virginia Commonwealth University
Molly M. Hyer
Virginia Commonwealth University
Zuby Okafor
Virginia Commonwealth University
Grace Young
Virginia Commonwealth University
Chloe Burns
Virginia Commonwealth University
Emilie Bjerring
Virginia Commonwealth University
Samya Dyer
Virginia Commonwealth University
Paul Howell
Virginia Commonwealth University
Susie Turkson
Virginia Commonwealth University
Tanja Jovanovic
Wayne State University
Gretchen N. Neigh
gretchen.mccandless@vcuhealth.org
Virginia Commonwealth University

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Abstract

Post-traumatic stress disorder (PTSD) is a stress and trauma-related disorder that is largely characterized by hyperarousal to fear-based cues. N-methyl-D-aspartate (NMDA) receptors are key mediators of fear learning behaviors. Altered NMDA receptor signaling is a hallmark of PTSD pathology that impacts fear memory learning and extinction processes. In the current study, we observed that exposing adolescent rats to a mixed modality stress paradigm of repeated restraint, social defeat, and individual housing (chronic adolescent stress; CAS) decreases immunoreactivity of the NMDA subunit NR2B in the central amygdala in adulthood. We sought to determine the impact of a peripherally administered NMDA agonist D-cycloserine (DCS) intervention to enhance extinction of learned fear in CAS animals. Upon reaching adulthood, CAS animals were exposed to a cued fear-potentiated startle paradigm. DCS was administered immediately following the first of two extinction sessions with the goal of enhancing the extinction learning. CAS females expressed blunted cued fear conditioning compared to non-stressed females, but were not influenced by the DCS intervention. DCS may have reconsolidated cued fear associations in CAS males, however, males generally were able to successfully learn fear extinction regardless of stress history. These data suggest that chronic stress during adolescence modifies NMDA receptor expression, but functional impacts on cued fear learning differ by sex and require additional approaches to better understand the mechanisms underlying stress-induced impairments in fear learning behaviors.

Introduction

Individuals with PTSD can exhibit exaggerated fear reactivity to stimuli they associate with a traumatic memory (Van Der Kolk, 2000). The amygdala orchestrates the encoding of associative fear learning and is strongly implicated in PTSD pathophysiology (Morey et al., 2012; Ousdal et al., 2020). PTSD is linked to a stronger connectivity of the basolateral amygdala (BLA) complex with prefrontal regions involved with emotional processing, which influences behaviors observed in people with PTSD (Brown et al., 2014; Delgado et al., 2008). The adolescent period is a critical window for brain development (Gogtay et al., 2004) during which the amygdala undergoes substantial dynamic development (Pattwell et al., 2016) and enhanced sensitivity to negative stimuli (Silvers et al., 2017; Vink et al., 2014). As a result, adolescents are particularly sensitive to the impacts of a traumatic experience on brain development increasing susceptibility to future stressors (Mancini et al., 2023) which can lead to altered cognitive function that is sustained into adulthood (Chaby et al., 2015; Hyer et al., 2021). Impairment in cognition may present as the inability to properly integrate new information (Espejo et al., 2016), as reflected in deficient learning of safety cues and behaviors with PTSD (Glover et al., 2015; Jovanovic et al., 2012).

The amygdala's role in processing fear memory consolidation and extinction can be disrupted by a history of adolescent stress, setting the groundwork for altered capacity to process trauma. Understanding the mechanisms by which adolescent stress serves as a precursor for susceptibility to PTSD will provide insights into vulnerability in adulthood. It is well-established that fear memory acquisition, consolidation, retrieval, and extinction learning are dependent on synaptic NMDA receptors

in the amygdala (Garelick & Storm, 2005; Lee et al., 2006; Mamou et al., 2006). Preclinical stress studies demonstrate that stress alters expression of NMDA receptors and decreases postsynaptic functionality, contributing to observed behavioral deficits across stress paradigms (Almeida-Santos et al., 2019; Nasca et al., 2015; Tse et al., 2019). The NMDA receptor consists of binding sites for the excitatory neurotransmitter glutamate at the NR1 subunit and a co-agonist at the NR2 subunit. Binding at both sites is required to activate the receptor on a postsynaptic neuron. The partial co-agonist D-cycloserine (DCS) can enhance activation of NMDA transmission. Efficacy of DCS in fear potentiated startle paradigms has been reproduced across a myriad of fear potentiated startle paradigms, indicating the role of NMDA receptors in fear acquisition and extinction (Lee et al., 2006; McCallum et al., 2010; D. L. Walker et al., 2002). In the clinic, DCS has been used as an adjuvant therapeutic to cognitive behavioral or exposure therapy and produces modest effects (De Kleine et al., 2012; Otto, Pollack, et al., 2016). For example, in a randomized clinical trial in Veterans with PTSD, DCS enhanced within-session extinction and reduced potentiated startle responses post treatment (Rothbaum et al., 2014). As such, DCS has potential to augment behavioral therapy, but additional research is required to elucidate proper timing of the therapeutic for clinic effectiveness. Furthermore, while there are known sex differences in stress responsivity (Bekhbat et al., 2020; Conrad et al., 2003; Shors et al., 2001), there is limited information on the effectiveness of treatments for PTSD based on sex.

Adolescent stress is associated with development of PTSD in adulthood (Bremner et al., 2003; Nemeroff et al., 2006) and the ability to integrate new information during reconsolidation of a retrieved memory is disrupted by a history of stress (Akirav et al., 2009; Espejo et al., 2016). Previous work from our laboratory indicates that a mixed modality chronic adolescent stress paradigm (CAS) in Wistar rats leads to a weakening of synaptic strength in adult females, attributed to glutamatergic synapse remodeling in the hippocampus. The study also demonstrated a modest decrease in labeling of NMDA1a immunoreactivity in stressed males (Hyer et al., 2021). Our observations in the aforementioned study were completed in the hippocampus, but we postulated that regions that coordinate processing of fear memory, such as the amygdala complex, were likely impacted by the CAS paradigm. Given the shifts in NMDA function following CAS and the importance of NMDA activity for conditioned fear learning and memory, we hypothesized that a history of CAS would lead to disruptions in the ability to eliminate previous associations of a negative stimulus with the presentation of a non-threatening cue in the well-established fear potentiated startle paradigm (Vega-Torres et al., 2018; D. Walker & Davis, 2002; D. L. Walker et al., 2002). Additionally, we sought to determine the capacity of a DCS intervention to enhance extinction learning in male and female Wistar rats with a history of chronic adolescent stress.

Methods

Animals

Male and female Wistar rats were born to timed pregnant females (Charles River, North Carolina). Upon birth, the litters were culled to four pups per sex (8 pups total). On postnatal day (PND) 22, pups were weaned and housed in same sex pairs. Animals were housed in the vivarium on a 14:10 light cycle, with

temperatures maintained between 20–23°C. All animals were provided food and water *ad libitum*. All experiments were approved and performed in accordance with the Institutional Animal Care and Use Committee of Virginia Commonwealth University (VCU), as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Chronic Adolescent Stress (CAS)

Animals underwent a 12-day mixed modality CAS paradigm that has previously been described by our group (Bourke & Neigh, 2011; Hyer et al., 2021; Wegener et al., 2024). Upon PND 35, rats assigned to the stress group were individually housed. Beginning on PND 38 until PND 49, a developmental period that corresponds to adolescence in humans (Sengupta, 2013), CAS rats experienced randomized sessions of physical restraint (6 days) and social defeat (6 days). For the restraint stress, animals were kept in narrow plastic restraint tubes for 60 minutes (Braintree Scientific, Braintree, MA) that did not compress the rat but prevented the rat from being able to turn around 180°. During the social defeat procedure, a perforated plastic barrier was placed in the home cage of a same sex adult Long Evans rat (Charles River, Morrisville, NC). The experimental adolescent animal was placed on the opposite side for two minutes to allow for exchange of sensory information without direct contact. The barrier was then removed, and the rats could interact for five minutes or until the Long Evans rat successfully pinned the experimental rat three times, whichever came first. A pin was defined as the Long Evans rat placing the Wistar rat into a supine position, temporarily immobilizing them. After the five minutes, or three pins, the barrier was replaced for an additional 25 minutes to allow for sensory exchange. All animals were then returned to their home cages. On PND 60, the non-stressed animals were individually housed, and all groups received enrichment compliant with the VCU Division of Animal Resource guidelines and consisted of a nylabone and cardboard tunnel.

Litters were counterbalanced across experiments. In experiment 1, non-stressed (NS) and CAS animals underwent the startle paradigm (described below): NS males n = 10, CAS males n = 10, NS females n = 8, CAS females n = 8. At the end of the startle paradigm a subset of experiment 1 animals was used to assess immunoreactivity of the NMDA subunit NR2B in the amygdala. Following review of that data, experiment 2 queried the use of a NMDA targeted intervention. In Experiment 2, NS and CAS animals received a saline or DCS intervention immediately following the first extinction session: NS saline-treated males n = 11, NS DCS-treated males n = 17, CAS saline-treated males n = 12, CAS DCS-treated males n = 19, NS saline-treated females n = 9, NS DCS-treated females n = 15, CAS saline-treated females n = 11, CAS DCS-treated females n = 15. Based on power calculations for females in Experiment 1, sample size was increased for Experiment 2.

Startle Paradigm

On PND 83–92 the animals were exposed to the fear-potentiated startle fear conditioning paradigm. Acoustic startle responses were measured with a Startle Response System (San Diego Instruments Inc.,

San Diego, CA) that maintains constant background white noise at 55dB. Inside the chamber there was a plastic tube in which the animals were placed during the experiment. The tube contains a metal grate to measure the force of pressure applied by the animal. Given the dependence of pressure on animal weight, all recorded startle outputs (mV) were normalized to animal body weight (grams).

Animals were habituated to the startle boxes with chamber lights on for five minutes on two consecutive days. Following habituation, the fear conditioning procedure was initiated and occurred across 6 consecutive days: Day 1 - baseline; Day 2 - fear conditioning, Day 3 - fear acquisition test, Day 4 and 5 - extinction training, and Day 6 – extinction recall (Fig. 1A).

Baseline acoustic startle was recorded following exposure to an acoustic probe of either low, medium, or high volume (90dB, 95dB, 105dB respectively) that played every thirty seconds. Each volume was assigned across 10 randomized trials (30 trials total) per animal on day 1. Acoustic startle response was normalized to the animal's body weight, and all cue level trials were averaged together to produce an average weight corrected value per animal.

On Day 2, the 20-minute fear conditioning session consisted of 10 trials of a flashing LED light (conditioned stimulus, CS) which preceded a foot shock of 0.6 mA (unconditioned stimulus, US) at random intertrial intervals. The following day, Day 3, fear acquisition was tested. Similar to the baseline day, an acoustic probe of low, medium, or high volume was randomized across 30 trials. For half of the trials, each acoustic probe was paired with the flashing LED light (CS+) used during fear conditioning. During the noise alone (NA) trials the acoustic probe was presented without the LED light. The fear-potentiated startle (FPS) was determined by dividing the differential startle response (average acoustic startle for CS + trials – average acoustic startle of the NA rials) by the average startle response for NA trials.

On days 4 and 5, animals completed extinction training. Animals were placed in the startle boxes with the LED light flashing on every thirty seconds for three seconds without a shock to train the animals to form a new (safety) association between the conditioned stimulus and absence of shock.

On the final day of the paradigm, extinction recall was assessed, and an average FPS value was determined per animal.

Immunostaining

The day after the safety learning test the animals were euthanized by rapid decapitation. One brain hemisphere was post-fixed in 4% paraformaldehyde for at least twenty-four hours before transfer to phosphate buffered saline (PBS) for 24 hours. A subset of brains from experiment 1 were saturated in 20% sucrose solution and sectioned at 40µm on a Leica CM1950 cryostat. Brain sections containing the amygdala were collected and heated in a citrate buffer, rinsed with PBS, then placed in blocking buffer at room temperature for an hour. Tissue was then incubated overnight with a monoclonal anti-NMDAR2B antibody (BioLegend Cat. No. 818701). On the following day, sections were rinsed in PBS, then incubated

overnight with Alexa Fluor 488 goat anti-mouse secondary antibody (ThermoFisher AB_2534088) at room temperature, rinsed again in PBS, and coverslipped with Vectashield HardSet Antifade Mounting Medium with DAPI (Vector, cat # H-1500).

Basolateral and central regions of the amygdala were imaged on a Zeiss LSM 700 by taking 10μm image stacks with 1μm intervals at 63x oil immersion. Regions were identified using Fig. 57 in the Paxinos and Watson Rat Brain Atlas (6th edition). Two image stacks were taken per section and two sections per region were acquired by an individual blinded to the experimental conditions, totaling four image stacks per region. Image stacks were analyzed using Volocity (Quorum Technology Inc., Ontario, Canada) to determine the density of NR2B as indicated by the volume of fluorescence within the image stack. Parameters within Volocity were set to exclude objects larger than 490μm^3 and to count intensity threshold between 30,345 – 65,535 for the BLA and 44,525 – 65,535 for the CeA. These settings were implemented based on negative control images to exclude blood vessel and background labeling, respectively. Volumes across the four images per region were averaged and each averaged value per animal was used for analysis.

D-cycloserine Intervention

In experiment 2, DCS (Sigma Aldrich, St. Louis, MO), a co-agonist at the NR2 subunit of the NMDA receptor which can enhance activation of NMDA transmission, was prepared in saline and administered at 15 mg/kg (or equal volume of saline in saline-treated animals) via subcutaneous injection immediately following day one of extinction training. This dosage has shown effectiveness in previous fear conditioning studies (Graham & Scott, 2018b; Lehner et al., 2010; Tang & Graham, 2019). The timing of the administration was chosen based on previous studies demonstrating efficacious impacts on safety learning (Ledgerwood et al., 2005; McCallum et al., 2010).

Statistical Analysis

All data were analyzed using GraphPad Prism Software (San Diego, CA). Four outliers were identified in Experiment 2 using a ROUT outlier assessment in Prism set at 1%: 1 NS-saline male and 1 CAS-saline male during fear conditioning test, as well as 1 NS-DCS and 1 CAS-DCS female during extinction recall. These animals were removed from the fear conditioning versus extinction recall comparisons. A two-way Analysis of Variance (ANOVA) was used to analyze for impacts of the variables stress and sex on immunohistochemistry, baseline acoustic startle, and the fear conditioning test. A repeated measures two-way ANOVA was used to compare the variables stress and session on fear conditioning versus extinction recall comparisons in Experiment 1. A repeated measures three-way ANOVA was used to compare the variables stress, treatment, and session on fear conditioning versus extinction recall test outcomes in Experiment 2. Significance was analyzed using an alpha level of 0.05.

Results

Body weights

Males and females were weighed every week for 8 weeks throughout the time course of the studies. Males gained weight over time ($F_{(2.2, 173)} = 3016$, $p < 0.0001$; Fig. 1B), but were impacted by a history of stress such that CAS males gained less weight over the course of the study ($F_{(1, 77)} = 7.727$, $p = 0.0068$). Females also gained weight over time ($F_{(2.8, 176)} = 1592$, $p < 0.0001$; Fig. 1C), but were not impacted by stress ($p > 0.05$). These sex specific impacts of CAS on body weight are consistent with our previous reports of CAS animals (Hyer et al., 2021; Wegener et al., 2024).

Experiment 1 Startle Paradigm

For baseline assessment of acoustic startle response, there was a main effect of sex such that females, regardless of stress history, had a higher average acoustic startle response compared to males ($F_{(1, 32)} = 4.328$, $p = 0.0456$; Fig. 2A). There was no effect of stress history on baseline acoustic startle response ($F_{(1, 32)} = 0.2696$, $p > 0.05$). There was no main effect of stress or sex on FPS during the Experiment 1 fear conditioning test ($p > 0.05$; Supp. Figure 1).

Due to the sex differences in baseline acoustic startle, FPS results from fear conditioning and extinction recall were directly compared within each sex to determine if stress history had an impact on an animal's ability to extinguish fear to the previously reinforced CS. In males, there was an effect of session such that FPS was lower during the extinction recall session as compared to the fear acquisition test, indicating successful fear extinction ($F_{(1, 18)} = 13.23$, $p = 0.0019$; Fig. 2B), but no main effect of stress ($F_{(1, 18)} = 0.916$, $p > 0.05$). In females, there was no main effect of session ($F_{(1, 14)} = 0.654$, $p > 0.05$; Fig. 2C) or stress history ($F_{(1, 14)} = 0.464$, $p > 0.05$) when comparing the average FPS values between sessions; however, there was a near interaction between session and stress ($F_{(1, 14)} = 3.403$, $p = 0.0863$). As a follow up, a paired t-test indicated that both non-stressed females and CAS females showed a lack of fear extinction, as there was no significant reduction in FPS from fear conditioning to extinction recall (non-stressed: $t = 1.928$, $df = 7$; $p = 0.0951$; CAS: $t = 0.7137$, $df = 7$; $p = 0.4985$). A power analysis of our data showed that females would need approximately 20 animals per group to detect a group difference with minimum power of 0.8 and sample sizes were increased for experiment 2.

Additionally, a percent change was calculated for each animal to determine the percent difference in FPS outcomes between the fear conditioning and extinction recall sessions : ((‘extinction recall’-‘fear conditioning’)/’fear conditioning’)*100. There was no impact of CAS of the percent change for males or females in Experiment 1 ($p > 0.05$; Supp. Figure 2A).

Immunostaining

A subset of animals was analyzed for changes in NR2B labeling to determine if sex differences observed in Experiment 1 were associated with NMDA subunit expression. In the central amygdala, animals with a history of stress had decreased labeling of the NR2B subunit ($F_{(1, 18)} = 5.423$, $p = 0.0317$; Fig. 2D). There

was no impact of sex in the central amygdala. Additionally, there was no effect of sex or stress on NR2B labeling in the basolateral amygdala ($p > 0.05$; Fig. 2E).

Experiment 2 Startle Paradigm

During the baseline acoustic startle assessment in Experiment 2, there was a main effect of stress with CAS animals exhibiting higher average acoustic startle ($F_{(1, 105)} = 4.441$, $p = 0.0375$; Fig. 3A), but there was not a main effect of sex on baseline outcomes ($F_{(1, 105)} = 1.943$, $p > 0.05$).

There was an interaction between stress history and sex during the fear acquisition test on FPS ($F_{(1, 101)} = 4.998$, $p = 0.027$; Supp. Figure 3). Each sex was then analyzed separately for a main of effect of stress using an unpaired t-test. There was no effect of stress observed in males ($p > 0.05$); however, there was marginal impact of stress in females such that CAS lowered FPS ($t = 1.831$, $df = 46$, $p = 0.0734$, Cohen's *d = 0.528*) suggesting a medium effect size despite failing to reach the designated alpha value.

FPS was then directly compared within each sex to compare the fear conditioning test to the extinction recall session. In males, there was a main effect of session ($F_{(1, 53)} = 11.65$, $p = 0.0012$; Fig. 3B) such that FPS decreased after extinction training. In addition, there was a main effect of intervention ($F_{(1, 53)} = 7.833$, $p = 0.0071$) such that DCS increased FPS compared to saline treated rats. There was not a main effect of stress in males ($F_{(1, 53)} = 0.659$, $p > 0.05$) or an interaction between stress and intervention ($F_{(1, 53)} = 1.445$, $p > 0.05$). A percent change was calculated for each animal to determine the difference in FPS during the fear conditioning versus extinction recall sessions: ((‘extinction recall’-‘fear conditioning’)/’fear conditioning’)*100. There was an interaction between stress and intervention in males ($F_{(1, 53)} = 4.777$, $p = 0.033$; Supp. Figure 2B). A follow-up t-test indicated that non-stressed males were not affected by DCS ($t = 0.553$, $df = 25$, $p > 0.05$). However, CAS males did exhibit an effect of DCS compared to saline males suggesting that DCS increased fear expression in stressed males ($t = 2.363$, $df = 28$, $p = 0.0253$).

In females, FPS was impacted by session ($F_{(1, 44)} = 7.115$, $p = 0.0107$; Fig. 3C) such that FPS values were higher during the fear conditioning session compared to extinction recall regardless of stress background or intervention. A main effect of stress ($F_{(1, 44)} = 9.402$, $p = 0.0037$) was observed for females such that FPS was lower in females with a history of CAS regardless of session (fear conditioning or extinction recall) or intervention (saline or DCS). No effect of DCS on FPS was observed in females ($F_{(1, 44)} = 0.141$, $p > 0.05$). Evaluation of percent change did not indicate an influence of stress or treatment on FPS ($p > 0.05$; Supp. Figure 2C).

Discussion

These data indicate that adolescent stress differentially influences male and female Wistar rat behavior in a cued fear-potentiated startle paradigm. Extinction of learned fear behavior was successfully established in males, but females exhibited variability in successful fear consolidation and extinction

recall sessions. Males were not impacted by a history of stress, but stressed females generally had an attenuated fear-potentiated startle response compared to their non-stressed counterparts. Despite observing an influence of CAS in females, as well as lowered NR2B expression in the CeA, DCS did not alter extinction recall in females. Interestingly, a subset of stressed males that received DCS may have reconsolidated cued fear associations as reflected by an increase in percent change in fear-potentiated startle outcomes during the extinction recall session compared to the initial fear conditioning assessment. Taken together, these studies suggest that chronic stress can decrease amygdala NR2B expression in both sexes, but the functional impact of glutamatergic signaling modifications in a fear-based learning paradigm differ per sex.

We observed that females had a higher acoustic startle response during the baseline session compared to males in Experiment 1. This outcome may have been influenced by the smaller sample size in Experiment 1, as it was not observed in Experiment 2, and following Experiment 1, we determined a need for higher sample size to fully evaluate effects of stress on fear-potentiated startle parameters. Further, previous reports have shown that Sprague Dawley males have a higher baseline startle amplitude compared to females (Russo & Parsons, 2021; Vazquez et al., 2024), suggesting that strain may interact with sex to influence acoustic startle response.

CAS increased baseline acoustic startle response for males and females in Experiment 2. As such, we were surprised to not observe elevated fear-potentiated startle in stressed rats during the fear conditioning recall session. Despite observing increased reactivity in response to an acoustic stimulus during the baseline session, CAS females exhibited attenuated FPS during the fear conditioning and extinction recall sessions compared to non-stressed females. As such, CAS females may have a fear association learning deficit that might be attributed to altered synaptic signaling.

Our results in Experiment 1 demonstrate that a history of CAS lowered NR2B expression in the CeA. The CeA is credited as the amygdala's output center for fear processing (Keifer et al., 2015). Glutamate receptors in the CeA exhibit roles in fear memory acquisition that are functionally distinct from the BLA (Zimmerman & Maren, 2010). We proposed that an intervention targeted at the NMDA receptor would facilitate enhanced extinction properties and elucidate if CeA NR2B expression influenced cued fear learning and memory properties, particularly in females. In both Experiments, NS and CAS males successfully extinguished the cued-fear associations following the extinction sessions. Randomization to drug treatment groups was not determined based on performance during the fear conditioning recall session and a confounding non-experimental effect emerged such that males assigned to the DCS groups generally had elevated FPS values compared to the saline males that pre-existed any drug treatments. Therefore, we also calculated the percent difference in FPS exhibited during the extinction recall versus the fear conditioning session to aid in interpretation of any potential effects of DCS while minimizing the impact of the pre-existing group differences in cued fear- potentiation. The percent difference values indicate that DCS resulted in a subset of CAS males with increased fear expression. Timing of DCS administration can increase memory stabilization, which might reconsolidate the memory (Lee et al., 2006). However, administration of DCS did not facilitate or impede extinction recall in NS or

CAS females. DCS can facilitate fear extinction, but repetitive extinction training days can also diminish expression of fear recall in a cued fear conditioning paradigm (D. L. Walker et al., 2002). As such, it is possible that we did not observe a significant impact of DCS in our studies because we employed two consecutive extinction sessions, and this may have been sufficient for CAS males and females to successfully uncouple the conditioned stimulus from the negative stimuli without a therapeutic intervention. Additionally, the behavioral impacts of DCS can depend on baseline differences in anxiety (Ho et al., 2005) and reproductive history in rats (Tang & Graham, 2019). These previous studies imply that variable responses to DCS are due to the complex and wide-reaching influence of glutamate on baseline behavior and pathways that have broad impacts on neural networks once augmented by DCS.

While we did not assess estrus stage on each day of the paradigm, we have previously reported that CAS does not disrupt cycling (Hyer et al., 2021) or plasma hormone levels in adult females (Bourke & Neigh, 2011; Pyter et al., 2013). Numerous studies denote circulating and exogenous hormones as a major regulator of extinction and memory recall in females (Chang et al., 2009; Graham & Daher, 2016; Graham & Scott, 2018a; Milligan-Saville & Graham, 2016). However, estrus cycle stage in female Wistar rats has also been shown to not influence the expression of fear-potentiated startle amplitude (Zhao et al., 2018) and sex specific differences may be specific to the metrics used to assess fear learning (Voulo & Parsons, 2019). Females use alternative expressions of fear, such as escaping which can manifest as darting behavior, a response not expressed readily by male rats (Gruene et al., 2015). Female rats may also express differences in ultrasonic vocalizations during fear conditioning and extinction based on their resilience to extinction learning (Tryon et al., 2021). Variability in startle response across female cohorts may be explained by the need to assess female rats differently than males to tease apart the influence of stress history on fear memory consolidation and extinction.

As the stress response occurs across a spectrum, individuals with a stress history can have an elevated or blunted response to aversive stimuli. The focus of the current study was to determine the influence of a history of adolescent stress on manifestation of PTSD-like symptoms in adulthood. We aimed to identify mechanisms by which sex differences in PTSD vulnerability emerge in traumatized adolescent populations. Future preclinical studies need to evaluate a range of behavioral outputs in female rodents to sufficiently detect cued fear learning memory deficits. The minimal influence of DCS in our studies is aligned with variability reported in clinical observations. Conflicting results are observed in clinic populations where DCS has been used to augment the treatment of anxiety, addiction, and other neuropsychiatric disorders (Hofmann et al., 2012; Otto, Kredlow, et al., 2016; Price et al., 2013). The use of DCS to augment behavioral therapy may only be effective on an individual basis, but the growing body of literature surrounding its use warrants continued focus to pinpoint appropriate therapeutic interventions.

Declarations

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Molly Hyer, Amy Wegener, Zuby Okafor, Grace Young, Chloe Burns, Emilie Bjerring, Samya Dyer, Paul Howell, and Susie Turkson. The first draft of the manuscript was written by Amy Wegener and Molly Hyer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

Data that support the findings of this study will be made available following request to the corresponding author.

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Figures

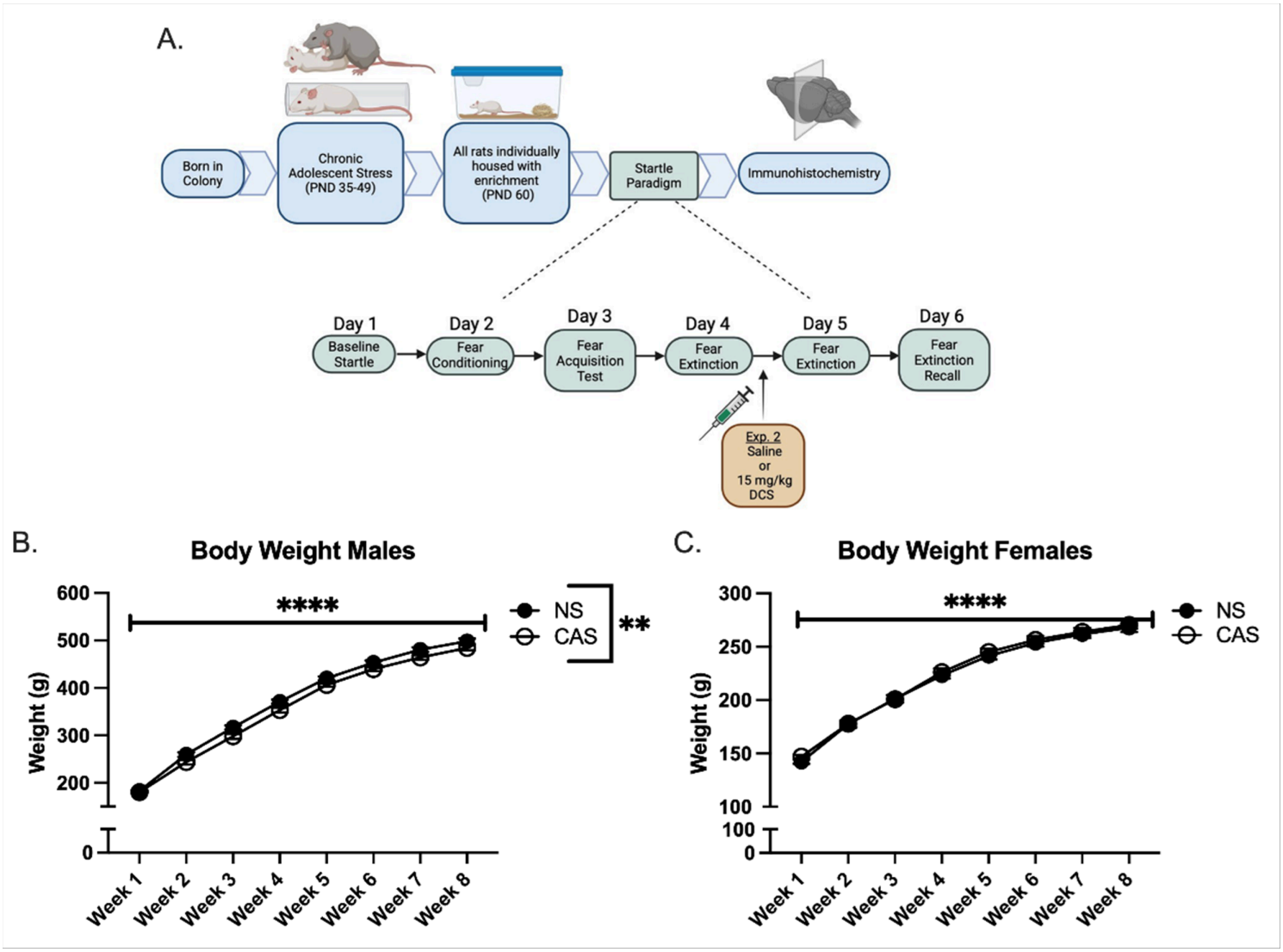

Figure 1

(A) Schematic representing the experimental timeline (Created with Biorender.com). Adolescent male and female Wistar rats were assigned to a non-stressed group or were exposed to the chronic mixed modality stress paradigm comprised of equal parts social defeat and physical restraint throughout adolescence. As adults, rats were subjected to a cued fear-potentiated startle paradigm. A subset of rats was given an intervention of DCS (or saline) following the first extinction session. Immunohistochemistry analysis of NR2B expression in the amygdala was completed after behavioral testing. (B) Males gained weight over time and CAS reduced weight gain in males. (C) Females gained weight over time and were not impacted by a history of stress. Lines and symbols represent Mean ± SEM; **p<0.01, ****p<0.0001.

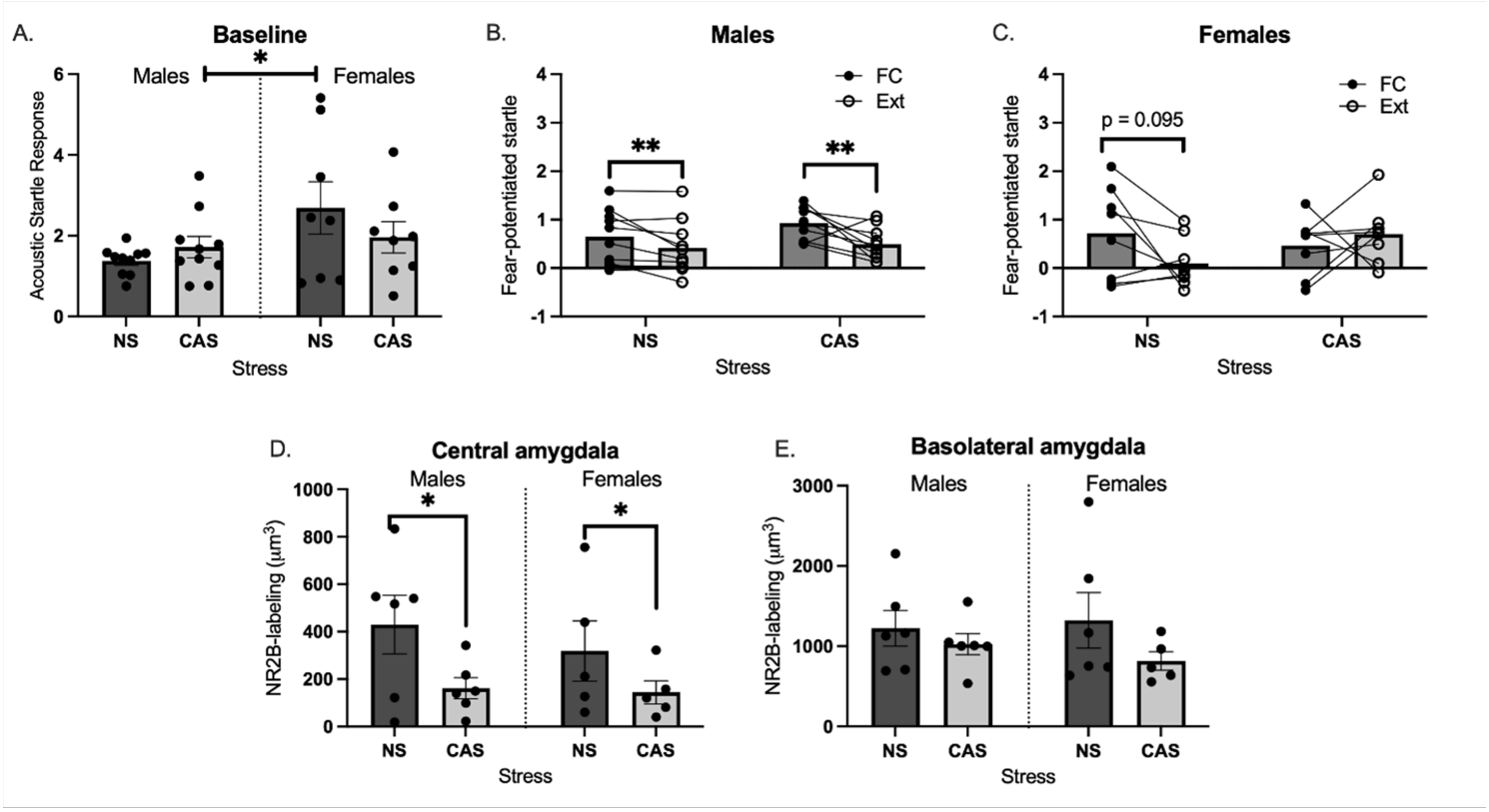

Figure 2

Experiment 1 startle paradigm and immunohistochemistry results. (A) Females exhibited a higher acoustic startle response compared to males during the baseline startle assessment. (B) Non-stressed and CAS males exhibited successful fear extinction, reflected by a lower fear-potentiated startle during fear extinction recall (Ext) compared to the fear conditioning (FC) session. (C) Females did not properly extinguish the learned cue as there was no statistically significant difference between FC and Ext recall sessions. (D) NR2B labeling quantified in the central amygdala was decreased in animals with a history of CAS. (E) NR2B labeling in the basolateral amygdala was not impacted by stress or sex. Bars represent Mean ± SEM;*p<0.05, **p<0.01.

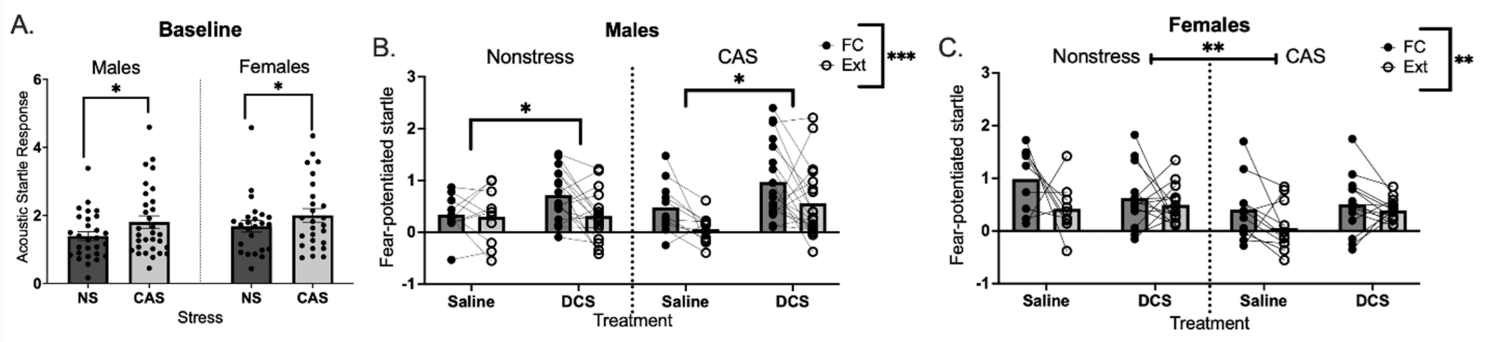

Figure 3

Experiment 2 startle paradigm results. (A) Rats with a history of CAS exhibited a higher acoustic startle response compared to non-stressed rats during the baseline startle assessment. (B) Non-stressed and

CAS males exhibited lower fear-potentiated startle during fear extinction (Ext) recall following extinction training compared to the fear conditioning (FC) session, but were not influenced by a history of stress. In males, DCS increased FPS values regardless of stress history. (C) Females also exhibited successful fear extinction recall. CAS females had an attenuated fear-potentiated startle compared to non-stressed females. Bars represent Mean ± SEM;*$p<0.05$, **$p<0.01$, and ***$p<0.001$.