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Chronic adolescent stress causes sustained impairment of cognitive flexibility and hippocampal synaptic strength in female rats

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ABSTRACT

Females that experience chronic stress during development, particularly adolescence, are the most vulnerable group to stress-induced disease. While considerable attention has been devoted to stress-induced manifestation of anxiety, depression, and PTSD, evidence indicates that a history of chronic stress is also a risk factor for cognitive decline and dementia – with females again in a higher risk group. This interplay between sex and stress history indicates specific mechanisms drive neural dysfunction across the lifespan. The presence of sex and stress steroid receptors in the hippocampus provides a point of influence for these variables to drive changes in cognitive function. Here, we used a rodent model of chronic adolescent stress (CAS) to determine the extent to which CAS modifies glutamatergic signaling resulting in cognitive dysfunction. Male and female Wistar rats born in-house remained non-stressed (NS), unmanipulated aside from standard cage cleaning, or were exposed to either physical restraint (60 min) or social defeat (CAS) each day (6 trials each), along with social isolation, throughout the adolescent period (PND 35–47). Cognition was assessed in adult (PND 80–130) male and female rats (n = 10–12) using the Barnes Maze task and the Attention Set-Shift task. Whole hippocampi were extracted from a second cohort of male and female rats (NS and CAS; n = 9–10) and processed for RNA sequencing. Brain tissue from the first cohort (n = 6) was processed for density of glutamatergic synaptic markers (GluA1, NMDA1a, and synaptophysin) or whole-cell patch clamping (n = 4) to determine glutamatergic activity in the hippocampus. Females with a history of chronic stress had shorter latencies to locate the goal box than NS controls during acquisition learning but showed an increased latency to locate the new goal box during reversal learning. This reversal deficit persisted across domains as females with a history of stress required more trials to reach criterion during the reversal phases of the Attention Set-Shift task compared to controls. Ovariectomy resulted in greater performance variability overall during reversal learning with CAS females showing worse performance. Males showed no effects of CAS history on learning or memory performance. Bioinformatic prediction using gene ontology categorization indicated that in females, postsynaptic membrane gene clusters, specifically genes related to glutamatergic synapse remodeling, were enriched with a history of stress. Structural analysis indicated that CAS did not alter glutamate receptor density in females. However, functionally, CAS females had a decreased AMPA/NMDA-dependent current ratio compared to controls indicating a weakening in synaptic strength in the hippocampus. Males showed only a slight change in density of NMDA1a labeling in the CA3 region with a history of stress. The data observed here suggest that females are at risk for impaired cognitive flexibility following a history of adolescent stress, possibly driven by changes in glutamatergic signaling.

1. Introduction

Chronic stress experienced during development is associated with increased risk for a myriad of conditions including psychiatric disease (Heim et al., 2008; Wulsin et al., 2016) and drug abuse (for review see Mukhara et al., 2018). Often, these diseases are accompanied by cognitive dysfunction that can worsen over time - implicating chronic stress as a risk factor for cognitive decline and dementia (Marazziti et al., 2010; Tran et al., 2010; Wu and Yan, 2017; Zhao et al., 2015). Compared to men, women are twice as likely to suffer from stress-induced

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disorders, such as depression and PTSD (Naninck et al., 2015; Piccinelli and Wilkinson, 2000) and are similarly vulnerable to cognitive impairments (Podcasy and Epperson, 2016; Yagi and Galea, 2018). This divergence suggests that sex confers a vulnerability to stress-related dysfunction that may manifest in cognitive decline. Life experiences, such as chronic stress and inflammation (Bekhbat et al., 2019), interact with genetic sex to contribute to cognitive decline, further complicating the prevention and treatment of neural dysfunction. Currently our understanding of the complex interactions between sex, developmental stress, and cognition are limited. Identifying the mechanisms by which chronic stress drives changes in cognition will further our understanding of how chronic stress exposure may drive sex-specific consequences in neural function.

Adolescence is a sensitive developmental timepoint due to the significant endocrine maturation and neuronal refinement that occur during this window (Green and Mccormick, 2016; Paus et al., 2010). This high level of plasticity makes the adolescent brain particularly vulnerable to stress-induced changes in neural circuitry that increase susceptibility to psychiatric disease (Sheth et al., 2017; Yohn and Blendy, 2017). Sex differences in psychiatric disorders are initially evident in adolescence (Brenhouse and Andersen, 2011), suggesting that developing endocrine mechanisms are a driving factor in sex-specific disease manifestation and cognitive function (McCormick and Mathews, 2007; Velazquez-Zamora et al., 2012). Animal models of adolescent stress have shown sex-specific, long-lasting stress-induced alterations to physiology and behavior. Social isolation in males and females (Weintraub et al., 2010) and restraint in males (Romeo et al., 2006) during adolescence shifts adult stress reactivity differently by sex and causes dynamic alterations to hippocampal, prefrontal, and amygdala circuitry in males (Pattwell et al., 2016). Adolescent psychosocial stress sex-dependently changes the hippocampal transcriptome (Rowson et al., 2019), alters the sensitivity of glucocorticoid signaling proteins (Bekhbat et al., 2019; Bourke et al., 2013), induces alterations in norepinephrine signaling (Bingham et al., 2010), and alters morphology of prefrontal cortical neurons (Urban et al., 2019). Corticosterone treatments during adolescence directly shift a host of somatic and neuroendocrine changes that are sex-specific (Kaplowitz et al., 2016). These changes in physiology are accompanied by a number of behavioral alterations including increased depressive- and anxiety-like behaviors (Hong et al., 2012; Pyter et al., 2013) and cognitive dysfunction (Chaby et al., 2015; Snyder et al., 2015). Taken together, chronic stress experienced during adolescence has a profound effect on reshaping the stress response leading to downstream consequences, such as compromised neural function, likely mediated by sex-steroid interactions.

The hippocampus is rich with steroid hormone receptors – both sex steroids and stress hormones (MacLusky et al., 1996). The density of glucocorticoid receptors throughout the hippocampal subregions – including the dentate gyrus (DG), CA3, and CA1 regions, as well as the interconnectivity with the hypothalamus (Han et al., 2005), have positioned the hippocampus to have considerable interaction with stress response circuitry. Further connections with the prefrontal cortex (PFC; Diorio et al., 1993) confer a unique susceptibly of the hippocampus to the consequences of stress, positioning this region as a locus for interaction between sex and stress hormones to drive stress-induced changes in cognition (Luine et al., 2017; McEwen, 1999). Learning and memory performance are mediated by several neurophysiological mechanisms within the hippocampal subregions and the PFC (Nicolle and Baxter, 2003), including changes in the composition of glutamatergic receptors at the level of the synapse that drive long term potentiation (LTP) and long-term depression (LTD). Chronic stress can have a direct impact on LTP and LTD by shifting synaptic composition (Shors et al, 2001, 2004) resulting in altered signaling patterns in the PFC (Urban et al., 2019) and hippocampal subregions (Hawley and Leasure, 2012) that can contribute to impaired neural function – possibly through hyperexcitability or neurotoxicity (Farrell et al., 2015). In addition, both sex and stress steroids, can alter subunit composition (Bredemann and McMahon, 2014; Lee et al., 2004) and membrane trafficking (Smith et al., 2009; Smith and Mcmahon, 2005) of glutamate receptors. Thus, hormone-dependent modifications of the glutamate receptor can dramatically alter the outcome of synaptic plasticity following stress. What remains unknown are the mechanisms by which chronic stress experienced during adolescence remodels synaptic circuitry manifesting in impaired neural function in adulthood.

Taken together, the above work indicates that chronic stress experienced during adolescence has a profound impact on physiology and behavior in a sex-specific manner. However, the extent to which chronic adolescent stress contributes to cognitive impairments in females, and the mechanism(s) responsible, remains unclear. The current study sought to identify the interactions between a history of chronic adolescent stress (CAS), specifically psychosocial stress and isolation, and sex hormones on cognitive function and mechanistic changes in synaptic function driving these consequences. We used a rodent model of chronic psychosocial stress during adolescence that has previously been shown to result in long-lasting, sex-specific changes in behavior, neural function, and inflammation (Bourke and Neigh, 2011; Pyter et al., 2013; Rowson et al., 2019). Here, adult female and male rats were assessed for cognitive function across different domains of learning and memory – both spatial and cue-based associations, followed by large-scale bioinformatic assessment of hippocampal genes that identified stress- and sex-dependent enrichment in glutamatergic synapses. Functional investigation of glutamate-dependent activity revealed sex-specific changes in signaling pattern and synaptic composition that may contribute to stress-induced alterations in cognition. Ovariectomy was used to eliminate circulating sex steroids to determine if stress history interacts with sex steroids to influence learning and memory. Taken together, the findings from these experiments reveal a potential sex-specific mechanism that confers vulnerability to stress-induced cognitive decline in females and further emphasizes the long-lasting changes to the brain and behavior induced by chronic stress experienced during adolescence.

2. Methods

2.1. Animals

Timed pregnant Wistar rats (n = 6) were obtained on gestational day 14 from Charles River (Morrisville, NC). Litters were culled on postnatal day (PND) 3 to a maximum of 8 pups with equal numbers of males and females. Pups were weaned on PND 22 and all experimental subjects were Wistar rats. To serve as defeaters in the stress paradigm, Long Evans rats (n = 8), retired breeder males and ovariectomized females, were obtained from Charles River (Morrisville, NC) and housed in opposite sex pairs. The Long-Evans strain has been used in social defeat paradigms for the defeaters due to increased aggressive behavior evident in this strain in both males and females (DeBold and Miczek, 1984). All animals were housed in static cages on a 14:10 light-dark cycle in a temperature (20–23 °C) and humidity (60%) controlled colony room. Animals were weighed weekly as well as at critical experimental timepoints and received food and water *ad libitum*. All experiments were approved and performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University, as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Chronic adolescent stress paradigm

Post-weaning, all animals were reared in-house and housed in same-sex, non-sibling pairs until the start of the chronic adolescent stress (CAS) paradigm. The mixed-modality, psychosocial CAS paradigm combines social isolation, social defeat, and restraint stress. This paradigm was originally adapted from (Bolhuis et al., 1984) and adaptations have been used as a stressor during adolescence by multiple groups

(Bourke and Neigh, 2011; McCormick and Mathews, 2007; Weathington et al., 2012). The version of the stress paradigm used in this study has been used in multiple previous studies (CITE THEM HERE- I THINK THERE ARE LIKE 6 – only ours are exactly his paradigm). Male and female rats were assigned to the stress group (CAS; n = 24) or to act as non-stress controls (NS; n = 20). On PND 35, CAS animals were isolate housed, while NS controls remained pair-housed for the duration of the experiment. Social defeat or restraint stress occurred daily from PND 37–49 between 10:00am and 12:00pm and all animals underwent six rounds of both defeat and restraint. During social defeat (Bourke and Neigh, 2011), each experimental rat (intruder), was placed into the home cage of a same-sex, mature Long Evans rat (defeater). For 2 min, the intruder and defeater were separated by a barrier that allowed sensory exposure with no physical contact. The barrier was removed, and the animals could freely interact for 5 min or three pins, whichever occurred first. A pin was defined as the intruder momentarily immobilized in the supine position by the defeater. The barrier was replaced allowing sensory exposure for an additional 25 min. During restraint stress, each experimental animal was placed into an acrylic rat restraint for 60 min within its own home cage. The restraints prevented 180° turns but did not compress the rat (Fig. 1).

2.3. Vaginal lavage

To track estrus cycle stage (Cora et al., 2015), female rats were gently restrained, and the vaginal canal was flushed with approximately 200 μL of phosphate buffered saline (PBS). The wet sample was imaged under a light microscope at 10×. The presence and quantity of round cells, needle cells, and/or neutrophils were used to determine cycle stage. Males were gently restrained at the same time to control for handling. To minimize the influence of lavage and handling on behavior (Walker et al., 2002), samples were collected daily at the same time for 14 days prior to the beginning of behavioral testing to establish a cycling pattern.

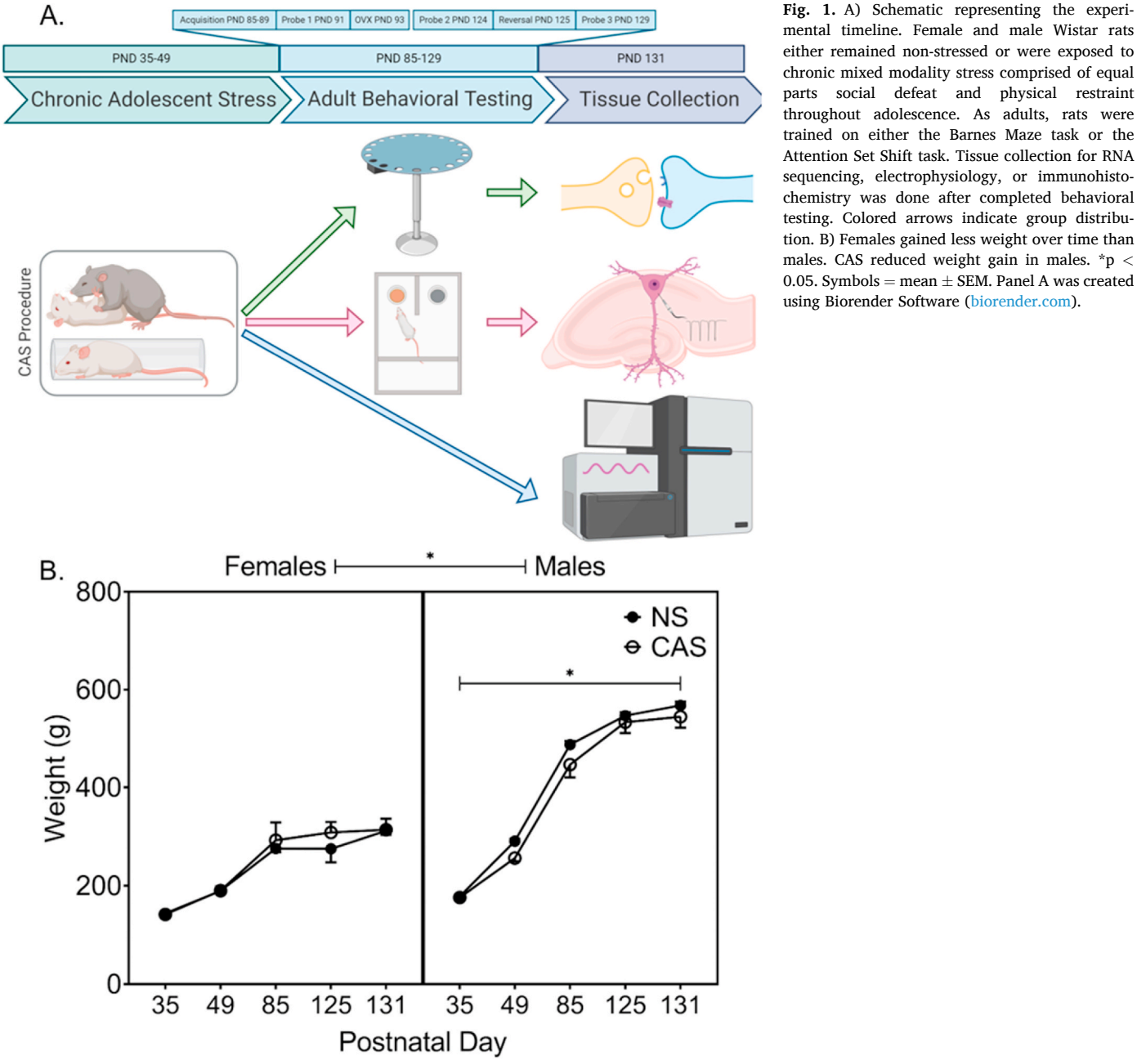


Fig. 1. A) Schematic representing the experimental timeline. Female and male Wistar rats either remained non-stressed or were exposed to chronic mixed modality stress comprised of equal parts social defeat and physical restraint throughout adolescence. As adults, rats were trained on either the Barnes Maze task or the Attention Set Shift task. Tissue collection for RNA sequencing, electrophysiology, or immunohistochemistry was done after completed behavioral testing. Colored arrows indicate group distribution. B) Females gained less weight over time than males. CAS reduced weight gain in males. *p < 0.05. Symbols = mean ± SEM. Panel A was created using Biorender Software (biorender.com).

2.4. Barnes Maze assessment

Spatial learning and memory were assessed using the Barnes Maze (protocol adapted from Sunyer et al., 2007). Testing occurred in a neutral room illuminated to 2500 lux on a black, circular table (121 cm in diameter) elevated 91.5 cm from the floor with 20 equidistant holes (10 cm in diameter) around the perimeter fitted with 19 false boxes and one goal box. Placement of the goal box was counterbalanced across groups. Prior to testing, rats were habituated to the room for 30 min a day for two days. All trials were recorded with an overhead camera and tracked using EthoVisionXT 13.0 (Noldus, Leesburg, VA).

Acquisition training occurred over five consecutive days between PND 85–89 (Fig. 1). Rats were placed in the center of the maze and given 3 min to locate the goal box. Immediately after the rat entered the goal box, the experimenter turned off the lights and covered the entry into the goal box for 2 min before a 3-min inter-trial interval in the home cage followed by a second trial. The apparatus was cleaned with 70% ethanol between every trial. Dependent variables included latency to the goal box, velocity, error rate (defined as the number of non-goal box visits prior to locating the goal box), and time spent in the periphery which served as a proxy for search strategy. A single 5-min memory probe took place 48 h following the final acquisition day on PND 91. A long-term memory probe was done four weeks later on PND 124. During probe testing, the goal box was replaced with a false box allowing for quantification of latency to locate the goal box location and number of revisits to the goal box location over a 5-min trial.

Reversal learning began 24 h after the second probe assessment (PND 125) to measure cognitive flexibility and was identical to that of acquisition learning with the goal box placed in the opposite quadrant from its initial location. Training occurred over three days. A reversal probe assessment was completed 48 h following the final reversal day on PND 129 to assess memory for the new and old goal box locations.

2.5. Attention set-shifting assessment

Assessment of cue-dependent learning was completed using the attention set-shift task (AST; adapted from Birrell and Brown, 2000). The AST consisted of a habituation and training stage, followed by a testing stage composed of seven trial types (Fig. 1; Fig. 5). Rats were restricted to 15 g of standard chow per day from PND 74 to the end of testing. Weight was monitored throughout testing to prevent body weight loss of more than 15%.

2.5.1. Habituation

Day one of habituation took place in the rat's home cage where they were presented with two unscented terra cotta pots (diameter: 6 cm, depth: 7 cm) filled with bedding and a Froot Loop quarter (Kellog's, Battle Creek, MI). The rat was allowed to explore each pot and consume the Froot Loops. With each subsequent trial, the Froot Loop was placed deeper within the bedding to associate digging with the reward. Trials were repeated until both rewards were retrieved in under 1 min for three consecutive trials. Day two took place in a Plexiglass cage (55 cm L x 27 cm W x 21 cm) fit with a removable barrier creating the start box and a fixed divider between the same two pots (Fig. 1). The rat was placed in the start box and the divider was lifted to allow exploration of the arena and discovery of the reward. Trials were repeated until both rewards were retrieved in under 1 min for three consecutive trials (average of 10 trials per animal).

2.5.2. Training

Phase I trained for simple odor discrimination using two differently scented pots but only one containing a quarter Froot Loop reward. The rat was placed in the start box and allowed to explore until the reward was consumed. Phase II trained for simple texture discrimination using unscented pots filled with different digging medias and only one pot containing the reward. For both phases, trials were repeated until the reward was retrieved on the first attempt for six consecutive trials to ensure correct association between the odor/media and the reward. Location of the baited pot was counterbalanced between trials.

2.5.3. Testing

Testing consisted of seven phases of different trial types (Fig. 5). Criterion for each group of trials was defined as digging in the rewarded pot on the first try for six consecutive trials. Trials were repeated until criterion was reached before moving to the next phase. Digging was defined as displacement of the media with the forepaws or snout. Placement of the rewarded pot was counterbalanced randomly across all trials. Phase I tested odor discrimination using two differently scented pots filled with bedding, one containing a Froot Loop reward. Phase II assessed complex discrimination using new digging media as a distractor with the same odors and target pot as Phase I. Phase III examined reversal learning by using the same media and odors as Phase II but moving the Froot Loop to the pot with the previously unrewarded odor. Phase IV assessed interdimensional shifting by replacing all odors and digging medias but still using odor as the target dimension. Phase V was a second reversal learning test using the same odors and media from Phase IV but the Froot Loop was placed in the previously unrewarded pot. Phase VI analyzed extradimensional shift by switching the relevant dimension to digging media by using the target scent from Phase IV applied to both pots with two, novel digging medias requiring the rat to distinguish between the medias and ignore the scent.

2.6. Ovariectomy

To determine the role of sex-steroids on reversal learning in the Barnes Maze task, a subset of females was ovariectomized 48 h following the first probe assessment. These females were tested for reversal learning 21 days later to assure total removal of circulating sex steroids. Surgery was performed as described in Harrell et al. (2014). Briefly, adult female Wistar rats (NS = 5, CAS = 5) were dosed with 100 μl/100 g of a ketamine/xylazine/acepromazine cocktail (25:5:1; original concentrations: ketamine 50 mg/ml; xylazine 20 mg/ml; acepromazine: 10 mg/ml) to induce anesthesia which was maintained with inhaled isoflurane. Each flank was shaved to the hip and cleaned with betadine and alcohol, and an abdominal incision was made to locate the ovaries. Ovaries were gently pulled out, ligated with a 3-0 silk suture, and cut. The abdominal cavity was closed with a 5–0 absorbable suture, surgical staples were used to close the skin, and betadine was applied to the staples. Rats were placed on a heating pad and monitored until mobile. Rats received oral meloxicam for one day and remained under daily observation until staple removal 5 days post-operation.

2.7. Tissue collection and processing for gene pathway enrichment

RNA sequencing data was provided by a cohort of rats generated by and reported in Rowson et al. (2019). Male and female Wister rats remained non-stressed or underwent the same stress paradigm described here (n = 9–10). On PND 120, hippocampal tissue was dissected following rapid decapitation and flash frozen before RNA extraction and RNA sequencing analysis (see Rowson et al., 2019 for methods). To compare differentially expressed genes following CAS, gene lists for females and males were generated using expression fold change of 1.3 and $p < 0.05$. Lists were uploaded into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8. The Functional Annotation Clustering tool was used to identify enriched, functionally related gene categories based on the gene ontology categories for biological process, molecular function, and cellular components. An Enrichment Score of 1.3 (equivalent to non-log scale of 0.05) was used as a cut-off to determine the probability of observing a certain number of transcripts in a given category (Huang et al., 2008).

2.8. Tissue collection and processing for immunohistochemistry

On PND 131, rats that had undergone the Barnes Maze task were sacrificed via transcardial perfusion following administration (i.p.) of phenytoin-pentobarbital (150 mg/kg ip; SomnaSol, Henry Schein Animal Health, Dublin, OH). Whole brains were post-fixed in 4% paraformaldehyde before cryoprotection in 30% sucrose. Brains were cryosectioned (Leica CM 1950) at 40 μM and slices placed in sodium azide at 4 °C for storage.

2.9. Immunohistochemistry

Markers for GluA1 and NMDA1a were selected as these subunits have previously been demonstrated to be altered by chronic stress (Pacheco et al., 2017; Kallarackal et al., 2013; Katsouli et al., 2014). Sections from the PFC (Paxinos and Watson Fig. 16), specifically the cingulate region as glutamate binding in this region directly contributes to impaired set-shift performance (Nicolle and Baxter, 2003), and hippocampus (Paxinos and Watson Fig. 65) were mounted on glass slides (Fisher Scientific, 12-550-15) to dry for 24 h. For antigen retrieval, 10 mM Citrate Buffer (pH 6) was heated to 95 °C, tissue was incubated in the citric acid for 2 min, then allowed to cool in solution for 20 min. Slides were washed in PBS and placed in blocking buffer solution of 5% Normal Goat Serum (NGS) (Vector Laboratories, S-1000) and 0.4% Triton X-100 (Invitrogen, ThermoFisher, HFH10) in PBS for 1 h. Slides were incubated in primary antibody with blocking buffer overnight at 4 °C. On day two, slides were rinsed with PBS then incubated in secondary antibody and PBS for 1 h. Slides were washed in PBS and cover slipped using VECTASHIELD HardSet Antifade Mounting Medium with DAPI (Vector Laboratories, H-1500). The following antibodies were used for each target: Synaptophysin: primary mouse anti-synaptophysin monoclonal antibody (1:1000; Synaptic Systems, 101 011) and goat anti-mouse IgG1 Alexa Fluor 594 (1:500; Invitrogen, ThermoFisher Scientific, A-21125; red), AMPA: primary rabbit anti-GluA1 polyclonal antibody (1:500; Abcam, ab31232) and goat anti-rabbit Alexa Fluor 488 (1:500; Abcam, ab150077; green), NMDA: primary rabbit anti-NMDA1ar (1:500; Abcam, ab17345) and goat anti-rabbit Alexa Fluor 488 (1:500; Abcam, ab150077; green).

2.10. Imaging and analysis

Image stacks (10 μm with 1 μm intervals) were taken using a laser-scanning confocal microscope (Zeiss 700, Carl Zeiss, Thornwood, NY) at 60× oil immersion. Four image stacks per region were taken; including the PFC, DG, CA3 apical region, CA3 basal region, CA1 apical region, and CA1 basal region. Image stacks were analyzed using Volocity 6.3 software (PerkinElmer, Inc. Waltham, MA) to measure volume of labeled puncta. Detection offset threshold and minimum puncta size were created and applied for each target and antibody and brain region using a negative control image (secondary antibody only) to control for background labeling.

2.11. Tissue collection and processing for electrophysiology

Brain slices were obtained by methods previously described in Goswamee and McQuiston (2019). Female rats that had undergone the set-shifting task were anesthetized then transcardially perfused with ice cold sucrose-saline (consisting of (in mM): Sucrose 230, KCl 2.5, CaCl2 2, MgCl2 6, NaHPO4 1, NaHCO3 25, glucose 25) and sacrificed by decapitation. The brain was removed, hemi-sected, and horizontal slices containing the mid temporal hippocampus were cut at 350 μm on a Leica VT1200 (Leica Microsystems, Buffalo Grove, IL). Sections were incubated in a holding chamber kept at 34 °C. The holding chamber solution consisted of artificial cerebrospinal fluid (aCSF) (in mM): NaCl 125, KCl 3.0, CaCl2 1.2, MgCl2 1.2, NaHPO4 1.2, NaHCO3 25, glucose 25 bubbled with 95% O2/5% CO2. Recordings were performed at 32–35 °C.

2.12. Electrophysiological measurements

Whole-cell patch clamp recordings from hippocampal CA1 principal cells were performed using patch pipettes (2–4 MΩ) pulled from borosilicate glass (8250 1.65/1.0 mm) on a Sutter P-1000 pipette puller and were filled with intracellular recording solution that contained a Cesium-based recording solution [(in mM): CsMeSO4 120, NaCl 8, Mg-ATP 2, Na-GTP 0.1, HEPES 10, Cs-BAPTA 10, QX-314 Chloride 10]. Currents were measured with a Model 2400 patch clamp amplifier (A-M Systems, Port Angeles, WA) and converted into a digital signal by a PCI-6040 E A/D board (National instruments, Austin, TX). The Schaffer collateral fibers were electrically stimulated by providing a single electrical pulse (100 μs pulse width), delivered via a bipolar platinum–iridium stimulating electrodes (approx. 100 kΩ, FHC Inc., Bowdoin, ME, United States) placed approximately 50 μm from the soma. All voltage clamp experiments where the access resistance changed by more than approximately 20% were discarded. WCP Strathclyde Software was used to store and analyze membrane potential and current responses on a PC computer (courtesy of Dr. J Dempster, Strathclyde University, Glasgow, Scotland).

2.13. Statistics

Weights and behavioral data (Barnes Maze and Set Shift) were analyzed using a repeated measure three-way analysis of variance (ANOVA) with the factors of sex, day/trial type, and stress with $\alpha < 0.05$. Three-way ANOVA was used to incorporate the ovariectomy variable with stress and training day on reversal learning experience. Within sex effects were determined using two-way ANOVA. Where appropriate, Tukey's post-hoc analyses were conducted to determine the factor driving significant interactions. Density of synaptic markers was analyzed using three-way ANOVAs across sex, stress history, and brain region ($\alpha < 0.05$). Tissue quality impacted image stack integrity causing some samples to be excluded from volumetric analysis of synaptic markers resulting in sample sizes for each group in each region ranging between $n = 5–7$. Correlations between estrus cycle stage and behavioral or synaptic metrics were conducted using a two-tailed Pearson's correlation with $\alpha < 0.05$. Pairwise comparisons for electrophysiology data was analyzed using a Student's T-test.

3. Results

3.1. Chronic adolescent stress slows weight gain in males but not females

Weight gain was greater in males compared to females ($F_{(1,190)} = 539.50$; $p < 0.0001$); 3-way ANOVA) and sex and stress interacted to influence weight ($F_{(1,190)} = 5.77$; $p = 0.02$; 3-way ANOVA; Fig. 1). While all males gained weight over time ($F_{(4,96)} = 308.5$; $p < 0.0001$; 2-way ANOVA), CAS impeded weight gain in males ($F_{(1,96)} = 6.71$; $p = 0.01$; 2-way ANOVA). CAS males deviated from the NS males at the end of the CAS paradigm ($t_{(20)} = 4.25$; $p = 0.0004$; 2-way ANOVA) but not at any later timepoints ($p > 0.05$) suggesting that following this deviation, CAS males compensated and gained weight at the same rate as the NS males over time. In females, chronic stress history did not alter weight gain ($F_{(1,94)} = 0.92$; $p = 0.34$; 2-way ANOVA) and all females gained weight consistently over time ($F_{(4,94)} = 41.95$; $p < 0.0001$; 2-way ANOVA; Fig. 1).

During the social defeat procedure, the number of pins was recorded. Pins increased over time for both males and females ($F_{(5,192)} = 3.15$; $p = 0.009$; 2-way ANOVA) and males experienced more pins than females ($F_{(1,192)} = 24.92$; $p < 0.0001$; 2-way ANOVA).

3.2. Chronic adolescent stress increases acquisition speed in female rats but not males

During acquisition training on the Barnes Maze task, sex did not alter latency to locate the goal box ($p > 0.05$) but females did move faster than males ($F_{(1,231)} = 29.24$; $p < 0.0001$; 3-way ANOVA; Fig. 2). In females, all animals successfully learned the location of the goal box, evident by decreasing latencies across each trial day ($F_{(4,141)} = 40.63$; $p < 0.0001$; 2-way ANOVA; Fig. 2). However, a history of chronic stress resulted in shorter latencies to the goal box in females compared to NS controls ($F_{(1,141)} = 6.71$; $p = 0.01$; 2-way ANOVA; Fig. 2). While velocity did increase over the course of training ($F_{(4,141)} = 21.47$; $p < 0.0001$; 2-way ANOVA; Fig. 2), there was no effect of CAS ($F_{(1,141)} = 1.90$; $p = 0.17$; 2-way ANOVA; Fig. 2), indicating speed was not altered. However, CAS did reduce error rate ($F_{(1,141)} = 4.01$; $p = 0.047$; 2-way ANOVA; Fig. 2) indicating that NS females committed significantly more errors than CAS females. Both groups declined in error rate with each training day ($F_{(4,141)} = 17.07$; $p < 0.0001$; 2-way ANOVA; Fig. 2) as they improved on the task.

Regardless of stress history ($F_{(1,18)} = 0.05$; $p = 0.82$; 2-way ANOVA; Fig. 2), all males were able to successfully learn the location of the goal box ($F_{(4,72)} = 34.44$; $p < 0.0001$; 2-way ANOVA; Fig. 2). Velocity was similar between NS and CAS males ($F_{(1,18)} = 0.02$; $p = 0.89$; 2-way ANOVA; Fig. 2) and increased over time ($F_{(4,72)} = 13.14$; $p < 0.0001$; 2-way ANOVA; Fig. 2) matching the reduction in latency to locate the goal box over the course of the training period. Males showed no effect of stress history on error rate ($F_{(1,18)} = 0.55$; 2-way ANOVA; $p = 0.46$) while number of errors was reduced over the training days ($F_{(4,72)} = 11.51$; $p < 0.0001$; 2-way ANOVA; Fig. 2).

3.3. Chronic adolescent stress impairs cognitive flexibility in adult female but not male rats

Reversal learning was assessed by moving the goal box to the opposite quadrant from the location learned during acquisition training. Females were faster to locate the location of the new goal box compared to males ($F_{(1,108)} = 7.90$; $p = 0.006$; 3-way ANOVA; Fig. 2) and committed less errors in their search ($F_{(1,108)} = 11.09$; $p = 0.0012$; 3-way ANOVA; Fig. 2). CAS females took significantly longer to learn the location of the new goal box than NS controls ($F_{(2,36)} = 7.26$; $p = 0.002$; 2-way ANOVA; Fig. 2). This deficit was due to an increased error rate in CAS females compared to controls ($F_{(1,54)} = 5.28$; $p = 0.03$; 2-way ANOVA; Fig. 2). Velocity increased over time ($F_{(2,36)} = 4.59$; $0 = 0.02$; 2-way ANOVA; Fig. 2) but did not differ with CAS ($F_{(1,18)} = 1.48$; $p = 0.24$; 2-way ANOVA; Fig. 2) indicating that both groups increased their speed over the course of training, matching the reduction in latency ($F_{(1,18)} = 4.9$; $p = 0.04$; 2-way ANOVA; Fig. 2).

To determine if reproductive hormone levels altered reversal performance in the Barnes Maze task, a subset of females (NS = 5; CAS = 5) underwent ovariectomy before reversal training. Overall, females that underwent ovariectomy exhibited longer latencies to locate the new goal box during Reversal Training ($F_{(1,77)} = 18.13$; $p < 0.0001$; 2-way ANOVA; Fig. 3) regardless of stress history ($F_{(1,23)} = 1.22$; $p = 0.28$; 2-way ANOVA; Fig. 3) suggesting that after ovariectomy, the effect of CAS on latency to locate the new goal box was attenuated while ovariectomy itself significantly impaired performance (Fig. 3). All females regardless of ovariectomy and CAS history showed improved error rate ($F_{(2,77)} = 4.03$; $p = 0.02$; 2-way ANOVA; Fig. 3) and velocity ($F_{(2,77)} = 9.48$; $p = 0.0002$; 2-way ANOVA; Fig. 3) overtime. There was a non-significant effect of ovariectomy on error rate ($F_{(1,77)} = 3.20$; $p = 0.08$; 2-way ANOVA; Fig. 3) with intact females committing fewer errors than ovariectomized females, matching the observed effect in latency.

All males learned the new goal box location across the three days of training ($F_{(2,36)} = 9.71$; $p = 0.0004$; 2-way ANOVA; Fig. 2) at the same rate regardless of stress history ($F_{(1,18)} = 0.11$; $p = 0.75$; 2-way ANOVA; Fig. 2). Furthermore, there was no effect of CAS on velocity ($F_{(1,18)} = 0.34$; $p = 0.57$; 2-way ANOVA; Fig. 2) or error rate ($F_{(1,18)} = 0.18$; $p = 0.67$; 2-way ANOVA; Fig. 2) in males while error rate decreased for both groups across the training days ($F_{(2,36)} = 6.6$; $p = 0.004$; 2-way ANOVA; Fig. 2).

3.4. Memory was not altered by chronic adolescent stress in either males or females

Neither chronic adolescent stress ($F_{(1,108)} = 0.003$; $p = 0.96$; 3-way ANOVA; Fig. 4) nor sex ($F_{(1,108)} = 0.32$; $p = 0.57$; 3-way ANOVA; Fig. 4) altered memory during the Barnes Maze probe assessments.

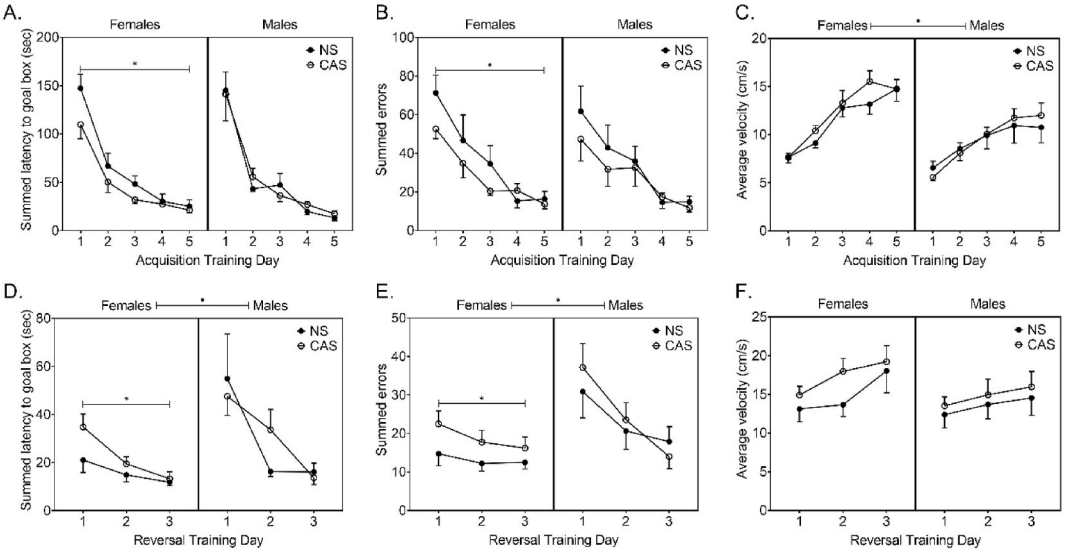


Fig. 2. A) During Acquisition training on the Barnes Maze task females with a history of chronic stress had shorter latencies to locate the goal box than non-stressed controls. B) This reduction was likely due to fewer errors committed by CAS females. C) There was no effect of CAS on speed during the task. D) During Reversal training, CAS females showed a reduced latency to locate the new goal box compared to controls. E) Similarly, CAS females had a higher error rate compared to NS females. F) Velocity remained unaltered by CAS history. A-F) Both Acquisition and Reversal training were unaltered by CAS in males. *$p < 0.05$. Symbols = mean ± SEM.

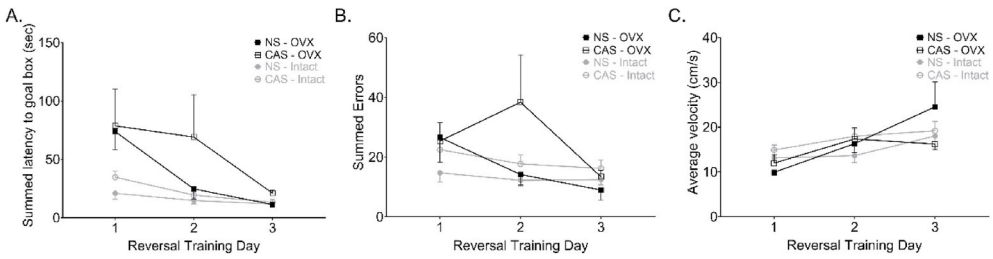


Fig. 3. Following Acquisition training, a subset of females was ovariectomized to determine the role of sex steroids in spatial reversal learning. A) Regardless of stress history, ovariectomy increased the latency to locate the new goal box during reversal learning while variability was increased for both NS and CAS females. There was no effect of ovariectomy on B) error rate or C) velocity. *$p < 0.05$. Symbols = mean ± SEM. Data for the intact females is copied from Fig. 4 and is represented in light gray for comparison.

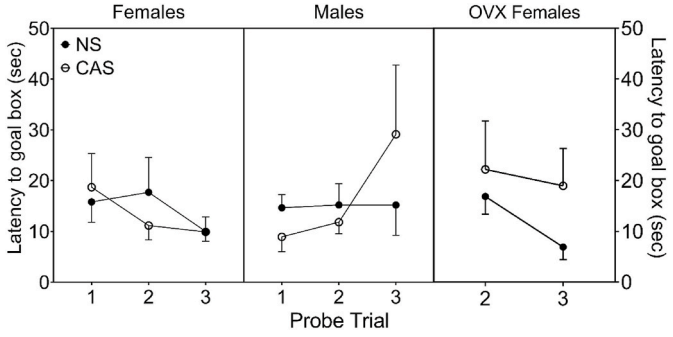


Fig. 4. There was no effect of sex, stress history, or ovariectomy during the probe trials suggesting these variables did not interact to shift memory performance. OVX females were intact for Probe Trial 1 so are included in that data set for that trial. Symbols = mean ± SEM.

3.5. Cognitive flexibility was impaired in the attention set-shift task in females but not males

Stress history altered the number of trials to criterion in the set-shift task ($F_{(1,78)} = 12.74$; $p = 0.0006$; 3-way ANOVA, Fig. 5). In females, stress history ($F_{(1,6)} = 8.31$; $p = 0.03$; 2-way ANOVA; Fig. 5) and trial type ($F_{(5,30)} = 13.91$; $p < 0.0001$; 2-way ANOVA; Fig. 5) interacted ($F_{(5,30)} = 2.75$; $p = 0.04$; 2-way ANOVA; Fig. 5) to impair performance on the set-shift task. Post-hoc analysis indicated that females with a history of CAS required more trials to reach criterion during reversal 1 ($p = 0.0001$) and reversal 2 ($p = 0.04$). Male rats showed altered performance based on trial type ($F_{(5,35)} = 4.62$; $p = 0.002$; 2-way ANOVA; Fig. 5) but CAS did not influence the number of trials needed to reach criterion ($F_{(1,7)} = 1.48$; 2-way ANOVA; $p = 0.26$; Fig. 5).

3.6. Gene enrichment was differentially altered by CAS in females and males

Bioinformatic prediction using gene ontology categorization indicated that in females, there were 12 enriched gene clusters (Enrichment Score (ES) > 1.3) following a history of CAS (Table 1). In males, we observed 18 enriched gene clusters after CAS exposure (Table 1). In females, the most enriched gene category was postsynaptic membrane-related genes. These genes further clustered into glutamatergic synaptic genes including genes for a number of receptor subunits such as *Grin2a, Grin2b, Grm3, Grm5, Gabrb1, Gabrb2, Gabrg3, and Gabrr2*.

3.7. Female and male rats with a history of chronic adolescent stress displayed region-specific alterations in synapse composition in the hippocampus

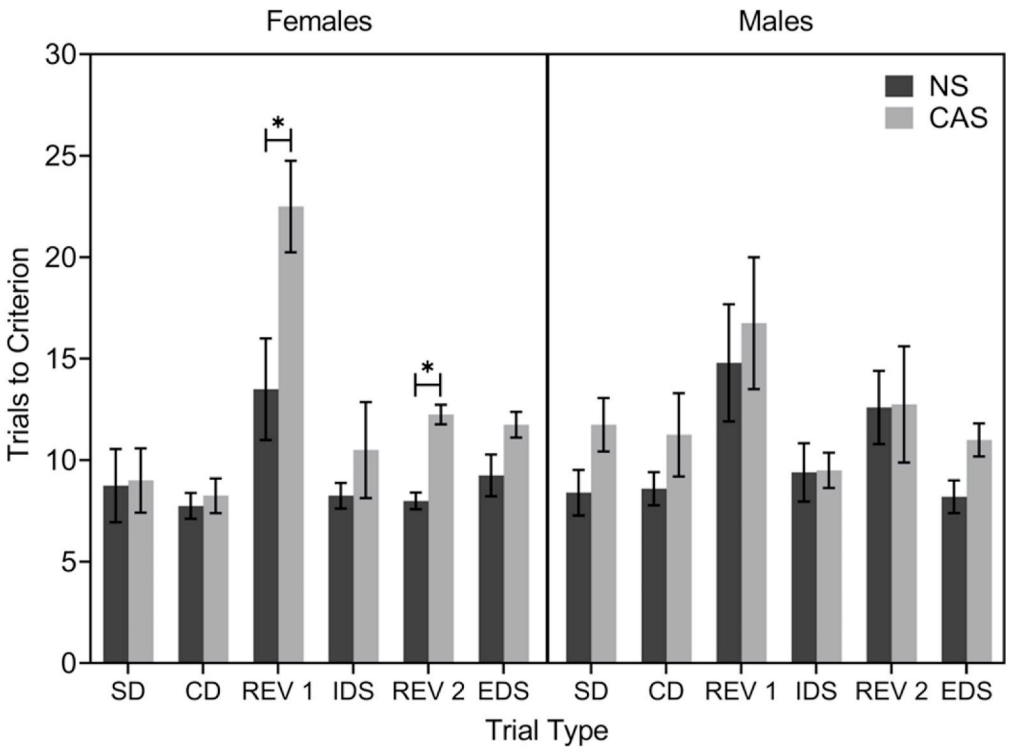
As bioinformatic prediction indicated glutamatergic synapses as a target of CAS, we investigated the extent to which synaptic markers and glutamate-specific receptors were altered following CAS in females and males (Fig. 6). Synaptophysin was used to label pre-synaptic terminals in the hippocampus and PFC to determine if synaptic density in general was shifted. There was an effect of sex ($F_{(1,120)} = 3.97$; $p = 0.05$; 3-way ANOVA; Fig. 6) and an interaction between sex and stress on synaptophysin density ($F_{(1,120)} = 4.43$; $p = 0.04$; 3-way ANOVA, Fig. 6). CAS females generally had a higher density of synaptophysin labeling ($F_{(1,60)} = 6.58$; $p = 0.01$; 2-way ANOVA; Fig. 6) largely driven by density along the basal dendrites of the CA1 region in the hippocampus compared to NS females ($p = 0.03$). Synaptophysin density varied by region ($F_{(5,60)} = 23.29$; $p < 0.01$; 2-way ANOVA; Fig. 6). In males, synaptophysin density was not altered by CAS history ($F_{(1,60)} = 0.25$; $p = 0.62$; 2-way ANOVA; Fig. 6) but did vary by region ($F_{(5,60)} = 14.47$; $p < 0.01$; 2-way ANOVA; Fig. 6).

To determine the density of excitatory receptors within these regions, GluA1a and NMDA1a were used to label receptors in the hippocampus and PFC. There was an interaction between sex and stress on GluA1 labeling ($F_{(1,121)} = 4.21$; $p = 0.04$; 3-way ANOVA; Fig. 6). GluA1a labeling was not altered due to stress history in females ($F_{(1,69)} = 1.91$; $p = 0.17$; 2-way ANOVA; Fig. 6) but showed region-specific differences in density ($F_{(5,69)} = 2.77$; $p = 0.03$; 2-way ANOVA; Fig. 6). GluA1a labeling was reduced by a history of CAS in males overall ($F_{(1,52)} = 3.92$; $p = 0.05$; 2-way ANOVA; Fig. 6) and varied by region ($F_{(5,52)} = 5.26$; $p < 0.01$; 2-way ANOVA; Fig. 6). Stress history altered the density of NMDA1a labeling ($F_{(1,119)} = 8.31$; $p = 0.005$; 3-way ANOVA, Fig. 6). NMDA1a density was not altered by CAS in female rats ($F_{(1,66)} = 0.89$; $p = 0.35$; 2-way ANOVA; Fig. 6) but did vary by region ($F_{(5,66)} = 18.37$; $p < 0.01$; 2-way ANOVA; Fig. 6). CAS history decreased density of NMDA1a labeling in males ($F_{(1,53)} = 16.57$; $p < 0.01$; 2-way ANOVA; Fig. 6) largely driven by a decrease along CA3 apical dendrites ($p < 0.01$). NMDA1a density varied by region in males ($F_{(5,53)} = 34.23$; $p < 0.01$; 2-way ANOVA; Fig. 6).

3.8. Chronic adolescent stress altered synaptically elicited ionotropic glutamate receptor responses in the hippocampus of female rats

To further investigate how CAS functionally impacted glutamatergic signaling in the hippocampus of female rats, we conducted whole-cell patch clamp experiments to determine the AMPA/NMDA ratio, an indicator of synaptic plasticity. For this, brain slices were perfused with aCSF supplemented with 25 μM Bicuculline, a potent and selective antagonist of the $GABA_A$ receptor. The cells were voltage clamped at +40 mV, and the total outward current in response to a single electrical pulse was measured. Following acquisition of the total outward current, bath applied 10 μM NBQX was used to eliminate the contribution of the AMPA current. The resulting outward current was mediated by activation of the NMDA receptor. The amplitude of the AMPA current was determined by digitally subtracting the NMDA current from the total current. Females displayed a difference in the AMPA/NMDA ratio with stress history ($t = 6.62$, $df = 14$; $p < 0.001$; Fig. 6). CAS females showed a lower ratio indicating reduced synaptic strength compared to NS controls.

A.



B.

Phase	Abbreviation	Dimensions		Exemplar Combinations	
		Relevant	Irrelevant	Rewarded	Unrewarded
Simple Discrimination	SD	Odor	(Bedding)	Orange	Peppermint
Complex Discrimination	CD	Odor	Digging Media	Orange / Crinkle Paper	Peppermint / Crinkle Paper
Reversal 1	REV 1	Odor	Digging Media	Peppermint / Crinkle Paper	Orange / Crinkle Paper
Intradimensional Shift	ID	Odor	Digging Media	Lavender / Small Stones	Almond / Small Stones
Reversal 2	REV 2	Odor	Digging Media	Almond / Small Stones	Lavender / Small Stones
Extradimensional Shift	ED	Digging Media	Odor	Shredded rubber / Almond	Cat Litter / Almond

Fig. 5. A) During the Attention Set Shift task females with a history of stress required more trials to reach criterion (six correct choices in a row) than NS females during Reversal 1 (REV 1) and Reversal 2 (REV 2) trials. CAS did not alter the number of trials to needed to reach criterion in the males. B) A table representing the experimental design for testing during the task with sample exemplar combinations. *$p < 0.05$. Symbols = mean ± SEM.

3.9. Estrus cycle stage did not influence behavioral metrics or neural outcomes

Lavage sampling was used to establish a pattern of cycling for each female. Chronic stress history did not alter the cycling pattern in females as all females displayed a four-day cycling pattern. Estrus cycle stage did not correlate with metrics of learning and memory, including latency to locate the goal box during acquisition training, latency to locate the goal box location in probe 1, probe 2, and probe 3, latency to locate the goal box during reversal training, or trials needed to reach criterion in the set-shift task ($p > 0.05$). Similarly, estrus cycle stage upon termination did not correlate with synaptic markers ($p > 0.05$).

4. Discussion

These data indicate that a history of chronic stress manifests in sex-specific alterations to glutamatergic signaling accompanied by altered cognitive performance in adulthood. Adult female rats that experienced chronic stress as adolescents displayed impaired reversal learning across learning domains. Following ovariectomy, this CAS-induced impairment was attenuated, despite a global decrease in performance and increase in variability. Gene enrichment analysis indicated alterations in the composition of hippocampal glutamatergic synapses in CAS females. Functional investigation indicated a shift in AMPA/NMDA ratio in the CA3-CA1 synaptic pathway indicative of reduced synaptic strength. While CAS females did not show a change in postsynaptic receptor composition, they did show increased density of presynaptic terminals along basal dendrites in the CA1 region. In males, NMDA receptor labeling was reduced throughout the CA3 region following CAS, despite no evident changes in behavior. Taken together, these data suggest stress-induced modifications in glutamatergic signaling are a mechanism by which chronic stress may act as a risk factor for impaired cognition in females – highlighting a potential sex-specific mechanism conferring vulnerability to females.

4.1. Chronic adolescent stress alters cognitive performance in females

Here, we observed impaired cognitive flexibility in female rats with a history of chronic stress. The females that experienced CAS took longer and committed more errors when attempting to learn the new location of a goal box during the reversal training phase of the Barnes Maze task. This impairment also manifested during the reversal phases of the

Table 1
Bioinformatic prediction indicated a number of enriched gene categories based on gene ontology for biological process, molecular function, and cellular components in females and males based on a history of chronic adolescent stress. Enrichment Scores (ES) > 1.3 = p < 0.05. The genes in the top category for females included *Grin2a, Grin2b, Grm3, Grm5, Gabrb1, Gabrb2, Gabrg3, and Gabrr2.* In males the top gene category included *Fras1, Col4a1, Col4a3, Col5a1, Col7a1, Col11a1, Col27a1, Fn1,* and *Vegfa.*

Females			Males		
Gene Category	Enrichment Score	Significance	Gene Category	Enrichment Score	Significance
postsynaptic membrane	ES = 2.62	p = 3.0E-3	extracellular matrix structural constituent	ES = 4.81	p = 5.2E-4
receptor-mediated endocytosis	ES = 1.42	p = 2.5E-3	chloride channel activity	ES = 2.8	p = 1.6E-5
membrane components	ES = 6.96	p = 3.6E-1	collagen trimer	ES = 2.71	p = 3.4E-4
symporter activity	ES = 3.62	p = 6.5E-6	transcription activity	ES = 2.16	p = 5.4E-3
anion transmembrane transporters	ES = 2.89	p = 1.0E-5	endothelial growth factor	ES = 1.96	p = 1.1E-3
ventricular morphogenesis	ES = 2.59	p = 4.1E-2	calmodulin binding	ES = 1.73	p = 2.8E-3
protein secretion	ES = 2.44	p = 1.2E-1	cadherins	ES = 1.67	p = 1.2E-1
potassium transport	ES = 2.18	p = 7.6E-4	regulation of endothelial cell proliferation	ES = 1.64	p = 2.1E-2
protein serine/threonine kinase	ES = 2.1	p = 1.9E-3	integrin-mediated signaling pathway	ES = 1.57	p = 7.7E-4
midbrain development	ES = 1.66	p = 4.2E-4	antigen and major histocompatibility complex processing	ES = 1.55	p = 1.2E-3
facial morphogenesis	ES = 1.59	p = 1.8E-3	GABA receptor complex	ES = 1.54	p = 6.8E-3
regulation of cell growth	ES = 1.57	p = 4.1E-3	sequence-specific DNA binding	ES = 1.51	p = 4.4E-6
			integrin complex	ES = 1.5	p = 9.2E-3
			DNA repair	ES = 1.42	p = 3.3E-1
			positive regulator of the BMP signaling pathway	ES = 1.4	p = 1.2E-3
			sodium ion transport	ES = 1.39	p = 1.42E-2
			intracellular signal transduction	ES = 1.32	p = 1.3E-2
			action potential	ES = 1.32	p = 8.4E-3

In males, the most enriched gene category was extracellular matrix structural constituents that included genes for a number of cellular structural components including *Fras1, Col4a1, Col4a3, Col5a1, Col7a1, Col11a1, Col27a1, Fn1,* and *Vegfa.*

Attention Set Shift task where a history of chronic adolescent stress required more trials to reach criterion in females. As stress history resulted in a slight improvement in learning during initial acquisition training, the impact of stress on cognitive performance reflects a distinct effect on cognitive flexibility as opposed to learning in general. Chronic stress during early life (Dandi et al., 2018; Lovic and Fleming, 2004; Morrison et al., 2016), adolescence (Snyder et al., 2015), and adulthood (Laclair et al., 2019) can impair cognitive flexibility but this varies by task and extent. Taken together, these findings indicate that stress-induced effects on cognition are dependent on the salience of the stressor as well as the developmental timing of the stress itself.

The improvement in initial learning observed in females with a history of CAS compared to NS counterparts during the Barnes Maze acquisition phase could be a result of increased arousal state. Learning is optimized during moderately heightened states of arousal (U curve) mediated by increases in epinephrine and glucocorticoids (Bowman et al., 2002; Luine et al., 1994). Barnes Maze acquisition is a period of heightened arousal due to the novelty of the environment and mild aversion to the exposure and bright lights (Barnes, 1979; Sunyer et al., 2007), suggesting that modifications to epinephrine or glucocorticoid signaling may contribute to this improvement. As reversal learning in both tasks used here required considerable habituation, arousal was attenuated, exposing the underlying CAS-induced impairment in cognition. Chronic stress-induced PTSD is known to be accompanied by impaired extinction memory, suggesting that the neural mechanisms which normally drive shifts in learning and memory towards relevant cues are compromised (Breslau, 2009; Myers et al., 2011). Thus, what may initially improve learning in females with a history of chronic stress, subsequently impairs learning when the circumstances have changed, leading to behavioral deficits such as PTSD. Taken together, these data elucidate a behavioral pattern of stress-induced modifications to learning and memory that contribute to the manifestation of psychiatric disease.

4.2. A history of chronic adolescent stress altered glutamatergic signaling in adult females

Bioinformatic analysis revealed enriched gene clusters contributing to glutamatergic synapse remodeling in CAS females. Glutamatergic neurotransmission is readily modulated by glucocorticoid activity, and, within the CA3 region, acute stress in female mice results in nearly identical changes in the genetic profile of glutamatergic synapses to those reported here (Marrocco et al., 2017). The trisynaptic pathway within the hippocampus is comprised of the DG, the CA3, and the CA1 (see Ribak and Shapiro, 2007). Granule cells in the DG receive external input and synapse with mossy fibers in the CA3. The CA3 region is extremely dense with interconnectivity allowing for a wealth of context to be combined to enhance learning and memory (for review see Scharfman and MacLusky, 2017). The CA3 connects to Schaffer collaterals in the CA1 region to finalize processing and begin consolidation before sending information to the PFC. The connectivity of this pathway facilitates learning and memory by anatomically allowing these regions to influence each other. Thus, anatomical changes observed in one region, may directly alter functionality of another. Here, females with a history of chronic adolescent stress displayed no change in glutamatergic receptor density despite altered synaptic strength in the CA3-CA1 synapse and overall increased presynaptic density. In the current study, receptor density was quantified following membrane permeabilization suggesting that the total observed density reflected epitope binding on both the plasma membrane and intracellular compartment. The AMPA/NMDA ratio is reflective of relative distribution of these receptors in the postsynaptic plasma membrane. Taken together, these data suggest that CAS females may have increased internalization of AMPA receptors resulting in reduced synaptic responses and increased potential of an amplified propensity for LTP production given appropriate conditions. Conversely, LTD may be limited in females with a history of CAS.

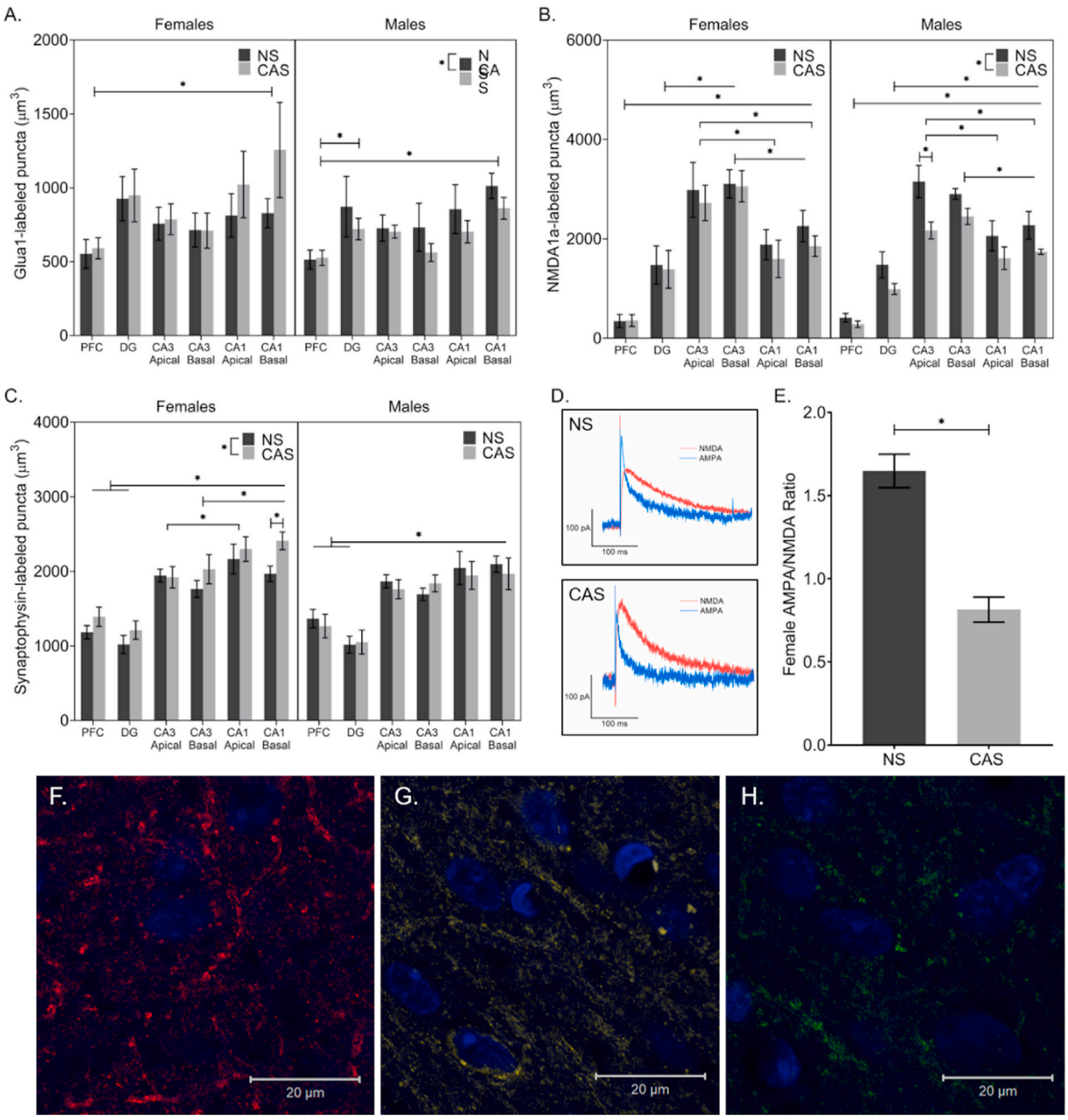


Fig. 6. A) CAS did not alter the density of GluA1a receptor expression in females but caused a global decrease in males. In females the PFC showed reduced receptor density compared to the CA1 basal region while in males both the PFC and DG has less labeling than all other regions. B) In males, CAS decreased NMDA1a receptor density overall with the CA3 apical region showing the most pronounced decline compared to NS males. In both sexes the PFC and DG showed reduced labeling while the CA3 region showed the highest density of receptor expression. C) Females with a history of stress had increased labeling of the presynaptic marker synaptophysin along basal dendrites of the CA1 region compared to controls. For both sexes the PFC and DG showed less labeling overall. D) Representative current traces showing AMPA and NMDA-mediated currents from a NS female (top) and CAS female (Bottom). E) Females that experienced CAS had a decreased AMPA/NMDA ratio compared to controls indicating a reduction in synaptic plasticity in the CA1 region of the hippocampus. Symbols = mean ± SEM. *$p < 0.05$. Hash marks on significance lines point to the specific groups while lines without hashmarks indicate a significant difference from all groups below the line. Representative images (63× oil) of F) GluA1a (red), G) NMDA1a (yellow), and H) synaptophysin (green) labeling. Blue is DAPI. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

These findings expand on previous work showing sex-divergent effects of stress on spine density and LTP and that in females these changes are modulated by estrogen (Shors et al., 2004). In males, early life stress impairs LTP well into adulthood via a CRF-dependent mechanism (Ivy et al., 2010). Optogenetic induction of LTP/LTD in the PFC of male rats ameliorates reversal learning deficits depending on stress history indicating the importance of sex and region on interactions between sex and cognition (Adler et al., 2020). In females, changes may be occurring at the presynaptic level as reported in a study which showed that shock-induced corticosterone activity increased the size of the readily releasable pool of glutamatergic vesicles (Treccani et al., 2014; Yuen et al., 2011). As we saw altered gene expression, signaling, and increased presynaptic density, it is likely that adolescent stress shifts the hippocampal glutamatergic profile, possibly through a steroid hormone-dependent mechanism, and that this change is sustained into adulthood in females. It is worth noting that the dorsal and ventral hippocampus are functionally distinct, with more dorsal regions mediating spatial and recognition memory and ventral regions regulating

emotions (Kheirbek et al., 2013). Here, the behavioral effects fell under the purview of the dorsal hippocampus thus receptor expression was analyzed in this region. Based on previous work in rodent models of adolescent stress that have shown stress-induced effects on anxiety- and depressive-like behavior in males and females (Kheirbek et al., 2013; Lukkes et al., 2017; Pyter et al., 2013), it is possible that the glutamatergic shifts are not region-specific and that the synaptic changes throughout the hippocampus (gene expression and possibly synaptic strength) contribute to the multidimensional consequences of a history of chronic stress. Taken together, these studies suggest a mechanism by which stress-induced shifts in glutamatergic signaling can result in altered cognitive performance.

Despite the observed deficit in reversal learning, we did not observe any changes in synaptic profiles within the PFC. While the PFC does play a prominent role in cognitive flexibility, the connectivity with the hippocampus is integral to this type of learning (Wu et al., 2015). The CA1, in particular, is necessary for retrieving long term memories from the PFC to incorporate into active learning in the hippocampus. This mechanism is dependent on both LTP and LTD which are driven by NMDA-dependent signaling. Here, increased NMDA-dependent current and altered glutamate receptor gene expression, may be contributing to both the improved acquisition learning and impaired reversal learning observed in females after chronic adolescent stress through LTP and LTD, respectively. A history of chronic adolescent stress enriched expression of genes for NMDA receptors (*Grin 2, Grin 3*), metabotropic glutamate receptors (*mGlur 2/3, mGlur 5*), as well as GABA receptor genes (*Gabra1*) – all of which are essential components of signal transmission between the CA1 and PFC. As mGluRs are responsible for negative feedback of glutamate signaling, it is possible that the change in gene expression contributed to the increase in presynaptic terminals, subsequently resulting in altered synaptic plasticity potential. The dysregulated signaling pattern may contribute to the decline in cognitive performance –through hyperexcitability and/or neurotoxicity (Farrell et al., 2015). In females, spine density and LTP are induced by increased estradiol signaling that is dependent on a higher ratio of NMDA to AMPA transmission (Smith and Mcmahon, 2005; Woolley and Mcewen, 1994). Given these findings, the role of CA1 glutamatergic signaling is integral to PFC-dependent learning behaviors. As estradiol can modify this interaction, females are particularly vulnerable to stress-induced modifications to these physiological mechanisms.

4.3. Ovariectomy increased variability in performance on the Barnes Maze task

Previous work from our group (Rowson et al., 2019) indicated that pathways mediated by ESR1, the gene for estrogen receptor α, are enriched following chronic adolescent stress, implicating ESR1 as a prominent transcription factor mediating the effects of adolescent stress in females. Here we observed that removing circulating estrogen through ovariectomy slightly attenuated the effect of chronic adolescent stress history on latency to locate the new goal box during reversal learning and was accompanied by a considerable increase in individual variability. Similar findings have been seen in ovariectomized female rats in the set-shift task (Kritzer et al., 2007), however, estradiol treatment in ovariectomized stressed rats will rescue radial arm maze performance (Bowman et al., 2002). As Rowson et al. (2019) observed alterations in ESR1-dependent pathways, it is possible that chronic stress during adolescence shifts the underlying ratio of ERα to ERβ, increasing the variability of estrogen-dependent effects that is exacerbated by estrogen deficiencies related to surgical ovariectomy or aging. Similarly, Marrocco et al., 2017 found that ovariectomy attenuated stress-induced impairments in behavior but that exogenous treatment with estradiol did not restore the effect and depended on genotype (heterozygous BDNF Val66Met carriers), suggesting a more complex interplay beyond circulating estradiol alone. Interestingly, evidence indicates that ovariectomy induces more male-typic performance on learning and memory tasks (Gibbs and Johnson, 2008; Heikkinen et al., 2002) suggesting a complex role for the endocrine system in cognitive function. Future work is needed to determine the extent to which chronic stress at vulnerable developmental timepoints, particularly for endocrine refinement, may shift the balance between sex-steroid receptors and circulating hormones and subsequent implications for neural function.

4.4. Despite appearing protected, males show CAS-induced deficits in other dimensions

In the current study, chronic adolescent stress exposure only moderately impacted male rats – specifically, AMPA and NMDA receptor densities were decreased throughout the hippocampus and PFC – likely related to the decrease in synaptophysin labeling. While glutamate receptors are essential for learning and memory, there were no evident deficits observed here. While the current study focused on the effects of chronic stress on glutamatergic signaling in females, males showed no indication that glutamatergic activity was altered. Importantly, the gene expression data indicated that GABAergic-related genes appear enriched in males following chronic stress (Table 1 and text). Previous work has shown that male rats exposed to chronic stress express anhedonic behaviors and display reduced probability of evoked GABA release in the hippocampus and an upregulation of extra-synaptic GABAa receptors (Holm et al., 2011). This stress-induced facilitation of LTD is not accompanied by alterations in LTP (Holderbach et al., 2007) and is possibly mediated by altered serotonergic signaling (Joëls et al., 2003). Chronic unpredictable stress does not alter AMPA/NMDA ratio in Schaffer Collateral synapses in adult Sprague-Dawley rats (Kallarackal et al., 2013). Taken together, these data indicate that chronic stress shifts the GABAergic profile in adult males that leads to downstream manifestation of anhedonic behaviors. Future studies are needed to parse out the specific sex-differences, and influence of developmental stress, in hippocampal glutamatergic and GABAergic activity in the hippocampus.

Previous studies, from our group and others, have highlighted that stress history, as opposed to recent stress exposure, in males establishes a latent vulnerability requiring a second challenge to manifest in behavioral dysfunction (Frank et al., 2012; Hudson et al., 2014; Munhoz et al., 2006) although evidence of the vulnerability can be observed in transcriptomic profiles (CITE SYDNEY). These observations are inline with the "two-hit" hypothesis which suggests that vulnerability can be unmasked with challenges experienced in adulthood – including acute stress or immune challenges. While both male and female rats with a history of CAS show alterations to the hippocampal transcriptome, as well as changes in DNA methylation (Rowson et al., 2019), males with a history of chronic adolescent stress show no deficits in the stress response (Bekhbat et al., 2019) or neuroimmune profile (Pyter et al., 2013) until receiving an immune challenge (Bekhbat et al., 2020). Furthermore, males received no manipulation of sex steroids in the current study, however, both testosterone (Kokras et al., 2017) and estradiol (Hyer et al., 2017; Ormerod et al., 2004) can influence cognition and hippocampal signaling (Skucas et al., 2013) irrespective of stress history in males. Taken together, these studies suggest that males may require a second perturbation to unmask any latent stress effects when far removed from the stressor – emphasizing the need to consider sex differences when looking at the effects of stress.

5. Conclusion and future directions

Here we observed sex-specific, long-lasting modifications to synaptic plasticity within the hippocampus that were accompanied by impaired cognitive performance after chronic adolescent stress. As females show increased susceptibility to stress-induced disorders across multiple species, including humans, it is essential to elucidate the sex-specific mechanisms that may confer this vulnerability. The complex interactions evident between stress and sex hormones, and their

considerable effects on the brain and behavior, indicate a complex relationship that is dependent on the salience of the stressor, the timing of the stressor, and the outcome in question. That the current work investigates consequences of developmental stress in animals that are far removed from the experience of the stress itself, suggests a profound and long-lasting ability of stress to modify glutamatergic transmission and the genetic profile of the hippocampus, manifesting in impaired cognition. As the endocrine system, particularly sex hormones, is being refined during adolescence, it is likely that chronic stress experienced during this developmental period increases susceptibility to cognitive dysfunction, as well as a myriad of other neurological conditions, that are dependent on glutamatergic signaling through alterations in hormone profiles. By identifying risk factors that can contribute to stress-induced disorders, prevention and intervention become accessible modes of treating these debilitating diseases with an unfair distribution among women.

CRediT authorship contribution statement

M.M. Hyer: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **G.A. Shaw:** Investigation, Writing - review & editing. **P. Goswamee:** Methodology, Investigation, Writing - review & editing. **S.K. Dyer:** Investigation, Writing - review & editing. **C.M. Burns:** Investigation, Writing - review & editing. **E. Soriano:** Investigation, Writing - review & editing. **C.S. Sanchez:** Investigation. **S.A. Rowson:** Investigation, Writing - review & editing. **A.R. McQuiston:** Conceptualization, Writing - review & editing, Funding acquisition. **G.N. Neigh:** Conceptualization, Resources, Writing - review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

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References

Adler, S.M., Girotti, M., Morilak, D.A., 2020. Optogenetically-induced long term depression in the rat orbitofrontal cortex ameliorates stress-induced reversal learning impairment. Neurobiol. Stress 13, 100258. https://doi.org/10.1016/j.ynstr.2020.100258.

Barnes, C.A., 1979. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. 93, 74–104.

Bekhbat, M., Howell, P.A., Rowson, S.A., Kelly, S.D., Tansey, M.G., Neigh, G.N., 2019. Chronic adolescent stress sex-specifically alters central and peripheral neuroimmune reactivity in rats. Brain Behav. Immun. 76, 248–257. https://doi.org/10.1016/J.BBI.2018.12.005.

Bekhbat, M., Mukhara, D., Dozmorov, M., Stansfield, J., Benusa, S., Hyer, M.M., Rowson, S.A., Kelly, S., Qin, Z., Dupree, J., Tharp, G., Tansey, M.G., Neigh, G.N., 2020. Adolescent stress sensitizes the adult neuroimmune transcriptome and leads to sex-specific microglial and behavioral phenotypes. Neuropsychopharmacology.

Bingham, B., Mcfadden, K., Zhang, X., Bhatnagar, S., Beck, S., Valentino, R., 2010. Early adolescence as a critical window during which social stress distinctly alters behavior and brain norepinephrine activity. Neuropsychopharmacology 36, 896–909. https://doi.org/10.1038/npp.2010.229.

Birrell, J.M., Brown, V.J., 2000. Medial frontal cortex mediates perceptual attentional set shifting in the rat. J. Neurosci. 20, 4320–4324. https://doi.org/10.1523/JNEUROSCI.20-11-04320.2000.

Bolhuis, J.J., Fitzgerald, R.E., Dijk, D.J., Koolhaas, J.M., 1984. The corticomedial amygdala and learning in an agonistic situation in the rat. Physiol. Behav. 32, 575–579. https://doi.org/10.1016/0031-9384(84)90311-1.

Bourke, C.H., Neigh, G.N., 2011. Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. Horm. Behav. 60, 112–120. https://doi.org/10.1016/J.YHBEH.2011.03.011.

Bourke, C.H., Raees, M.Q., Malviya, S., Bradburn, C.A., Binder, E.B., Neigh, G.N., 2013. Glucocorticoid sensitizers Bag 1 and Ppid are regulated by adolescent stress in a sex-dependent manner. Psychoneuroendocrinology 38, 84–93. https://doi.org/10.1016/j.psyneuen.2012.05.001.

Bowman, R.E., Ferguson, D., Luine, V.N., 2002. Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience 113, 401–410.

Bredemann, T.M., McMahon, L.L., 2014. 17β Estradiol increases resilience and improves hippocampal synaptic function in helpless ovariectomized rats. Psychoneuroendocrinology 42, 77–88. https://doi.org/10.1016/J.PSYNEUEN.2014.01.004.

Brenhouse, H.C., Andersen, S.L., 2011. Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. Neurosci. Biobehav. Rev. 35, 1687–1703.

Breslau, N., 2009. The epidemiology of trauma, PTSD, and other posttrauma disorders. Trauma Violence Abuse 10, 198–210. https://doi.org/10.1177/1524838009334448.

Chaby, L.E., Cavigelli, S.A., Hirrlinger, A.M., Lim, J., Warg, K.M., Braithwaite, V.A., 2015. Chronic stress during adolescence impairs and improves learning and memory in adulthood. Front. Behav. Neurosci. 9, 327. https://doi.org/10.3389/fnbeh.2015.00327.

Cora, M.C., Kooistra, L., Travlos, G., 2015. Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears. Toxicol. Pathol. 1–18 https://doi.org/10.1177/0192623315570339.

Dandi, E., Kalamari, A., Touloumi, O., Lagoudaki, R., Nousiopoulou, E., Simeonidou, C., Spandou, E., Tata, D.A., 2018. Beneficial effects of environmental enrichment on behavior, stress reactivity and synaptophysin/BDNF expression in hippocampus following early life stress. Int. J. Dev. Neurosci. 67, 19–32. https://doi.org/10.1016/j.ijdevneu.2018.03.003.

DeBold, J.F., Miczek, K.A., 1984. Aggression persists after ovariectomy in female rats. Horm. Behav. 18, 177–190. https://doi.org/10.1016/0018-506X(84)90041-2.

Diorio, D., Viau, V., Meaney, M.J., 1993. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J. Neurosci. 13, 3839–3847. https://doi.org/10.1523/jneurosci.13-09-03839.1993.

Farrell, M.R., Gruene, T.M., Shansky, R.M., 2015. The influence of stress and gonadal hormones on neuronal structure and function. Horm. Behav. 76, 118–124. https://doi.org/10.1016/J.YHBEH.2015.03.003.

Frank, M.G., Thompson, B.M., Watkins, L.R., Maier, S.F., 2012. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. Brain Behav. Immun. 26, 337–345. https://doi.org/10.1016/j.bbi.2011.10.005.

Gibbs, R.B., Johnson, D.A., 2008. Sex-specific effects of gonadectomy and hormone treatment on acquisition of a 12-arm radial maze task by Sprague Dawley rats. Endocrinology 149, 3176–3183. https://doi.org/10.1210/en.2007-1645.

Goswamee, P., McQuiston, A.R., 2019. Acetylcholine release inhibits distinct excitatory inputs onto hippocampal CA1 pyramidal neurons via different cellular and network mechanisms. Front. Cell. Neurosci. 13, 267. https://doi.org/10.3389/fncel.2019.00267.

Green, M.R., Mccormick, C.M., 2016. Sex and stress steroids in adolescence: gonadal regulation of the hypothalamic-pituitary-adrenal axis in the rat. Gen. Comp. Endocrinol. 234, 110–116. https://doi.org/10.1016/j.ygcen.2016.02.004.

Han, F., Ozawa, H., Matsuda, K.I., Nishi, M., Kawata, M., 2005. Colocalization of mineralocorticoid receptor and glucocorticoid receptor in the hippocampus and hypothalamus. Neurosci. Res. 51, 371–381. https://doi.org/10.1016/j.neures.2004.12.013.

Harrell, C.S., Burgado, J., Kelly, S.D., Neigh, G.N., 2014. Ovarian steroids influence cerebral glucose transporter expression in a region- and isoform-specific pattern. J. Neuroendocrinol. 26, 217–225. https://doi.org/10.1111/jne.12139.

Hawley, D.F., Leasure, J.L., 2012. Region-specific response of the hippocampus to chronic unpredictable stress. Hippocampus 22, 1338–1349. https://doi.org/10.1002/hipo.20970.

Heikkinen, T., Puoliva, J., Liu, L., Rissanen, A., Tanila, H., 2002. Effects of ovariectomy and estrogen treatment on learning and hippocampal neurotransmitters in mice. Horm. Behav. 41, 22–32. https://doi.org/10.1006/hbeh.2001.1738.

Heim, C., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2008. The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology 33, 693–710. https://doi.org/10.1016/j.psyneuen.2008.03.008.

Holderbach, R., Clark, K., Moreau, J.L., Bischofberger, J., Normann, C., 2007. Enhanced long-term synaptic depression in an animal model of depression. Biol. Psychiatr. 62, 92–100. https://doi.org/10.1016/j.biopsych.2006.07.007.

Holm, M.M., Nieto-Gonzalez, J.L., Vardya, I., Henningsen, K., Jayatissa, M.N., Wiborg, O., Jensen, K., 2011. Hippocampal GABAergic dysfunction in a rat chronic mild stress model of depression. Hippocampus 21, 422–433. https://doi.org/10.1002/hipo.20758.

Hong, S., Flashner, B., Chiu, M., Ver Hoeve, E., Luz, S., Bhatnagar, S., 2012. Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats. Physiol. Behav. 105, 269–275. https://doi.org/10.1016/j.physbeh.2011.08.036.

Hudson, S.P., Jacobson-Pick, S., Anisman, H., 2014. Sex differences in behavior and pro-inflammatory cytokine mRNA expression following stressor exposure and re-exposure. Neuroscience 277, 239–249. https://doi.org/10.1016/j.neuroscience.2014.07.007.

Hyer, M.M., Khantsis, S., Venezia, A.C., Madison, F.N., Hallgarth, L., Adekola, E., Glasper, E.R., 2017. Estrogen-dependent modifications to hippocampal plasticity in paternal California mice (Peromyscus californicus). Horm. Behav. 96, 147–155. https://doi.org/10.1016/j.yhbeh.2017.09.015.

Ivy, A.S., Rex, C.S., Chen, Y., Dubé, C., Maras, P.M., Grigoriadis, D.E., Gall, C.M., Lynch, G., Baram, T.Z., 2010. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J. Neurosci. 30, 13005–13015. https://doi.org/10.1523/JNEUROSCI.1784-10.2010.

Joëls, M., Verkuyl, J.M., Van Riel, E., 2003. Hippocampal and hypothalamic function after chronic stress. In: Annals of the New York Academy of Sciences. New York Academy of Sciences, pp. 367–378. https://doi.org/10.1196/annals.1286.036.

Kallarackal, A.J., Kvarta, M.D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A.M., Thompson, S.M., 2013. Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. J. Neurosci. 33, 15669–15674. https://doi.org/10.1523/JNEUROSCI.2588-13.2013.

Kaplowitz, E.T., Savenkova, M., Karatsoreos, I.N., Romeo, R.D., 2016. Somatic and neuroendocrine changes in response to chronic corticosterone exposure during adolescence in male and female rats. J. Neuroendocrinol. 28 https://doi.org/10.1111/jne.12336 n/a-n/a.

Kheirbek, M.A., Drew, L.J., Burghardt, N.S., Costantini, D.O., Tannenholz, L., Ahmari, S.E., Zeng, H., Fenton, A.A., Hen, R., 2013. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. Neuron 77, 955–968. https://doi.org/10.1016/j.neuron.2012.12.038.

Kokras, N., Pastromas, N., Papasava, D., De Bournonville, C., Cornil, C.A., Dalla, C., 2017. Sex differences in behavioral and neurochemical effects of gonadectomy and aromatase inhibition in rats. https://doi.org/10.1016/j.psyneuen.2017.10.007.

Kritzer, M.F., Brewer, A., Montalmant, F., Davenport, M., Robinson, J.K., 2007. Effects of gonadectomy on performance in operant tasks measuring prefrontal cortical function in adult male rats. Horm. Behav. 51, 183–194. https://doi.org/10.1016/J.YHBEH.2006.07.005.

Laclair, M., Febo, M., Nephew, B., Gervais, N.J., Poirier, G., Workman, K., Chumachenko, S., Payne, L., Moore, M.C., King, J.A., Lacreuse, A., 2019. Sex differences in cognitive flexibility and resting brain networks in middle-aged marmosets. eNeuro 6. https://doi.org/10.1523/ENEURO.0154-19.2019.

Lee, S.J., Romeo, R.D., Svenningsson, P., Campomanes, C.R., Allen, P.B., Greengard, P., Mcewen, B.S., 2004. Estradiol affects spinophilin protein differently in gonadectomized males and females. J. Neurosci. 127, 983–988. https://doi.org/10.1016/j.neuroscience.2004.05.049.

Lovic, V., Fleming, A.S., 2004. Artificially-reared female rats show reduced prepulse inhibition and deficits in the attentional set shifting task—reversal of effects with maternal-like licking stimulation. Behav. Brain Res. 148, 209–219. https://doi.org/10.1016/S0166-4328(03)00206-7.

Luine, V., Gomez, J., Beck, K., Bowman, R., 2017. Sex differences in chronic stress effects on cognition in rodents. Pharmacol. Biochem. Behav. 152, 13–19. https://doi.org/10.1016/J.PBB.2016.08.005.

Luine, V., Villegas, M., Martinez, C., McEwen, B.S., 1994. Repeated stress causes reversible impairments of spatial memory performance. Brain Res. 639, 167–170. https://doi.org/10.1016/0006-8993(94)91778-7.

Lukkes, J.L., Meda, S., Thompson, B.S., Freund, N., Andersen, S.L., 2017. Early life stress and later peer distress on depressive behavior in adolescent female rats: effects of a novel intervention on GABA and D2 receptors. Behav. Brain Res. 330, 37–45. https://doi.org/10.1016/j.bbr.2017.04.053.

MacLusky, N.J., Yuan, H., Elliott, J., Brown, T.J., 1996. Sex differences in corticosteroid binding in the rat brain: an in vitro autoradiographic study. Brain Res. 708, 71–81. https://doi.org/10.1016/0006-8993(95)01310-5.

Marazziti, D., Consoli, G., Picchetti, M., Carlini, M., Faravelli, L., 2010. Cognitive impairment in major depression. Eur. J. Pharmacol. 626, 83–86. https://doi.org/10.1016/j.ejphar.2009.08.046.

Marrocco, J., Petty, G.H., Ríos, M.B., Gray, J.D., Kogan, J.F., Waters, E.M., Schmidt, E.F., Lee, F.S., McEwen, B.S., 2017. A sexually dimorphic pre-stressed translational signature in CA3 pyramidal neurons of BDNF Val66Met mice. Nat. Commun. 8, 808. https://doi.org/10.1038/s41467-017-01014-4.

McCormick, C.M., Mathews, I.Z., 2007. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacol. Biochem. Behav. 86, 220–233. https://doi.org/10.1016/j.pbb.2006.07.012.

McEwen, B.S., 1999. Stress and hippocampal plasticity. Annu. Rev. Neurosci. 22, 105–122. https://doi.org/10.1146/annurev.neuro.22.1.105.

Morrison, K.E., Narasimhan, S., Fein, E., Bale, T.L., 2016. Peripubertal stress with social support promotes resilience in the face of aging. Endocrinology 157, 2002–2014. https://doi.org/10.1210/en.2015-1876.

Mukhara, D., Banks, M.L., Neigh, G.N., 2018. Stress as a risk factor for substance use disorders: a mini-review of molecular mediators. Front. Behav. Neurosci. https://doi.org/10.3389/fnbeh.2018.00309.

Munhoz, C.D., Lepsch, L.B., Kawamoto, E.M., Malta, M.B., De Sá Lima, L., Avellar, M.C.W., Sapolsky, R.M., Scavone, C., 2006. Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-κB in the frontal cortex and hippocampus via glucocorticoid secretion. J. Neurosci. 26, 3813–3820. https://doi.org/10.1523/JNEUROSCI.4398-05.2006.

Myers, K.M., Carlezon, W.A., Davis, M., 2011. Glutamate receptors in extinction and extinction-based therapies for psychiatric illness. Neuropsychopharmacology. https://doi.org/10.1038/npp.2010.88.

Naninck, E.F.G., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lazic, S.E., Lucassen, P.J., Korosi, A., 2015. Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. Hippocampus 25, 309–328. https://doi.org/10.1002/hipo.22374.

Nicolle, M.M., Baxter, M.G., 2003. Glutamate receptor binding in the frontal cortex and dorsal striatum of aged rats with impaired attentional set-shifting. Eur. J. Neurosci. 18, 3335–3342. https://doi.org/10.1111/j.1460-9568.2003.03077.x.

Ormerod, B.K., Lee, T.T.Y., Galea, L.A.M., 2004. Estradiol enhances neurogenesis in the dentate gyri of adult male meadow voles by increasing the survival of young granule neurons. Neuroscience 128, 645–654. https://doi.org/10.1016/j.neuroscience.2004.06.039.

Pattwell, S.S., Liston, C., Jing, D., Ninan, I., Yang, R.R., Witztum, J., Murdock, M.H., Dincheva, I., Bath, K.G., Casey, B.J., Deisseroth, K., Lee, F.S., 2016. Dynamic changes in neural circuitry during adolescence are associated with persistent attenuation of fear memories. Nat. Commun. 7, 1–9. https://doi.org/10.1038/ncomms11475.

Paus, T., Keshavan, M., Giedd, J.N., 2010. Why do many psychiatric disorders emerge during adolescence? Nat. Rev. Neurosci. 9, 947. https://doi.org/10.1038/nrn2513.

Piccinelli, M., Wilkinson, G., 2000. Gender differences in depression. Critical review. Br. J. Psychiatry 177, 486–492. https://doi.org/10.1192/BJP.177.6.486.

Podcasy, J.L., Epperson, C.N., 2016. Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin. Neurosci. 18, 437–446.

Pyter, L.M., Kelly, S.D., Harrell, C.S., Neigh, G.N., 2013. Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. Brain Behav. Immun. 30, 88–94. https://doi.org/10.1016/j.bbi.2013.01.075.

Ribak, C.E., Shapiro, L.A., 2007. Ultrastructure and synaptic connectivity of cell types in the adult rat dentate gyrus. Prog. Brain Res. 163, 155–166. https://doi.org/10.1016/S0079-6123(07)63009-X.

Romeo, R.D., Bellani, R., Karatsoreos, I.N., Chhua, N., Vernov, M., Conrad, C.D., McEwen, B.S., 2006. Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal Axis plasticity. Endocrinology 147, 1664–1674. https://doi.org/10.1210/en.2005-1432.

Rowson, S.A., Bekhbat, M., Kelly, S.D., Binder, E.B., Hyer, M.M., Shaw, G., Bent, M.A., Hodes, G., Tharp, G., Weinshenker, D., Qin, Z., Neigh, G.N., 2019. Chronic adolescent stress sex-specifically alters the hippocampal transcriptome in adulthood. Neuropsychopharmacology 1–9. https://doi.org/10.1038/s41386-019-0321-z, 0.

Scharfman, H.E., MacLusky, N.J., 2017. Sex differences in hippocampal area CA3 pyramidal cells. J. Neurosci. Res. 95, 563–575. https://doi.org/10.1002/jnr.23927.

Sheth, C., McGlade, E., Yurgelun-Todd, D., 2017. Chronic stress in adolescents and its neurobiological and psychopathological consequences: an RDoC perspective. Chronic Stress 1. https://doi.org/10.1177/2470547017715645, 247054701771564.

Shors, T.J., Chua, C., Falduto, J., 2001. Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. J. Neurosci. 21, 6292–6297. https://doi.org/10.1523/JNEUROSCI.21-16-06292.2001.

Shors, T.J., Falduto, J., Leuner, B., 2004. The opposite effects of stress on dendritic spines in male vs. female rats are NMDA receptor-dependent. Eur. J. Neurosci. 19, 145–150. https://doi.org/10.1046/j.1460-9568.2003.03065.x.

Skucas, V.A., Duffy, A.M., Harte-Hargrove, L.C., Magagna-Poveda, A., Radman, T., Chakraborty, G., Schroeder, C.E., MacLusky, N.J., Scharfman, H.E., 2013. Testosterone depletion in adult male rats increases mossy fiber transmission, LTP, and sprouting in area CA3 of hippocampus. J. Neurosci. 33, 2338–2355. https://doi.org/10.1523/JNEUROSCI.3857-12.2013.

Smith, C.C., Mcmahon, L.L., 2005. Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J. Neurosci. 25, 7780–7791. https://doi.org/10.1523/JNEUROSCI.0762-05.2005.

Smith, C.C., Vedder, L.C., McMahon, L.L., 2009. Estradiol and the relationship between dendritic spines, NR2B containing NMDA receptors, and the magnitude of long-term potentiation at hippocampal CA3–CA1 synapses. Psychoneuroendocrinology 34, S130–S142. https://doi.org/10.1016/J.PSYNEUEN.2009.06.003.

Snyder, K., Barry, M., Plona, Z., Ho, A., Zhang, X.-Y., Valentino, R.J., 2015. The impact of social stress during adolescence or adulthood and coping strategy on cognitive function of female rats. Behav. Brain Res. 286, 175–183. https://doi.org/10.1016/J.BBR.2015.02.047.

Sunyer, B., Patil, S., Höger, H., Luber, G., 2007. Barnes maze, a useful task to assess spatial reference memory in the mice. Protoc. Exch. 198, 58–68. https://doi.org/10.1038/nprot.2007.390.

Tran, T.T., Srivareerat, M., Alkadhi, K.A., 2010. Chronic psychosocial stress triggers cognitive impairment in a novel at-risk model of Alzheimer's disease. Neurobiol. Dis. 37, 756–763. https://doi.org/10.1016/J.NBD.2009.12.016.

Treccani, G., Musazzi, L., Perego, C., Milanese, M., Nava, N., Bonifacino, T., Lamanna, J., Malgaroli, A., Drago, F., Racagni, G., Nyengaard, J.R., Wegener, G., Bonanno, G., Popoli, M., 2014. Acute stress rapidly increases the readily releasable pool of glutamate vesicles in prefrontal and frontal cortex through non-genomic action of corticosterone. Mol. Psychiatr. https://doi.org/10.1038/mp.2014.20.

Urban, K.R., Geng, E., Bhatnagar, S., Valentino, R.J., 2019. Age-and sex-dependent impact of repeated social stress on morphology of rat prefrontal cortex pyramidal neurons. Neurobiol. Stress 10, 100165. https://doi.org/10.1016/j.ynstr.2019.100165.

Velazquez-Zamora, D.A., Garcia-Segura, L.M., González-Burgos, I., 2012. Effects of selective estrogen receptor modulators on allocentric working memory performance and on dendritic spines in medial prefrontal cortex pyramidal neurons of ovariectomized rats. Horm. Behav. 61, 512–517. https://doi.org/10.1016/j.yhbeh.2012.01.010.

Walker, Q., Nelson, C.J., Smith, D., Kuhn, C.M., 2002. Vaginal lavage attenuates cocaine-stimulated activity and establishes place preference in rats. Pharmacol. Biochem. Behav. 73, 743–752. https://doi.org/10.1016/S0091-3057(02)00883-3.

Weathington, J.M., Arnold, A.R., Cooke, B.M., 2012. Juvenile social subjugation induces a sex-specific pattern of anxiety and depression-like behaviors in adult rats. Horm. Behav. 61, 91–99. https://doi.org/10.1016/j.yhbeh.2011.10.008.

Weintraub, A., Singaravelu, J., Bhatnagar, S., 2010. Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity. Brain Res. 1343, 83–92. https://doi.org/10.1016/j.brainres.2010.04.068.

Woolley, C.S., Mcewen, B.S., 1994. Estradiol regulates hippocampal dendritic spine density via an N-Methy-D-Aspartate receptor-dependent mechanism. J. Neurosci. 74, 7660–7667.

Wu, J., Yan, J., 2017. Editorial: stress and cognition. Front. Psychol. 8, 970. https://doi.org/10.3389/fpsyg.2017.00970.

Wu, M.V., Sahay, A., Duman, R.S., Hen, R., 2015. Functional differentiation of adult-born neurons along the septotemporal axis of the dentate gyrus. Cold Spring Harb Perspect Biol 7, a018978. https://doi.org/10.1101/cshperspect.a018978.

Wulsin, A.C., Wick-Carlson, D., Packard, B.A., Morano, R., Herman, J.P., 2016. Adolescent chronic stress causes hypothalamo–pituitary–adrenocortical hypo-responsiveness and depression-like behavior in adult female rats. Psychoneuroendocrinology 65, 109–117. https://doi.org/10.1016/j.psyneuen.2015.12.004.

Yagi, S., Galea, L.A.M., 2018. Sex differences in hippocampal cognition and neurogenesis. Neuropsychopharmacology 1. https://doi.org/10.1038/s41386-018-0208-4.

Yohn, N.L., Blendy, J.A., 2017. Adolescent chronic unpredictable stress exposure is a sensitive window for long-term changes in adult behavior in mice. Neuropsychopharmacology 42, 1670–1678. https://doi.org/10.1038/npp.2017.11.

Yuen, E.Y., Liu, W., Karatsoreos, I.N., Ren, Y., Feng, J., McEwen, B.S., Yan, Z., 2011. Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. Mol. Psychiatr. 16, 156–170. https://doi.org/10.1038/mp.2010.50.

Zhao, Y., Bhattacharjee, S., Dua, P., Alexandrov, P.N., Lukiw, W.J., 2015. microRNA-based biomarkers and the diagnosis of alzheimer's disease. Front. Neurol. 6, 162. https://doi.org/10.3389/fneur.2015.00162.