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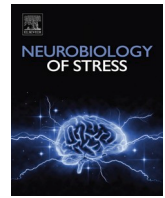
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Juvenile exposure to acute traumatic stress leads to long-lasting alterations in grey matter myelination in adult female but not male rats

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ABSTRACT

Stress early in life can have a major impact on brain development, and there is increasing evidence that childhood stress confers vulnerability for later developing psychiatric disorders. In particular, during peri-adolescence, brain regions crucial for emotional regulation, such as the prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HPC), are still developing and are highly sensitive to stress. Changes in myelin levels have been implicated in mental illnesses and stress effects on myelin and oligodendrocytes (OLs) are beginning to be explored as a novel and underappreciated mechanism underlying psychopathologies. Yet there is little research on the effects of acute stress on myelin during peri-adolescence, and even less work exploring sex-differences. Here, we used a rodent model to test the hypothesis that exposure to acute traumatic stress as a juvenile would induce changes in OLs and myelin content across limbic brain regions. Male and female juvenile rats underwent 3 h of restraint stress with exposure to a predator odor on postnatal day (p) 28. Acute stress induced a physiological response, increasing corticosterone release and reducing weight gain in stress-exposed animals. Brain sections containing the PFC, AMY and HPC were taken either in adolescence (p40), or in adulthood (p95) and stained for markers of OLs and myelin. We found that acute stress induced sex-specific changes in grey matter (GM) myelination and OLs in both the short- and long-term. Exposure to a single stressor as a juvenile increased GM myelin content in the AMY and HPC in p40 males, compared to the respective control group. At p40, corticosterone release during stress exposure was also positively correlated with GM myelin content in the AMY of male rats. Single exposure to juvenile stress also led to long-term effects exclusively in female rats. Compared to controls, stress-exposed females showed reduced GM myelin content in all three brain regions. Acute stress exposure decreased PFC and HPC OL density in p40 females, perhaps contributing towards this observed long-term decrease in myelin content. Overall, our findings suggest that the juvenile brain is vulnerable to exposure to a brief severe stressor. Exposure to a single short traumatic event during peri-adolescence produces long-lasting changes in GM myelin content in the adult brain of female, but not male, rats. These findings highlight myelin plasticity as a potential contributor to sex-specific sensitivity to perturbation during a critical window of development.

1. Introduction

Stress early in life can have a major impact on brain development and behavior. In particular, stressful experiences from infancy through adolescence are associated with an increased risk of later developing

psychiatric disorders (Carr et al., 2013; Hughes et al., 2017; Ventriglio et al., 2015). For example, childhood trauma increases the risk for developing depression and anxiety (Heim and Nemeroff, 2001). Yet, individuals who experience similar stressful environments can have very different responses to stress; only a subpopulation demonstrates

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vulnerability, while others demonstrate resilience (Compas and Phares, 1991; Kessler et al., 1995; McEwen and Stellar, 1993). In part, these individual differences may be explained by biological sensitivities to context and the environment (Ellis and Boyce, 2008). Furthermore, other factors, such as sex, may play a role. For example, females are more susceptible to developing post-traumatic stress disorder (PTSD) and anxiety (Breslau, 2009; Kessler et al., 1995; McLean and Anderson, 2009). Thus, a major goal is not only to understand the neurobiological effects of early life stress, but also to understand the biological factors that contribute to individual variability.

Experiencing early life stress, encompassing infancy through adolescence, leads to physiological changes in the body and in the central nervous system (Bolton et al., 2019; Pagliaccio et al., 2014; Van Bodegom, Homberg and Henckens, 2017). In particular, stressors experienced during peri-adolescence may have a significant impact on brain maturation and development. Adolescence, defined by the onset of puberty (~age 10 in humans, ~postnatal day 36 in rodents), is a major period of experience-dependent plasticity and thus, the brain is particularly sensitive to environmental stimuli such as stressors (Piekarski et al., 2017). This in turn could contribute towards the onset of psychiatric disorders such as anxiety and depression, which often appear around this time (Eiland and Romeo, 2013; Gee and Casey, 2015; Kessler et al., 2005, 2007). Importantly, brain regions that play a role in the stress response, such as the amygdala (AMY), hippocampus (HPC) and prefrontal cortex (PFC), are still developing during peri-adolescence and are highly sensitive to stress (McEwen et al., 2015; Popoli et al., 2012; Roozendaal et al., 2009; Spear, 2000).

In humans, stress early in life leads to alterations in multiple brain regions, including in the HPC, AMY and PFC. For example, early life stress reduced HPC and AMY volume and led to alterations in the frontal cortex and anterior cingulate cortex (Andersen et al., 2008; R. A. Cohen et al., 2006; Hanson et al., 2013; Luby et al., 2013; Teicher et al., 2006). In one specific longitudinal study of children who experienced maltreatment, high cortisol levels and PTSD symptoms were correlated with subsequent reductions in HPC volume (Carrion et al., 2007). In addition to structural changes in grey matter (GM), early life stress also changes functional connectivity. In particular, there is elevated AMY reactivity to emotional stimuli and weaker AMY – PFC connectivity after early stress (Gee et al., 2013; McCrory et al., 2013; Nooner et al., 2013; Tottenham and Galván, 2016). In rodents, structural and connectivity changes in the HPC, AMY and PFC are also observed following early life stress (M. M. Cohen et al., 2013; Gutman and Nemeroff, 2002; Honeycutt et al., 2020; Johnson et al., 2018; Meaney et al., 1988). While the majority of rodent studies have utilized early postnatal stressors, resembling infancy in humans, later early-life periods such as the juvenile and/or peri-adolescent period, resembling childhood and pre-teen years in humans, have only recently been explored (Eiland et al., 2012; Eiland and Romeo, 2013; Isgor et al., 2004; Oztan et al., 2011; Spear, 2000). In 2012, Eiland and colleagues found that chronic restraint stress during the peri-adolescent period (p20-41) in male and female rats reduced pyramidal neuron complexity in the PFC and HPC but increased neuronal complexity in AMY. Furthermore, these changes were associated with elevated depressive-like behaviors (Eiland et al., 2012). Thus, in both humans and rodents, there is a growing body of literature suggesting that stress exposure throughout early life leads to changes in developing limbic brain regions, including the HPC, AMY and PFC.

Much of the peri-adolescent literature has focused on the effects of stress on neurons and neuronal plasticity. However, stress effects on glia are beginning to be explored. Specifically, oligodendrocytes (OLs) and the myelin they produce are sensitive to stress, not only in white matter, but also in GM regions (Chetty et al., 2014; Makinodan et al., 2012; Monje, 2018; Saul et al., 2015). In addition, OLs and myelin have been implicated in a number of mental health disorders, including schizophrenia, depression and PTSD, suggesting they play a functional role in mood (Birey et al., 2017; Chao et al., 2015; Falkai et al., 2016; Fields,

2008; Lee and Fields, 2009; Ma et al., 2007; Nave and Ehrenreich, 2014; Regenold et al., 2007; Sokolov, 2007; Tham et al., 2011). Chronic stress early in life can also alter myelination in both humans and rodents. In humans, both institutionalization and early child abuse are associated with alterations in white matter in the PFC; furthermore, these changes correlate with cognitive deficits (Hanson et al., 2013; Lutz et al., 2017). In mice, chronic social isolation during peri-adolescence leads to increased depressive-like behaviors, reduced myelin basic protein (MBP) and hypomyelination of the PFC (Leussis and Andersen, 2008; Makinodan et al., 2012). This effect is only observed when the stressor occurs during the juvenile period, following weaning but prior to puberty, suggesting there may be a critical window for stress effects on PFC myelination (Makinodan et al., 2012). Together, these data indicate that altered myelination may be a novel and underappreciated mechanism by which psychopathologies emerge.

While the majority of prior work has focused on chronic stress effects on myelin and OLs, less is known about the effects of acute traumatic events. Experiencing trauma in childhood leads to increased risk for developing psychiatric disorders, including anxiety, depression and PTSD; these same disorders are also associated with alterations in OLs and myelin (Fields, 2008; Heim and Nemeroff, 2001; Yehuda et al., 2001). A critical unanswered question then is whether changes in OLs and myelin are observed following acute traumatic stress during the juvenile period. In particular, the PFC, AMY and HPC are key limbic brain regions of interest, as they are highly plastic during peri-adolescence and chronic stress robustly alters myelin across these regions. Thus, in this study, we sought to explore whether experiencing acute stress during peri-adolescence will induce changes in OLs and GM myelin content across limbic regions involved in stress and emotional regulation. Furthermore, we aimed to assess both short and long-term consequences. As peri-adolescence is a period of heightened experience-dependent plasticity, we predicted that acute stress would result in altered myelination in developing limbic brain regions. Specifically, in line with the chronic stress literature, we hypothesized that there would be decreased myelination following acute stress. Lastly, little is known about how myelin and OLs relate to individual differences following trauma. Therefore, we sought to address if physiological responses to traumatic stress were associated with myelin and OLs in these regions. In addition, we examined whether there are sex differences in these measures following exposure to acute severe stress. Sex is an important biological factor that contributes to individual variation in response to stress. To test these questions, we first exposed male and female juvenile rats to an acute, severe stressor. We then analyzed OL and myelin markers in the PFC, AMY and HPC in order to examine the effects of stress on glial plasticity. Tissue was taken either from adolescent or adult animals in order to test for short- or long-term changes respectively. Lastly, we assessed whether myelin and OL markers correlated with corticosterone responses throughout stress exposure, with the hypothesis that animals with the greatest physiological changes would also display the greatest changes in myelin, relative to controls.

2. Methods

2.1. Animals

Sixty-four male and female Sprague Dawley rats were used for these experiments. All rats were bred in-house in order to minimize stressful experiences such as shipping prior to testing. The average litter size was 12 rats per litter. Rats were weaned at p21, pair-housed, given *ad libitum* access to food and water and kept on a 12/12 h light/dark cycle. As best as possible, rats from a single litter were balanced among multiple conditions in order to control for litter effects (Table S1). All procedures were approved by UC Berkeley's Animal Care and Use Committee.

2.2. Stress

Each cage of rats was randomly assigned to either a stress or control condition. For animals in the stress condition (n = 32), at postnatal day 28 (p28), juvenile male and female Sprague Dawley rats were exposed to 3 h of severe stress (immobilization with exposure to a predator odor; n = 16 males, n = 16 females; Fig. 1A). We chose a combinatorial stressor in order to induce a severe stress response. Our lab and others have shown this paradigm produces a robust, stress response (Long et al., 2021; Morrow et al., 2000; Muroy et al., 2016; Zoladz and Diamond, 2016), while in contrast, prior work in our lab has identified a single 3 h immobilization trial alone as a moderate stressor with beneficial effects (Kirby et al., 2013; Muroy et al., 2016). Specifically, rats were restrained in plastic Decapicone bags (Braintree Scientific, Inc, Braintree, MA) and placed in a clean cage with a predator odor inside a fume hood. The cage

contained a cotton ball infused with 1 mL of synthetic fox urine (Red Fox Urine, Trap Shack Company, Arcadia, WI) taped approximately 1 inch from the animal's nose. Cagemates were placed side by side in the cage for the extent of the stressor. Blood samples from the tail vein were collected at three time points (see details below). All stress testing was conducted between the hours of 8am and noon. At the end of the 3-h stressor, cagemates were returned to a clean cage and allowed to self-groom. All animals in the stress condition were kept in a separate housing room for three days prior to being returned to their normal housing room, in order to minimize stress transmission to other rats.

2.3. Weight collections

Animals in both conditions were weighed at p28. Animals in the stress condition were weighed immediately prior to placement in the

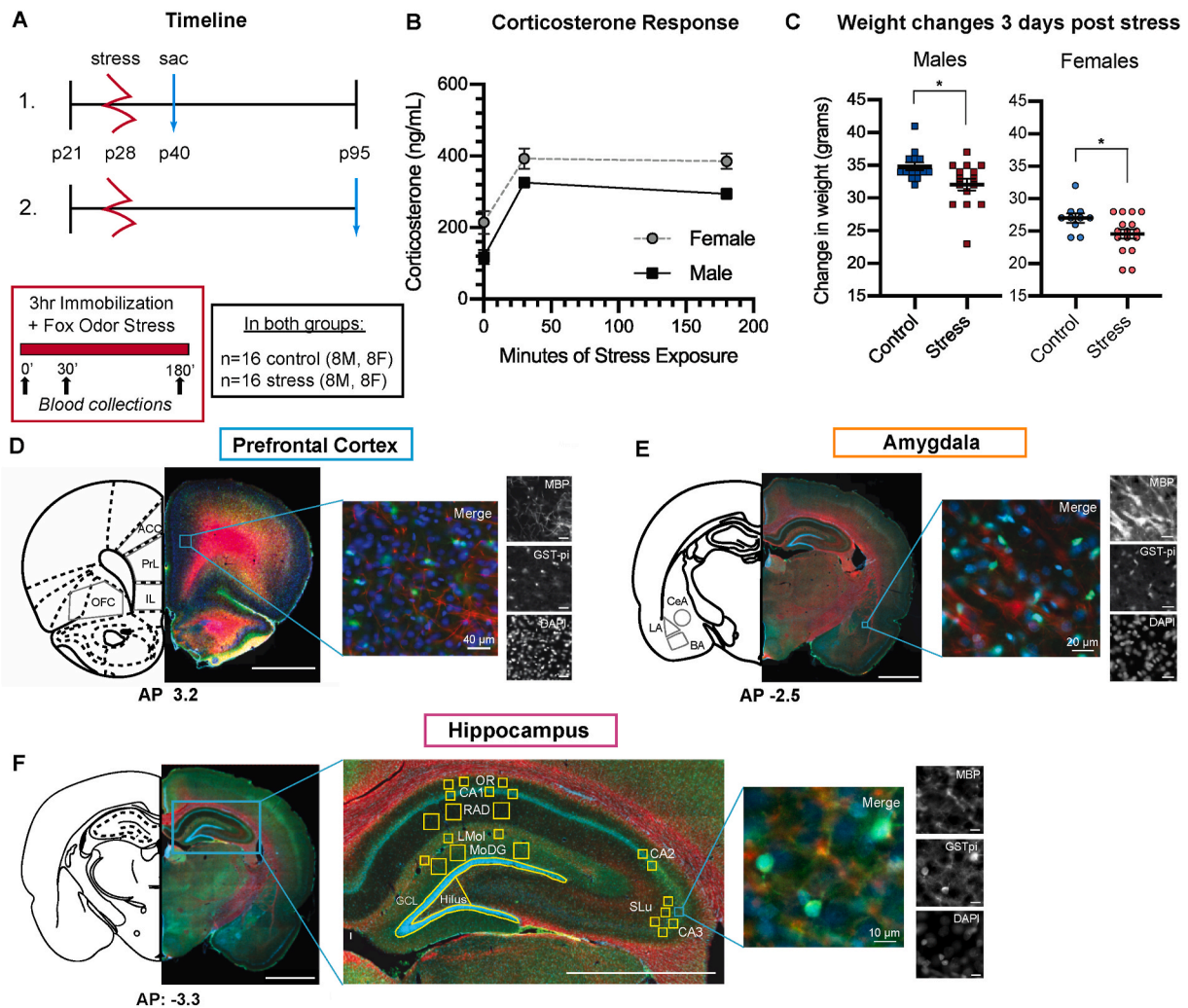


Fig. 1. Methods and Physiological responses to acute traumatic stress.

A) Experimental Timeline. Animals were divided into two cohorts: one tested in the short term (1) and one tested in the long term (2). For both cohorts, animals underwent 3 h of immobilization stress with exposure to fox urine (stress) at postnatal day 28 (p28). (n = 8 males, n = 8 females in each cohort). Blood was collected at three timepoints: just prior to stress (0'), 30 min into the stress (30'), and at the end of stress at 180 min (180'). An additional control group remained in their home cage (n = 8 males, n = 8 females in each cohort). One cohort of animals was sacrificed (sac) at p40, while another cohort was sacrificed at p95 when they were adults. B) Corticosterone responses to acute traumatic stress. Corticosterone levels (ng/mL) robustly increased during exposure to the stressor for both male and females, with higher corticosterone at 30 and 180 min over baseline values. C) Weight changes three days post stress for animals in both cohorts. Statistically significant differences are marked with asterisks (*p < 0.05). D-F: Example prefrontal cortex (PFC), amygdala (AMY) and hippocampal (HPC) coronal sections stained for MBP (red), GST-pi (green) and DAPI (blue). All coordinates are anterior-posterior (AP) from bregma. D) Example PFC section. Subregions analyzed are labeled in grey. Scale bar 2 mm. On right: Representative staining in the prelimbic cortex. E) Example AMY section. Subregions analyzed are labeled in grey. Scale bar 2 mm. On right: Representative staining in the basal amygdala. F) Example HPC section. Scale bar 2 mm. Center: A zoom in on the hippocampus. Subregions analyzed are labeled in yellow. Scale bar 1 mm. On right: Representative staining in the CA3 subregion. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

stress manipulation, while animals in the control condition ($n = 32$, 16 males, 16 females) were weighed in the housing room and otherwise remained in their home cage undisturbed. On the day stress animals were returned to their original housing room (three days post-stress exposure), rats in all conditions were weighed again. Weights from six female control animals were excluded in analyses due to inaccurate measurements.

2.4. Serum sampling for corticosterone analysis

Once restrained, tail blood was collected from each rat at 0 min, 30 min into, and at the end (3 h) of the acute traumatic stressor. Specifically, a sterile scalpel was used to remove a small segment at the end of the tail, and approximately 0.1–0.2 mL of blood was collected at each time point. For the baseline time point at the start of the stressor, we collected blood within the first 2 min to avoid detecting elevations in corticosterone due to the restraint itself. All collected samples were kept on ice throughout the stressor. Blood clots were then removed, and samples were centrifuged at 9391 g for 20 min at 4 °C. Serum was then extracted and stored in clean tubes at –80 °C. Samples were assayed using a Corticosterone EIA kit (Arbor Assays, Ann Arbor, MI), with 2 replicates per sample. Samples were compared against a standard curve to obtain the concentration of corticosterone within each sample. Using corticosterone values from the three time points throughout stress exposure (0, 30 and 180 min), we calculated an area under the curve (AUC) for each animal. This provides us with an overall measure of the corticosterone response.

2.5. Perfusions and Brain Extractions

Rats were euthanized either at p40 ($n = 32$; 16 control, 16 stress) or p95 ($n = 32$, 16 control, 16 stress) to test for short- or long-term effects, respectively. Animals were weighed, then deeply anesthetized with sodium pentobarbital 200 mg/kg (Euthasol®, Vibrac AH Inc.) and transcardially perfused with ice-cold 0.9% saline followed by freshly made 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were extracted, post-fixed for 24 h at 4 °C in 4% PFA and sunk in 30% sucrose in 0.1 M PBS over several days. Brains were stored at –80 °C until they were ready to be sliced.

2.6. Histology and immunohistochemistry

Frozen brains were cryosectioned at 40 μm on an NX70 CryoStar Cryostat (ThermoFischer Scientific). Free-floating sections were stored in 12 tubes with antifreeze, with every 12th slice placed in the same tube. Samples were stored at –20 °C prior to staining. Immunohistochemical (IHC) staining was conducted in order to quantify oligodendrocyte (OLs) and myelin markers. Specifically, IHC was used to detect myelin basic protein (MBP), one of the essential proteins in the myelin sheath (Hamano et al., 1996) and glutathione s-transferase pi (GST-pi), a marker for immature to mature OLs (Tansey and Cammer, 1991). Tissue slices from one vial of tissue (every 12th slice) were stained. Slices were first washed in tris-buffered saline (TBS), and blocked with 3% normal donkey serum (NDS) in TBS with 0.3% Triton-X100 for 1 h at room temperature. Slices were then incubated overnight at 4 °C with the following primary antibodies: rat anti-MBP (1:500, Abcam ab7349) and rabbit anti-GST-pi (1:5000, MBL 311). All antibodies were diluted in 0.3% TritonX-TBS containing 1% NDS. On day two, following three rinses in TBS (3×5 min), sections were incubated for 2 h with the following secondary antibodies: AlexaFluor 488 donkey anti-rabbit and Cy3 donkey anti-rat (1:500, 711-545-152 and 712-165-153 respectively, Jackson ImmunoResearch Labs Inc). Sections were then rinsed in TBS (3×5 min) and incubated for 10 min with DAPI (1:40,000 in 1xPBS), followed by three more rinses in TBS. Lastly, sections were mounted on glass slides and cover slipped using 1,4 diazabicyclo [2.2.2]octane (DABCO).

2.7. Fluorescent microscopy and image analysis

All tissue was imaged on an AxioScan Slide Scanner at 20x (AxioScan.Z1, Zeiss, Oberkochen, Germany). During analysis, experimenters were blind to animal condition. Regions of interest were chosen and exported as TIFFs using ZEN blue imaging software (Zeiss). For analysis of the prefrontal cortex, five PFC sections from AP 3.7 to AP 1.7 were used for analysis. Four regions of interest (ROIs) of the PFC were quantified: anterior cingulate cortex (ACC), prelimbic cortex (PL), infralimbic cortex (IL) and orbitofrontal cortex (OFC; Figure S1). For analysis of the amygdala, four sections from AP -2.00 to –3.24 were quantified, with ROIs for the basal amygdala (BA), lateral amygdala (LA) and central (CeA) amygdala (Figure S2). For the PFC and amygdala, all ROIs were hand-drawn in Fiji (Schindelin et al., 2012). For analysis of the hippocampus, six sections from AP -2.92 to –4.20 were used for analysis. Ten regions of the hippocampus were quantified: the CA1, CA2, CA3, lacunosum moleculare (LMol), molecular layer dentate gyrus (MoDG), oriens layer hippocampus (Or), radiatum layer hippocampus (Rad), stratum lucidum hippocampus (SLu), granule cell layer (GCL) and Hilus (Figure S3). The GCL and Hilus ROIs were hand-drawn in Fiji, however all of the other ROIs were analyzed by placing squares of varying sizes throughout the hippocampus. Squares were either 75 x 75 or 150 x 150 μm . All ROIs were placed based on consistent anatomical markers.

For all regions, GM myelin content was measured by using the integrated fluorescence intensity of MBP expression, normalized by area. OLs (GST-pi + cells) were quantified using a custom written Fiji script, which included background subtraction, automated thresholding and particle analysis. The same parameters were used for all animals and all tissue regions. In addition, parameters were chosen such that the script counted within 10% of counts obtained by human experimenters. OL cell density is presented as the number of cells within a given ROI per mm^2 . In addition, cell density was calculated as a % of all DAPI + cells; these data corresponded well with cell density by area, so all data presented are shown as cell density/area. Measures for each ROI were averaged across the anterior-posterior extent of sections and from both hemispheres.

2.8. Statistical tests

All data are presented as the mean, plus or minus the standard error of the mean (SEM). Outliers were removed if they were more than two standard deviations above the group mean. For all 8 groups, across the 17 subregions and both markers (MBP and GST-pi), only 31 values were excluded. For analysis of corticosterone, we used a repeated measures ANOVA, followed by a Sidak's post-hoc test for multiple comparisons. For analysis of GM myelin content and OLs, two-way ANOVA tests were used to compare male and female rats in the stress and control conditions at each age time point. An additional two-way ANOVA compared control vs. stress animals across different subregions of interest. Bonferroni post-hoc analyses were used to correct for multiple comparisons. Unpaired independent sample student's t-tests were used to directly compare changes between control and stressed animals within each sex, when appropriate. Pearson correlations were used to identify relationships between corticosterone AUC and OL or myelin markers. In all tests, the alpha value was set at 0.05. All analyses were performed with GraphPad Prism version 8.4 (GraphPad Software, San Diego, California USA) and RStudio (RStudio Team, 2018).

3. Results

3.1. Male and female juvenile rats exhibit a robust physiological response following exposure to acute, severe stress

First, we examined the physiological response of male and female juvenile rats to acute stress exposure, in order to confirm our paradigm

was indeed a potent stressor at this age. Previously, our lab and others have shown that predator scent coupled with immobilization produces a large corticosterone response in adult rats (Long et al., 2021; Morrow et al., 2000; Muroy et al., 2016; Zoladz and Diamond, 2016). Here, exposure to 3 h of immobilization stress in the presence of fox urine odor (Fig. 1A) produced robust increases in serum corticosterone over baseline levels in both male and female juvenile rats (Fig. 1B). The physiological increase in corticosterone observed 30 min into stress exposure persisted throughout the duration of the 3-h stressor. A 2-way repeated measures ANOVA yielded a main effect of time ($F(1.346, 40.39) = 90.49, p < 0.0001$), and a main effect of sex ($F(1, 30) = 11.49, p < 0.002$), with no interaction between the two. A Sidak's post-hoc test identified robust differences between corticosterone levels at baseline (0 min) compared to 30 and 180 min for both males and females ($p < 0.0001$). Female rats demonstrated significantly higher levels of corticosterone relative to males at all time points, including at baseline (Fig. 1B). This is consistent with previously reported findings that adult female rats exhibit higher baseline corticosterone levels (Kalil et al., 2013; Mitsushima et al., 2003). In addition to corticosterone, we assessed changes in body weight following acute stress exposure. Changes in body weight are strong physiological indicators of stress

(Harris et al., 1998; Pulliam et al., 2010). Three days after stress, both male and female juveniles gained less weight relative to controls (males, $t(28) = 2.133, p = 0.042$; females, $t(24) = 2.246, p = 0.034$); Fig. 1C). Collectively, these data suggest that acute, severe stress elicits a rapid physiological stress response in juvenile rats, increasing corticosterone levels and decreasing weight gain.

3.2. Myelin and oligodendrocytes are altered in limbic brain regions following acute juvenile stress in both the short and long term, with regional and sex specific differences

We sought to address whether acute severe juvenile stress affects grey matter (GM) myelin and oligodendrocytes (OLs) within three major limbic brain regions: the prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HPC). In order to identify both short- and long-term effects of juvenile stress, one group of animals was sacrificed 12 days post stress exposure (at p40), while another was sacrificed almost two months post stress exposure, at an adult age (p95) (Fig. 1A). We stained brain tissue for two markers: Glutathione S-transferase pi (GST-pi), a marker of immature to mature OLs, and myelin basic protein (MBP), a marker of myelination. In the PFC, we analyzed four separate

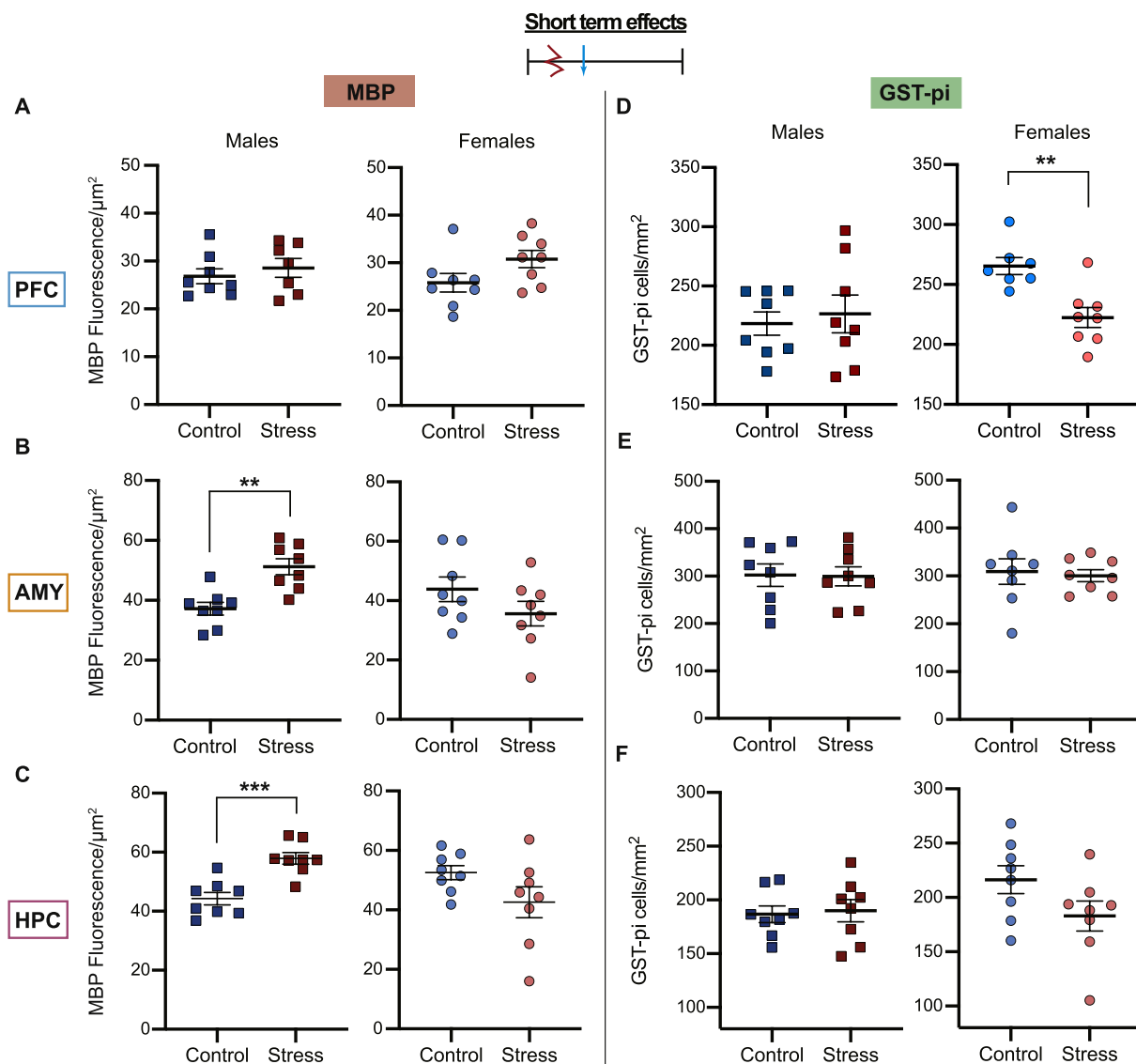


Fig. 2. Short term effects of stress-exposure on myelin and oligodendrocytes. A–C: MBP fluorescence intensity results in the (A) PFC, (B) AMY and (C) HPC. D–F: GST-pi + cell density results in the (D) PFC, (E) AMY and (F) HPC. Statistically significant differences are marked with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

subregions: the anterior cingulate cortex (ACC), the prelimbic (PL) and infralimbic (IL) cortices, and the orbitofrontal cortex (OFC) (Fig. 1D). In the AMY, we analyzed three subregions: the lateral amygdala (LA), basal amygdala (BA), and central amygdala (CeA) (Fig. 1E). In the HPC, we analyzed ten subregions of interest: the CA1, CA2, CA3, lacunosum moleculare (LMol), molecular layer dentate gyrus (MoDG), oriens layer hippocampus (OR), radiatum layer hippocampus (RAD), stratum lucidum hippocampus (SLu), granule cell layer (GCL), and hilus regions (Fig. 1F). Measures of OLs and GM myelin in the PFC, AMY and HPC were averaged across subregions.

3.2.1. Acute severe stress drives short-term increases in grey matter myelin in the amygdala and hippocampus of male rats

In all three brain regions (PFC, AMY and HPC), there were short term effects of stress on GM myelin, measured by MBP. In the PFC, there were no differences in MBP fluorescence intensity in males. For females, while we did not see an effect of stress within the whole PFC ($p = 0.08$; Fig. 2A), there was increased MBP, on average, relative to controls (Control = 25.8 ± 1.9 fluorescence/ μm^2 , Stress = 30.8 ± 1.8 fluorescence/ μm^2) and a 2-way ANOVA (subregion x condition) revealed a main effect of stress ($F(1,56) = 11.04$, $p = 0.0016$; Table 1; Figure S4A). Stress-exposed female animals displayed higher levels of MBP in all subregions of the PFC compared to their respective controls, indicating the main effect of stress was not driven by any one PFC subregion (Table 1; Figure S4A). For both the AMY and HPC, while there was no main effect of sex, there was an interaction between sex and condition, with increased MBP intensity in stress-exposed males and decreased MBP intensity in stress-exposed females (AMY: $F(1,28) = 10.78$, $p = 0.0028$; HPC: $F(1,28) = 13.71$, $p = 0.0009$; Fig. 2B–C). In the AMY, MBP intensity significantly increased in stress-exposed males relative to controls (Males: Control = 37.2 ± 6.1 fluorescence/ $\pm \mu\text{m}^2$, Stress = 51.193 ± 7.5 fluorescence/ μm^2 , $t(14) = 4.095$, $p = 0.0011$; Fig. 2B). This effect of stress on MBP appeared across all of the AMY ROIs. Bonferroni multiple comparisons tests revealed significant increases in MBP in the BA, LA, and CeA for stress-exposed males (BA: $p = 0.0097$; LA: $p = 0.0104$; CeA: $p = 0.0477$; Figure S4B). In contrast to the males, stress-exposed females displayed, on average, reduced MBP; although this effect was not statistically significant averaged across the AMY ($p = 0.10$), there was a main effect of condition when testing across all AMY subregions ($F(1,42) = 5.68$, $p = 0.022$; Table 1). In the HPC, there was a statistically significant increase in MBP in stress-exposed males compared to controls and a trend for decreased MBP relative to controls in stress-exposed females (Males: $t(14) = 4.745$, $p = 0.000313$; Females: $t(14) = 1.75$, $p = 0.10$; Fig. 2C). This increased MBP in stress-exposed males occurred in the majority of individual subregions of the HPC, including in the CA1, CA2, CA3, MoDG, RAD, SLu and GCL (CA1: $p = 0.0123$; CA2: $p = 0.0112$; CA3: $p = 0.0417$; MoDG: $p = 0.0066$; RAD: $p = 0.0228$; SLu: $p = 0.0142$; GCL: $p = 0.0307$; Figure S5A). Interestingly, in stress-exposed females, there was a main effect of condition ($F(1,138) = 21.57$, $p < 0.0001$; Table 1) and multiple comparisons tests

revealed significant decreases in MBP in the following HPC subregions: CA1, CA2, OR and RAD (CA1: $p < 0.0001$; CA2: $p = 0.0470$; OR: $p = 0.0044$; RAD: $p = 0.0009$; Figure S5B).

3.2.2. Acute severe stress drives short term decreases in oligodendrocytes in the PFC of female rats

Acute severe juvenile stress also produced short term changes in OL density across limbic regions in female rats. In the PFC, while there was no main effect of sex, there was an interaction between sex and condition ($F(1,27) = 5.3$, $p = 0.0292$). Stress-exposed females had reduced density of GST-pi + cells in the PFC relative to controls (Control = 265 ± 7.1 cells/ mm^2 , Stress = 222 ± 8.4 cells/ mm^2 , $t(13) = 3.842$, $p = 0.002$, Fig. 2D). No change was observed in stress-exposed males. Across the individual PFC subregions, there was again a main effect of stress for females ($F(1,52) = 24.72$, $p < 0.0001$; Table 1), but not males. A multiple comparisons test identified statistically significant decreases in GST-pi in the IL and OFC regions for stress-exposed females ($p = 0.0318$ and $p = 0.0378$ respectively; Figure S4C). In contrast, there were no changes in GST-pi density for males and females, both in the AMY as a whole and in all AMY subregions (Fig. 2E, Figure S4D). Lastly, in the HPC, stress-exposed females tended to have decreased GST-pi + density relative to controls (Control = 216 ± 36.205 cells/ mm^2 , Stress = 182 ± 38.77 cells/ mm^2 , $p = 0.09$, Fig. 2F) and indeed, there was a main effect of condition when looking across all HPC ROIs ($F(1,135) = 8.28$, $p = 0.0047$; Table 1). Yet, there was also a large amount of variance in GST-pi across subregions (Figure S5). Decreased GST-pi density was seen across many subregions, and most robustly in the RAD ($p = 0.032$). Yet other regions displayed no change or even increases relative to controls, such as in the CA2 and CA3 (Figure S5C–D). No changes were observed in males, either in the HPC as a whole or in any particular HPC ROIs.

Together, these findings suggest that acute severe stress leads to short term increases in GM myelin content, with increased myelin in the PFC in females and increased myelin in the AMY and HPC for males. In contrast, OL density remained unchanged shortly following stress in all regions for males, while there were significant decreases in the HPC and PFC for females.

3.2.3. Acute severe stress drives long term decreases in grey matter myelin across all limbic regions for female, but not male rats

We next looked at the long-term effects of acute severe stress exposure on GM myelin. At p95, two months after stress exposure, animals are well into adulthood. In all three brain regions, there were long term effects of juvenile stress on GM myelin. In particular, stress-exposed females, but not males, had reduced MBP fluorescence intensity in the PFC, AMY, and HPC compared to controls (PFC: $t(11) = 2.378$, $p = 0.036$, AMY: $t(14) = 3.362$, $p = 0.0046$, HPC: $t(14) = 4.262$, $p = 0.0008$, Fig. 3A–C). In each of these areas, all subregions showed a female specific decrease in MBP intensity compared to control animals, highlighting main effects of stress (PFC: $F(1,56) = 20.07$, $p < 0.0001$, AMY: $F(1,42) = 28.81$, $p < 0.0001$, HPC: $F(1,139) = 112.9$, $p < 0.0001$;

Table 1

Main effects of condition on MBP and GST-pi in limbic subregions of interest. Two-way ANOVAs (subregion x condition) were conducted for each major limbic region, testing for a main effect of condition (control or stress) on myelin and oligodendrocytes. F statistics and p-values are reported here. Statistically significant main effects are in bold.

	Short Term (p40)				Long Term (p95)			
	MBP		GST-pi		MBP		GST-pi	
	Male	Female	Male	Female	Male	Female	Male	Female
PFC	$F(1,52) = 1.49$ $p = 0.23$	$F(1,56) = 11.04$ **$p = 0.0016$	$F(1,55) = 0.77$ $p = 0.38$	$F(1,52) = 24.72$ ***$p < 0.0001$	$F(1,56) = 0.52$ $p = 0.47$	$F(1,56) = 20.07$ ***$p < 0.0001$	$F(1,56) = 5.43$ *$p = 0.023$	$F(1,54) = 0.04$ $p = 0.84$
AMY	$F(1,42) = 43.23$ ***$p < 0.0001$	$F(1,42) = 5.68$ *$p = 0.022$	$F(1,42) = 0.01$ $p = 0.92$	$F(1,42) = 0.16$ $p = 0.69$	$F(1,42) = 0.05$ $p = 0.83$	$F(1,42) = 28.81$ ***$p < 0.0001$	$F(1,41) = 19.15$ ***$p < 0.0001$	$F(1,41) = 6.53$ *$p = 0.014$
HPC	$F(1,138) = 98.64$ ***$p < 0.0001$	$F(1,138) = 21.57$ ***$p < 0.0001$	$F(1,137) = 0.35$ $p = 0.56$	$F(1,135) = 8.28$ **$p = 0.0047$	$F(1,140) = 16.79$ ***$p < 0.0001$	$F(1,139) = 112.9$ ***$p < 0.0001$	$F(1,138) = 3.20$ $p = 0.08$	$F(1,136) = 7.09$ **$p = 0.0087$

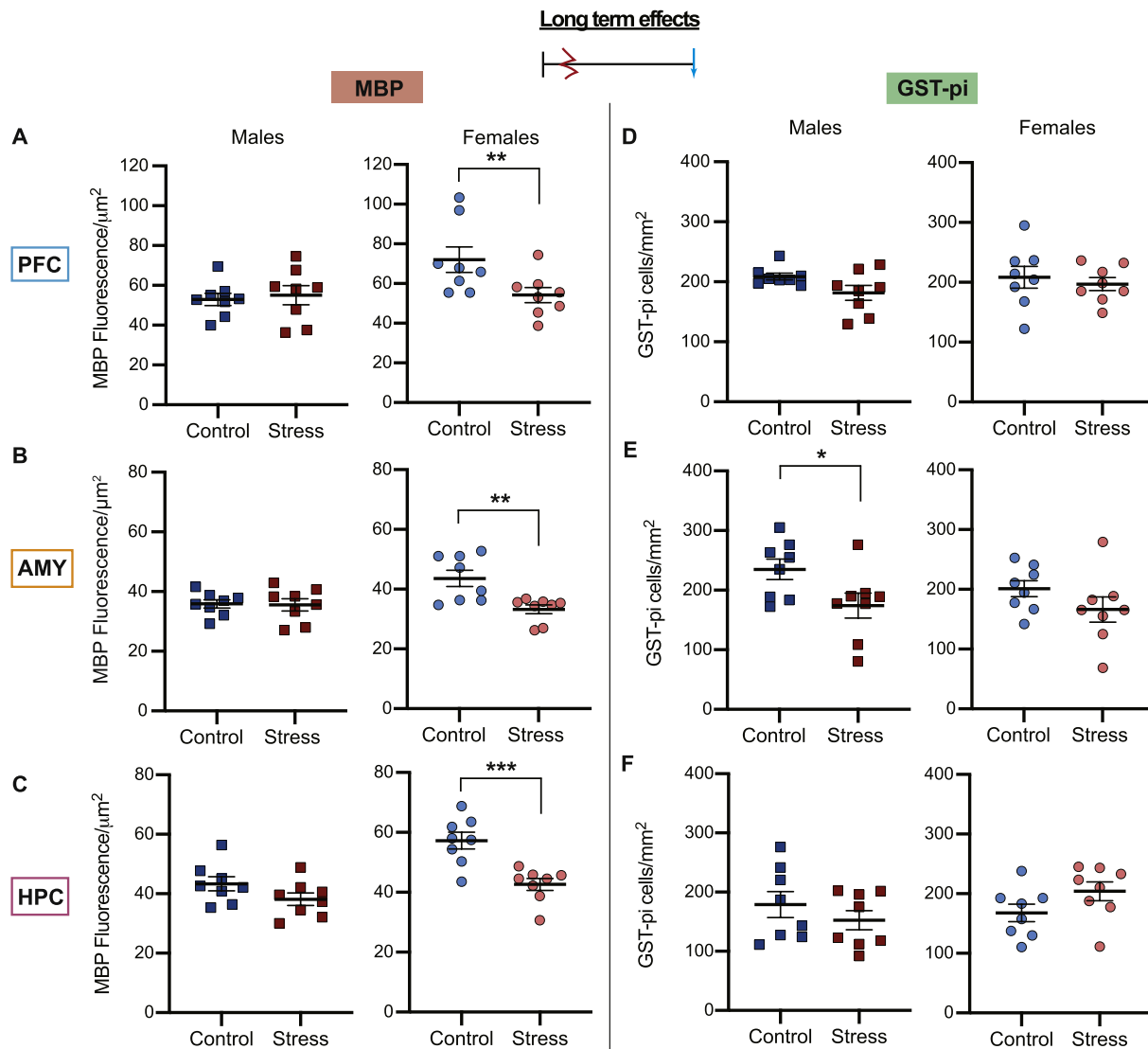


Fig. 3. Long term effects of stress-exposure on myelin and oligodendrocytes. A-C: MBP fluorescence intensity results in the (A) PFC, (B) AMY and (C) HPC. D-F: GST-pi + cell density results in the (D) PFC, (E) AMY and (F) HPC. Statistically significant differences are marked with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Table 1). In the PFC, a Bonferroni multiple comparisons test identified significant decreases in MBP in the OFC region ($p = 0.022$, Figure S6A). In the AMY, all three ROIs showed significant decreases in MBP (BA: $p = 0.0014$; LA: $p = 0.0070$; CeA: $p = 0.0042$; Figure S6B). Decreases in adult female HPC MBP also consistently appeared across multiple subregions of interest; all regions except the OR and SLU showed a significant decrease in MBP fluorescence intensity in stress compared to control animals (CA1: $p = 0.0005$; CA2: $p = 0.0016$; CA3: $p = 0.0002$; LMol: $p = 0.0017$; MoDG: $p = 0.0008$; RAD: $p = 0.0137$; GCL: $p = 0.0010$; Hilus: $p < 0.0001$, Figure S7A-B). In contrast with females, no differences in average MBP were observed between stress-exposed and control adult males in any of three limbic regions (Fig. 3A-C). Despite significant interactions between sex and condition in the PFC ($F(1,28) = 4.4$, $p = 0.0436$) and the AMY ($F(1,28) = 6.490$, $p = 0.0166$; Fig. 3A-B), none of the PFC or AMY subregions showed differences in MBP between stress exposed and control males. In HPC subregions however, there was a main effect of condition ($F(1,140) = 16.79$, $p < 0.0001$; Table 1) and the OR showed a significant decrease in MBP levels in stress-exposed males ($p = 0.012$; Figure S7A-B). Overall, juvenile acute stress produced robust long-term decreases in GM myelin in females, while males remained largely unaffected.

3.2.4. Acute severe stress decreases oligodendrocyte density in the long term only in the CeA

Unlike effects identified in the short term, acute severe juvenile stress did not lead to consistent global changes in OL density across limbic regions. However, despite the lack of global effects, there were several significant changes in OL density at p95. In the PFC, stress-exposed males displayed a trend towards decreased GST-pi density ($p = 0.067$; Fig. 3D) that reflected a statistically significant main effect of stress on PFC subregions ($F(1,56) = 5.4$, $p = 0.0234$; Table 1). This mean decrease was observed for all subregions of the PFC (Figure S6C). In contrast, no changes in PFC GST-pi density were observed for adult females. In the AMY, there was a significant decrease in GST-pi density for adult male rats (Male: $t(14) = 2.242$, $p = 0.0417$; Fig. 3E), but not stress-exposed females. GST-pi density differed highly across subregions. Interestingly, GST-pi density was significantly lower in the CeA for both male and female stress-exposed animals relative to controls (Males: $p = 0.0059$; Females: $p = 0.0118$; Figure S6D). Lastly, in the HPC, no long-term change in average GST-pi density was observed when directly comparing controls to stress-exposed animals for either sex (Fig. 3F). However, when looking at HPC subregions, there was a main effect of stress on GST-pi for females ($F(1,136) = 7.09$, $p = 0.0087$; Table 1), and

on average, stress exposed females had a higher density of GST-pi + cells relative to controls (Figure S7C-D).

Overall, for females, acute, severe stress as a juvenile leads to long term decreases in GM myelin content in limbic regions. In contrast, stress exposed males display no long-term changes in myelin levels. OL density is also largely unaffected, except for in the CeA, where both males and females display decreased OL density.

3.3. PFC, AMY and HPC myelin levels shortly after stress correlate with corticosterone responses

All juvenile animals exposed to acute stress demonstrated marked increases in corticosterone levels while undergoing the 3-h stressor, indicating a physiological stress response (Fig. 1B). Here, we explored whether these physiological changes in corticosterone corresponded with individual changes in GM myelin content and OL cell density. We assessed overall corticosterone levels utilizing the area under the curve (AUC). In the short term, at p40, AUC corticosterone was significantly correlated with myelin levels (MBP) in several groups (Fig. 4). Specifically, in stress-exposed males, PFC myelin content was inversely correlated with corticosterone ($r = -0.84, p = 0.019$; Fig. 4A). Animals

with the highest levels of corticosterone during stress-exposure had the lowest levels of PFC myelin at p40. In contrast, AMY and HPC myelin levels were positively correlated with AUC corticosterone in male animals. This effect was statistically significant in the AMY and trending in the HPC (AMY: $r = 0.89, p = 0.003$; HPC: $r = 0.67, p = 0.07$; Fig. 4B-C). In p40 stress-exposed females however, AUC corticosterone responses during stress-exposure were negatively correlated with myelin content in all three regions, though this relationship was only statistically significant in the HPC ($r = -0.81, p = 0.016$; Fig. 4C). For the AMY and HPC, these opposing correlational directions for males and females mimics the opposing directionality of group-wide changes in MBP; stress-exposed males had increased AMY and HPC myelin content at p40, while stress-exposed females had decreased AMY and HPC myelin content on average, relative to controls. Importantly, corticosterone levels at timepoint 0, which served as a baseline, were not significantly correlated with GM myelin content in p40 males or females for any of the brain regions analyzed. This indicates that corticosterone responses during stress exposure, and not basal corticosterone, was associated with subsequent myelin levels.

Although corticosterone was significantly associated with p40 MBP levels, there were no statistically significant correlations of

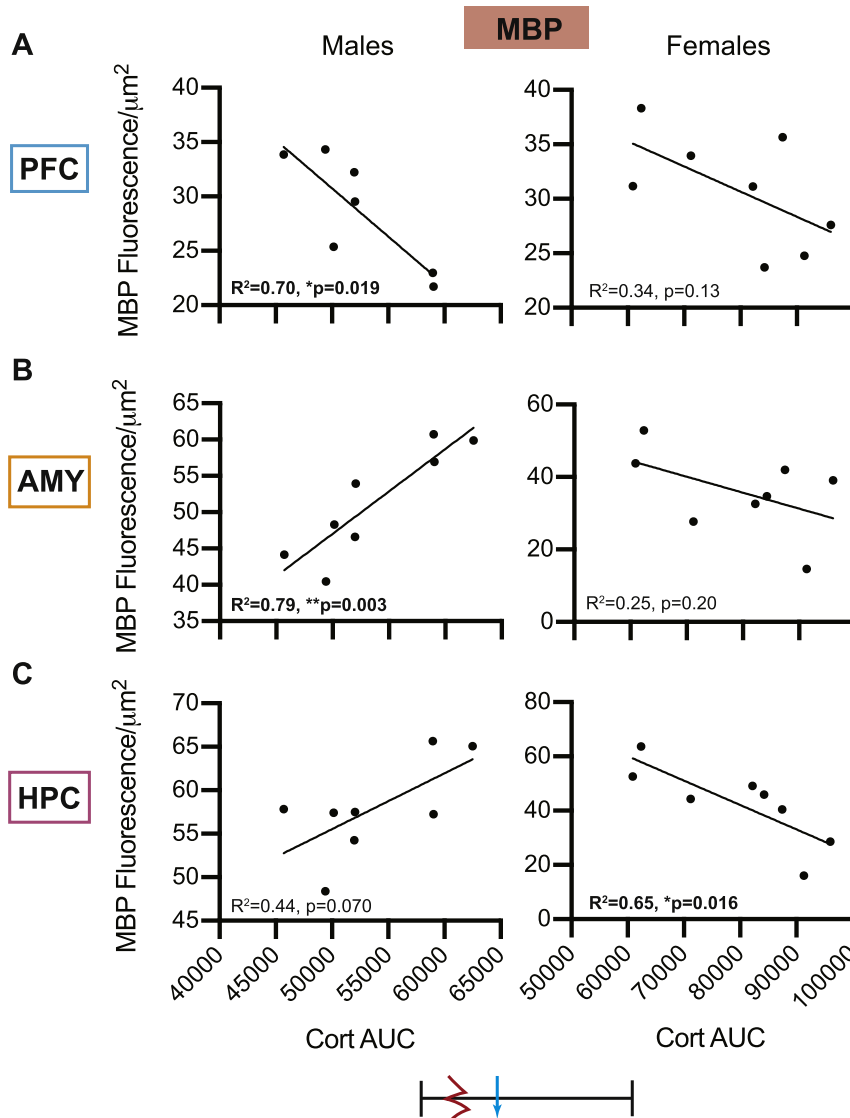


Fig. 4. Correlations of corticosterone with short term myelin. Pearson's correlations of MBP fluorescence intensity with area under the curve (AUC) for corticosterone in the (A) PFC, (B) AMY and (C) HPC of male and female rats tested at p40.

corticosterone with GST-pi at p40, nor were there significant correlations of corticosterone with either MBP or GST-pi in the long term, in either sex (Figure S8). In addition, weight changes following stress did not strongly correlate with GM myelin content at either p40 or p95 (Figure S9). Overall, these data provide evidence that corticosterone levels during acute stress exposure may be associated with short-term PFC, AMY and HPC myelin levels.

4. Discussion

Changes in myelin and oligodendrocytes (OLs) are beginning to emerge as a novel mechanism that may contribute towards stress-induced pathologies (Chao et al., 2015; Chetty et al., 2014; Gibson et al., 2018). Here, we aimed to fill a gap in the literature surrounding myelin and OL plasticity following juvenile trauma. In particular we focused on changes in the prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HPC) - regions that are highly plastic and sensitive to stress during the peri-adolescent period (Spear, 2000). Furthermore, we sought to address if stress-induced alterations in grey matter (GM) myelin and OLs are long-lasting, and whether sex differences are observed. To our knowledge, this is the first study to examine changes in myelination in these regions after a single, acute stressor during this critical developmental window.

In this study, we exposed male and female juvenile rats to an acute traumatic stressor. First, we confirmed that juvenile stress exposure induced physiological changes; trauma increased serum corticosterone levels and reduced weight gain relative to controls in both males and females. Next, we tested whether exposure to acute severe stress as a juvenile produced subsequent short- or long-term changes in GM myelin and OLs in the PFC, AMY and HPC. Most interestingly, stress exposure altered myelin and OLs in all three brain regions, with changes occurring in a sex-specific manner. Further, exposure to juvenile stress had different effects on male and female myelin content and OLs across time. These changes are summarized in Table 2. In the short term, myelination in the PFC increased for stress exposed females, while AMY and HPC myelination increased for stress exposed males. Juvenile stress also reduced OL density in the PFC and HPC in p40 females but not males. In contrast to the short term increases in myelin, in the long term, juvenile stress decreased myelination in all three limbic regions exclusively in females. In the majority of brain regions, OL density remained largely unchanged in the long term. Altogether, our findings suggest that myelin and OLs in the PFC, AMY and HPC are sensitive to acute stress during the peri-adolescent time period of development. We conclude that experiencing a single acute severe stress as a juvenile alters myelin and OLs in a sex- and region-specific manner, with lasting impacts.

4.1. Short-term changes following juvenile stress-exposure

In the short-term, stress-exposed females displayed increased levels of MBP across the PFC and males displayed increased MBP across the AMY and HPC. These findings supports the possibility that stress drives early maturation of limbic circuits (Bath et al., 2016; Callaghan and Richardson, 2011; Gee et al., 2013; Honeycutt et al., 2020; Ono et al.,

2008; Thomas et al., 2016). For example, in male mice, early life stress due to fragmented maternal care drives an earlier rise in MBP in the HPC (Bath et al., 2016), and early weaning prompts precocious myelination in the AMY (Ono et al., 2008). During development, myelination acts to inhibit axonal sprouting and spine turnover, thereby acting as a brake on plasticity (Fields, 2008). Therefore, early myelination of the PFC, AMY or HPC could lead to impaired circuit functioning. In particular, plasticity within the PFC is especially critical for behaviors such as cognitive flexibility and decision making (Thomas et al., 2016). Alternatively, rather than driving early maturation to adult levels, stress may be altering developmental trajectories in the brain in a more transient manner (Thomas et al., 2020).

Our finding that myelination increases immediately following acute juvenile trauma stands in contrast with findings following exposure to chronic post-natal or juvenile stress. For example, chronic maternal separation reduced markers of myelin in the mPFC and HPC (Bordner et al., 2011; Wei et al., 2015; Yang et al., 2017). Furthermore, juvenile social isolation or social defeat reduced both myelin thickness and MBP levels in the mPFC (Liu et al., 2012; Zhang et al., 2016). Although GM myelination increased in several conditions shortly following exposure to acute juvenile stress, OL cell density either decreased or remained unchanged in the short term. In particular, OL density decreased in the PFC and HPC for stress exposed females, suggesting juvenile stress may impair OL proliferation or survival in those regions. A decrease in OL density following acute stress exposure is in line with reports of chronic early life adversity. For example, reductions in OLs are observed in the PFC of male mice following maternal separation (Bordner et al., 2011; Yang et al., 2017).

4.2. Long-term changes following juvenile stress-exposure

We observed a long-term reduction in PFC, AMY and HPC MBP in females, but limited changes in males. In prior work, adolescent social defeat did not change HPC myelin in the long term in male mice (Xu et al., 2020). In addition, in a study of adult stress in male mice, chronic social stress did not alter myelin related transcripts in the AMY (Liu et al., 2018). Thus, the lack of long-term changes in AMY and HPC myelin in male animals are not unexpected. Long term changes in AMY and HPC myelin in female animals, however, have never before been described, making our study the first to report a long lasting, acute stress-induced reduction in AMY and HPC myelination. A long-term reduction in PFC myelination is also in line with prior studies of juvenile chronic stress. For example, social isolation as a juvenile (from p21 to p35) led to reductions in PFC myelination, alterations in OL morphology and changes in mPFC-mediated behaviors when tested at p65 (Makinodan et al., 2012). Social isolation outside of this critical time window did not lead to such changes. We also found no changes, on average, in adult PFC OL cell number following juvenile stress. This is in line with a number of studies with stressors both in adolescence and adulthood that demonstrate reductions in PFC myelin levels but not OLs (Lehmann et al., 2017; Liu et al., 2012; Makinodan et al., 2012; Xu et al., 2020; Zhang et al., 2016). A key difference, however, is that in these prior studies, changes in PFC myelination were observed in male, but

Table 2

Summary of stress-exposure effects on MBP and GST-pi across all conditions.

Arrows denote direction of change. Bold arrows indicate statistically significant effects when comparing both the average of the region and the subregions of interest. Dashed arrows indicate effects that were trending when comparing the average of the region but showed a statistically significant main effect of condition when comparing the subregions of interest.

	Short Term (p40)				Long Term (p95)			
	MBP		GST-pi		MBP		GST-pi	
	Male	Female	Male	Female	Male	Female	Male	Female
PFC	–	↑	–	↓	–	↓	↓	–
AMY	↑	↓	–	–	–	↓	↓	↓
HPC	↑	↓	–	↓	↓	↓	–	↑

not female animals, while here we observe the opposite effects. For example, one study found that social isolation (p30-p35) reduced MBP protein levels, quantified by western blots, in male but not female rats (Leussis and Andersen, 2008). Here, in contrast, in both the short and long-term, we found that robust effects of traumatic stress on PFC myelination and OLs were limited to females. Different stressor types (social isolation or physical restraint stress), the timing of stress (whether before or after puberty onset), or the method of MBP assessment (immunohistochemistry or Western blot), could all contribute to these observed dissimilarities. The majority of prior work focuses only on male rodents, and there remains a great need for side-by-side comparison of male and female animals using the same paradigms. Indeed, testing different stress paradigms may be especially useful, as long-term sex differences in myelin and OLs could arise due to known differential sensitivities of males and females to particular stressors (Bourke and Neigh, 2011; Toledo-Rodriguez and Sandi, 2007; Weintraub et al., 2010). Understanding sex differences are especially important, as females are more likely to experience trauma during the peri-adolescent period, and trauma during this time has long-lasting consequences on mental health, such as increased risk for developing PTSD (Cuffe et al., 1998; Garza and Jovanovic, 2017; Udwin, Boyle, Yule, Bolton, & O’Ryan, 2000).

4.3. Relating short-term and long-term effects of juvenile stress-exposure

The most striking result in this study is that in females, but not in males, exposure as a juvenile to an acute traumatic event led to drastic and wide-spread alterations in the adult brain, measured as reductions in GM myelination across all three brain regions. The observed short-term decrease in OLs in females may partially explain this longer-term decrease in MBP; less immature and mature OLs at p40 might lead to fewer mature, myelinating OLs in adulthood as OLs undergo maturation over time (Noble et al., 2006). Male animals exposed to stress also display changes in GM myelination, with contrasting changes in the short- and long-term; for example, HPC myelin content was increased at p40 but decreased at p95, relative to controls. One possibility is that acute stress alters developmental trajectories in GM myelination, perhaps leading to an earlier peak and an earlier decline. In our dataset, the same animals could not be examined at both time points due to the nature of our method; however, it would be interesting for future studies to analyze myelin *in vivo* in a longitudinal manner, perhaps with MRI imaging. Furthermore, adding more timepoints would also improve resolution in the dataset, allowing us to map a curve of both GST-pi and MBP trajectories following stress, providing a deeper understanding into how stress alters myelin and OLs across time.

4.4. Associations of myelin with corticosterone responses during stress

Intriguingly, corticosterone responses during acute stress were correlated with myelin levels 12 days later at p40. The limbic system contains a high density of glucocorticoid receptors (GRs) and is highly sensitive to glucocorticoid signaling. OLs in these regions also express GRs (Chiba et al., 2012; Holsboer, 2000). Although a detailed mechanism remains unknown, there is literature to suggest glucocorticoids affect oligodendrogenesis and myelination in the adult brain (Chetty et al., 2014; Masters et al., 1994; Miyata et al., 2011; Wennström et al., 2006). Specifically, exposing neural stem cells to corticosterone both *in vitro* and *in vivo* increased expression of the OL transcription factors Olig1 and Olig2 and increased the percentage of MBP⁺ cells 1–2 weeks later. This effect was dependent on activation of GR signaling (Chetty et al., 2014). Corticosterone-induced changes in transcriptional signaling may therefore provide a possible mechanism by which stress alters myelination in the short-term. Of note, here we observed corticosterone correlated with short-term, but not long-term, myelin content. Although long-term changes in myelin did not correlate with the initial corticosterone response, a lack of correlation does not necessarily

indicate that corticosterone is not mechanistically driving these long-term changes. Any stress response at all, regardless of the level of corticosterone, might lead to downstream effects that ultimately alter myelin. However, other hormone or signaling pathways activated during the initial stress response could also be responsible for these long-term changes; these possibilities are underexplored and will be an area for future work.

In the current study, in the PFC, for both males and females, animals with higher corticosterone responses showed the lowest levels of PFC myelination at p40. Activation of GRs in the PFC may therefore either indirectly or directly contribute towards reduced myelination, although a causal role cannot be identified here. A different relationship was observed for AMY and HPC myelin. In males, higher corticosterone responses were associated with higher levels of AMY and HPC myelination at p40. This is in line with prior work in our lab showing that corticosterone enhances oligodendrogenesis in the HPC of adult male rats (Chetty et al., 2014). It is possible that corticosterone may be driving increased myelination in these regions, leading to the group-wide short-term increase in AMY and HPC MBP in males. Yet, this correlation was reversed in female animals; animals with higher corticosterone showed reduced AMY and HPC myelin. This sex difference is interesting and merits further exploration in future studies. Females have a higher ratio of GRs to mineralcorticoid receptors (MRs), which could contribute towards this sex difference (Brydges et al., 2014). More broadly, males and females display different corticosterone responses following adolescent stress, perhaps helping explain some of the observed long-term sex differences. For example, adult female rats exposed to chronic stress during adolescence have higher basal corticosterone levels compared to non-stressed controls (Barha et al., 2011). In contrast, chronic adolescent stress has no lasting impacts on basal corticosterone levels in adult male animals (Barha et al., 2011). This long-lasting change in basal corticosterone levels in females may be adaptive, or it may be suggestive of female-specific risk. Indeed, we observed here that adult females but not males, displayed reduced myelination across all limbic regions. Whether these changes are adaptive or not would need to be assessed through future work.

4.5. Limitations and future directions

While some limitations of the current study have already been discussed, there are several additional considerations. Firstly, although group effects were observed, the sample sizes were too low to reliably explore potential subgroups and inter-individual variations. Secondly, in the current study we observed changes in MBP measured via fluorescence intensity. Increases in MBP intensity could reflect either an increase in the number of axons being myelinated, a thickening of already existing myelinated axons, or some combination of the two. Future work could use higher resolution imaging and/or electron microscopy to pull apart these possibilities and to address whether there are changes in OL morphology following acute juvenile stress. Higher resolution could also be applied to the local microcircuits within these limbic regions. For example, the PFC is a diverse brain region, with many subregions and local microcircuits across cortical layers (Dalley et al., 2004; Kolb, 1984). Although we looked at specific subregions of the PFC, each known to be involved in different aspects of behavior, we did not look at specific layers of the PFC. Prior literature has also focused on only deep layers of cortex (layers 5 + 6) where there are primarily pyramidal projection neurons (Lehmann et al., 2017; Makinodan et al., 2012). This is yet another difference between our study and others. Future work should aim to look at layer specific changes with a more detailed anatomical eye. Lastly, here, we looked at two specific markers: one for OLs and one for myelin. Future work could add additional markers of OLs to assess stress-induced changes across the OL lineage. OLs develop from oligodendrocyte progenitor cells (OPCs). OPCs persist throughout the postnatal period and continue to divide and generate myelinating OLs throughout their lifespan (Bergles and Richardson, 2016). While our

study focused on immature and mature myelinating OLs, stress can also affect the progenitor pool in these limbic regions (Qiao et al., 2020; Saul et al., 2015; Teissier et al., 2020). For example, repeated variable stress selectively reduced the number of proliferating OPCs in the AMY in juvenile male mice, while leaving proliferating neurons unchanged (Saul et al., 2015). Although we did not measure OPCs in the current study, it is possible that acute stress-exposure reduced OPC density in the short term, contributing towards long-term decreases in GM myelin. Indeed, OPCs may play an important role in stress-related behaviors and should be analyzed in future work. For example, loss of OPCs in the PFC was sufficient to phenocopy depressive-like behaviors driven by chronic social stress (Birey et al., 2015).

OLs and myelin across limbic regions are now being recognized for their role in stress-associated behaviors. For example, adult PTSD patients have increased HPC myelin compared to trauma exposed controls (Chao et al., 2015). Interestingly, this increase in HPC myelin is positively correlated with PTSD symptom scores, suggesting that vulnerability to stress-induced disorders is related to HPC myelin. In a recent study from our lab, similar findings were observed in adult male rats. Specifically, OLs and GM myelin in the dentate gyrus of the HPC positively correlated with avoidance behaviors following exposure to acute severe stress. In addition, increased myelin levels in the AMY were associated with enhanced fear behavior following trauma (Long et al., 2021). Together, these findings suggest that, in adults, OLs and myelin may be associated with individual vulnerability following acute stress. An important direction will be to assess whether changes in OLs and myelin following acute juvenile trauma correspond with subsequent changes in behavior. Acute severe stress in rodents also models another component of human PTSD; most animals will be resilient to stress, while a subset will display long lasting increases in fear and anxiety-like behaviors (Kessler et al., 1995; Russo et al., 2012; Zovkic et al., 2013). Of particular interest then will be to see if changes in myelin and OLs are associated with susceptibility to juvenile acute stress. Indeed, some of the animals in this study may have displayed behavioral resilience following acute stress exposure, and one possibility is that the changes in the myelin and OLs observed here reflect a mechanistic adaptation to stress exposure, ultimately promoting resilience. Alternatively, observed changes in myelin and OLs may reflect detrimental changes in behavior. As exposure to acute stress leads to wide variability in subsequent behavior, future studies will require large group sizes to meaningfully assess these possibilities. In addition, animals exposed to juvenile stress may have altered vulnerability to later stressors; thus, testing behavior following a second stressor in adulthood will be an interesting area for future study. While the current study has shown significant changes in myelin and OLs following acute stress exposure, we are limited in statistical power to assess the behavioral implications of these changes.

The functional implications of changes in myelin and OLs should be examined across a wide range of behaviors, and in both sexes. In a recent study, only animals susceptible to chronic social defeat had reduced myelin protein and fewer mature OLs in the mPFC. In addition, focal mPFC demyelination decreased social preference, implicating a causal role for myelin in social behavior (Bonnefil et al., 2019). Indeed, many prior studies measuring stress-induced changes in myelin and OLs have focused on associations with depressive-like behaviors, including social interaction (Birey et al., 2015; Lehmann et al., 2017; Leussis and Andersen, 2008; Liu et al., 2012, 2016; Tang et al., 2019). It will be interesting for future work to assess a diverse array of behaviors, covering the social, reward, fear and avoidance domains, to uncover how different behaviors affected by acute stress might be associated with myelin or OLs in a given limbic region. Finally, sex differences should be explored in a behavioral context as well. Indeed, males and females exposed to juvenile stress display differential behavioral symptoms in adulthood, with females selectively showing increased anhedonia (Horowitz et al., 2014). Thus, along with testing a range of behavioral domains, future work should specifically test if long-term changes in myelin in female rats correspond with changes in

depressive-like behaviors.

The findings described here should also be considered within a broader circuit context. For example, the PFC, AMY and HPC are all highly interconnected, and function together to regulate emotions (Ishikawa and Nakamura, 2003; Roozendaal et al., 2009). Connectivity between these regions is also altered following stress, in both humans and in rodents (Gee et al., 2013; Grandjean et al., 2016; Honeycutt et al., 2020). Myelination of axons corresponds with conduction velocity and synchronization across these brain regions (Monje, 2018; Pajevic and Basser, 2013), making the study of myelin critical in understanding changes in connectivity. In humans, studies using diffusion tensor imaging (DTI) detect changes in specific white matter tracts following early life adversity (Choi et al., 2009; Eluvathingal et al., 2006; Jackowski et al., 2008). For example, early socioemotional deprivation reduced diffusion coefficients in the uncinate fasciculus, which connects the anterior temporal lobe to the frontal lobe (Eluvathingal et al., 2006). In addition, experiencing verbal abuse as a child leads to altered white matter tracts connecting the medial temporal lobe to the PFC (Choi et al., 2009). Yet detailed mapping of myelin tracts connecting specific brain regions remains challenging in humans. Thus, future studies in rodents should aim to identify changes in myelination within a specific circuit, focusing on specific axonal projections between brain regions (for example, IL projections to the AMY). Understanding how and if particular axonal projections are preferentially myelinated following stress will further our knowledge of circuit plasticity and its connection to behavior. A recent study found that pharmacological stimulation of neurons led to increases in myelination in an axon-specific manner. In addition, and relevant to the current study, juveniles showed higher sensitivity to stimulation than adults (Mitew et al., 2018). This suggests that neural activity, whether driven by stress or otherwise, may drive circuit specific modulation of myelin. Lastly, a critical area for future study will be to go beyond correlation and into causation, and to manipulate myelin and OLs directly within a brain region or circuit in order to assess their functional contribution to stress pathology and behaviors.

5. Conclusions

The findings presented here have important implications for understanding stress-sensitive developmental periods. There is increasing evidence that stress during late childhood and early adolescence may confer vulnerability for developing psychiatric disorders later in life (Carr et al., 2013; Heim and Nemeroff, 2001; Nemeroff, 2004a, 2004b; Ventriglio et al., 2015). Exposing rats to peri-adolescent stress is used to model the detrimental effects of childhood and early-adolescent trauma in humans (Tsoory et al., 2007; Tsoory and Richter-Levin, 2006). The majority of prior studies have tested chronic stressors during the peri-adolescent time period; however, many traumatic experiences are often acute in nature and can lead to long-lasting changes to the brain and behavior (Carrion and Wong, 2012; Nemeroff et al., 2006; Tsoory et al., 2007; Tsoory and Richter-Levin, 2006). Further, understanding acute trauma provides us with detailed knowledge about vulnerable windows during development when the brain is most sensitive to stress. Many studies have suggested that peri-adolescence is a sensitive period of development in which there is significant remodeling of limbic regions following stress (Spear, 2000). Our work here adds to the growing literature demonstrating that myelin and OLs are sensitive to stress early in life, providing an additional mechanism by which stress remodels the brain (Bath et al., 2016; Demaestri et al., 2020; Leussis and Andersen, 2008; Makinodan et al., 2012). Many psychiatric disorders, including schizophrenia, depression and PTSD, are characterized by alterations in myelination (Fields, 2008; Hamidi et al., 2004; Lee and Fields, 2009; Lutz et al., 2017; Regenold et al., 2007; Tanti et al., 2018). However, whether changes in myelin contributes to vulnerability to these disorders, or whether they are simply a biomarker remains to be determined. Understanding stress-induced plasticity of PFC, AMY and HPC myelin

and OLS may contribute to our understanding of these psychiatric disorders, as well as the vulnerability to developing pathology following early life stress. Studying sex differences in response to early life trauma is also important. In particular, females are known to have increased risk of developing stress-associated pathology, including PTSD (Gater et al., 1998; Kessler et al., 2005; Weissman et al., 2005). While behavior was not included in the current study, the selective long-term reductions in myelination in female animals is suggestive of female-specific risk. Overall, findings in rodents will inform our knowledge of how traumatic stressors may impact human brain development and mental health.

CRedit authorship contribution statement

Jocelyn M. Breton: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Matthew Barraza:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Kelsey Y. Hu:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Samantha Joy Frias:** Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Kimberly L. P. Long:** Formal analysis, Methodology, Writing – review & editing. **Daniela Kaufer:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jynstr.2021.100319>.

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