OXFORD

Cerebral Cortex, 2023, **33**, 6382–6393
https://doi.org/10.1093/cercor/bhac511
Advance access publication date 4 January 2023
Original Article

The waiting game: investigating the neurobiological transition from acute to persistent pain in adolescent rats

Sabrina Salberg[1], Angela Doshen[2], Glenn R. Yamakawa[1], Jillian Vinall Miller[3], Melanie Noel[4], Luke Henderson[2], Richelle Mychasiuk[1,*]

[1]Department of Neuroscience, Monash University, 99 Commercial Road, Melbourne, VIC, 3004, Australia,
[2]School of Medical Sciences (Neuroscience), Brain and Mind Centre, University of Sydney, 94 Mallett St, Camperdown, NSW, 2050, Australia,
[3]Department of Anesthesiology, Perioperative & Pain Medicine, Cumming School of Medicine, University of Calgary, 29 Street NW, Calgary, AB, T2N 2T9, Canada,
[4]Department of Psychology, Alberta Children's Hospital Research Institute, Hotchkiss Brain Institute, The University of Calgary, 3330 Hospital Dr NW, Calgary, AB, T2N 4N1, Canada

*Corresponding author: Department of Neuroscience, Central Clinical School, Monash University, 6th Floor, 99 Commercial Road, Melbourne VIC, 3004. Email: Richelle.mychasiuk@monash.edu

Persistent postsurgical pain affects 20% of youth undergoing a surgical procedure, with females exhibiting increased prevalence of chronic pain compared with males. This study sought to examine the sexually-dimorphic neurobiological changes underlying the transition from acute to persistent pain following surgery in adolescence. Male and female Sprague Dawley rats were randomly allocated to a sham or injury (plantar-incision surgery) condition and assessed for pain sensitivity while also undergoing magnetic resonance imaging at both an acute and chronic timepoint within adolescence. We found that injury resulted in persistent pain in both sexes, with females displaying most significant sensitivity. Injury resulted in significant gray matter density increases in brain areas including the cerebellum, caudate putamen/insula, and amygdala and decreases in the hippocampus, hypothalamus, nucleus accumbens, and lateral septal nucleus. Gray matter density changes in the hippocampus and lateral septal nucleus were driven by male rats whereas changes in the amygdala and caudate putamen/insula were driven by female rats. Overall, our results indicate persistent behavioral and neurobiological changes following surgery in adolescence, with sexually-dimorphic and age-specific outcomes, highlighting the importance of studying both sexes and adolescents, rather than extrapolating from male adult literature.

Key words: gray matter density; neuroimaging; nociception; persistent postsurgical pain; sex differences.

Introduction

Adolescent chronic pain (i.e. debilitating pain lasting for 3 months or more) is a growing public health epidemic with devastating effects across the lifespan, costing countries billions of dollars annually (Groenewald et al. 2014). Up to 38% of youth report chronic headaches, abdominal pain, or musculoskeletal pain, which peaks in adolescence and is most prevalent in girls (Perquin et al. 2000; Blyth et al. 2001; Stanford et al. 2008; King et al. 2011). Of utmost concern, pediatric chronic pain is not limited to adolescence. Up to two thirds of adolescents with chronic pain will become adults with chronic pain (Walker et al. 2012), which will cost society even more. For this reason, pediatric chronic pain has been coined "a modern public health disaster" (Clinch and Eccleston 2009). Given that adolescence is characterized by significant brain maturation and remodeling (Paus et al. 1999; Mengler et al. 2014), understanding the neurobiological pathologies underlying the transition from acute to chronic pain during adolescence is critical if we are to help adolescents maintain healthy developmental trajectories and disrupt the often inevitable persistence of chronic pain into adulthood.

From an evolutionary perspective, acute pain is critical for survival as it signals threat and urges protection from further harm (Broom 2001). In this sense, acute pain is a healthy, essential process. When healthy acute pain becomes chronic, this pain signaling system becomes dysfunctional. Contrary to acute pain, chronic pain is not a normal, necessary response to a nociceptive stimulus, and often results in persistent activation of stress and inflammatory pathways, cell death, and neurodegeneration (Koch et al. 2007; Johansson et al. 2008; Berliocchi et al. 2012). Interestingly, there is evidence that as pain transitions from an acute to a chronic state there becomes less overall activity levels within sensory regions of the brain, such as the thalamus and somatosensory cortex, and greater activation within brain regions integrally involved in memory and emotion, such as the medial prefrontal cortex, amygdala, and hippocampus (Apkarian et al. 2005; Brooks and Tracey 2005; Tracey and Mantyh 2007; Hashmi et al. 2013). However, there is evidence that the pattern, not overall level, of activity in sensory processing regions such as the thalamus and somatosensory cortices are altered in chronic neuropathic pain, raising the prospect of different mechanisms emerging in different brain circuits (Alshelh et al. 2016).

Within both the preclinical and clinical literature, when "adults" have persistent pain, the transition from acute to chronic pain has been associated with hallmark brain changes that

Received: August 24, 2022. **Revised:** December 1, 2022. **Accepted:** December 2, 2022

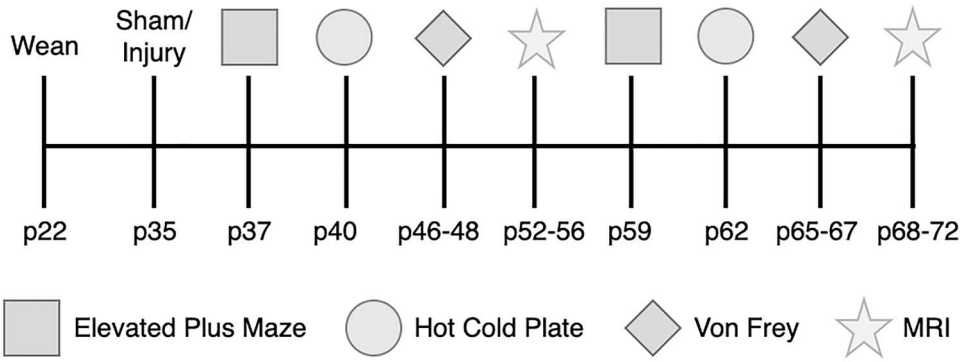


Fig. 1. Experimental timeline of study. (p) Indicates postnatal day, with the acute timepoint battery depicted from p37 to p56 and the chronic timepoint from p59 to p72.

include reduced frontal cortex and anterior cingulate volumes, changes in gray matter density, as well as in white matter integrity (Jasmin et al. 2004; DaSilva et al. 2007; Seminowicz et al. 2009; Gustin et al. 2011). This suggests that there are neurobiological factors above and beyond sensory pain pathways involved in the maintenance and chronification pain. However, to our knowledge, the neurobiological changes responsible for this transitory process have not been demonstrated in adolescents, a population at increased risk for the onset and development of chronic pain, with 20% of youth that undergo a surgical procedure suffering from persistent postsurgical pain (Pagé et al. 2013; Rabbitts et al. 2017). Therefore, the purpose of this study was to use advanced magnetic resonance imaging (MRI) to characterize the underlying, and possibly sexually-dimorphic, neurobiological changes that occur in response to a mild injury (plantar-incision surgery) and facilitate the transition from acute to chronic pain in adolescence. This was modeled using an acute and chronic timepoint following surgery, mimicking persistent pain sensitivity from a surgical procedure.

Methods

Animals

All experiments were carried out under the approval of the Alfred Medical Research and Education Precinct Animal Ethics Committee and in accordance with the Precinct Animal Centre (PAC; E/1928/2019/M). Sprague Dawley rats were acquired from the Monash Animal Research Platform and all animals were kept in a temperature-controlled facility (PAC; 21 °C) on a 12:12 h light:dark cycle. Rat dams and sires were mated, allowing for in-house breeding of all pups used in this study. Male and female pups were left undisturbed with their mother until weaning at postnatal day (p) 22. Pups were randomly assigned to either a sham or injured condition on p35, followed by a battery of behavior tests (elevated plus maze, von Frey, and hot cold plate) and MRI, with the researcher blinded to experimental conditions. These tests were performed at both an acute and chronic timepoint within adolescence. Adolescence in the Sprague Dawley rat is believed to span p31–70 and is characterized by similar maturational changes as those that occur in human adolescence (Kolb et al. 2012; Mychasiuk and Metz 2016). For this study, the acute timepoint included the first round of behavioral tests occurring between p37 and p48 and the acute MRI at ~p54, whereas the chronic timepoint encompassed the second set of behavioral tests from p59 to p67 and the chronic MRI scan occurring at ~p70. See Fig. 1 for experimental timeline. This resulted in 4 groups: male sham ($n = 10$), male injured ($n = 8$), female sham ($n = 8$), female injured ($n = 7$).

Injury

On p35, a plantar-incision surgery was performed on half of the pups using the Brennan model and as previously described (Brennan et al. 1996). Briefly, animals were anesthetized until unresponsive with 5% isoflurane at 1 L/min O_2, and then transferred to a nosecone with 2% isoflurane at 1 L/min O_2 for the duration of the procedure. The left hind paw was sterilized and a 1-cm incision made longitudinally in the skin; the underlying plantaris was then incised longitudinally 3×. The skin was sutured with 2 simple interrupted sutures and the wound cleaned before the animal was placed back into its' home-cage to recover. For the sham procedure, the other half of the animals were anesthetized with 5% isoflurane at 1 L/min O_2 until unresponsive, then returned to their home-cage.

Elevated plus maze

There is high comorbidity between chronic pain and mental health disorders, with a bidirectional relationship between pain and anxiety (Gatchel 2004). Given this, the elevated plus maze (EPM) was run on p37 and p59, as a measure of anxiety-like behavior. The apparatus consisted of 2 enclosed arms and 2 open arms (each 51 cm × 11 cm), intersecting to form a "+" shape. A single 5-min trial was run and tracked by TopScan software, with time spent in the open and closed arms recorded. The less time spent in the open arms of the maze indicates increased anxiety-like behavior.

Von Frey

The von Frey is the primary measure for measuring mechanical sensitivity in rats (Deuis et al. 2017) and was therefore completed on p48 and p67 as a measure of mechanical nociceptive thresholds. The testing apparatus consisted of a small wire grid base, with boxes to enclose the rat, placed on top (Salberg et al. 2020, 2021). The task was run over the course of 3 days, with the first 2 being for habituation, whereby the rat was placed in the box for 20 min then returned to its' home-cage. On the third day the animal was again habituated for 20 min before being tested, which involved increasing size filaments being applied to each hind paw 5×. The number of retractions of the hind paw was observed and testing was discontinued once a 5/5 reaction is recorded. The larger the filament size recorded, the higher the threshold of the animal.

Hot cold plate

The hot cold plate is a common test for measuring thermal sensitivity in rats (Le Bars et al. 2001) and thus was conducted on p40 and p62 as a secondary measure of nociception (thermal nociceptive thresholds). The hot cold testing apparatus was comprised of a temperature-controlled plate that was enclosed with a cylinder to contain the rat. The task was run over 3 days, with the first 2 being for habituation whereby the rat was placed on the plate at room temperature for 2 min before being returned to its' home-cage. On the third day, the plate was set to hot (52 °C) and the animal placed inside with latency to react recorded. The animals were given > 1 h in their home-cage before being placed back on the plate set to cold 2 °C, with latency to react recorded. Shorter latencies to react indicate increased sensitivity.

MRI collection

On approximately p55 and p69, animals were induced with 5% isoflurane at 1 L/min of O_2, then transferred to a nosecone with 2–3% isoflurane at 1 L/min of O_2 to maintain anesthesia throughout the scan. The temperature and respiration rate of the rat were monitored for the duration of the scan using a rectal and chest probe, respectively. Adjustments in the warm water bath running underneath the animal and the rate of anesthesia were made as needed in response to changes in body temperature and respiration. The animal was positioned in ear bars to keep movement to a minimum for the scan, and eye gel was applied to prevent drying of the eyes. A 9.4T Bruker MRI with actively decoupled volume transmit and room temperature-cooled surface array coils was used for imaging. For volumetric analysis, a 3D T2-weighted image was acquired using a RARE sequence (repetition time = 4,500 ms; effective echo time (TE) = 45 ms; RARE factor = 8; raw voxel size 0.16 × 0.16 × 0.16 mm).

Voxel based morphometry analysis

To allow the use of standard neuroimaging tools, each T2-weighted image was resized by a factor of 10 in x, y, and z directions to create brain and voxel sizes similar to humans. This scaling resulted in images with voxels that were 1.6 × 1.6 × 1.6 mm in size. These resized T2-weighted images were then rigid-body aligned to the stereotactic template space (also scaled). Using Statistical Parametric Mapping 12 (SPM12), the images were normalized and segmented into probability maps of gray matter, white matter and cerebrospinal fluid using the unified segmentation approach (Ashburner and Friston 2005). This process included normalization to the template with an affine coregistration followed by a nonlinearly spatially warping to the SIGMA template. The signal intensity of normalized gray matter of each voxel was modulated by the determinant of the Jacobian and resliced into 1.2 × 1.2 × 1.2-mm voxels, as per the original "optimized" VBM procedure (Good et al. 2001). The resulting gray matter density images were then smoothed using a 4 mm (0.4 mm in native space) full-width-at-half-maximum Gaussian filter.

Total brain and gray matter volumes were calculated for the acute and chronic time points and significant differences between sham and injured and between males and females determined ($P < 0.05$ 2-sample, 2-tailed t-tests). Significant differences in regional gray matter density between sham and injured rats were determined at a voxel-by-voxel level by placing the gray matter density brain maps into a second level full factorial analysis with 3 factors and 2 levels in each factor; injury (sham: injured), sex (male: female), timepoint (acute: chronic) ($P < 0.05$, false discovery rate (FDR)-corrected, minimum cluster size 20 contiguous voxels). Time was entered as an independent variable and injury and sex as dependent variables. Total brain volume was added as a nuisance variable. Significantly different clusters were overlaid onto a mean T2 weighted image set. The locations of significant clusters were identified using a rat brain atlas (Paxinos and Watson 2014).

Gray matter densities were extracted from significant clusters and mean ± SEM values plotted. To determine if areas identified as being altered by the injury also displayed sex-related differences, gray matter densities were calculated for all rats, male only rats, and female only rats, and significant differences between males and females determined using post-hoc t-tests ($P < 0.05$, corrected for multiple comparisons). Finally, significant relationships between gray matter densities and mechanical, hot, and cold withdrawal thresholds were determined for all, male only, and female only, for 8 clusters determined as displaying a significant effect of injury (Pearson's correlations, $P < 0.05$, corrected for multiple comparisons). Significant differences in correlation coefficients between the male and female only groups were also determined ($P < 0.05$, 2-tailed).

Statistics

All statistics were carried out in SPSS 27.0 for Mac. Within-subjects 2-way repeated measures analysis of variance (ANOVAs) were run for all behavioral tests, with injury (sham: injured) and sex (male: female) as factors, accounting for timepoint (acute: chronic). When Mauchley's test indicated sphericity was violated, Greenhouse–Geisser corrections were performed. When necessary, post-hoc pairwise comparisons (Tukey) were completed. Statistical significance was considered at $P < 0.05$. All data can be obtained at the opensource framework repository—https://osf.io/zs8vk/?view_only=8543f146cb2a4a2292c081218a0a106.

Results

Behavioral assessment of nociception and anxiety-like behavior

The within-subjects, 2-way repeated measures ANOVA for the von Frey task revealed a significant injury by sex by timepoint interaction, whereby injured rats were more sensitive at both the acute and chronic timepoints, $F(1, 29) = 6.714$, $P = 0.015$. See Fig. 2. Pairwise comparisons also demonstrated differences between sham males and sham females at the acute timepoint, $P = 0.002$. With respect to the hot plate, the within-subjects 2-way repeated measures ANOVA demonstrated a significant timepoint by injury interaction, $F(1, 29) = 4.654$, $P = 0.038$, where both male and female animals in the injured groups exhibited decreased thermal sensitivity at the acute and chronic timepoints. There were no significant findings for thermal sensitivity within the cold plate. Finally, the within-subjects 2-way repeated measures ANOVA for the elevated plus maze demonstrated a significant timepoint by injury interaction, $F(1, 29) = 4.846$, $P = 0.036$, where animals in the injured groups exhibited increased anxiety-like behavior when compared with the sham animals. Post-hoc analyses also identified a significant sex difference whereby males were more anxious than females, but the persistence of anxiety was greater in females than males, $P = 0.004$. See Fig. 2.

To summarize, both males and females that were injured exhibited persistent changes in mechanical nociception and thermal nociception (hot plate only) 30 days post-injury, providing evidence for the presence of persistent pain. There were sex differences in the mechanical nociception of shams, with males

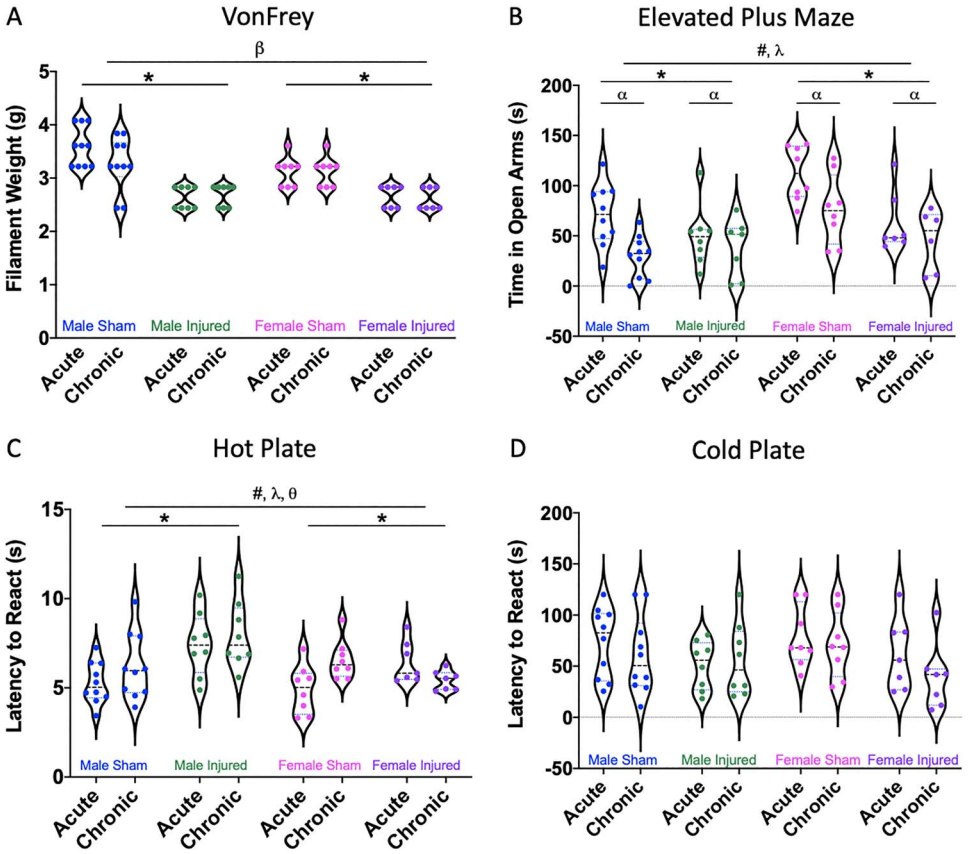


Fig. 2. Violin plots of behavioral results. (#) Indicates a main effect of sex, (*) indicates a main effect of injury, (α) indicates a main effect of timepoint, (β) indicates a significant sex by injury by timepoint interaction, (λ) indicates a significant timepoint by injury interaction, and (θ) indicates a significant sex by injury interaction; P's < 0.05. A) Average filament weight from the von Frey task, whereby the interaction observed was sex by injury by timepoint where injured rats were more sensitive at both the acute and chronic timepoints, and sham females were more sensitive than sham males at the acute timepoint; B) average time spent in the open arms of the elevated plus maze, whereby interaction observed was timepoint by injury where injured animals exhibited increased anxiety-like behavior compared with sham animals; C) average latency to react on the hot plate, whereby interactions observed were timepoint by injury, and sex by injury where animals in the injured groups exhibited altered thermal sensitivity at the acute and chronic timepoints; D) average latency to react on the cold plate.

exhibiting lowered sensitivity. Persistent changes in anxiety-like behavior associated with the injury was greater in females than in males. See Table 3 for summary of key results.

Gray matter assessment

Overall changes

At the acute time point there were no significant differences between sham and injured rats as a single group, or when divided into male and female groups, with respect to total brain volume or total gray matter volume (Fig. 3; Table 1). In contrast, at the chronic time point, although there were no significant differences between sham and injured total and gray matter volumes for all and male groups, injured female rats displayed greater total and gray matter volumes compared with female sham rats.

Regional differences in gray matter density

Voxel-by-voxel assessment of gray matter densities revealed that injury had a significant effect in a number of brain regions (Fig. 4 and Table 2). Gray matter densities were significantly greater in injured compared with sham rats in the region encompassing the left and right caudate/putamen (CPu) extending into the insula, right (contralateral to injury) amygdala, and the cerebellar cortex. In contrast, significantly reduced gray matter densities in injured compared with sham rats occurred in the area encompassing the left and right dorsomedial hypothalamus (DMH), right hippocampus, left perirhinal and entorhinal cortices, left nucleus accumbens (NAc), right lateral septal nucleus, and in the right lateral orbital cortex. Voxel-by-voxel analysis of the effect of sex and time revealed no significant differences in regional gray matter densities.

Although a voxel-by-voxel analysis revealed no effect of sex on regional gray matter densities, extraction of gray matter densities from the effect of injury significant clusters revealed more subtle sex-related effects (Fig. 5). Gray matter density changes in the amygdala and CPu/insula were driven largely by the female rats, whereas gray matter density changes in the hippocampus and lateral septal nucleus were driven by the male rats. See Table 3 for summary of key results.

In addition to regional differences, we assessed the relationships between gray matter density and mechanical, hot, and cold nociceptive sensitivity at the acute and chronic time point, for all, male and female rats in each of the 8 clusters. Interestingly, at the acute time point, we found significant correlations between all sham rats and mechanical thresholds in all 8 clusters except for the amygdala (Fig. 6). Injury appeared to disrupt these relationships with gray matter density in the hypothalamus and lateral orbital cortex being significant in the injured group. Furthermore, time appears to affect the relationship with no cluster displaying a significant relationship with mechanical threshold at the chronic timepoint. In contrast, there were no significant relationships

Table 1. Total brain and total gray matter volumes.

	Total brain volume (mean ± SEM mm^3)		Total gray matter volume (mean ± SEM mm^3)	
	Sham	*Injured*	*Sham*	*Injured*
Acute				
All	1722.66 ± 25.39	1736.33 ± 19.53	1183.59 ± 17.58	1193.36 ± 15.63
Male	1792.97 ± 23.44	1791.02 ± 5.85	1234.38 ± 17.58	1238.28 ± 5.85
Female	1791.01 ± 13.67	1671.88 ± 25.39	1111.33 ± 9.77	1142.58 ± 21.48
Chronic				
All	1798.83 ± 27.34	1812.50 ± 15.63	1222.66 ± 19.53	1234.38 ± 11.72
Male	1876.95 ± 25.39	1853.52 ± 9.77	1281.25 ± 19.53	1261.72 ± 9.77
Female	1679.27 ± 17.58	**1765.63 ± 17.58***	1150.39 ± 11.72	**1201.17 ± 13.67***

* $P < 0.05$ sham versus injured. Bold values indicate significant effects.

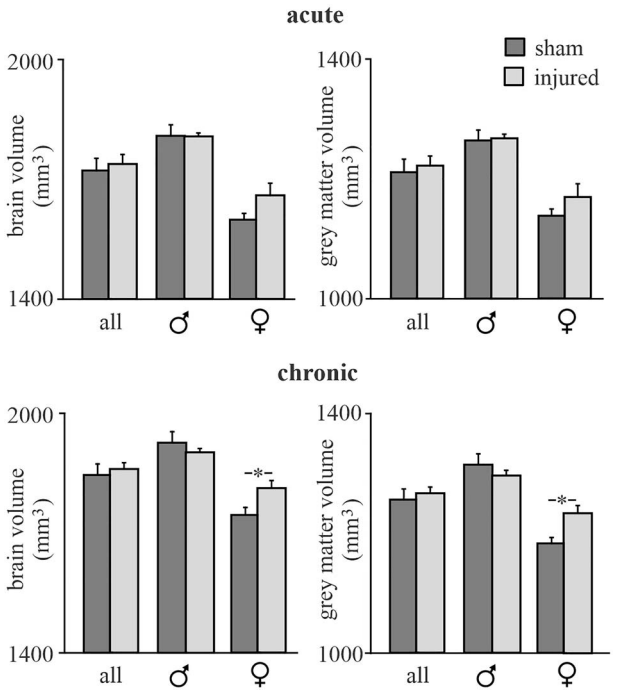


Fig. 3. Brain and gray matter volume changes. Plots of mean (±SEM) total brain and gray matter volumes at the acute (postnatal day 52–56) and chronic (postnatal day 68–72) time points for all rats, males only, and females only. * $P < 0.05$ 2-sample *t*-test. Female injured rats had increased brain and gray matter volumes compared with female shams at the chronic timepoint.

between either hot or cold thresholds in any group in the lateral septal nucleus, CPu/insula, or nucleus accumbens. Significant relationships between hot thresholds and gray matter density occurred in the cerebellar cortex, hippocampus, hypothalamus, and lateral orbital cortex and cold thresholds were significantly correlated in only the hypothalamus and amygdala. Finally, significant differences between male and female correlations occurred in injured groups within the lateral orbital cortex, amygdala, cerebellar cortex, and hypothalamus.

Discussion

As pain persists in adults, there is recruitment of various brain regions outside of the primary somatosensory pathways (Apkarian 2011), including prefrontal and cingulate cortices (DaSilva et al. 2007; Seminowicz et al. 2009; Gustin et al. 2011). However, to our knowledge whether similar changes also occur in adolescents, a population at increased risk for the onset and development of chronic pain, has not been demonstrated (King et al. 2011). Alarmingly, ~20% of youth that undergo a surgical procedure will suffer from chronic postsurgical pain (Pagé et al. 2013; Rabbitts et al. 2017), with a significantly increased prevalence of chronic pain in girls compared with boys (Blyth et al. 2001; Stanford et al. 2008). Previous research from our laboratory has demonstrated that the neuroinflammatory and systemic response to chronic pain is prominent in adolescent males, but nearly absent in females (Salberg et al. 2023). Our previous studies were unable to ascertain the pathophysiological mechanisms responsible for female presentation of more pain, both behaviorally and clinically. Therefore, this study sought to examine the sexually-dimorphic neurobiological changes that underly the transition from acute to chronic pain following a minor surgery in adolescence.

We found that, behaviorally, both males and females demonstrated altered mechanical and thermal nociceptive sensitivity following surgery, with these changes being persistent across time. In addition, we observed sex differences in these outcomes whereby female shams exhibited greater mechanical sensitivity than male sham animals, along with increased thermal sensitivity in females compared with males. These sex-dependent changes were consistent with MRI measures, whereby (i) total gray matter volume was increased at the chronic timepoint in female injured animals and (ii) regional gray matter density increases were driven by females whereas regional density decreases were driven by males. Acutely, injury resulted in altered gray matter density in the cerebellar cortex, hippocampus, amygdala, CPu/insula, NAc, hypothalamus, and lateral septal nucleus, with all of these changes being maintained at the chronic time point.

Results from the von Frey task exemplify the persistence of pain in our surgery animals, as they demonstrated little to no recovery from the acute to chronic timepoint. This persistence of pain behavior was also observed on the hot plate, with surgery reducing thermal nociception across time. Interestingly, our results contradict findings of the laboratory from which the injury model was developed. Brennan et al. found that this surgery model induced consistent mechanical and thermal hyperalgesia, however these deficits were transient, resolving within 5–10 days postsurgery (Brennan et al. 1996; Brennan 1999, 2011). The discrepancy in results may be due to the age and sex of the animals, as the initial studies by Brennan and colleagues were conducted in male adult rats, whereas our

Table 2. Brain region, *F* value, cluster size and gray matter density for clusters that displayed significant gray matter density changes due to injury.

Brain region	*F* value	Cluster size	Gray matter density (mean ± SEM prob x vol[x10^1])					
			All		**Males**		**Females**	
			Sham	*Injured*	*Sham*	*Injured*	*Sham*	*Injured*
Injured > sham								
Cerebellar cortex	19.29	917						
Acute			7.05 ± 0.11	**7.48 ± 0.12** #	7.28 ± 0.13	7.81 ± 0.12	6.72 ± 0.10	7.10 ± 0.10
Chronic			7.46 ± 0.14	**7.85 ± 0.09** #	7.76 ± 0.16	8.09 ± 0.10	7.03 ± 0.12	**7.58 ± 0.08***
Amygdala	32.00	16,165						
Acute			7.98 ± 0.11	**8.29 ± 0.09** #	8.29 ± 0.11	8.51 ± 0.07	7.55 ± 0.06	**8.04 ± 0.13***
Chronic			8.35 ± 0.13	**8.66 ± 0.08** #	8.68 ± 0.14	8.81 ± 0.06	7.87 ± 0.05	**8.49 ± 0.13***
CPu/Insula								
Left	27.03	4767						
Acute			8.54 ± 0.12	**8.79 ± 0.10** #	8.87 ± 0.12	9.06 ± 0.05	8.07 ± 0.06	8.49 ± 0.13
Chronic			8.93 ± 0.14	**9.19 ± 0.07** #	9.30 ± 0.14	9.34 ± 0.05	8.40 ± 0.06	**9.03 ± 0.12***
Right	32.00	16,165						
Acute			7.98 ± 0.11	**8.29 ± 0.09** #	8.29 ± 0.11	8.51 ± 0.07	7.55 ± 0.06	**8.04 ± 0.13***
Chronic			8.35 ± 0.13	**8.66 ± 0.08** #	8.68 ± 0.01	8.81 ± 0.06	7.87 ± 0.05	**8.49 ± 0.13***
Injured < sham								
Perirhinal cortex	25.28	339						
Acute			2.20 ± 0.07	**2.02 ± 0.07** #	2.31 ± 0.09	2.13 ± 0.06	2.05 ± 0.06	1.89 ± 0.12
Chronic			2.29 ± 0.07	**2.09 ± 0.06** #	2.43 ± 0.08	2.16 ± 0.09	2.08 ± 0.04	2.01 ± 0.09
V1/V2	18.08	202						
Acute			3.93 ± 0.11	**3.66 ± 0.12** #	4.11 ± 0.15	3.99 ± 0.06	3.66 ± 0.09	3.28 ± 0.15
Chronic			4.14 ± 0.12	**3.82 ± 0.09** #	4.38 ± 0.16	3.94 ± 0.13	3.80 ± 0.07	3.69 ± 0.10
Entorhinal cortex	20.41	98						
Acute			3.77 ± 0.09	**3.48 ± 0.09** #	3.90 ± 0.12	3.66 ± 0.06	3.57 ± 0.08	3.26 ± 0.14
Chronic			3.91 ± 0.07	**3.65 ± 0.07** #	4.03 ± 0.09	3.73 ± 0.12	3.74 ± 0.06	3.57 ± 0.06
Hippocampus	20.70	202						
Acute			5.98 ± 0.11	**5.67 ± 0.11** #	6.29 ± 0.09	5.98 ± 0.06	5.53 ± 0.06	5.36 ± 0.15
Chronic			6.15 ± 0.13	**5.84 ± 0.09** #	6.53 ± 0.09	**6.09 ± 0.04***	5.61 ± 0.08	5.56 ± 0.11
Thalamus	21.25	37						
Acute			2.93 ± 0.08	**2.69 ± 0.06** #	3.14 ± 0.07	**2.86 ± 0.04***	2.63 ± 0.10	2.50 ± 0.04
Chronic			2.95 ± 0.09	**2.69 ± 0.07** #	3.15 ± 0.09	2.88 ± 0.05	2.65 ± 0.08	2.48 ± 0.06
Hypothalamus	23.14	57						
Acute			5.00 ± 0.09	**4.67 ± 0.08** #	5.12 ± 0.11	4.85 ± 0.10	4.84 ± 0.12	4.47 ± 0.07
Chronic			5.22 ± 0.08	**4.75 ± 0.11** #	5.29 ± 0.09	4.83 ± 0.18	5.12 ± 0.13	4.66 ± 0.13
Lateral septal nucleus	19.92	61						
Acute			3.11 ± 0.07	**3.00 ± 0.07** #	3.18 ± 0.09	3.13 ± 0.07	3.02 ± 0.09	2.96 ± 0.052.
Chronic			3.24 ± 0.06	**2.93 ± 0.04** #	3.32 ± 0.06	**2.90 ± 0.07***	3.13 ± 0.11	86 ± 0.10
Nucleus accumbens	13.37	22						
Acute			4.96 ± 0.09	**4.69 ± 0.11** #	5.10 ± 0.12	4.83 ± 0.12	4.76 ± 0.12	4.54 ± 0.20
Chronic			5.27 ± 0.08	**4.87 ± 0.09** #	5.32 ± 0.12	4.92 ± 0.13	5.19 ± 0.11	4.82 ± 0.13
Lateral orbital cortex	18.49	380						
Acute			8.83 ± 0.13	**8.65 ± 0.09** #	9.19 ± 0.11	8.90 ± 0.07	8.32 ± 0.10	8.37 ± 0.08
Chronic			9.04 ± 0.14	**8.87 ± 0.08** #	9.43 ± 0.13	9.08 ± 0.08	8.49 ± 0.11	8.63 ± 0.07

Gray matter density changes at both the acute and chronic timepoints are shown. CPu: caudate putamen, V1/V2: primary/secondary visual cortices. # $P < 0.05$ voxel-by-voxel analysis, * $P < 0.05$ between sexes post-hoc 2-sample *t*-test. Bold values indicate significant effects.

Table 3. Summary of key results.

Behavior tests	Female shams demonstrated greater mechanical nociceptive sensitivity than male shams acutely
	Females demonstrated increased persistence of anxiety-like behavior
Main effect of injury	Gray matter density increases in the cerebellum, amygdala, and CPu/insula
	Gray matter density decreases in the hippocampus, hypothalamus, lateral septal nucleus, nucleus accumbens and lateral orbital cortex
Effects of sex on injury related changes	Gray matter density changes in the amygdala and CPu/insula were driven by females
	Gray matter density changes in the hippocampus and lateral septal nucleus were driven by males

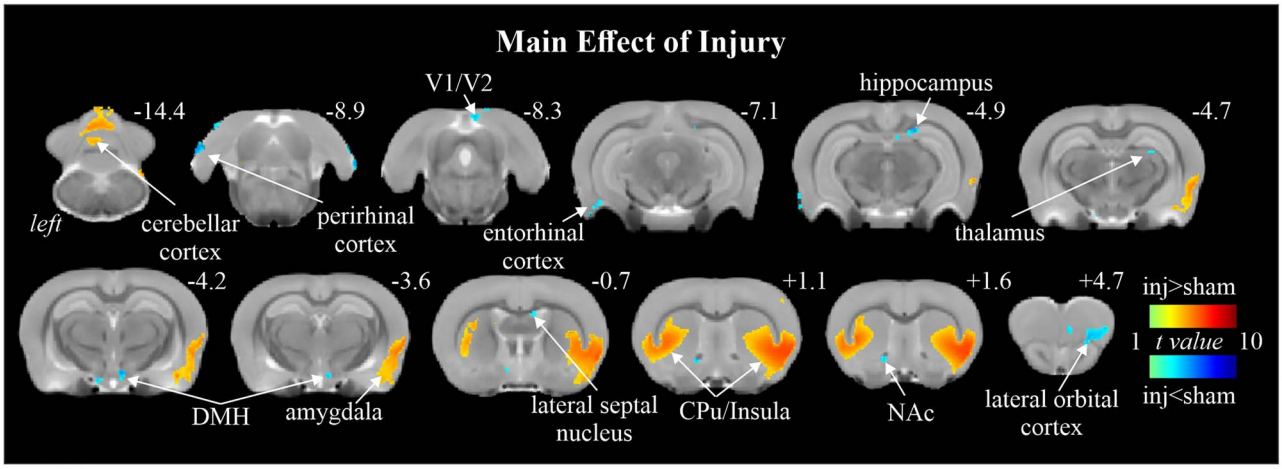


Fig. 4. Main effect of injury on gray matter density changes. The hot and cool color scales indicate regions where injury resulted in significant increases or decreases in gray matter density in injured rats compared with sham rats. Gray matter changes are overlaid onto a mean T2-weighted anatomical image set. The approximate location relative to bregma is indicated to the top right of each coronal slice. Gray matter density increases in the cerebellum, amygdala, and CPu/insula. Gray matter density decreases in the hippocampus, hypothalamus, lateral septal nucleus, nucleus accumbens, and lateral orbital cortex.

study utilized both male and female adolescent rats. Given that adolescence is a critical window of development with a high prevalence of chronic pain presentation, it is possible that the sustained response observed is indicative of the increased risk for persistent pain following surgery. This theory is supported by a further study that employed the plantar-incision model in neonatal rats, showing mechanical and thermal hypersensitivity in adolescent male rats > 25 days post-injury (Burke and Trang 2017). Thus, the age at which the surgery is performed has a significant influence on pain-related outcomes. In addition to direct pain outcomes, we observed increases in anxiety-like behavior from the acute to chronic timepoint. This outcome was measured as pain and anxiety are highly comorbid clinically, with a bidirectional relationship whereby pain can increase anxiety and anxiety further exacerbates pain (Gatchel 2004; Vinall et al. 2016). Similar to the prevalence rates of pain, anxiety is reported significantly more frequently in females compared with males (McLean et al. 2011). Our results corroborate this statistic, with females demonstrating a greater persistence in anxiety-like behavior following the surgery.

Interestingly, results uncovered neurobiological changes that differed between the sexes, with female surgery animals presenting with increased brain and gray matter densities compared with shams at the chronic timepoint. Although much of the literature shows decreased gray matter density in chronic pain populations, these outcomes were dependent on various other characteristics, such as the source of the chronic pain and the specific brain regions analyzed (Apkarian et al. 2004; Baliki et al. 2011; Gustin et al. 2011; May 2011; Smallwood et al. 2013; Cauda et al. 2014; Henssen et al. 2019; Kang et al. 2019). Broadly, gray matter density decreases have been observed in association with chronic pain in the medial prefrontal cortex (PFC), thalamus, anterior cingulate cortex, insular cortex, primary somatosensory cortex, and spinal trigeminal nucleus, whereas increases have been shown in the periaqueductal gray (PAG), caudate, and cerebellum (Apkarian et al. 2004; Gustin et al. 2011; Ruscheweyh et al. 2011; Smallwood et al. 2013; Cauda et al. 2014; Emerson et al. 2014; Wilcox et al. 2015; Henssen et al. 2019; Kang et al. 2019). Whole brain gray matter has been reduced in chronic back pain patients, whereas regional decreases in osteoarthritis and chronic back pain patients have been shown in the hippocampus, insula, cingulum, and somatosensory cortices (Baliki et al. 2011). Complex regional pain syndrome (CRPS), however, has a distinct pain signature, with decreases found in the anterior insula and orbital frontal cortex (Baliki et al. 2011).

Previously, decreases in gray matter density associated with pain were thought to be caused by irreversible neurodegeneration, however studies have shown that at least in non-neuropathic pain conditions, pain relief can result in gray matter normalization (Gwilym et al. 2010). Although the precise cellular changes underpinning gray matter density changes remain unknown, given evidence from human and experimental animal investigations of regional gliosis and neural death, it is likely they reflect a combination of both neural and glial changes (Scholz et al. 2005; Shi et al. 2012; Loggia et al. 2015). In addition, it has been suggested that subtle changes in brain anatomy may also reflect changes in dendritic morphology and given the significant synaptic plasticity that occurs during adolescence, pain could dysregulate neurodevelopment during this period (Gustin et al. 2014). For example, microglia, that rapidly respond to injury and orchestrate neural repair responses, are also required for normal synaptic pruning and homeostatic regulation (Schafer et al. 2012). Research has demonstrated that other traumatic adolescent experiences that result in chronic inflammation modify functioning of microglia leading to over- or under-pruning during this critical window of development (Pyter et al. 2013; Eyolfson et al. 2022). Furthermore, these studies have also highlighted differences in microglia response between adult and adolescent rodents. To the best of our knowledge, this is the first study to examine the neurobiological transition of acute to persistent pain in adolescence, which will provide valuable insight into the role of age in the chronification of pain.

At the acute and chronic timepoints, both sexes exhibited increases in gray matter density in the cerebellar cortex and decreases in the hypothalamus. It is known that the cerebellum has a role in acute pain, with functional MRI (fMRI) studies consistently showing activation with painful stimuli, and stimulation of nociceptive fibers producing activity in the cerebellum (Apkarian et al. 2005; Moulton et al. 2010). However, there is limited research into the mechanisms behind this relationship, with researchers

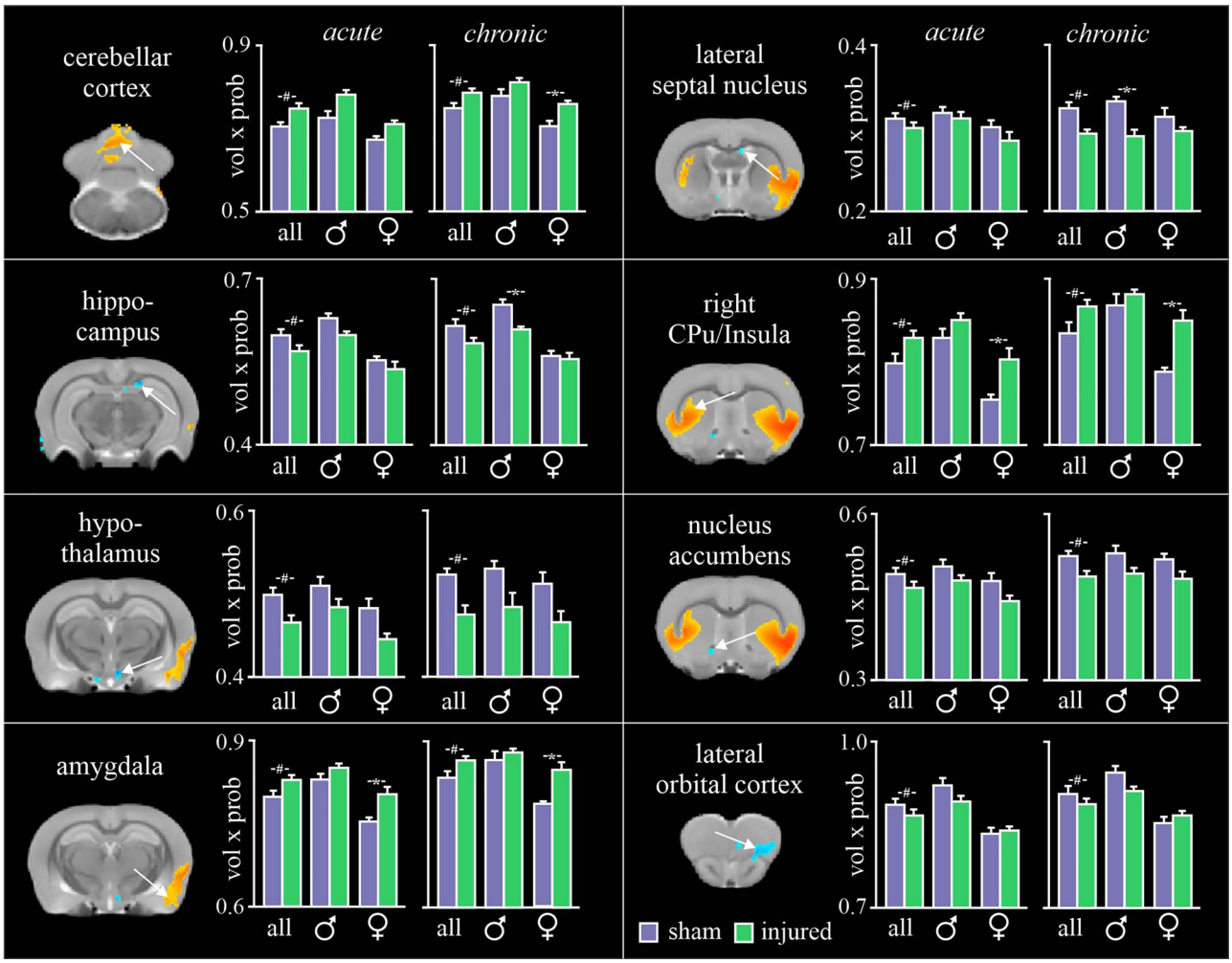
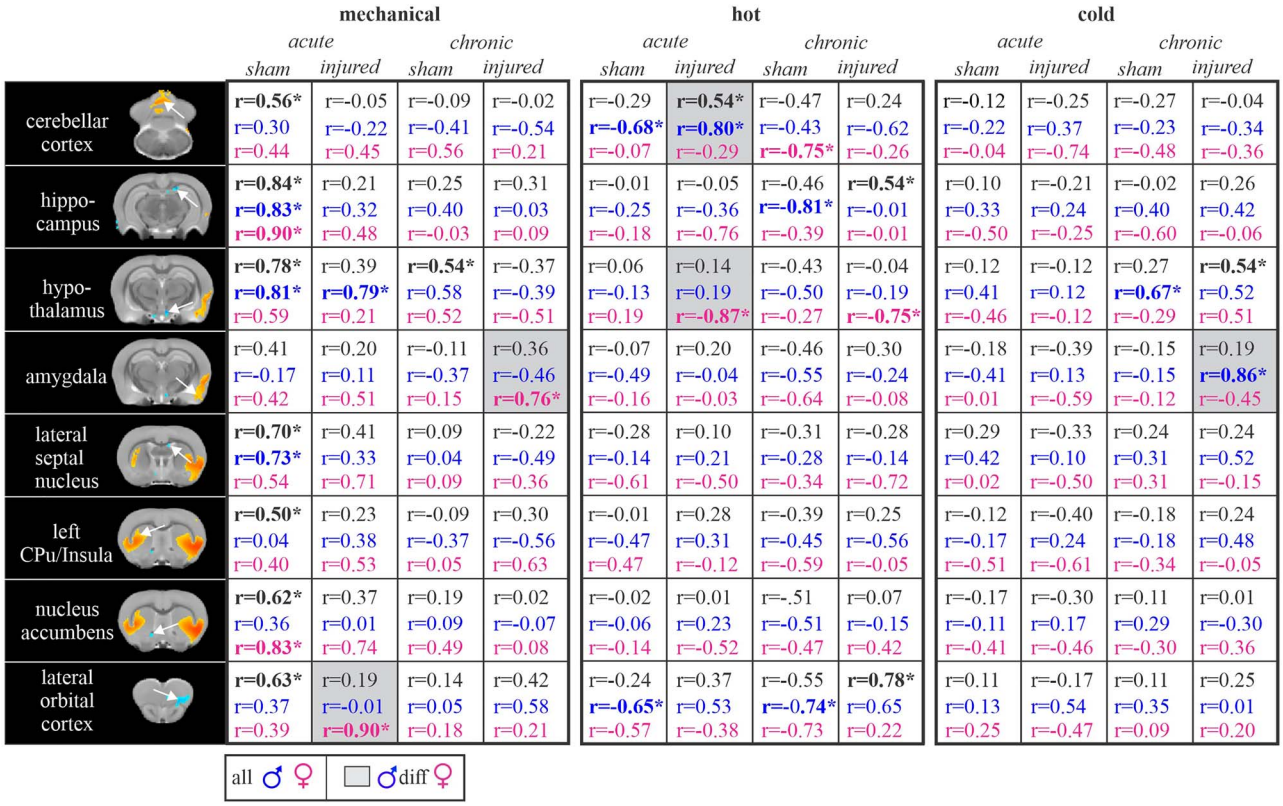


Fig. 5. Main effect of sex on gray matter density changes. Plots of mean (±SEM) gray matter density extracted from numerous significant clusters from the main effect of injury analysis. Plots are shown for all rats, males only, and females only, and at both the acute and chronic timepoints. CPu: caudate putamen; prob: probability; # $P < 0.001$ voxel-by-voxel analysis, * $P < 0.05$ post-hoc 2-sample *t*-test. Gray matter density changes in the amygdala and CPu/insula were driven by females. Gray matter density changes in the hippocampus and lateral septal nucleus were driven by males.

hypothesizing that the cerebellum integrates affective and sensorimotor aspects of pain processing (Moulton et al. 2010). Of importance, the neuroendocrine corticotropin response to pain is under the control of the hypothalamus, which is responsible for maintaining homeostasis through endocrine and autonomic responses thus restoring balance following stressors that disrupt it, such as pain (Bernard 2007; Cortelli and Pierangeli 2007). Hypothalamic orexin and dopaminergic neurons also aid in the control of pain, inhibiting pain transmission and modulating pain signals (Puopolo 2019; Fakhoury et al. 2020). The persistent reduction in hypothalamic volume following exposure to the pain stimuli may represent a change in homeostatic regulation of pain responsivity and signaling.

Overall, regional gray matter density increases were driven by females and gray matter decreases by males. Female driven gray matter increases as a consequence of injury were observed at the acute and chronic timepoints in the amygdala and right CPu/insula. It is not surprising that these areas were affected by injury, as these structures are considered key regions of the pain matrix and are involved in the processing of pain. Contrary to the dearth of research on the cerebellum and pain, there is an abundance of literature demonstrating the association between the amygdala and the emotional, affective, and cognitive processing of pain (Neugebauer et al. 2004; Simons et al. 2014). Along with the hippocampus and hypothalamus, the amygdala makes up a part of the limbic system that regulates behavior and emotion (Rajmohan and Mohandas 2007). This system has been shown to be involved in the emotional processing of pain, with more significant activation occurring as pain transitions to chronic stages (Brooks and Tracey 2005; Tracey and Mantyh 2007; Hashmi et al. 2013). The caudate putamen is activated during pain and is involved in the motor processing of pain (Starr et al. 2011; Azqueta-Gavaldon et al. 2020). With connections to regions involved in sensory, affective, and memory processing of pain, such as the anterior cingulate cortex, insula, thalamus, amygdala, and hippocampus, it may also contribute to these socio-emotional aspects of pain (Starr et al. 2011). The insula plays a role in the sensory and affective integration of pain information (Brooks and Tracey 2007) and is the most frequently reported region activated in fMRI studies of pain, with stimulation of the region producing pain on the contralateral side of the body (Ostrowsky et al. 2002; Apkarian et al. 2005; Brooks and Tracey 2007). In addition, the insula gives significance to the pain experiences, with damage in the region producing pain asymbolia (Brooks and Tracey 2007).

Sex-dependent alterations between groups were also observed in males, with male injured animals showing decreases in



	mechanical acute sham	mechanical acute injured	mechanical chronic sham	mechanical chronic injured	hot acute sham	hot acute injured	hot chronic sham	hot chronic injured	cold acute sham	cold acute injured	cold chronic sham	cold chronic injured
cerebellar cortex	**r=0.56***	r=-0.05	r=-0.09	r=-0.02	r=-0.29	**r=0.54***	r=-0.47	r=0.24	r=-0.12	r=-0.25	r=-0.27	r=-0.04
	r=0.30	r=-0.22	r=-0.41	r=-0.54	**r=-0.68***	**r=0.80***	r=-0.43	r=-0.62	r=-0.22	r=0.37	r=-0.23	r=-0.34
	r=0.44	r=0.45	r=0.56	r=0.21	r=-0.07	r=-0.29	**r=-0.75***	r=-0.26	r=-0.04	r=-0.74	r=-0.48	r=-0.36
hippo-campus	**r=0.84***	r=0.21	r=0.25	r=0.31	r=-0.01	r=-0.05	r=-0.46	**r=0.54***	r=0.10	r=-0.21	r=-0.02	r=0.26
	r=0.83*	r=0.32	r=0.40	r=0.03	r=-0.25	r=-0.36	**r=-0.81***	r=-0.01	r=0.33	r=0.24	r=0.40	r=0.42
	r=0.90*	r=0.48	r=-0.03	r=0.09	r=-0.18	r=-0.76	r=-0.39	r=-0.01	r=-0.50	r=-0.25	r=-0.60	r=-0.06
hypo-thalamus	**r=0.78***	r=0.39	**r=0.54***	r=-0.37	r=0.06	r=0.14	r=-0.43	r=-0.04	r=0.12	r=-0.12	r=0.27	**r=0.54***
	r=0.81*	**r=0.79***	r=0.58	r=-0.39	r=-0.13	r=0.19	r=-0.50	r=-0.19	r=0.41	r=0.12	**r=0.67***	r=0.52
	r=0.59	r=0.21	r=0.52	r=-0.51	r=0.19	**r=-0.87***	r=-0.27	**r=-0.75***	r=-0.46	r=-0.12	r=-0.29	r=0.51
amygdala	r=0.41	r=0.20	r=-0.11	r=0.36	r=-0.07	r=0.20	r=-0.46	r=0.30	r=-0.18	r=-0.39	r=-0.15	r=0.19
	r=-0.17	r=0.11	r=-0.37	r=-0.46	r=-0.49	r=-0.04	r=-0.55	r=-0.24	r=-0.41	r=0.13	r=-0.15	**r=0.86***
	r=0.42	r=0.51	r=0.15	**r=0.76***	r=-0.16	r=-0.03	r=-0.64	r=-0.08	r=0.01	r=-0.59	r=-0.12	r=-0.45
lateral septal nucleus	**r=0.70***	r=0.41	r=0.09	r=-0.22	r=-0.28	r=0.10	r=-0.31	r=-0.28	r=0.29	r=-0.33	r=0.24	r=0.24
	r=0.73*	r=0.33	r=0.04	r=-0.49	r=-0.14	r=0.21	r=-0.28	r=-0.14	r=0.42	r=0.10	r=0.31	r=0.52
	r=0.54	r=0.71	r=0.09	r=0.36	r=-0.61	r=-0.50	r=-0.34	r=-0.72	r=0.02	r=-0.50	r=0.31	r=-0.15
left CPu/Insula	**r=0.50***	r=0.23	r=-0.09	r=0.30	r=-0.01	r=0.28	r=-0.39	r=0.25	r=-0.12	r=-0.40	r=-0.18	r=0.24
	r=0.04	r=0.38	r=-0.37	r=-0.56	r=-0.47	r=0.31	r=-0.45	r=-0.56	r=-0.17	r=0.24	r=-0.18	r=0.48
	r=0.40	r=0.53	r=0.05	r=0.63	r=0.47	r=-0.12	r=-0.59	r=-0.05	r=-0.51	r=-0.61	r=-0.34	r=-0.05
nucleus accumbens	**r=0.62***	r=0.37	r=0.19	r=0.02	r=-0.02	r=0.01	r=-.51	r=0.07	r=-0.17	r=-0.30	r=0.11	r=0.01
	r=0.36	r=0.01	r=0.09	r=-0.07	r=-0.06	r=0.23	r=-0.51	r=-0.15	r=-0.11	r=0.17	r=0.29	r=-0.30
	r=0.83*	r=0.74	r=0.49	r=0.08	r=-0.14	r=-0.52	r=-0.47	r=0.42	r=-0.41	r=-0.46	r=-0.30	r=0.36
lateral orbital cortex	**r=0.63***	r=0.19	r=0.14	r=0.42	r=-0.24	r=0.37	r=-0.55	**r=0.78***	r=0.11	r=-0.17	r=0.11	r=0.25
	r=0.37	r=-0.01	r=0.05	r=0.58	**r=-0.65***	r=0.53	**r=-0.74***	r=0.65	r=0.13	r=0.54	r=0.35	r=0.01
	r=0.39	**r=0.90***	r=0.18	r=0.21	r=-0.57	r=-0.38	r=-0.73	r=0.22	r=0.25	r=-0.47	r=0.09	r=0.20

Fig. 6. Gray matter density and behavior correlations. Relationships between Gray matter densities and mechanical, hot and cold nociceptive thresholds for 8 clusters in all rats (black text), male only rats (blue text) and female only rats (pink text). To the left are overlays of the 8 clusters derived from the main effect of injury analysis, overlaid onto coronal T2-weighted anatomical images. Pearson's correlation *r* values in bold with a * are significant ($P < 0.05$). Gray shaded boxes indicate significant differences between male and female correlations. CPu: Caudate putamen. At the acute timepoint, most clusters showed a correlation between gray matter density and mechanical thresholds in sham animals. Correlations between gray matter densities and hot thresholds were observed acutely in the cerebellum of males and hypothalamus of females.

the hippocampus and lateral septal nucleus. Given that the hippocampus is critical in learning and memory, in the context of chronic pain its' role in contextual conditioning and extinction can become dysregulated (Phillips and LeDoux 1992; Mutso et al. 2012). This can cause abnormalities such as density decreases through reduced neurogenesis and altered synaptic plasticity (Mutso et al. 2012; Fasick et al. 2015). Interestingly, the lateral septal nucleus receives extensive input from the hippocampus and projects to regions such as the hypothalamus, ventral tegmental area, nucleus accumbens, and PAG (Swanson and Cowan 1979). Thus, the lateral septal nucleus provides a major relay from the hippocampus to various subcortical regions. Given its location and connections, the lateral septal nucleus forms a critical part of the limbic system, integrating a variety of emotional and cognitive information to regulate behavioral responses, reward, feeding, anxiety, fear, and sociability (Rizzi-Wise and Wang 2021). It is therefore not surprising that it also has a role in pain, with the circuit from the paraventricular hypothalamus to the lateral septal region being identified as necessary and sufficient for regulating visceral pain (Li et al. 2022). Furthermore, the septal nucleus has been shown to be involved in acute thermal pain processing (Somade et al. 2019).

Another significant finding from our results was the correlation between nociceptive behavior and brain regional density. Although most clusters displayed a significant correlation between gray matter density and mechanical thresholds in sham animals at the acute timepoint, these relationships did not occur in the injured animals. This suggests that at least at the acute timepoint, injury disrupted the relationship between mechanical sensitivity and gray matter density in numerous brain regions. One area that did not display significant relationships between gray matter density and either mechanical, hot, or cold thresholds, at either the acute or chronic timepoint in either group was the amygdala. However, the amygdala did display a unique pattern in injured rats at the chronic timepoint, i.e. a positive correlation with mechanical thresholds in females only, but positive correlations with cold thresholds in males only. These amygdala-dependent sex differences in nociception are not limited to rodents. Clinical participants with chronic abdominal pain exhibit sex differences in connectivity of the emotional-arousal network (including the amygdala) in response to visceral stimulation (Labus et al. 2008). Furthermore, Kogler et al. demonstrated differential resting state functional connectivity (rsFC) within the amygdala of males and females (Kogler et al. 2016). Interestingly, in this study, cortisol was negatively associated with rsFC in females, but positively correlated with cortisol in males. These studies suggest that there are innate sex differences in amygdala-dependent responses to noxious stimuli.

In addition, at the acute time point in injured animals, the cerebellum displayed a significant correlation with hot thresholds in male rats only whereas the hypothalamus displayed a significant correlation with hot thresholds in females only. This suggests that the cerebellum is involved in the pathways responsible for the increased nociception observed, with a more significant influence on male behavior. As stated previously, the cerebellum's role in pain is not fully elucidated, but is known to be involved in the sensorimotor processing of pain (Moulton et al. 2010). This finding highlights the need for further research into this

relationship, as the cerebellum likely plays a key role in altering pain sensitivity.

Overall, both males and females exhibited changes within the pain matrix, however the presentation of structures involved differed between the sexes. Although the pain response was similar for males and females, they were manifested through different physiological and neurobiological pathways. This is consistent with previous research in our laboratory (Salberg et al. 2023), and others (Hagiwara et al. 2021) that demonstrated significant sex differences in the mechanisms associated with pain outcomes observed and could be due to a variety of factors, such as the presence of varied sex hormones, or the delayed neuromaturation rate in males compared with females. These results again emphasize the need to include both sexes in pain studies as the vast majority of mechanisms driving the manifestation and persistence of pain appear to be sexually-dimorphic.

Conclusion

Our study found that a minor surgical procedure "in adolescence" resulted in persistent nociceptive sensitivity across time in both sexes, however females exhibited greater persistent sensitivity. The surgery also modified brain structure in a sex-dependent manner that we were able to identify with MRI, whereby the persistence of pain increased gray matter volume in female animals, but not males. In addition, the surgery altered gray matter density in a number of brain regions, with sex-related differences in the hippocampus, amygdala, lateral septal nucleus, and CPu/insula. Our study highlights the importance of studying adolescence as a population in its own right, as although many of the brain regions affected with the persistence of pain were outside of the sensory pain pathways, similar to adults, changes were often in opposite directions, with increases in gray matter density with persistent pain as opposed to decreases in adults. Overall, both males and females exhibited changes within the pain matrix, however the structures involved differed between the sexes. These results emphasize the need to include both sexes in pain studies in order to provide more targeted treatment options, as the neurobiological mechanisms driving the manifestation and persistence of pain appear to be sexually-dimorphic.

Acknowledgments

The authors acknowledge the facilities and scientific and technical assistance of the National Imaging Facility (NIF), a National Collaborative Research Infrastructure Strategy (NCRIS) capability at Monash Biomedical Imaging (MBI), a Technology Research Platform at Monash University.

Funding

This work was supported by the National Health and Medical Research Council (NHMRC; RM-AP1173565); and the Canadian Institute of Health Research (CIHR; RMPJT153051).

Conflicts of interest statement: The authors of no conflicts of interest to declare.

References

Alshelh Z, Di Pietro F, Youssef AM, Reeves JM, Macey PM, Vickers ER, Peck CC, Murray GM, Henderson LA. Chronic neuropathic pain: it's about the rhythm. *J Neurosci*. 2016:36(3):1008–1018.

Apkarian AV. The brain in chronic pain: clinical implications. *Pain Management*. 2011:1(6):577–586.

Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci*. 2004:24(46):10410–10415.

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain*. 2005:9(4):463–463, 484.

Ashburner J, Friston KJ. Unified segmentation. *NeuroImage*. 2005:26(3):839–851.

Azqueta-Gavaldon M, Youssef AM, Storz C, Lemme J, Schulte-Göcking H, Becerra L, Azad SC, Reiners A, Ertl-Wagner B, Borsook D, et al. Implications of the putamen in pain and motor deficits in complex regional pain syndrome. *Pain*. 2020:161(3):595–608.

Baliki MN, Schnitzer TJ, Bauer WR, Apkarian AV. Brain morphological signatures for chronic pain. *PLoS One*. 2011:6(10):e26010.

Berliocchi L, Russo R, Tassorelli C, Morrone L, Bagetta G, Corasanti M. Death in pain: peripheral nerve injury and spinal neurodegenerative mechanisms. *Curr Opin Pharmacol*. 2012:12(1):49–54.

Bernard JF. *Hypothalamus and nociceptive pathways. Encyclopedia of pain*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2007. pp. 944–948

Blyth F, March L, Brnabic A, Jorm L, Williamson M, Cousins M. Chronic pain in Australia: a prevalence study. *Pain*. 2001:89(127–134):127–134.

Brennan TJ. Postoperative models of nociception. *Inst Lab Anim Res J*. 1999:40(3):129–136.

Brennan TJ. Pathophysiology of postoperative pain. *Pain*. 2011:152(3 Suppl):S33–S40.

Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. *Pain*. 1996:64(3):493–502.

Brooks J, Tracey I. From nociception to pain perception: imaging the spinal and supraspinal pathways. *J Anat*. 2005:207(1):19–33.

Brooks JCW, Tracey I. The insula: a multidimensional integration site for pain. *Pain*. 2007:128(1):1–2.

Broom DM. Evolution of pain. *Vlaams Diergeneeskundig Tijdschrift*. 2001:70(1):17–21.

Burke NN, Trang T. Neonatal injury results in sex-dependent nociceptive hypersensitivity and social behavioral deficits during adolescence, without altering morphine response. *J Pain*. 2017:18(11):1384–1396.

Cauda F, Palermo S, Costa T, Torta R, Duca S, Vercelli U, Geminiani G, Torta DM. Gray matter alterations in chronic pain: a network-oriented meta-analytic approach. *NeuroImage*. 2014:4:676–686.

Clinch J, Eccleston C. Chronic musculoskeletal pain in children: assessment and management. *Rheumatology (Oxford)*. 2009:48(5):466–474.

Cortelli P, Pierangeli G. Hypothalamus and headaches. *Neurol Sci*. 2007:28(2):S198–S202.

DaSilva AF, Granziera C, Snyder J, Hadjikhani N. Thickening in the somatosensory cortex of patients with migraine. *Neurology*. 2007:69(21):1990–1995.

Deuis JR, Dvorakova LS, Vetter I. Methods used to evaluate pain behaviors in rodents. *Front Mol Neurosci*. 2017:10:284.

Emerson NM, Zeidan F, Lobanov OV, Hadsel MS, Martucci KT, Quevedo AS, Starr CJ, Nahman-Averbuch H, Weissman-Fogel I, Granovsky Y, et al. Pain sensitivity is inversely related to regional grey matter density in the brain. *Pain*. 2014:155(3):566–573.

Eyolfson E, Carr T, Fraunberger E, Khan A, Clark I, Mychasiuk R, Lohman A. Repeated mild traumatic brain injuries in mice cause age- and sex-specific alterations in dendritic spine density. *Exp Neurol*. 2022;357:114172.

Fakhoury M, Salman I, Najjar W, Merhej G, Lawand N. The lateral hypothalamus: an uncharted territory for processing peripheral neurogenic inflammation. *Front Neurosci*. 2020: 14:101.

Fasick V, Spengler RN, Samankan S, Nader ND, Ignatowski TA. The hippocampus and TNF: common links between chronic pain and depression. *Neurosci Biobehav Rev*. 2015:53:139–159.

Gatchel RJ. Comorbidity of chronic pain and mental health disorders: the biopsychosocial perspective. *Am Psychol*. 2004:59(8):795–805.

Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *NeuroImage*. 2001:14(1):21–36.

Groenewald CB, Essner BS, Wright D, Fesinmeyer MD, Palermo TM. The economic costs of chronic pain among a cohort of treatment-seeking adolescents in the United States. *J Pain*. 2014:15(9): 925–933.

Gustin SM, Peck CC, Wilcox SL, Nash PG, Murray GM, Henderson LA. Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. *J Neurosci*. 2011:31(16):5956–5964.

Gustin SM, McKay JG, Petersen ET, Peck CC, Murray GM, Henderson LA. Subtle alterations in brain anatomy may change an individual's personality in chronic pain. *PLoS One*. 2014:9(10):e109664.

Gwilym SE, Filippini N, Douaud G, Carr AJ, Tracey I. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheumat*. 2010:62(10):2930–2940.

Hagiwara H, Sakimura K, Abe M, Itoi K, Kamiya Y, Akema T, Funabashi T. Sex differences in pain-induced modulation of corticotropin-releasing hormone neurons in the dorsolateral part of the stria terminalis in mice. *Brain Res*. 2021:1773:147688.

Hashmi JA, Baliki MN, Huang L, Baria AT, Torbey S, Hermann KM, Schnitzer TJ, Apkarian AV. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain*. 2013:136(9):2751–2768.

Henssen D, Dijk J, Knepflé R, Sieffers M, Winter A, Vissers K. Alterations in grey matter density and functional connectivity in trigeminal neuropathic pain and trigeminal neuralgia: a systematic review and meta-analysis. *NeuroImage: Clinical*. 2019:24:102039.

Jasmin L, Burkey A, Granato A, Ohara P. Rostral agranular insular cortex and pain areas of the central nervous system: a tract-tracing study in the rat. *J Comp Neurol*. 2004:468(3):425–440.

Johansson A, Gunnarsson L, Linton S, Bergkvist L, Stridesberg M, Nilsson O, Cornefjord M. Pain, disability and coping reflected in the diurnal cortisol variability in patients scheduled for lumbar disc surgery. *Eur J Pain*. 2008:12(5):633–640.

Kang D, McAuley JH, Kassem MS, Gatt JM, Gustin SM. What does the grey matter decrease in the medial prefrontal cortex reflect in people with chronic pain? *Eur J Pain*. 2019:23(2):203–219.

King S, Chambers CT, Huguet A, MacNevin RC, McGrath PJ, Parker L, MacDonald AJ. The epidemiology of chronic pain in children and adolescents revisited: a systematic review. *Pain*. 2011:152(12): 2729–2738.

Koch A, Zacharowski K, Boehm O, Lipfert P, von Giessen H, Wolf A, Freynhagen R. Nitric oxide and pro-inflmmatory cytokines correlate with pain intensity in chronic pain patients. *Inflamm Res*. 2007:56(1):32–37.

Kogler L, Müller VI, Seidel EM, Boubela R, Kalcher K, Moser E, Habel U, Gur RC, Eickhoff SB, Derntl B. Sex differences in the functional connectivity of the amygdalae in association with cortisol. *NeuroImage*. 2016:134:410–423.

Kolb B, Mychasiuk R, Muhammad A, Li Y, Frost DO, Gibb R. Experience and the developing prefrontal cortex. *Proc Natl Acad Sci*. 2012:109(supplement_2):17186–17193.

Labus JS, Naliboff BN, Fallon J, Berman SM, Suyenobu B, Bueller JA, Mandelkern M, Mayer EA. Sex differences in brain activity during aversive visceral stimulation and its expectation in patients with chronic abdominal pain: a network analysis. *NeuroImage*. 2008:41(3):1032–1043.

Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev*. 2001:53(4):597–652.

Li YC, Wang Q, Li MG, Hu SF, Xu GY. A paraventricular hypothalamic nucleus input to ventral of lateral septal nucleus controls chronic visceral pain. *Pain*. 2022:Publish Ahead of Print.

Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, Hooker JM. Evidence for brain glial activation in chronic pain patients. *Brain*. 2015:138(3):604–615.

May A. Structural brain imaging: a window into chronic pain. *Neuroscientist*. 2011:17(2):209–220.

McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res*. 2011:45(8):1027–1035.

Mengler L, Khmelinskii A, Diedenhofen M, Po C, Staring M, Lelieveldt B, Hoehn M. Brain maturation of the adolescent rat cortex and striatum: changes in volume and myelination. *NeuroImage*. 2014:84:35–44.

Moulton EA, Schmahmann JD, Becerra L, Borsook D. The cerebellum and pain: passive integrator or active participator? *Brain Res Rev*. 2010:65(1):14–27.

Mutso AA, Radzicki D, Baliki MN, Huang L, Banisadr G, Centeno MV, Radulovic J, Martina M, Miller RJ, Apkarian AV. Abnormalities in hippocampal functioning with persistent pain. *J Neurosci*. 2012:32(17):5747–5756.

Mychasiuk R, Metz GA. Epigenetic and gene expression changes in the adolescent brain: what have we learned from animal models? *Neurosci Biobehav Rev*. 2016:70:189–197.

Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. *Neuroscientist*. 2004:10(3):221–234.

Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F. Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. *Cereb Cortex*. 2002:12(4):376–385.

Pagé MG, Stinson J, Campbell F, Isaac L, Katz J. Identification of pain-related psychological risk factors for the development and maintenance of pediatric chronic postsurgical pain. *J Pain Res*. 2013:6:167.

Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd J, Rapoport J, Evans A. Structural maturation of neural pathways in children and adolescents: in vivo study. *Science*. 1999:283(5409): 1908–1911.

Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. London: Academic Press; 2014

Perquin CW, Hazebroek-Kampschreur AA, Hunfeld JA, Bohnen AM, van Suijlekom-Smit LW, Passchier J, Van Der Wouden JC. Pain in children and adolescents: a common experience. *Pain*. 2000:87(1): 51–58.

Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci*. 1992:106(2):274–285.

Puopolo M. The hypothalamic-spinal dopaminergic system: a target for pain modulation. *Neural Regen Res*. 2019:14(6):925–930.

Pyter LM, Kelly SD, Harrell CS, Neigh GN. Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. *Brain Behav Immun*. 2013:30:88–94.

Rabbitts JA, Fisher E, Rosenbloom BN, Palermo TM. Prevalence and predictors of chronic postsurgical pain in children: a systematic review and meta-analysis. *J Pain*. 2017:18(6):605–614.

Rajmohan V, Mohandas E. The limbic system. *Indian J Psychiatry*. 2007:49(2):132–139.

Rizzi-Wise CA, Wang DV. Putting together pieces of the lateral septum: multifaceted functions and its neural pathways. *ENeuro*. 2021:8(6):ENEURO.0315–ENEU21.2021.

Ruscheweyh R, Deppe M, Lohmann H, Stehling C, Flöel A, Ringelstein EB, Knecht S. Pain is associated with regional grey matter reduction in the general population. *Pain*. 2011:152(4):904–911.

Salberg S, Noel M, Burke N, Vinall J, Mychasiuk R. Utilization of a rodent model to examine the neurological effects of early life adversity on pain sensitivity. *Dev Psychobiol*. 2020:62(3):386–399.

Salberg S, Yamakawa GR, Griep Y, Bain J, Berveridge J, Sun M, McDonald S, Shultz S, Brady R, Wright D, et al. Pain in the developing brain: early life factors alter nociception and neurobiological functioni in adolescent rats. *Cerebr Cortex Commun*. 2021:2(2):tgba014.

Salberg S, Yamakawa GR, Beveridge JK, Noel M, Mychasiuk R. A high-fat, high sugar diet and adversity early in life modulate pain outcomes at the behavioural and molecular level in adolescence: the role of sex. *Brain Behav Immun*. 2023:108:57–79.

Schafer D, Lehrman E, Kautzman A, Koyama R, Mardinly A, Yamasaki R, Ransohoff R, Greenberg M, Barres B, Stevens B. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012:74(4):691–705.

Scholz J, Broom DC, Youn DH, Mills CD, Kohno T, Suter MR, Moore KA, Decosterd I, Coggeshall RE, Woolf CJ. Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. *J Neurosci*. 2005:25(32):7317–7323.

Seminowicz D, Laferriere A, Millecamps M, Yu J, Coderre T, Bushnell M. MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain. *NeuroImage*. 2009:47(3):1004–1014.

Shi Y, Gelman BB, Lisinicchia JG, Tang SJ. Chronic-pain-associated astrocytic reaction in the spinal cord dorsal horn of human immunodeficiency virus-infected patients. *J Neurosci*. 2012:32(32):10833–10840.

Simons LE, Moulton EA, Linnman C, Carpino E, Becerra L, Borsook D. The human amygdala and pain: evidence from neuroimaging. *Hum Brain Mapp*. 2014:35(2):527–538.

Smallwood RF, Laird AR, Ramage AE, Parkinson AL, Lewis J, Clauw DJ, Williams DA, Schmidt-Wilcke T, Farrell MJ, Eickhoff SB, et al. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. *J Pain*. 2013:14(7):663–675.

Somade PM, Chopade AR, Chitale MS, Patil PA, Brid SV. Evaluation of role of septal nuclei in modulation of pain in selected pain models. *J Pharm Sci Res*. 2019:11(6):2233–2239.

Stanford EA, Chambers CT, Biesanz JC, Chen E. The frequency, trajectories and predictors of adolescent recurrent pain: a population-based approach. *Pain*. 2008:138(1):11–21.

Starr CJ, Sawaki L, Wittenberg GF, Burdette JH, Oshiro Y, Quevedo AS, McHaffie JG, Coghill RC. The contribution of the putamen to sensory aspects of pain: insights from structural connectivity and brain lesions. *Brain*. 2011:134(7):1987–2004.

Swanson LW, Cowan WM. The connections of the septal region in the rat. *J Comp Neurol*. 1979:186(4):621–655.

Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. *Neuron*. 2007:55(3):377–391.

Vinall J, Pavlova M, Asmundson GJ, Rasic N, Noel M. Mental health comorbidities in pediatric chronic pain: a narrative review of epidemiology, models, neurobiological mechanisms and treatment. *Child Aust*. 2016:3(4):40.

Walker LS, Sherman AL, Bruehl S, Garber J, Smith CA. Functional abdominal pain patient subtypes in childhood predict functional gastrointestinal disorders with chronic pain and psychiatric comorbidities in adolescence and adulthood. *Pain*. 2012:153(9):1798–1806.

Wilcox SL, Gustin SM, Macey PM, Peck CC, Murray GM, Henderson LA. Anatomical changes at the level of the primary synapse in neuropathic pain: evidence from the spinal trigeminal nucleus. *J Neurosci*. 2015:35(6):2508–2515.