

# Hippocampal and parahippocampal volumes vary by sex and traumatic life events in children

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**Background:** Childhood trauma is reliably associated with smaller hippocampal volume in adults; however, this finding has not been shown in children, and even less is known about how sex and trauma interact to affect limbic structural development in children. **Methods:** Typically developing children aged 9 to 15 years who completed a trauma history questionnaire and structural  $T_1$ -weighted MRI were included in this study ( $n = 172$ ; 85 female, 87 male). All children who reported 4 or more traumas ( $n = 36$ ) composed the high trauma group, and all children who reported 3 or fewer traumas ( $n = 136$ ) composed the low trauma group. Using multivariate analysis of covariance, we compared FreeSurfer-derived structural MRI volumes (normalized by total intracranial volume) of the amygdalar, hippocampal and parahippocampal regions by sex and trauma level, controlling for age and study site. **Results:** We found a significant sex  $\times$  trauma interaction, such that girls with high trauma had greater volumes than boys with high trauma. Follow-up analyses indicated significantly increased volumes for girls and generally decreased volumes for boys, specifically in the hippocampal and parahippocampal regions for the high trauma group; we observed no sex differences in the low trauma group. We noted no interaction effect for the amygdalae. **Limitations:** We assessed a community sample and did not include a clinical sample. We did not collect data about the ages at which children experienced trauma. **Conclusion:** Results revealed that psychological trauma affects brain development differently in girls and boys. These findings need to be followed longitudinally to elucidate how structural differences progress and contribute to well-known sex disparities in psychopathology.

## Introduction

A substantial literature associates childhood psychological trauma with smaller limbic structural volumes in adults, consistent with the importance of these regions in emotion recognition, fear processing and episodic memory.<sup>1</sup> Teicher and colleagues<sup>1</sup> have suggested that trauma during childhood may alter the trajectory of brain development, which may lead to volumetric differences and the emergence of psychiatric symptoms in adolescence or young adulthood. However, evidence supporting this chain of events is scarce, and longitudinal studies are needed. In the current study, we focused on threat-detection and threat-processing regions, with the broad hypothesis that exposure to early threat may result in attenuated threat-processing during development and subsequent alterations in regional brain volumes. We focused on limbic brain regions — specifically the hippocampal, parahippocampal and amygdalar regions — because limbic structures have been commonly implicated in the neuroscience of trauma, although we acknowledge that a

growing literature demonstrates the importance of trauma in diverse cortical regions.<sup>1-6</sup>

Summarizing most of the available adult literature, a major meta-analysis ( $k = 49$  studies,  $n = 2720$  adults) found that maltreatment and other traumatic events during childhood (especially multiple traumatic events) was associated with reduced hippocampal volumes.<sup>7</sup> These findings were consistent with previous meta-analyses of adult hippocampal volumes<sup>1-6,8,9</sup> and may highlight the role of trauma and the associated stress response during periods of heightened synaptogenesis (e.g., childhood and early adolescence). The effect of trauma on adult amygdalar volumes appears weaker, which may suggest that the aforementioned attenuation is more related to threat-processing regions than to the biologically essential function of basic threat detection. One relevant meta-analysis<sup>10</sup> found no significant amygdalar differences between people with and without posttraumatic stress disorder (PTSD); another suggested a smaller left amygdala in adults with PTSD but no significant difference on the right.<sup>2</sup> Recent results from the ENIGMA-PGC large-scale

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neuroimaging consortium ( $n = 1868$  participants) found smaller hippocampi and marginally smaller amygdalae in people with PTSD compared to healthy controls; 15 of the 16 ENIGMA sites reported adult data.<sup>11</sup>

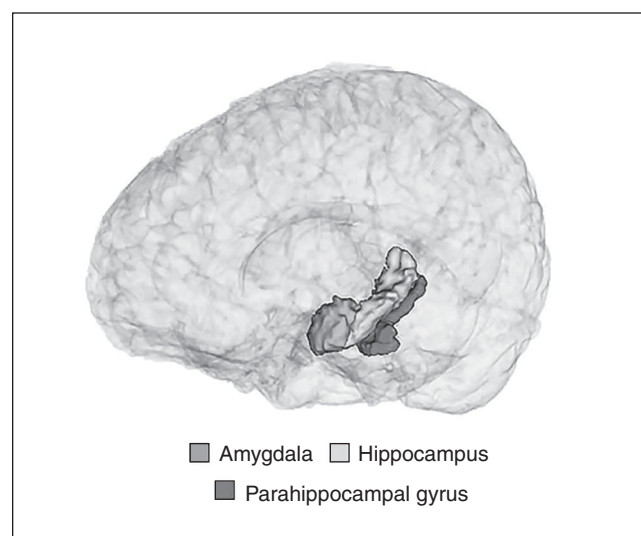
Despite strong evidence for smaller hippocampal volumes in adults who have experienced traumatic events, meta-analyses reporting regional volumes measured in childhood have found no significant effect of trauma history on hippocampal or amygdalar volume,<sup>1-8</sup> perhaps because environmental influences take some time to alter the trajectory of brain development, and/or the impact on synaptogenesis is cumulative and takes time to reach a detectable level. A lack of support for reduced hippocampal volumes in childhood marks an important distinction from the large body of literature noting consistently smaller hippocampal size in adult survivors of childhood adversity. A major review concluded that reduced hippocampal volume is reliably observed in adults who experienced childhood maltreatment but is not apparent in children.<sup>12</sup> Normal subcortical development for both males and females follows a course of continual increases in amygdalar and hippocampal volume during puberty<sup>13</sup>; finding reduced hippocampal size in adults but not in children after adverse childhood events is surprising but consistent with a developmental model in which such volumetric differences do not become apparent until adulthood.<sup>2</sup> Rodent studies place the emergence of this reduction in hippocampal volume during the transition between puberty and adulthood,<sup>14</sup> but human imaging studies during childhood are clearly warranted.

Importantly, the common emergence during adolescence of psychiatric diagnoses typically associated with childhood maltreatment (e.g., depression, anxiety, PTSD), especially for girls, highlights the importance of studying potential differences in neural development for girls and boys that may precede the emergence of these disorders. Adolescence is the developmental period during which it is common to see the onset of emotional disorders, and these disorders are more prevalent in females than males. Specifically, childhood rates of depression are comparable for boys and girls, but as individuals progress through adolescence,<sup>15</sup> the ratios of women to men with depression, PTSD and many anxiety disorders increase to 2:1 by adulthood.<sup>16</sup> Thus, the culmination of the central role of the limbic system in emotional processing and memory, the noted disconnect in hippocampal volumes between adults and children following adverse childhood events, and the clear evidence for sex-related disparities in the prevalence of psychiatric diagnoses mean that attention needs to be paid to potential sex differences in limbic development.

Previous meta-analyses with adult samples that examined sex differences have not consistently supported a sex effect on limbic volumes for adults. Logue and colleagues<sup>11</sup> did not find a significant sex  $\times$  trauma interaction in adults. Woon and Hedges<sup>17</sup> concluded that sex does not moderate hippocampal volume in adults with PTSD. However, Karl and colleagues<sup>2</sup> reported smaller effect sizes for reduced hippocampal volume in women than in men, and Calem and colleagues<sup>18</sup> found that an effect of childhood adversity on hippocampal size

weakened when accounting for sex, suggesting that sex is an important confounder. Recently, an adult study reported reduced hippocampal volume in men who experienced childhood adverse events, but not in women,<sup>19</sup> emphasizing the importance of considering sex as a biological variable of interest in accordance with new initiatives from the National Institutes of Health in the United States (see NOT-OD-15-102; <https://grants.nih.gov/grants/guide/notice-files/not-od-15-102.html>) and with regard to disparate prevalence rates in adult psychiatric diagnoses. Unfortunately, even less is known about children and possible sex-based structural differences in limbic volumes after trauma exposure. We were unable to locate any meta-analyses considering sex as a variable in MRI studies of trauma effects conducted during childhood. In fact, the handful of studies that considered sex  $\times$  trauma differences were generally limited by methodological issues, including lack of control for total brain volume<sup>20</sup> or very small sample sizes.<sup>21,22</sup>

The current study explores trauma-related sex differences in the limbic volumes of typically developing children with high and low levels of traumatic life events to specifically identify the possibility of a sex  $\times$  trauma-level interaction effect on amygdalar, hippocampal and parahippocampal volumes during childhood. Given previous research, we identified the hippocampi as our primary region of interest. We also included the amygdalae and parahippocampal gyri because of their proximity to the hippocampi in the brain (Fig. 1), and because stressful life events have been linked to alterations in all 3 brain regions.<sup>23</sup> The role of the amygdala is well established in stress-related conditions.<sup>2,10,24</sup> Functional evidence supports the parahippocampal gyri in distinguishing people with clinically significant PTSD from those without.<sup>24</sup> In addition, the parahippocampal gyri display hypoconnectivity with the prefrontal and occipital regions,<sup>25</sup> as well as with the amygdala,<sup>26</sup> in people with PTSD, suggesting its role in processing traumatic memories. To this end, we derived



**Fig. 1:** Freesurfer (Desikan–Killiany atlas) map of the amygdala, hippocampus and parahippocampal gyrus.

bilateral hippocampal, parahippocampal and amygdalar volumes from 172 children and then probed those findings for significant sex  $\times$  trauma interactions. Current sociocultural etiological explanations for sex differences in psychopathology that are not present during childhood, but emerge strongly during adolescence are widely regarded as inadequate. Hormonal differences could alter brain development during this critical period and lead to the clear shift in prevalence of psychological symptoms, but evidence for this is lacking. Because we had a study sample of outwardly psychologically healthy children who had experienced significant traumatic events, we assumed that they were still in the process by which trauma and related hormone changes guide ecophenotypic expression. Therefore, we anticipated finding a sex  $\times$  trauma interaction in limbic region brain volumes that might precede sex differences in the trauma-related psychopathology that eventually emerges.

## Methods

### *Participants*

We enrolled typically developing children ( $n = 183$ ), aged 9 to 15 years, in the Developmental Chronnecto-Genomics study of healthy brain development (supported by the National Science Foundation of the United States after obtaining written parental permission and child assent to participate in the study. The current study included all enrolled children who completed a modified version of the UCLA Trauma History Profile<sup>27</sup> and had acceptable structural MRI data ( $n = 172$ ). All children who self-reported 4 traumas or more ( $n = 36$ ) composed the high trauma group, and the remaining children, who reported 0 to 3 traumas ( $n = 136$ ), composed the low trauma group. Our resultant sample included 85 female (15 high trauma) and 87 male (21 high trauma) children. We selected the high trauma threshold of 4 traumas or more based on the original Adverse Childhood Experiences study<sup>28</sup> and a recent large-scale meta-analysis,<sup>29</sup> which showed that childhood exposure to 4 or more adverse events has been associated with numerous health risks, and that a dose-dependent relationship exists between the number of childhood adverse events and psychological difficulties in adulthood. Children were excluded from the study if their parents reported that they had ever had a diagnosis of any psychiatric or behavioural disorder, had a history of traumatic brain injury or other neurologic condition, or had metallic implants (e.g., orthodontia). The study was approved by the university institutional review boards, and all research was conducted according to ethical principles, including obtaining fully informed written parental consent and child assent. Data collection occurred at the University of Nebraska Medical Center in Omaha, Nebraska, and at the Mind Research Network in Albuquerque, New Mexico.

### *Measures*

The Barratt Simplified Measure of Social Status<sup>30,31</sup> is a measure of socioeconomic status based on marital status, employ-

ment status, educational achievement and occupational merit. The measure was administered to parents, who reported their occupation and highest level of education completed for themselves, their spouses, and their parents. A total score was calculated by adding the total education score to the total occupation score.

The Modified UCLA Trauma History Profile is a modified self-report version of the UCLA Trauma History Profile,<sup>27</sup> used to assess the number of traumatic life events encountered by children. Children answered yes or no to whether or not they had experienced each of 12 potentially traumatic events. We shortened the original 15 event measures to exclude items about sexual abuse or about physical abuse that occurred specifically in the home, so that participation would be considered low risk by the institutional review boards, given that participation did not offer a direct benefit to the children. However, we still assessed personal experiences of violence and witnessing violence to family members. The items used in the current study included the following: having someone close to them die; being hit, punched or kicked very hard; seeing a family member hit, punched or kicked very hard; seeing or hearing about violence to a loved one; being a victim of community violence; being in a war; being in a disaster; being in a bad accident; having a painful or scary medical procedure; seeing a dead body not at a funeral; and having anything else very scary or upsetting happen.

The Trauma Symptom Checklist for Children–A is a self-report measure for children aged 8 to 16 years who have experienced traumatic life events; it consists of 5 clinical scales. The measure uses statements such as, “Remembering things I don’t want to remember,” and “Getting into fights,” followed by a Likert scale of 0 (never) to 3 (almost all of the time). The clinical scales assess anxiety, depression, anger, posttraumatic stress and dissociation, and the measure has strong reliability and validity as psychometric support.<sup>32</sup> Cronbach’s  $\alpha$  for the clinical scales in this sample were sound, ranging from 0.79 to 0.88. See Mills and colleagues<sup>33</sup> for a detailed description of relationships among these and other psychometric scales with trauma level in the Developmental Chronnecto-Genomics sample, which also demonstrated that children are better reporters of psychological distress than their parents, at least in this nonclinical sample.

### *Magnetic resonance imaging*

We acquired structural  $T_1$ -weighted MRI images using a Siemens 3 T Skyra (University of Nebraska Medical Center) and Siemens 3 T TRIO (Mind Research Network) scanner, both with 32-channel head coils. We used a 3-dimensional magnetization-prepared rapid gradient echo sequence with the following parameters: repetition time 2400 ms, echo time 1.94 ms, flip angle 8°, field of view 256 mm, slice thickness 1 mm, base resolution 256, 192 slices, voxel size 1.0  $\times$  1.0  $\times$  1.0 mm. We processed the  $T_1$ -weighted structural brain images of all participants using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu>). We computed regional volumes for the 70 Desikan–Killiany atlas regions (34 regions per hemisphere, plus left and right hemisphere).<sup>34</sup> We followed the ENIGMA

protocol for quality assurance, which included performing visual checks of all cortical segmentations (<http://enigma.usc.edu/protocols/imaging-protocols>) and checking for motion, among other artifacts. Participants with large motion artifacts were excluded. In addition, histograms of all regional values were computed for visual inspection. All volumes were normalized by dividing each regional volume by the total intracranial volume (TIV) per participant to avoid the bias of head size in the volumetric measurements.

### Statistical analysis

We conducted a multivariate analysis of covariance (MANCOVA), with fixed factors of sex (male, female) and trauma level (high, low), and dependent variables for each brain region of interest entered separately as right and left volumes (amygdala, hippocampus, parahippocampus) for a total of 6 brain regions, controlling for age at scan and study site as a potential nuisance variable. We conducted follow-up between-participant analyses of covariance (ANCOVAs) for each brain region as indicated by the main analysis, and subjected them to conservative Bonferroni corrections. This analysis strategy — conducting a single main analysis (MANCOVA) and following up only its interpretable findings while also correcting for multiple tests — protected against potential bias.<sup>35</sup> Finally, we also considered the relationship between psychological symptoms and trauma exposure by sex using the same methodological approach.

## Results

The total sample included 87 boys and 85 girls, with a mean age  $\pm$  standard deviation (SD) of  $11.77 \pm 1.85$  years in the high trauma group and  $11.98 \pm 1.70$  years in the low trauma group ( $p = 0.51$ ). Both groups were predominantly white (low trauma, 85.3%; high trauma, 80.6%); 30.6% of children in the high trauma group indicated that their ethnicity was Hispanic or Latinx, compared with 23.5% in the low trauma group. The trauma groups did not differ according to socioeconomic status: both groups indicated average socioeco-

omic status (low trauma,  $44.54 \pm 13.66$ ; high trauma,  $44.28 \pm 12.28$ ;  $p = 0.91$ ). The mean number of traumatic events reported by children in the low trauma group was  $1.40 \pm 1.04$  (range 0 to 3), and these events focused on knowing someone who had died. The mean number of traumatic events reported by children in the high trauma group was  $5.08 \pm 1.00$  (range 4 to 7). Knowing someone who died was also the event reported most often in this group, followed closely by experiencing and/or bearing witness to violence. See Table 1 for the frequencies of events experienced by each trauma group.

The main study analysis was a MANCOVA of FreeSurfer-derived structural MRI volumes from the right and left amygdalar, hippocampal and parahippocampal regions, compared by sex (female, male) and trauma level (high, low), while controlling for the effects of age and site (University of Nebraska Medical Center, Mind Research Network). Table 2 reports regional volumes normalized by TIV. Despite differing cell sizes, Box's M for equality of covariance and Levene's test for equality of error variances were insignificant, allowing interpretation of the MANCOVA to continue. The MANCOVA sex  $\times$  trauma interaction was significant (Wilks  $\lambda = 0.86$ ;  $F_{6,161} = 4.53$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.15$ ), such that none of the volumes differed by sex in low trauma youth, but high trauma girls had larger limbic regional volumes than high trauma boys. This sex  $\times$  trauma interaction effect superseded interpretation of the sex main effect, which indicated that girls had larger volumes than boys (Wilks  $\lambda = 0.82$ ;  $F_{6,161} = 5.95$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.18$ ). Also pointing to the importance of the interaction, the trauma level main effect itself was not significant (Wilks  $\lambda = 0.99$ ;  $F_{6,161} = 0.24$ ;  $p = 0.96$ ;  $\eta_p^2 = 0.0088$ ). The MRI site nuisance variable was a significant covariate for the MANCOVA (Wilks  $\lambda = 0.88$ ;  $F_{6,161} = 3.36$ ;  $p = 0.002$ ;  $\eta_p^2 = 0.12$ ). Follow-up ANCOVAs indicated that scanner site was a significant covariate for the left ( $F_{1,166} = 17.40$ ;  $p < 0.001$ ) and right ( $F_{1,166} = 8.43$ ;  $p = 0.004$ ) hippocampi, but we observed no site effect for the other limbic regions ( $p > 0.23$ ). However, given that sex was evenly distributed by site, this nuisance variable could not account for the observed sex  $\times$  trauma interaction effect.

**Table 1: Traumatic life experiences by trauma group\***

| Traumatic event                                      | High trauma, <i>n</i> (%) | Low trauma, <i>n</i> (%) |
|--|---------------------------|--------------------------|
| Death of a loved one                                 | 26 (72.2)                 | 81 (59.6)                |
| Hit, punched, or kicked very hard                    | 24 (66.7)                 | 18 (13.2)                |
| Saw or heard about a violent death or serious injury | 24 (66.7)                 | 24 (17.6)                |
| Saw someone assaulted, shot at, or killed            | 24 (66.7)                 | 24 (17.6)                |
| Saw a family member hit, punched, or kicked          | 17 (47.2)                 | 3 (2.2)                  |
| Victim of community violence                         | 11 (30.6)                 | 5 (3.7)                  |
| Painful or scary medical treatment                   | 10 (27.8)                 | 7 (5.1)                  |
| Disaster   | 8 (22.2)                  | 10 (7.4)                 |
| Serious accident                                     | 7 (19.4)                  | 6 (4.4)                  |
| Saw a dead body                                      | 5 (13.9)                  | 4 (2.9)                  |
| War  | 1 (2.8)                   | 0 (0.0)                  |
| Other  | 31 (86.1)                 | 30 (22.1)                |

\*High trauma group, *n* = 36 children; low trauma group, *n* = 136 children.

**Table 2: Regional volumes normalized by total intracranial volume**

| Brain region                | Sex | Trauma group | Children, <i>n</i> | Mean volume $\pm$ SD, mm <sup>3</sup> |
|-----------------------------|-----|--------------|--------------------|---------------------------------------|
| Left amygdala               | F   | Low          | 70                 | 0.0011 $\pm$ 0.00013                  |
|                             |     | High         | 15                 | 0.0011 $\pm$ 0.00012                  |
|                             | M   | Low          | 66                 | 0.0010 $\pm$ 0.00011                  |
|                             |     | High         | 21                 | 0.0010 $\pm$ 0.00012                  |
| Right amygdala              | F   | Low          | 70                 | 0.0011 $\pm$ 0.00012                  |
|                             |     | High         | 15                 | 0.0011 $\pm$ 0.00014                  |
|                             | M   | Low          | 66                 | 0.0011 $\pm$ 0.00011                  |
|                             |     | High         | 21                 | 0.0010 $\pm$ 0.00012                  |
| Left hippocampus            | F   | Low          | 70                 | 0.0028 $\pm$ 0.00035                  |
|                             |     | High         | 15                 | 0.0030 $\pm$ 0.00024                  |
|                             | M   | Low          | 66                 | 0.0027 $\pm$ 0.00031                  |
|                             |     | High         | 21                 | 0.0025 $\pm$ 0.00037                  |
| Right hippocampus           | F   | Low          | 70                 | 0.0029 $\pm$ 0.00028                  |
|                             |     | High         | 15                 | 0.0030 $\pm$ 0.00019                  |
|                             | M   | Low          | 66                 | 0.0028 $\pm$ 0.00023                  |
|                             |     | High         | 21                 | 0.0027 $\pm$ 0.00031                  |
| Left parahippocampal gyrus  | F   | Low          | 70                 | 0.0018 $\pm$ 0.00032                  |
|                             |     | High         | 15                 | 0.0020 $\pm$ 0.00031                  |
|                             | M   | Low          | 66                 | 0.0018 $\pm$ 0.00033                  |
|                             |     | High         | 21                 | 0.0016 $\pm$ 0.00032                  |
| Right parahippocampal gyrus | F   | Low          | 70                 | 0.0016 $\pm$ 0.00026                  |
|                             |     | High         | 15                 | 0.0019 $\pm$ 0.00027                  |
|                             | M   | Low          | 66                 | 0.0016 $\pm$ 0.00019                  |
|                             |     | High         | 21                 | 0.0015 $\pm$ 0.00022                  |
| Total intracranial volume   | F   | Total        | 85                 | 1 447 728.59 $\pm$ 117 262.98         |
|                             | M   | Total        | 87                 | 1 595 890.80 $\pm$ 125 375.65         |

F = female; M = male; SD = standard deviation.

Age was not a significant covariate in the MANCOVA (Wilks  $\lambda$  = 0.96;  $F_{6,161}$  = 1.19;  $p$  = 0.31;  $\eta_p^2$  = 0.04), perhaps because the data had already taken TIV into account.

Regarding the key sex  $\times$  trauma interaction effect, univariate ANCOVAs with a Bonferroni corrected  $\alpha$  level of 0.008 revealed that the sex  $\times$  trauma interaction held for the left hippocampus ( $F_{1,166}$  = 9.17;  $p$  = 0.003;  $\eta_p^2$  = 0.052), but not for the right hippocampus ( $F_{1,166}$  = 2.96;  $p$  = 0.09;  $\eta_p^2$  = 0.018). The interaction effect was clearly noted in both the left ( $F_{1,166}$  = 9.55;  $p$  = 0.002;  $\eta_p^2$  = 0.054) and right ( $F_{1,166}$  = 15.42;  $p$  < 0.001;  $\eta_p^2$  = 0.085) parahippocampal regions (Fig. 2). However, the sex  $\times$  trauma interaction was not significant for the left ( $p$  = 0.13;  $\eta_p^2$  = 0.014) or right ( $p$  = 0.13;  $\eta_p^2$  = 0.014) amygdalae.

To examine the role of psychological symptoms in this sample, we also ran a MANCOVA with fixed factors of sex (male, female) and trauma level (high, low), age as a covariate, and psychological variables (PTSD, anxiety, depression, dissociation and anger) as dependent variables. Despite differing cell sizes, Box's M for equality of covariance and Levene's test for equality of error variances were insignificant, allowing interpretation of the MANCOVA to continue. In this analysis, both the trauma level main effect (Wilks  $\lambda$  = 0.92;  $F_{5,163}$  = 9.39;  $p$  < 0.001;  $\eta_p^2$  = 0.22) and the sex main effect (Wilks  $\lambda$  = 0.78;  $F_{5,163}$  = 2.99;  $p$  = 0.013;  $\eta_p^2$  = 0.084) were significant, but the sex  $\times$  trauma interaction was not significant

( $p$  = 0.30), and neither was the covariate of age ( $p$  = 0.10). The trauma effect held for each type of psychological difficulty in follow-up analyses subjected to Bonferroni correction (ranging from  $p$  < 0.001 for PTSD to  $p$  = 0.007 for anger), but no sex effects survived Bonferroni correction in the follow-up ANCOVAs. Psychological symptoms were greater in the high trauma group than the low trauma group for both boys and girls across the board.

Finally, as a way to test whether psychological symptoms contributed to observed volume effects reported in the main analysis, we repeated the original MANCOVA of FreeSurfer-derived structural MRI volumes in the bilateral amygdalae, hippocampal and parahippocampal regions compared by sex (female, male) and trauma level (high, low) while controlling for the effects of age and site (University of Nebraska Medical Center, Mind Research Network) and adding PTSD symptom severity. We used PTSD symptom severity as a covariate because this was the psychological variable most affected by trauma in this sample. Symptoms of PTSD did not serve as a significant covariate ( $p$  = 0.23) when added to the original analysis, nor did the inclusion of psychological symptoms change the results reported above, indicating the key finding of a strong sex  $\times$  trauma interaction on limbic brain volumes, specifically in the parahippocampal and hippocampal regions.

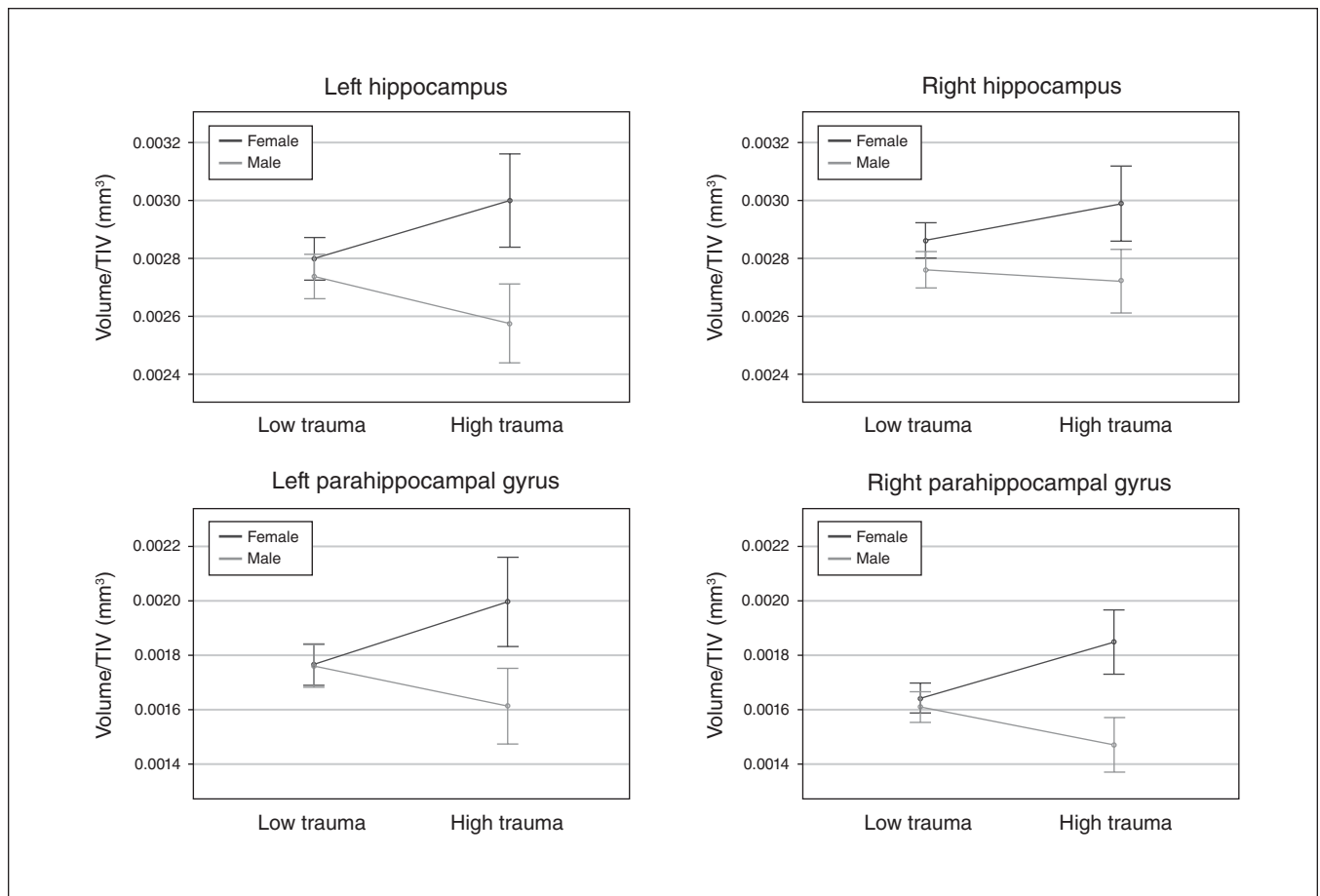
## Discussion

Our findings partially contradict the adult literature, which suggests that the hippocampi of traumatized children would be smaller than those of children who did not experience trauma. Our key finding was a significant sex  $\times$  trauma interaction in the structural volumes of limbic regions, which accounted for an impressive 15% of the variance in these areas. The boys in our high trauma group tended to have decreased parahippocampal and hippocampal volumes compared to the low trauma group, which was consistent with past adult literature,<sup>1-9</sup> but the girls in our high trauma group had increased parahippocampal and hippocampal volumes compared to the low trauma group. Volumes in our study did not differ by sex in the low trauma group, so the observed sex differences in the high trauma group were especially intriguing.

Notably, we did not find a significant trauma main effect on regional volumes in our child sample. Taken on its own, this appears consistent with most published literature on child development.<sup>1-8</sup> However, unlike the current study,

these previous studies examined the effect of trauma on brain volume without considering sex-based differences. We wonder if previous volumetric studies in child samples may have found sex  $\times$  trauma interaction effects if they had included sex in their analyses. Perhaps some of the volumetric inconsistencies in the child literature are related to missed sex effects on subcortical limbic brain regions in trauma-exposed children. In the current study, we corrected regional volumes using the TIV of each participant. Future work could examine the effect of TIV versus subcortical volume or total brain volume correction approaches on regional volumes in this age range. Interestingly, TIV tends to stabilize in early adolescence and does not show differences between adults with and without PTSD;<sup>36</sup> however, these effects have not been evaluated during childhood to the best of our knowledge.

Bridging the child to adult literature on hippocampal size, Paquola and colleagues<sup>37</sup> found that right hippocampal growth was diminished in participants aged 14 to 28 years who had experienced childhood maltreatment, offering a potential lower age range at which a reduction in hippocampal



**Fig. 2:** Sex  $\times$  trauma interaction graphs for the left and right hippocampal gyri and left and right parahippocampi. Trauma level is on the x-axes, and girls and boys are represented by separate lines. The y-axes show regional volumes ( $\text{mm}^3$ ) corrected by total intracranial volume ( $\text{mm}^3$ ) to control for individual differences in brain size and allow for appropriate comparisons between girls and boys. Covariates appearing in the model are evaluated at age = 11.94, site = 1.51. Error bars are set at  $\pm 2$  standard errors, denoting 95% confidence intervals.

size might be observed. For comparison, the average age of our sample was 11.9 years, and this hippocampal reduction did not occur in girls (who had increased hippocampal volumes) — it was evident only for the left hippocampus in boys. One longitudinal study found that male adolescents who experienced childhood maltreatment showed a trend toward slowed hippocampal development and suggested that this may represent a vulnerability for the onset of psychiatric disorders later in adolescence,<sup>38</sup> but longitudinal human data are sparse. Such slowed hippocampal development could reflect aberrant synaptogenesis in these adolescents. Animal models have demonstrated the causal importance of early-life stress on brain development. Specifically, rodent studies showed a delayed effect of traumatic events on hippocampal density that did not appear until early adulthood,<sup>14</sup> and suggested that male rodent hippocampi may be more sensitive to stress and prone to underdevelopment than female rodent hippocampi.<sup>39</sup>

Given the clear sex  $\times$  trauma interactions in hippocampal volume in our study, and the general null finding for the effect of trauma on hippocampal volume in the child literature, we conducted a systematic literature review of child studies of hippocampal volume by trauma and sex to fully explore the issue and found 12 relevant studies. More than half of the studies did not examine sex differences<sup>40–43</sup> or control for sex.<sup>38,44,45</sup> Quite surprisingly, only 1 study<sup>20</sup> reported separate hippocampal volumes by sex, eliminating the possibility of conducting a meta-analysis of potential sex effects here.

Tupler and De Bellis<sup>20</sup> reported larger hippocampal volumes for their child PTSD sample (from child abuse or witnessing domestic violence) than their healthy comparison sample — an important finding about development — but they did not find a significant sex  $\times$  group interaction. However, the study did not correct for TIV before analysis (which would have maximized power), but instead entered cerebral volume as a highly significant covariate. Another study did not find a hippocampal sex  $\times$  group interaction, but they did find a sex  $\times$  group interaction for lateral ventricular volumes, with boys having larger ventricles.<sup>46</sup> Two other studies did not find sex  $\times$  trauma differences, but their trauma sample sizes were very small — 4 girls and 5 boys,<sup>21</sup> and 6 girls and 8 boys<sup>22</sup> — greatly limiting their likelihood of finding sex-based differences.

Our findings did not support the existence of sex  $\times$  trauma interaction effects for the amygdalae, but we did find that volumes in girls were larger than those in boys. In general, the amygdala has been associated with developmental changes in which responsivity to emotional cues increases during childhood, peaks in adolescence and declines in adulthood.<sup>47</sup> Two structural studies found that children raised in orphanages in early childhood, presumably experiencing traumatic neglect, had larger amygdala volumes in adulthood, but no hippocampal differences compared with those who did not experience childhood adversity.<sup>22,43</sup> However, such amygdala effects are not usually noted in adulthood, suggesting the possibility of early amygdala hypertrophy and eventual atrophy by adulthood.<sup>48</sup> Tottenham and Sheridan<sup>23</sup> concluded that stressful events may exert early

effects on amygdalar development that precede their impact on hippocampal development.

Based on the high interconnectivity of amygdala, parahippocampal gyrus and hippocampus, we were intrigued — but not surprised — by the strong sex  $\times$  trauma interactions noted in the bilateral parahippocampal gyri. This region is activated in tasks of episodic memory and visuo-spatial processing and is important in processing contextual associations.<sup>49</sup> The parahippocampus has been associated with disruptions in autobiographical recall,<sup>50</sup> and a recent study noted sex differences in the parahippocampus during recall in patients with depression, suggesting that such differences may be linked to autobiographical memory overgenerality, which is correlated with depressive symptoms in women but not men.<sup>51</sup> Very interestingly, parahippocampal volume has been positively correlated with ruminative tendencies in women with depression.<sup>52</sup> We suspect that the volumetric differences we observed were related to sex differences in traumatic memory processing in this association region, placing girls at an increased risk for developing emotional disorders. Van Dam and colleagues<sup>53</sup> found that childhood adversity was associated with decreased grey matter volumes in the bilateral parahippocampal gyri and the left hippocampus in adults, but we are not aware of any evidence of parahippocampal volume differences by sex and trauma in children.

### Limitations

Regarding the first limitation of our study, we assessed a community sample of healthy children with and without significant traumatic life events, but we did not include a clinical sample of children diagnosed with PTSD. A pooled data reanalysis revealed larger hippocampal volumes in children with PTSD compared with children who did not experience maltreatment,<sup>20</sup> but another study reported increased hippocampal volumes in maltreated youth without PTSD and smaller volumes in those with PTSD.<sup>54</sup> Regardless, other research has shown that trauma exposure in healthy individuals is associated with lower hippocampal but not amygdalar volumes in adults, even though such effects tend to be smaller than in samples with known psychopathology.<sup>18</sup> Future research should evaluate these neural structures in children with PTSD, children with traumatic life events but not PTSD, and healthy children to tease out the effects of trauma from psychopathology, ideally assessed by structured clinical interviews and abuse assessments. In a sample of at-risk children without psychiatric diagnoses, sex-based differences were found in correlations between self-reported child maltreatment scores and regional brain volumes, such that maltreatment was associated with regions involved in impulse control for boys (i.e., reductions in the rostral prefrontal cortex and caudate) and emotion regulation for girls (i.e., reductions in rostral prefrontal cortex, orbitofrontal cortex, amygdala, and hippocampus).<sup>55</sup> In our sample, both boys and girls in the high trauma group reported significantly greater levels of PTSD, anxiety, depression, dissociation

and anger than children in the low trauma group, but we observed no sex  $\times$  trauma interaction effect for these psychological symptoms. This combination of findings hints that sex-related structural differences may precede sex-based disparities in diagnostic prevalence rates subsequent to trauma in adults.

The second limitation of our study was that we did not know the specific age at which traumatic life events occurred. Our study depended upon child self-report, suggesting a preponderance of traumas in middle childhood (ages 3–4 to puberty) between the end of childhood amnesia and data collection. However, future research should carefully examine the timing of traumatic experiences on neural development, given that reduced hippocampal volume in adulthood seems to be most significant when the maltreatment occurs in middle childhood,<sup>7</sup> and some studies suggest that very early childhood trauma may be most important for amygdalar volume.<sup>2,22</sup> We are not aware of any longitudinal studies tracking parahippocampal development with respect to trauma exposure.

## Conclusion

Childhood trauma is a known neurobiological risk factor for a variety of psychological disorders,<sup>11,56</sup> and changes to the hippocampi and amygdalae may serve as nonspecific risk factors linking trauma to psychopathology. From a clinical perspective, previous epidemiological research has documented that experiencing traumatic life events during childhood is a significant risk factor for childhood, adolescent and adult psychopathology,<sup>57–59</sup> and our psychiatric data support these conclusions.

As clinicians and neuroscientists, we are dismayed at the dearth of volumetric imaging studies exploring potential sex effects during childhood, especially considering that the prevalence of PTSD, depression and anxiety are significantly greater in women than men, while the prevalence of substance use disorders is much greater in men than women.<sup>16</sup> Limbic regions have been linked to the development of depressive disorders, specific phobias and PTSD in longitudinal trauma research,<sup>60</sup> and rates of emotional disorders increase during adolescence, especially for girls. Given that pubertal development influences subcortical brain development in areas such as the amygdala, hippocampus and putamen in both sexes,<sup>13</sup> again we are surprised by the lack of attention to sex differences in the imaging literature. Longitudinal neuroimaging studies with regard to sex differences and trauma exposure are clearly needed.

Our current findings raise a number of important issues in understanding limbic development subsequent to traumatic life events. First, our findings were consistent with other literature demonstrating that children do not display reduced hippocampal volume following childhood trauma as is commonly observed in adults. Second, our study calls into question previous null findings about the effect of trauma on childhood limbic volumes in studies that did not examine sex-related effects, by demonstrating that boys' and girls' volumes were differentially affected by trauma in

the hippocampal and parahippocampal regions. Third, given that regional volumes did not differ by sex in the low trauma group, it seems unlikely that pre-existing neural differences were responsible for volumetric differences in traumatized adults. Finally, our results hinted at a sexually dimorphic split during the development of the limbic system that may be consistent with patterns of rumination and overgeneralization in girls and avoidance in boys after experiencing traumatic life events, although this is speculative and future longitudinal work is clearly needed. Future research should also attend to the role of association regions such as the parahippocampal gyri as they relate to the processing of troubling autobiographical memories as a way of understanding the effects of trauma on psychopathology, especially given that both traumatized girls and boys in this healthy sample had elevated levels of mood, anxiety, behavioural and stress-related symptoms. Future studies should also examine the neurobiological origin of volumetric changes in the context of trauma. In this developmental sample, we would propose that synaptogenesis changes following trauma exposure may play a critical role, but again this is speculative.

In summary, we anticipate that these observed sex  $\times$  trauma interaction effects will spark new interests in the role that neural development may have on sex-based differences in psychopathology. Future cross-sectional and especially longitudinal studies should track the role of trauma with careful attention to sex differences in brain structure, function and connectivity in both children and adults. Future research should embrace and carefully study the clear neural differences between adults and children, considering the role that puberty and associated hormones may play in regional brain development. Girls and boys are biologically different, and attention to these differences in neural development should enhance the currently lacking explanations for striking differences in prevalence rates between the sexes with respect to stress-related, anxious, depressive and substance use disorders.

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