**ORIGINAL CONTRIBUTION**

# Increased right amygdala metabolite concentrations in the absence of atrophy in children and adolescents with PTSD

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## Abstract

Previous studies have shown that posttraumatic stress disorder (PTSD) is associated with dysfunction of the limbic system, in which the amygdala plays an important role. The purpose of this study was to evaluate whether the neurochemical concentrations assessed by proton magnetic resonance spectroscopy ($^1$H-MRS) in the amygdala are abnormal in children and adolescents with PTSD. Twenty-eight pediatric PTSD patients (11 boys, 17 girls) and 24 matched trauma-exposed control subjects (9 boys, 15 girls) underwent magnetic resonance brain imaging and $^1$H-MRS of the bilateral amygdalae. The concentrations of $N$-acetylaspartate (NAA), myo-inositol (mI), total creatine (tCr) and total choline (tCho) in the right amygdala were significantly increased in PTSD patients compared with trauma-exposed control subjects. There were significant group-by-age interactions in the left amygdala NAA and right amygdala mI concentrations: older pediatric patients with PTSD had higher left amygdala NAA concentration and younger patients had higher right amygdala mI concentration than trauma-exposed control subjects. There was also a significant correlation between right mI concentration and time since trauma in PTSD patients. Finally, there was significant group-by-age interaction in the left amygdala volume; intragroup analysis revealed that the right amygdala volume was significantly lower than the left in the PTSD group, but not in the control group. These neurochemical abnormalities of the amygdala may indicate that dysfunctions of both neurons and glial cells are involved in the pathology of pediatric PTSD.

**Keywords** PTSD · Proton magnetic resonance spectroscopy · Children and adolescents · Amygdala · Development · Psychoradiology

## Introduction

Exposure to a traumatic event, such as earthquake, war or sexual assault, may result in posttraumatic stress disorder (PTSD) [1]. Estimates of the prevalence of PTSD in children and adolescents exposed to trauma range from 5% [2] to 16% [3]. The core features of PTSD include persistence of intense, distressing and avoided reactions to reminders of the triggering event, alteration of mood and cognition, a pervasive sense of imminent threat, disturbed sleep and hypervigilance [4]; these are regarded as conditioned fear responses to traumatic stimuli and represent pathological sequelae to traumatic experiences [5]. Both structural and functional neuroimaging studies have revealed abnormalities of the limbic system in PTSD patients which may reflect a neurobiological substrate for these symptoms [6, 7]. The amygdala, being part of the limbic system, which plays an important role in the acquisition and consolidation of fear

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extinction [8, 9], has received particular attention: although its possible role in the pathophysiology of PTSD has been much discussed [10–12], neuroimaging evidence is still not conclusive.

Proton magnetic resonance spectroscopy ($^1$H-MRS) is an important psychoradiologic technique (http://radiopeadia.org/articles/psychoradiology) [13–16] and can noninvasively measure selected neurochemicals in vivo. The most easily detectable signal in the spectrum of normal human brain, the acetyl group of *N*-acetylaspartate (NAA), is often referred to as a neuronal marker since it is observed almost exclusively in neurons [17, 18]. Choline compounds (tCho) are involved in membrane synthesis and degradation, and occur at a particularly high concentration in glial cells [19]. Creatine and phosphocreatine (together detectable as total creatine, tCr) play an important role in cellular energy metabolism, acting as a buffer for adenosine triphosphate. Myo-inositol (mI) is a component of the inositol phosphate intracellular second messenger system, whose exact pathological role is only partially understood.

A meta-analysis of 16 MRS studies of PTSD, mainly targeting the hippocampus and anterior cingulate cortex (ACC), revealed decreased NAA concentration in the bilateral hippocampi, increased Cho/Cr ratio in the left hippocampus and decreased NAA in the ACC [20]. However, the neurochemical changes of the amygdala in PTSD have not been much studied. The finding in a rat PTSD model of increased NAA/Cr ratio in bilateral amygdalae [21] led us to hypothesize that NAA in the amygdala might show detectable concentration increases in human PTSD.

A limitation of most previous studies is the use of metabolite/Cr ratios rather than absolute metabolite concentrations, whose interpretation assumes that Cr concentration remains unaffected by development or pathophysiology: however, a number of studies have shown that this is not necessarily true [22–26]. Another limitation is that, although adult PTSD patients have been frequently investigated, there are only three MRS studies, all focusing on ACC, of children and adolescents with PTSD [5, 27, 28]; thus, the neurochemistry in pediatric PTSD remains largely unknown.

Volumetric changes of the amygdala in PTSD have been the subject of several investigations, with inconsistent findings [29–32]. A review of 12 studies including both adults and children reported smaller bilateral amygdalae [33]. A meta-analysis combining studies of adults and children [34] reported smaller left amygdala. However, another meta-analysis driven by three studies found no significant changes of amygdala volume in pediatric PTSD related to maltreatment [35]. A possible reason for these differences is developmental variation in amygdala volumes of young subjects [36]. In another study, although no significant group difference was found, there was a significant group-by-age interaction in predicting right amygdala volumes

[37]. This points to the importance of using age as an interactive variable in pediatric neuroimaging research.

The present study was designed to remedy the relative lack of attention paid to the in vivo neurochemistry and volumetry of the amygdala in pediatric PTSD. Our patients developed PTSD after a single event (an earthquake) and to provide an optimal baseline for comparison the control subjects came from the same region and had been exposed to the same traumatic event. Absolute metabolite concentrations were calculated to avoid the interpretative ambiguities of metabolite ratios. Volumetric changes were evaluated automatically and age was tested as an interactive variable. The aim was to define the neurochemical and volumetric changes in the amygdala of pediatric PTSD patients and to compare them to previous reports in adult PTSD.

## Methods

### Participants

A total of 4200 earthquake survivors underwent screening 8–15 months after the 8.0-magnitude earthquake in Sichuan, China, in May 2008. All participants were interviewed and screened using the PTSD checklist (PCL) [38]; those who scored > 35 on the PCL were administered the Clinician-Administered PTSD Scale (CAPS) [39] by an experienced psychiatrist. Those participants who scored > 50 on the CAPS were diagnosed with PTSD; those who scored < 30 on PCL were considered as trauma-exposed control subjects (non-PTSD controls) and were not assessed with the CAPS. Important exclusion criteria included (1) a major Axis I diagnosis (other than depression), (2) contraindication to MRI, (3) substance dependence, (4) traumatic brain injury, (5) gross obesity or growth failure, (6) left-handedness and (7) age older than 18 years.

In total, we recruited 28 pediatric subjects who met DSM-IV criteria for PTSD and 24 non-PTSD controls. Subjects were matched by age (mean age $13.1 \pm 1.8$ years for the PTSD group and mean $13.0 \pm 1.3$ years for controls; age range 10–16 years) and by sex (11 males and 17 females for the PTSD group and 9 males and 15 females for controls). Handedness was assessed using the Revised Physical and Neurological Examination for Subtle Signs inventory [40]. All subjects, PTSD and control, were right-handed. After a full description of the study procedures, all subjects and their parents/guardians provided written informed consent. The study was approved by the Institutional Review Board at the Health Center of Sichuan University, China. More information about the demographics and clinical data of participants is given in Table 1.

**Table 1** Clinical and demographic data

| | PTSD ($n=28$) | Non-PTSD ($n=24$) | $t$/Chi square | $P$ |
|---|---|---|---|---|
| Gender (male/female) | 11/17 | 9/15 | 0.017 | 0.90 |
| Age (years) | 13.14 ± 1.76 (10–16) | 13.04 ± 1.33 (11–16) | 0.231 | 0.82 |
| Handedness (right/left) | 28/0 | 24/0 | – | – |
| Years of education | 7.18 ± 1.74 (4–10) | 6.92 ± 1.38 (5–10) | 0.593 | 0.57 |
| Time since trauma (months) | 10.63 ± 1.54 (8–12) | 13.38 ± 1.26 (10–15) | 1.539 | 0.13 |
| PCL[a] | 54.64 ± 5.05 (40–65) | 23.79 ± 2.98 (19–35) | 25.498 | <0.001 |
| CAPS[b] | 65.23 ± 6.75 (60–86) | – | – | – |

Unless otherwise noted, data are means ± standard deviations, with ranges in parentheses

Age, years of education and time since trauma were reported by participants' parents or guardians at the time of MR imaging

[a]Posttraumatic Stress Disorder Checklist

[b]Clinician-Administered PTSD Scale

## MRI and proton MRS acquisition and analysis

MR measurements were performed using a 3.0 T MR scanner with an eight-channel phased-array head coil (GE Signa Excite). All subjects underwent routine brain MR imaging (MRI) examinations and pads were used to minimize head motion. First, high-resolution T1-weighted images [three-dimensional FSPGR; repetition time (TR) 8.5 ms, echo time (TE) 3.4 ms; flip angle 12°, reconstruction thickness 1 mm, matrix 256 × 256] were acquired and reconstructed for three-dimensional localization of the spectral voxels. Second, single voxel proton MRS was performed using point-resolved echo spectroscopy sequence (PRESS) with spectral editing (TR/TE 2000 ms/30 ms, spectral bandwidth 2000 Hz, 1024 data points, 128 average repetition times, sampling time 5 min 4 s). To enable secure placement of voxels wholly within the amygdala,

we chose a small voxel size of 12 mm × 10 mm × 12 mm. Placement of regions of interest (ROIs) was verified on the reconstructed sagittal and coronal planes, as described in detail elsewhere [41]. Field shimming and water suppression were automatically accomplished by the GE spectral software package. Pre-saturation bands for outer volume suppression were placed around the ROIs to avoid contamination from neighboring cerebrospinal fluid or scalp fat.

The MRS raw data were analyzed with LCModel (Fig. 1) [42]. Metabolite concentrations were reported as mmol/kg wet weight, using the unsuppressed water signal as an internal reference. Quality control was accomplished by rejecting peaks with a full width at half maximum (FWHM) > 8 Hz, metabolite concentrations with an estimation uncertainty (expressed as Cramer–Rao lower-bounds, CRLB) ≥ 15% and when spectra were visibly of poor quality, or contained obvious artifacts.
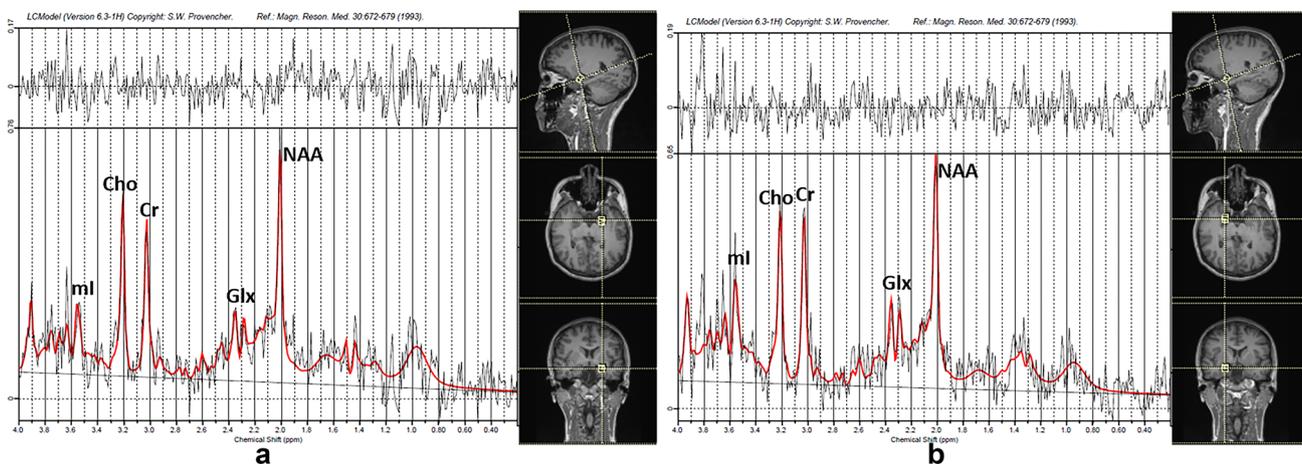


**Fig. 1** Location of the voxels in the bilateral amygdalae and LCModel processing results. **a** Left amygdala. **b** Right amygdala

## Volume measurements of amygdala

Automated amygdala segmentation was performed with the FreeSurfer (version 5.3.0) software package (http://surfer.nmr.mgh.harvard.edu) in all except three PTSD participants whose images showed movement artifact or were otherwise of poor quality. The processing stream was used for automatic subcortical segmentation from 3D T1-weighted images [43]. We applied several processing steps: skull stripping, Talairach transforms, atlas registration and spherical surface maps. Parcellations were initialized with common information from the within-subject template, which increases reliability and statistical power [43]. Quality control was performed by checking for outliers using visual inspection as described in a recent protocol (http://enigma.loni.ucla.edu/protocols/), manually repairing any segmentation error.

## Statistical analysis

All demographic, clinical, metabolic and volumetric data were tested for normality and homogeneity of variance: all peak–area ratio data were normally distributed by Shapiro–Wilks $W$ test. The statistical significance of differences in metabolite concentrations between pediatric PTSD patients and control subjects, and the effects of group on volume changes in the left and right amygdala corrected for intracranial volume, were tested using the two-sample independent $t$ test. Pearson correlations were performed to investigate the association between the metabolite concentrations and volumes and time since trauma, the PCL-C scores and CAPS-CA scores. We corrected alpha levels of metabolite concentrations and volumes by the false discovery rate (FDR) procedure for multiple comparisons.

Testing age as an interactive variable has been strongly recommended in pediatric and psychiatric neuroimaging research [36, 37, 44]. We therefore conducted additional analysis with hierarchical linear models (HLM) to test for interaction between age and amygdala volumes or metabolites [45]. Specifically, for volume models, level 1 included age and total intracranial volume, and level 2 included diagnostic group (PTSD vs non-PTSD controls) and sex. For metabolite models, the analyses nested metabolites as a function of age (on level 1) and group and sex (on level 2). These analyses also flexibly test other possible variables and covariates, such as time since trauma or PCL scores. Follow-up analyses with Pearson's $r$ correlation were also conducted to test correlations between age and metabolites or amygdala volumes by group. To enable post hoc probing of moderational effects, we decomposed the interactions by group status (PTSD vs non-PTSD) and by age at the 84th percentile (older pediatric patients) and the 16th percentile (younger pediatric patients) [46, 47] using PROCESS Version 3.1 for SPSS [48]. We used an alpha level of 0.05, two-tailed.

## Results

The two groups were well matched for age, gender and education years. Mean time since trauma of PTSD patients and controls was $10.6 \pm 1.5$ months and $13.4 \pm 1.3$ months, respectively, not significantly different ($P > 0.05$). Pediatric PTSD patients scored significantly higher than controls on PCL-C scores ($P < 0.05$). Patients' CAPS-AC scores reflect PTSD symptoms in the moderate-to-severe range (Table 1).

The results of statistical analysis are summarized in Table 2. As shown in Fig. 2, compared with controls, pediatric PTSD patients had significantly higher concentrations of NAA [$P = 0.022$, Cohen's $d = 1.003$], mI [$P = 0.020$, Cohen's $d = 1.240$], tCho [$P = 0.024$, Cohen's $d = 0.963$] and tCr [$P = 0.007$, Cohen's $d = 1.110$] in the right amygdala, but showed no significant differences in the left amygdala. In pediatric PTSD patients there was significant correlation between right amygdala mI concentration and the time since trauma ($P = 0.046$), but not with PCL-C and CAPS-CA scores ($P > 0.05$) (Table 3). Between the pediatric PTSD patients and controls, neither the total intracranial volume

**Table 2** Group metabolite differences in MRS between PTSD patients and controls, mean ± SD

| ROI | MRS/variable | PTSD | Controls | $t$ | $df$ | Cohen's $d$ | $P$ | $P$(FDR) |
|---|---|---|---|---|---|---|---|---|
| L-amygdala | NAA | $9.31 \pm 1.16$ | $9.13 \pm 0.76$ | 0.451 | 21 | 0.197 | 0.657 | 0.786 |
| | mI | $8.57 \pm 1.36$ | $7.65 \pm 1.04$ | 1.660 | 17 | 0.805 | 0.115 | 0.184 |
| | tCho | $2.83 \pm 0.34$ | $2.87 \pm 0.35$ | −0.365 | 30 | −0.133 | 0.718 | 0.786 |
| | tCr | $8.25 \pm 1.44$ | $8.39 \pm 1.45$ | −0.274 | 31 | −0.098 | 0.786 | 0.786 |
| R-amygdala | NAA | $9.44 \pm 1.35$ | $8.22 \pm 1.08$ | 2.457 | 24 | 1.003 | 0.022* | 0.048* |
| | mI | $9.57 \pm 1.15$ | $8.09 \pm 1.25$ | 2.556 | 17 | 1.240 | 0.020* | 0.048* |
| | tCho | $2.90 \pm 0.41$ | $2.54 \pm 0.35$ | 2.408 | 25 | 0.963 | 0.024* | 0.048* |
| | tCr | $8.70 \pm 1.62$ | $7.13 \pm 1.10$ | 2.936 | 28 | 1.110 | 0.007* | 0.048* |

*L* left, *R* right, *SD* standard deviation, *ROI* region of interests, *FDR* false discovery rate

*$P < 0.05$
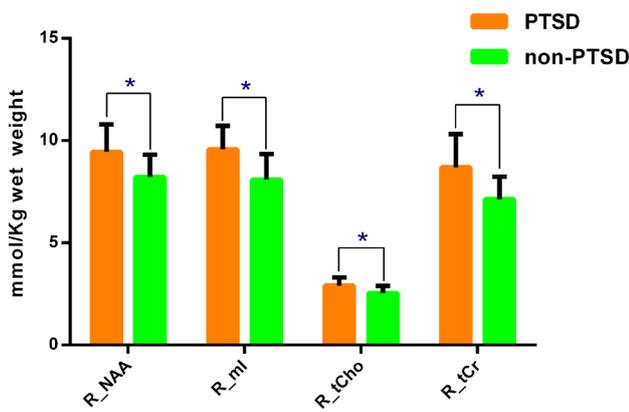
**Fig. 2** Differences in NAA, mI, tCho and tCr concentrations in the right amygdala in pediatric PTSD patients compared to controls. Data are presented as mean ± SD. Asterisk indicates a significant difference between pediatric PTSD patients and non-PTSD controls ($P < 0.05$)

**Table 3** Correlation of clinical variables and right amygdala metabolite levels found to differ between groups

| Variables | Statistics | Duration | Age | PCL | CAPS |
|---|---|---|---|---|---|
| NAA (PTSD) | $r$ | −0.244 | 0.022 | −0.029 | 0.094 |
| | $P$ | 0.381 | 0.937 | 0.932 | 0.784 |
| mI (PTSD) | $r$ | 0.763 | −0.512 | 0.431 | −0.314 |
| | $P$ | 0.046* | 0.240 | 0.469 | 0.607 |
| tCho (PTSD) | $r$ | 0.004 | 0.057 | 0.117 | 0.077 |
| | $P$ | 0.988 | 0.833 | 0.703 | 0.802 |
| tCr (PTSD) | $r$ | 0.206 | −0.037 | −0.099 | −0.103 |
| | $P$ | 0.412 | 0.885 | 0.747 | 0.739 |
| NAA (non-PTSD) | $r$ | 0.431 | 0.437 | −0.249 | – |
| | $P$ | 0.185 | 0.179 | 0.460 | – |
| mI (non-PTSD) | $r$ | −0.336 | 0.434 | −0.263 | – |
| | $P$ | 0.285 | 0.159 | 0.409 | – |
| tCho (non-PTSD) | $r$ | −0.322 | −0.152 | 0.304 | – |
| | $P$ | 0.334 | 0.656 | 0.363 | – |
| tCr (non-PTSD) | $r$ | 0.430 | 0.119 | −0.302 | – |
| | $P$ | 0.163 | 0.714 | 0.340 | – |

*r* correlation coefficient, *PCL* Posttraumatic Stress Disorder Checklist, *CAPS* Clinician-Administered PTSD Scale

*$P < 0.05$

nor the bilateral amygdalae volumes corrected for total intracranial volume were significantly different ($P > 0.05$) (Table 4). Within the pediatric PTSD group, the volume of right amygdala was less than the left ($P = 0.021$), while no difference was found in controls ($P > 0.05$).

HLM analysis on the left amygdala NAA concentration and right amygdala mI concentration found significant effects of group as well as the predicted age-by-group interaction. Results are summarized in Tables 5 and 6. In the follow-up analyses, a positive correlation between age and left amygdala NAA concentration ($r = 0.533$, $P = 0.091$) in PTSD and a negative correlation in non-PTSD ($r = -0.112$, $P = 0.728$) did not reach statistical significance, nor did the similar but inverse results in the right amygdala mI concentration (PTSD, $r = -0.512$, $P = 0.240$; non-PTSD, $r = 0.434$, $P = 0.159$). Fisher's $R$-to-$Z$ tests demonstrated that the differences in the correlation between age and these two metabolites were not statistically significant (left NAA, $z$ for $r = 1.455$, $P = 0.147$ two-tailed; right mI, $z$ for $r = -1.714$, $P = 0.087$ two-tailed) between the two groups.

The same analyses on amygdala volumes found no significant group differences (L-amygdala, $P = 0.353$; R-amygdala, $P = 0.382$), but did reveal significant age-by-group interaction in the left amygdala volume ($P = 0.009$), although not in the right amygdala volume ($P = 0.070$) (Tables 7, 8). Follow-up analyses found no statistically significant correlation between age and bilateral amygdalae volume. Specifically, we found a positive correlation between age and left amygdala volume with controlling for total intracranial volume ($r = 0.047$, $P = 0.829$) in PTSD and a negative correlation in non-PTSD ($r = -0.332$, $P = 0.122$), and similar results were found in right amygdala volume (PTSD, $r = 0.262$, $P = 0.215$; non-PTSD, $r = -0.156$, $P = 0.476$). Fisher's $R$-to-$Z$ tests demonstrated that the differences in the correlation between age and these two amygdala volumes were not statistically significant (left amygdala volume, $z$ for $r = 1.285$, $P = 0.197$ two-tailed; right amygdala volume, $z$ for $r = 1.395$, $P = 0.165$ two-tailed) between the two groups.

In the post hoc probing analyses, the effect of PTSD group at older and younger ages was the focus. These analyses showed that older pediatric patients (84th percentile

**Table 4** Group volume differences in bilateral amygdalae between PTSD patients and control subjects, mean ± SD

| Variable | PTSD (mm³) ($n = 25$) | Controls ($n = 24$) | $t$ | $df$ | Cohen's $d$ | $P$ |
|---|---|---|---|---|---|---|
| Total intracranial volume | 1,404,380.18 ± 141,328.97 | 1,394,600.12 ± 108,144.09 | 0.271 | 47 | 0.079 | 0.787 |
| L-amygdala | 1385.55 ± 252.35 | 1339.40 ± 275.68 | 0.612 | 47 | 0.179 | 0.544 |
| R-amygdala | 1249.96 ± 323.01 | 1393.18 ± 317.82 | −1.564 | 47 | −0.456 | 0.125 |
| L/R (PTSD) | – | – | 2.460 | 24 | 1.004 | 0.021* |
| L/R (non-PTSD) | – | – | −0.780 | 23 | −0.325 | 0.443 |

*L* left, *R* right, *SD* standard deviation

*$P < 0.05$

**Table 5** Hierarchical linear model predicting left NAA

| Fixed effect | Coefficient | Standard error | t ratio | df | P value |
|---|---|---|---|---|---|
| For intercept, $\beta_0$ | | | | | |
| Intercept, $\gamma_{00}$ | 12.537 | 7.027 | 1.784 | 20 | 0.090 |
| PTSD vs non-PTSD, $\gamma_{01}$ | 4.264 | 1.731 | 2.463 | 20 | 0.023 |
| Sex, $\gamma_{02}$ | − 7.481 | 6.992 | − 1.070 | 20 | 0.297 |
| For age slope, $\beta_1$ | | | | | |
| Intercept, $\gamma_{10}$ | − 0.284 | 0.570 | − 0.498 | 20 | 0.624 |
| PTSD vs non-PTSD, $\gamma_{11}$ | − 0.343 | 0.138 | − 2.488 | 20 | 0.022 |
| Sex, $\gamma_{12}$ | 0.618 | 0.566 | 1.091 | 20 | 0.288 |

**Table 6** Hierarchical linear model predicting right mI

| Fixed effect | Coefficient | Standard error | t ratio | df | P value |
|---|---|---|---|---|---|
| For intercept, $\beta_0$ | | | | | |
| Intercept, $\gamma_{00}$ | 8.375 | 6.544 | 1.280 | 16 | 0.219 |
| PTSD vs non-PTSD, $\gamma_{01}$ | − 9.690 | 3.721 | − 2.604 | 16 | 0.019 |
| Sex, $\gamma_{02}$ | 7.058 | 5.997 | 1.177 | 16 | 0.256 |
| For age slope, $\beta_1$ | | | | | |
| Intercept, $\gamma_{10}$ | 0.089 | 0.478 | 0.187 | 16 | 0.854 |
| PTSD vs non-PTSD, $\gamma_{11}$ | 0.616 | 0.257 | 2.396 | 16 | 0.029 |
| Sex, $\gamma_{12}$ | − 0.507 | 0.442 | − 1.146 | 16 | 0.268 |

**Table 7** Summary of hierarchical linear model predicting left amygdala volumes

| Fixed effect | Coefficient | Standard error | t ratio | df | P value |
|---|---|---|---|---|---|
| For intercept, $\beta_0$ | | | | | |
| Intercept, $\gamma_{00}$ | 1075.342 | 638.490 | 1.684 | 46 | 0.099 |
| PTSD vs non-PTSD, $\gamma_{01}$ | 961.597 | 1024.971 | 0.938 | 46 | 0.353 |
| Sex, $\gamma_{02}$ | − 2493.048 | 924.567 | − 2.696 | 46 | 0.010 |
| For age slope, $\beta_1$ | | | | | |
| Intercept, $\gamma_{10}$ | 70.530 | 49.216 | 1.433 | 43 | 0.159 |
| PTSD vs non-PTSD, $\gamma_{11}$ | − 101.724 | 36.936 | − 2.754 | 43 | 0.009 |
| Sex, $\gamma_{12}$ | − 43.497 | 52.680 | − 0.826 | 43 | 0.414 |
| For total brain volumes slope, $\beta_2$ | | | | | |
| Intercept, $\gamma_{20}$ | − 0.000380 | 0.000647 | − 0.588 | 43 | 0.560 |
| PTSD vs non-PTSD, $\gamma_{21}$ | 0.000184 | 0.000708 | 0.260 | 43 | 0.796 |
| Sex, $\gamma_{22}$ | 0.002175 | 0.000741 | 2.935 | 43 | 0.005 |

of age) with PTSD had significantly higher left amygdala NAA concentration [$t(21) = − 2.272$, $P = 0.036$ controlling for gender], whereas younger pediatric patients (16th percentile of age) showed lower NAA concentration than non-PTSD controls [this was not significant controlling for gender $t(21) = 0.963$, $P = 0.349$]. For right amygdala mI, younger pediatric patients with PTSD have significantly higher mI concentration [$t(17) = − 3.384$, $P = 0.005$], whereas older pediatric patients showed lower mI concentration [this was not significant $t(17) = 0.225$, $P = 0.826$]. Nevertheless, younger pediatric patients with PTSD showed smaller left amygdala volume [$t(47) = 0.451$, $P = 0.654$] and older pediatric patients showed larger volume than non-PTSD controls [$t(47) = − 1.717$, $P = 0.093$], although the differences of both were not significant controlling for total brain volumes and gender. The Johnson–Neyman technique [49], embedded in PROCESS [48], revealed that the PTSD group had significantly higher right amygdala mI concentration ($P < 0.05$) than non-PTSD controls below the age of 14.3 years and significantly larger left amygdala volume ($P < 0.05$) than non-PTSD controls above 14.4 years; left amygdala NAA concentration was significantly ($P < 0.05$) lower than controls below 10.1 years, but significantly ($P < 0.05$) higher above 15.5 years.

**Table 8** Summary of hierarchical linear model predicting right amygdala volumes

| Fixed effect | Coefficient | Standard error | $t$ ratio | $df$ | $P$ value |
|---|---|---|---|---|---|
| For intercept, $\beta_0$ | | | | | |
| Intercept, $\gamma_{00}$ | 365.123 | 631.042 | 0.579 | 46 | 0.566 |
| PTSD vs non-PTSD, $\gamma_{01}$ | 868.724 | 983.750 | 0.883 | 46 | 0.382 |
| Sex, $\gamma_{02}$ | $-17.658$ | 980.547 | $-0.018$ | 46 | 0.986 |
| For age slope, $\beta_1$ | | | | | |
| Intercept, $\gamma_{10}$ | 193.821 | 62.912 | 3.081 | 43 | 0.004 |
| PTSD vs non-PTSD, $\gamma_{11}$ | $-87.569$ | 47.134 | $-1.858$ | 43 | 0.070 |
| Sex, $\gamma_{12}$ | $-161.959$ | 67.749 | $-2.391$ | 43 | 0.021 |
| For total brain volumes slope, $\beta_2$ | | | | | |
| Intercept, $\gamma_{20}$ | $-0.001150$ | 0.000791 | $-1.454$ | 43 | 0.153 |
| PTSD vs non-PTSD, $\gamma_{21}$ | 0.000262 | 0.000663 | 0.395 | 43 | 0.695 |
| Sex, $\gamma_{22}$ | 0.001539 | 0.000857 | 1.797 | 43 | 0.079 |

## Discussion

The main finding of this study was that, when compared with non-PTSD controls, pediatric PTSD patients had increased absolute NAA, mI, tCho and tCr concentrations in the right amygdala, in the absence of significant reduction of amygdala volume. HLM analyses provided supplementary evidence of age-by-group interaction in the left amygdala NAA concentration and right amygdala mI concentration, as well as in the left amygdala volume. It is worth noting that each metabolite has a varying degree of freedom due to the inconsistent CRLBs which may lower the power to detect statistically significant effects from the overall sample. To our knowledge, this is the first reported MRS study of pediatric PTSD patients which focused on the amygdala.

### Increased NAA in the amygdala

Increased NAA concentration has been reported in autistic disorders [50, 51]. Our finding of increased NAA in the right amygdala in pediatric PTSD is consistent with the report of increased NAA/Cr in amygdala after exposure to a single prolonged stress in an animal model of PTSD which prompted our study [21] (see "Introduction"). We speculate that this increase of NAA may be related to exaggerated fear responses in PTSD.

Opposite to our finding in the right amygdala, several studies of PTSD have reported decreased NAA concentration or NAA/Cr ratio in the hippocampus [52, 53] and in ACC [5, 54–56]. It is of course possible that NAA, like any other aspect of brain structure and function, may show different pathological changes in different brain regions [51].

The amygdala is critically involved in the behavioral activation and inhibition system that characterizes emotional responses such as fear and anxiety [57]. This process may be exaggerated in PTSD [58]. This idea seems to be supported by functional MRI studies in which PTSD patients showed diminished ACC responsivity [6, 59, 60], but exaggerated amygdala responses [6, 61–63] in the presence of emotionally negative stimuli, especially where the functional response in the ACC was negatively correlated with that in the amygdala [6]. Another consideration is that we acquired MRS data from the bilateral amygdalae, including only gray matter. In other studies, differences in measured metabolites may be influenced by different proportions in the ROI of gray and white matter, which in general do not have identical concentrations of NAA, Cho and Cr [64].

Age effects have been noted in previous studies measuring NAA concentration [65]. Normally at birth, NAA is low while Cho and mI concentrations are high, and over the first a few years of life there is a gradual normalization toward adult values in most regions, not complete until about 20 years of age [66]. Our study is the first to report significant age-by-group interaction of NAA concentration in the left amygdala, which supports the principle that developmental variation cannot be ignored in pediatric PTSD. Specifically, findings indicated that pediatric patients with PTSD might have relatively higher (older) and relatively lower (younger) left amygdala NAA concentrations than controls, although the difference in younger patients was not significant controlling for gender. The lack of effect on the r-to-z finding in the correlation between age and NAA level may not only be related to the different sample characteristics, but also to the differing sample sizes between the two groups. Only longitudinal studies can ultimately clarify this important issue.

### Other metabolite differences

We also found significantly increased mI, tCho and tCr concentrations in the pediatric PTSD patients. We briefly note here the possible causal implications.

Myo-inositol is a pentose sugar, part of inositol triphosphate, an intracellular second messenger [66]. It is often

taken as a marker of glial cells, which have higher mI concentrations than neurons [67]. Increased mI concentration is usually interpreted as evidence of glial cell proliferation, such as in low-grade glioma, trauma and other diseases [68–70]. One study found significantly increased mI/Cr ratios in ACC, suggesting that elevated mI concentration may represent a marker for PTSD rather than abuse per se [71]. We found significant positive correlations between mI concentrations in the right amygdala and time since trauma in the PTSD group, which may suggest an aspect of developmental timing related to PTSD symptoms. The significant group-by-age interaction of mI concentration in the right amygdala also reinforces the importance of using age as an interactive variable. Similar to the left NAA, the lack of effect on the *r*-to-*z* finding in the correlation between age and mI level may also be related to the differing sample sizes between the two groups.

Choline-containing compounds are involved in membrane synthesis and degradation, and increased Cho is usually taken as a marker of accelerated membrane turnover [66]; glial cells have been reported to have high concentrations of Cho [19]. Unlike the NAA findings discussed above, the increased tCho concentration in our PTSD group accords with reports of increased tCho in ACC in PTSD [71]. The significance of these findings for the neurobiology of PTSD is yet to be determined.

Cr is crucially involved in energy metabolism. In vitro, glial cells contain two to four times higher Cr concentration than neurons [72]. Our finding of increased Cr in the right amygdala is in contrast to reports of decreased Cr in the right hippocampus and occipital white matter in two previous studies [73, 74]. In any case, our findings add to the list of conditions and situations in which Cr is not necessarily constant. All the evidence suggests that glial dysfunction in the right amygdala may be involved in the pathology of pediatric PTSD. Again, different regions may well show different metabolic pathologies.

## Volumetric differences

In contrast to these metabolic differences, pediatric PTSD patients had no significant abnormality in the bilateral amygdalae volume compared to non-PTSD controls. Similarly, a study on combat-related PTSD also found metabolite alterations, but no apparent atrophy of the hippocampus [73]. Our findings are in line with a meta-analysis which found no significant differences in amygdala volume in children with maltreatment-related PTSD [35]. However, we did find interhemispheric volumetric differences: the volume of the right amygdala was smaller than that of the left in our subjects with pediatric PTSD. A similar asymmetry has been reported in adult PTSD [29]. In contrast, a meta-analysis found that the right amygdala was significantly larger than

the left, both in PTSD and in the control group [75]. It may be hypothesized that psychotropic medication affects neuroanatomical outcome: the patients in our study and in the adult study [29] were both drug free, whereas patients in the later meta-analysis were mixed [75]. Additional research is needed to clarify this.

The significant group-by-age interaction for the left amygdala volume in the HLM analysis accords with the theory proposed by Weems et al. [36, 37, 44] that normal developmental variation in amygdala volumes may be altered under conditions of severe stress. Similarly, we also found a positive correlation between age and bilateral amygdala volumes in the PTSD group and a negative correlation in the non-PTSD group, though these correlations did not reach statistical significance. This phenomenon may represent a delay in pruning, atypical or prolonged growth in the PTSD group [37]. However, unlike Weems et al., we did not find significant group effects. This discrepancy might be due to different study populations: Weems compared youths with PTSD and with healthy controls, which allows the possibility that volume difference might reflect traumatic stress exposure rather than PTSD itself [37]; by contrast in our study, both PTSD patients and controls experienced the same trauma in the same place and at the same time. Although previous studies have emphasized the importance of the developmental timing of traumatic stress [44, 76], we found no significant group-by-time since trauma interaction, probably because time since trauma is relatively short in our two trauma-exposed groups. All this further reinforces the age-related effects we found and the importance of testing for effects of age and development. There is a report that amygdala volumes did not differ between institutionalized children compared with matched children adopted from orphanage care, while amygdala volumes were increased in those who spent greater than 15 months in institutionalized care [77]. One again, our results probably reflect PTSD itself, rather than simply trauma exposure.

## Limitations

The present study had some limitations. Firstly, the sample size was small. Secondly, it was cross-sectional; longitudinal studies will be needed to define the neurochemical abnormalities of pediatric PTSD at different stages. Thirdly, inclusion of a group of healthy subjects without trauma experience might have helped to define any effects of trauma itself, independent of consequent PTSD and further verify age-by-group interaction.

In summary, our findings add to the evidence for dysfunction of the amygdala, specifically the right amygdala, in pediatric PTSD. The increase in NAA concentration, considered a neural marker, may perhaps be related to the exaggerated fear responses characteristic of PTSD, while

the increase in Cho, Cr and mI concentrations may point to an associated pathology of glial cells.

**Author contributions** QY and QG conceived the project. WW and HS designed the protocol and wrote the main manuscript. WW, XS and SZ obtained the data. HS, QT, CX, GK and QG analyzed and interpreted the results. All authors reviewed the manuscript. GK and QY revised the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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