Title:

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

Authors:

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Short title:

Abnormal Metabolites and Amygdala Volume in Early PTSD

Abstract

Background: Previous studies of posttraumatic stress disorder (PTSD) were mainly of patients at a chronic stage, focussing on brain regions outside the amygdala. The goals of this study were to investigate the early biochemical and structural changes of anterior cingulate cortex (ACC) and amygdala in patients with PTSD and to explore their relationships. **Methods**: Seventy-eight PTSD subjects and 71 non-PTSD control subjects were enrolled, all of whom had suffered the same earthquake less than one year before. Single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) was performed and absolute metabolite concentrations in ACC and bilateral amygdalae were estimated with LCModel. Bilateral amygdalae were manually outlined and their volumes were calculated and corrected for the total intracranial volume. **Results**: The PTSD group showed significantly increased N-acetylaspartate (NAA) concentrations in the ACC, increased creatine (Cr) concentrations in the left amygdala, and increased myo-inositol (mI) concentrations in the right amygdala, compared to non-PTSD controls. The NAA concentrations in ACC were negatively correlated with the time since trauma. The PTSD group showed significantly decreased volume of bilateral amygdalae compared to non-PTSD controls, but amygdala volumes were not correlated with metabolite concentrations. **Conclusions:** This concurrent increase in some metabolite concentrations and decrease of amygdala volumes may represent a pattern of biochemical and morphological changes in recent-onset PTSD which is different

from that reported in chronic PTSD.

Keywords: posttraumatic stress disorder, brain, magnetic resonance, spectroscopy, metabolites, morphometry

Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder affecting people who are exposed to extraordinary distress events, whose main symptoms are reliving the traumatic event, avoiding trauma-related cues, negative alterations in thinking and feeling, and hyperarousal (1). The lifetime prevalence of PTSD is reported as 2% to 9% (2). Patients with PTSD are at increased risk of suicide, which is as high as 13% in one study of 431 veterans (3, 4).

A growing number of neuroimaging studies have investigated potential neurocircuitry models of PTSD (5). The two main hypotheses have been hyper-responsivity of the amygdala to threat-related stimuli, and deficient regulation by the ACC (6-9). Functional studies have supported the two hypotheses that hyper-activation of the amygdala contributes to the exaggerated fear response and persistence of traumatic memories, and that hypo-activation of ACC is important in the exaggerated threat detection and fear learning in PTSD (7, 9). In addition, resting-state connectivity studies have shown increased coupling within the salience network, including the amygdala and the ACC, which is associated with attention to external stimuli (10).

Proton magnetic resonance spectroscopy (^{1}H-MRS), a non-invasive method to measure localized brain metabolite concentrations, offers a way to probe cellular properties and metabolism. A number of MRS studies have reported abnormal metabolite levels in several brain regions in adult PTSD, but their findings have been

inconsistent (11). In the ACC there have been reports of decreased N-acetylaspartate (NAA) (12-15) and conversely of no abnormality of NAA (16-18). Creatine (Cr) has often been used as an internal reference for estimating the absolute concentrations of other metabolites in MRS studies, based on the assumption that its own concentration in relatively constant (19). However, decreased absolute Cr concentrations have been reported in the hippocampus of PTSD patients (20). Furthermore, there have been no published studies focusing on metabolic changes in the amygdala in adult PTSD patients, although its role in the regulation of emotion has been widely acknowledged.

There are similar inconsistencies among reports of structural imaging studies. In some the amygdala volume is decreased in PTSD patients compared to controls (21-23), and negatively associated with the severity of PTSD (21, 22); other studies have found no significant inter-group differences (24-26).

Most PTSD patients are studied a long time after the precipitating trauma, so early changes have not been well explored. Vietnam combat veterans with long disease durations have been widely studied by MRS (14, 27, 28). In other studies of PTSD patients trauma types were mixed (29, 30). Control subjects also differ between studies: in some, healthy subjects (13), but in others, non-PTSD participants with trauma experience (18), or mixed (16).

In the present study, we explored the structural and neurobiochemical changes of the ACC and the amygdalae in patients with PTSD who survived an extraordinary

earthquake several months before. The control subjects came from the same region and were exposed to the same traumatic event. We used absolute metabolite quantification and manual volumetric methodology to measure metabolite concentrations and amygdala volumes, respectively. We aimed to define abnormalities in the ACC and the amygdalae in recent-onset, medication-free patients with PTSD, and to compare these with reported abnormalities in the more often studied condition of chronic PTSD.

Methods and Materials

Subjects

All participants in this study had suffered from a devastating earthquake in May 2008 in Wenchuan County, Sichuan Province, the People's Republic of China. The prevalence of suspected PTSD in some stricken areas was as high as 46% (31). In all 184 adult survivors were recruited between January 2009 and August 2009, and all of them completed MRI and MRS examinations. The PTSD Checklist and the Clinician-Administered PTSD Scale (CAPS) were applied to assess traumatic severity and PTSD symptoms of the subjects (32, 33). The participants were interviewed by an experienced psychiatrist and classified based on the diagnostic criteria of the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition)(34). Those who met the criteria for PTSD related to this trauma were classified as the PTSD patient group, while those who did not meet the criteria were enrolled as the control group (non-PTSD). Exclusion criteria for all subjects were as follows: other Axis I psychiatric diagnosis;

medicine and/or alcohol abuse; any significant medical or neurological conditions or a history of head injury; left-handedness; any contraindication to MR imaging; age less than 18 or more than 65 years. Finally 78 drug-naive first-episode PTSD patients (20 males, 58 females, mean age 43.0±10.4 years) and 71 control subjects (23 males, 48 females, mean age 43.4±9.9 years) were included in the study. All participants were right-handed. Table 1 summarises the detailed demographic and clinical characteristics of these subjects. This study was approved by the Research Ethics Committee of the West China Hospital of Sichuan University. All participants gave written informed consent.

Magnetic Resonance Imaging Spectroscopy

All MR examinations were performed using a 3.0 T MR imaging system (Excite; GE Healthcare, Milwaukee, Wis) and an eight-channel phase-array head coil. All subjects underwent routine brain MR imaging (MRI) examinations, firmly padding the head to minimize motion. High-resolution T1 weighted images (3-dimensional spoiled gradient recall sequence) were acquired and reconstructed for localization of the MR spectroscopy voxels. The sequence parameters were as follows: repetition time (TR) 8.5 ms, echo time (TE) 3.4 ms; field of vie 24cm×24cm; flip angle 12°; 156 axial slices; thickness 1.0 mm; matrix size 512×512. Single voxel ^{1}H-MRS was performed using PRESS (Point Resolved Spectroscopy) sequence, with parameters as follows: TR 2000ms, TE 30ms, spectral bandwidth 1200Hz, 1024 data points, 128 signals average.

Volumes of interest (VOIs) were placed in the ACC and bilateral amygdalae (example spectra are shown in Fig 1). The voxel size varied with the shape and size of the ROI. To avoid contamination from neighboring cerebrospinal fluid or scalp fat, 6 pre-saturation bands for outer volume suppression were placed around the voxel. The built-in spectral GE software automatically accomplished field shimming and water suppression, and data acquisition started only when the prescan showed that the full width at half maximum (FWHM) of the water peak was < 7Hz and water suppression was > 95%.

MRS Data Post-processing

MRS data were excluded as of low quality if they met one of the following criteria: a. signal to noise ratio (SNR) for Cr < 15, and FWHM > 0.08ppm. The MRS raw data were exported and analysed u LCModel (Version 6.3-1H) using a basis set yielded by the same sequence and the same parameters (35). Eddy-current correction and water reference scaling were performed to improve spectral quality. Metabolites selected for further analysis were: NAA, glutamine plus glutamate (Glx), creatine plus phosphocreatine (Cr), choline (Cho), and myo-inositol (mI). Only values with a fitting error < 15% were accepted for further statistical analysis.

Measurement of Amygdala Volume

Volume of bilateral amygdalae was measured by manual tracing on 3-dimensional T1-weighted MR images (1 mm section thickness) which were reconstructed from axial

T1-weighted MR images. To visualize the amygdalae and separate them from nearby brain structures, oblique coronal images were reconstructed perpendicular to the long axis of the hippocampus (36). The positional normalizations were performed using MATLAB R2012b. The amygdala was manually segmented using ITK-SNAP (http://www.itksnap.org/pmwiki/pmwiki.php, version 3.6.0) (37), an open-source multi-platform software for tissue segmentation. Two radiologists blinded to the group membership traced the amygdala volumes independently. Inter-operator reliability was confirmed by correlation coefficients for the left and the right amygdala of 0.909 and 0.912, respectively.

The anatomic boundaries of the amygdala were defined in oblique coronal images and set as follows (38) (Fig 2):

- The anterior border was taken where the lateral sulcus closes to form the entorhinal sulcus.
- The posterior border was defined as the point where grey matter first starts to appear superior to the alveus of the hippocampus and lateral to the hippocampus head; if the alveus was not visible, the inferior horn of lateral ventricle was used as a border.
- The medial border included the uncus, while entorhinal cortex inferior to the uncal notch was excluded; if the uncal notch was not clear, a line was drawn from the most inferior point to the most medial aspect of the amygdala.

- The lateral border was defined by the inferior horn of the lateral ventricle, or adjacent white matter.
- For the superior border, the anterior part was defined by a straight line laterally from the entorhinal sulcus to the fundus of the inferior portion of the circular sulcus of the insular cortex; the posterior part was defined by a straight line laterally from the superolateral aspect of optic tract to the fundus of circular sulcus of insular cortex;
- The inferior border was defined by the uncal recess of lateral ventricle, such as the temporal horn, or by white matter.

The outline of the amygdala in each slice was traced with a manually driven mouse cursor. Delineated voxels were automatically summed by the software to calculate overall amygdala volumes.

Amygdala volumes were adjusted for total intracranial volume (TIV) (39, 40), which corrects for variation in head size (41, 42). Rapid automatic estimation of TIV was performed using the FreeSurfer image analysis suite and its library tool 'recon-all' (http://surfer.nmr.mgh.harvard.edu/) (23). The measured amygdalae volumes were normalized according to the following equation (40):

$$Volume_{i,\,adjusted} = Volume_{i,\,observed} - B(TCV_i - \overline{TCV}),$$

where TCV_i is the individual's total intracranial volume, $\overline{TCV}$ is the overall average total intracranial volume in the control group, and B is the slope of the regression line

of amygdala volume against total intracranial volume.

Statistical Analysis

All demographic characteristics, clinical, metabolite and volumetric data were tested for normality and homogeneity of variance. The independent samples *t*-test was used to analyse differences in quantitative variables between the two groups. First, demographic and clinical characteristics of PTSD and non-PTSD groups were compared. Then metabolite concentrations of all regions and volumes of bilateral amygdalae were compared between the PTSD and non-PTSD groups. Metabolites which demonstrated significant intergroup difference were further examined for their correlations with clinical variables or volume within the PTSD group using Pearson's correlation coefficient. For the multiple comparisons of metabolite concentrations and volumes, alpha levels were corrected using the FDR (False Discovery Rate) procedure (43). The significant level after correction (P_{adj}) was set at 0.05. Effect sizes were calculated via Cohen's d (0.2 = small, 0.5 = medium, 0.8 = large) (44). Values were expressed as mean±SD.

Results

Demographic and Clinical Variables

The PTSD and non-PTSD groups showed no significant differences in age, sex, education, or time since trauma ($P > 0.05$). Both groups showed a similar predominance

of female subjects. As expected PTSD patients scored significantly higher on PCL and total CAPS scores ($P<0.05$) (Table 1).

Metabolite Concentrations and Brain Volumes

Results of statistical analysis are given in Table 2. In the ACC, PTSD patients had significantly increased NAA concentrations compared to non-PTSD controls ($t = 3.63$, $P_{adj} < 0.001$, $d = 0.76$), but other metabolites (mI, Cr, Cho and Glx) showed no significant intergroup difference ($P_{adj} \geq 0.05$). For amygdalae, PTSD patients had significantly increased Cr concentration in the left amygdala and increased mI concentrations in the right amygdala compared to controls (Cr of left amygdala: $t=3.53$, $P_{adj}=0.004$, $d=0.89$; mI of right amygdala: $t=2.58$, $P_{adj}=0.045$, $d=0.91$) (Fig 3).

Both the left and right amygdalae volumes of PTSD patients were significantly decreased compared with controls (left: $t= -3.44$, $P_{adj} =0.004$, $d = -0.58$; right: $t= -4.32$, $P_{adj}<0.001$, $d = -0.73$) as was, total amygdala volume ($t= -4.51$, $P_{adj}<0.001$, $d = -0.76$). Effect sizes for significant inter-group differences of metabolites and volumes were medium or strong (Cohen's $d = 0.58–0.91$).

Correlations of metabolite concentrations

Table 3 shows the correlations between the metabolite concentrations and other variables in PTSD subjects. NAA concentrations in the ACC were negatively correlated with the time since trauma ($r = -0.30$, $P=0.02$) (Fig 4). No significant correlations were

found between clinical variables or amygdala volume and either Cr concentrations of left amygdala or mI concentrations of right amygdala (Cr concentrations with left amygdala volume: r =0.002; mI concentrations with right amygdala volume: r =-0.009; P>0.05).

Discussion

Compared with non-PTSD controls, the PTSD patients showed 4 significant abnormalities: 1) NAA concentrations were significantly increased in the ACC and negatively correlated with the time since trauma; 2) Cr concentrations were significantly increased in the left amygdala; 3) mI concentrations were significantly increased in the right amygdala; and 4) volumes of bilateral amygdalae were decreased.

To our knowledge, this is the first reported MRS study of the amygdala as well as the first to report increased NAA in adult patients with PTSD. We now discuss the possible pathophysiological significance of these abnormalities.

Metabolic abnormalities in the ACC

In contrast to our finding of increased NAA concentration in the ACC, two previous studies of PTSD have reported decreased NAA in the ACC (13, 45): Ham et al (13) in PTSD patients examined 15.0 ± 1.1 months after a fire accident, and Meyerhoff et al (45) in PTSD patients exposed to trauma events about 10 years previously. In the present study the mean time was shorter, only 10.5±1.8 months. These findings

suggested that PTSD patients may demonstrate different metabolic changes at different stages. In support of this, we found that the NAA concentrations in the ACC were negatively correlated with time since trauma, suggesting that NAA might be on track to decrease below normal when the disease progresses to the chronic stage. Furthermore, similar metabolic changes have been reported in other psychiatric disorders. A recent study exploring prefrontal metabolite concentrations at different stages of psychotic disorders found that NAA decreased during illness progression (46). Similarly, a longitudinal analysis found that depressed patients presented a progressive decrease of NAA/Cr ratio (47). A study of schizophrenia reported decreased frontal NAA in patients with chronic schizophrenia, but not in first-episode schizophrenia (48). In the light of these reports we suggest that our results might reflect the early changes of PTSD, while other reports (13, 45) may reflect later changes. Although there is no clear pathophysiological explanation for the increased NAA in PTSD, a similar finding has been reported in social anxiety disorder, where it was speculated that increased NAA in the ACC may be associated with increased activity in the emotional processing network (49).

Metabolic and volume abnormalities in the amygdalae

In our study PTSD patients had a significantly increased Cr concentration in the left amygdala, while Cr in the right amygdala demonstrated a trend towards increase which did not reach statistical significance (P and P_{adj} were 0.06 and 0.14, respectively).

Although Cr concentration has generally been believed to be unaffected in neurodegenerative processes, some studies have demonstrated otherwise (20, 50-52). Decreased Cr concentrations have been reported in the right hippocampus of PTSD patients (20) and in the ACC of depressive patients (51). Of more direct relevance to our study, increased Cr concentration has been reported in the left amygdala in patients with borderline personality disorder (50), and in the frontal brain in depressed patients (52).

Given the involvement of Cr in cellular energy metabolism (53), it is tempting to speculate that the increased Cr concentrations in the amygdala reflect more 'active' local energy metabolism (in some sense) in recent-onset PTSD, perhaps as part of an attempted protective response to trauma (54).

However, another possibility is glial proliferation. Glial cells are stress responsive, and glial abnormalities reportedly play a role in the pathophysiology of mood disorders (55). In a rodent model, acute stress can modulate glial cell activity (56). mI, being primarily located in glial cells, is a putative glial cell marker (57). An MRS study of myotonic dystrophy reported increases in both Cr and mI, and suggested that both might be explained by increased glial content (58). We observed a significant increase in mI, but only in the right amygdala. An increased mI/Cr in ACC has been reported previously in PTSD, and taken to be associated with glial proliferation (16). It is tempting to suggest that reactive proliferation of glial cells in response to trauma

accounts for both the mI and Cr abnormalities in early-stage PTSD subjects. Against this, our PTSD patients showed no significant change in mI concentration in the left amygdala, nor in Cr concentration in the right amygdala. This may reflect some functional difference or asymmetry between the left and the right amygdala, which are known to respond differently to emotional stimuli (59). MRS studies of other psychiatric diseases have also reported differing metabolite abnormalities in the two amygdalae (50).

We also demonstrated volume decrease of bilateral amygdalae in the PTSD patients. Previous studies of amygdala volume in PTSD have been inconsistent (21-24, 36, 60, 61). Some studies have reported normal or increased amygdala volumes (24, 36, 60), although patients in these studies tend to have a long illness duration , and one study was complicated by a high level of comorbidity with MDD (36). Other studies of PTSD have reported decreased amygdala volume compared with controls (21-23, 61), as we found. As to the mechanism of this structural abnormality, in a rodent model acute stress led to hypotrophy in basolateral amygdala (62), while chronic stress caused extensive spinogenesis and persistent dendritic elongation and induced basolateral amygdala hypertrophy (63, 64). Thus the acute stress might account for the volume decrease in amygdala in our patients, who were not comorbid with MDD, and who were examined at an early stage of their disease.

A reactive astrogliosis can be viewed as a defensive reaction against acute stress (65).

We tentatively suggested above that the neurochemical abnormalities we observed in the amygdala might be a consequence of such a gliosis, although the findings do differ between the two sides. Acute stress can lead to basolateral amygdala hypotrophy (62), which makes this an attractive causal explanation for the decrease we observed in bilateral amygdala volume. Brain atrophy and gliosis co-exist in many neurological diseases, such as frontal lobe dementia (66) and traumatic brain injury (67). Furthermore, coexistent hippocampal atrophy and glial proliferation has previously been reported in PTSD (20). How acute stress leads to gliosis and brain atrophy remains unclear, and further studies are needed to clarify it.

Limitations

There are two main limitations to our study. Firstly, the volume measurement was performed only on the bilateral amygdalae. Manual boundaries the ACC have no standard definition (68, 69), which makes it very difficult to estimate accurate volumes. Secondly, the present study only examined in the early stage of PTSD. Chronic changes in these patients were not explored because they received psychotherapy and medication (as clinically indicated) after the examination. Longitudinal studies are needed to explore the metabolic and structural changes of PTSD at different stages.

In conclusion, this study of recent-onset PTSD patients showed significantly increased NAA in ACC, increased Cr in left amygdala and increased mI in right amygdala, along with decreased volume decrease in bilateral amygdalae. This appears

to be a different pattern of biochemical and morphological changes to that reported in chronic PTSD. These findings confirm that the ACC and amygdala participate in the pathophysiology of PTSD, although elucidating the mechanisms will require further research.

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FIGURES AND TABLES

Table 1. Clinical and Demographic Data in PTSD Patients and Control Subjects (mean ± SD)

Group and analysis	Characteristic					
	Age (years)	Male/ female	Years of education	Months since trauma	CAPS	PCL
PTSD	43.0±10.4	20/58	7.0±3.3	10.6±2.4	56.3±15.7	48.8±13.0
Non-PTSD	43.4±9.9	23/48	7.0±3.4	11.3±2.0	21.5±11.0	27.8±7.2
P value	0.78	0.36	0.94	0.14	0.02	0.00

Non-PTSD, control subjects; PTSD, posttraumatic stress disorder; CAPS: Clinician-Administered PTSD Scale; PCL, PTSD Checklist

Table 2. Volumetric and Metabolic Measurements in the ACC and Bilateral Amygdalae in PTSD Patients and Control Subjects (mean ± SD)

VOI	Group	Metabolite levels（mmol/kg wet weight）					Volume（cm^3）
		mI	NAA	Cho	Cr	Glx	
ACC	PTSD	8.00±1.51	9.12±1.29	2.38±0.47	8.30±1.12	15.79±2.24	ND
	Non-PTSD	7.05±1.17	8.35±1.22	2.52±0.37	8.19±1.33	16.32±3.02	ND
	P (P_{adj})	0.02 (0.05)	<0.001	0.25 (0.30)	0.23 (0.30)	0.40 (0.45)	-
	Cohen's d	0.70	0.76	-0.24	0.23	-0.19	-
L-AM	PTSD	10.07±1.83	9.79±1.63	3.16±0.57	9.03±1.56	18.29±2.61	1.27±.0.13

	Non-PTSD	9.41±1.14	9.19±1.40	2.89±0.59	7.82±1.14	16.92±3.17	1.35±0.14
	P (P_{adj})	0.19 (0.29)	0.17 (0.29)	0.07 (0.14)	0.001 (0.004)	0.18 (0.29)	0.001 (0.004)
	Cohen's d	0.43	0.39	0.47	0.89	0.47	-0.58
R-AM	PTSD	10.38±2.10	9.15±1.55	2.98±0.43	8.65±1.65	16.89±2.44	1.31±0.11
	Non-PTSD	8.60±1.81	9.35±2.32	2.82±0.50	7.89±1.20	17.04±3.35	1.40±0.14
	P (P_{adj})	0.015 (0.045)	0.74 (0.78)	0.24 (0.30)	0.06 (0.14)	0.90 (0.90)	0.00
	Cohen's d	0.91	-0.10	0.33	0.53	-0.05	-0.73

ACC, anterior cingulate cortex; L-AM, left amygdala; R-AM, right amygdala; mI, myo-inositol; NAA, N-acetylaspartate; Cho, choline; Cr, creatine + phosphocreatine; Glx, glutamate + glutamine; P_{adj}, adjusted P value; ND, not done.

Table 3. Correlation of Clinical Variables and Metabolite Levels Found to Differ Between Groups after Adjusting Alpha Levels

Clinical variables	Metabolites and volumes differing between groups			
		CAPS	Age (years)	Time since trauma (months)
NAA in ACC	r	0.25	-0.08	-0.30
	p	0.08	0.54	0.02*
Cr in L-AM	r	0.07	0.02	0.92
	p	0.70	0.18	0.14
mI in R-AM	r	0.39	0.52	-0.28
	p	0.29	0.40	0.31

*P<0.05

ACC: anterior cingulate cortex; L-AM, left amygdala; R-AM, right amygdala;

CAPS: Clinician-Administered PTSD Checklist

Figure 1. Results of analysis of spectral data in a single subject in three brain regions: A, anterior cingulate cortex; B, left amygdala; C, right amygdala. The data are fitted using the LCModel, from which absolute metabolite concentrations are calculated using the unsuppressed water signal as an internal reference.

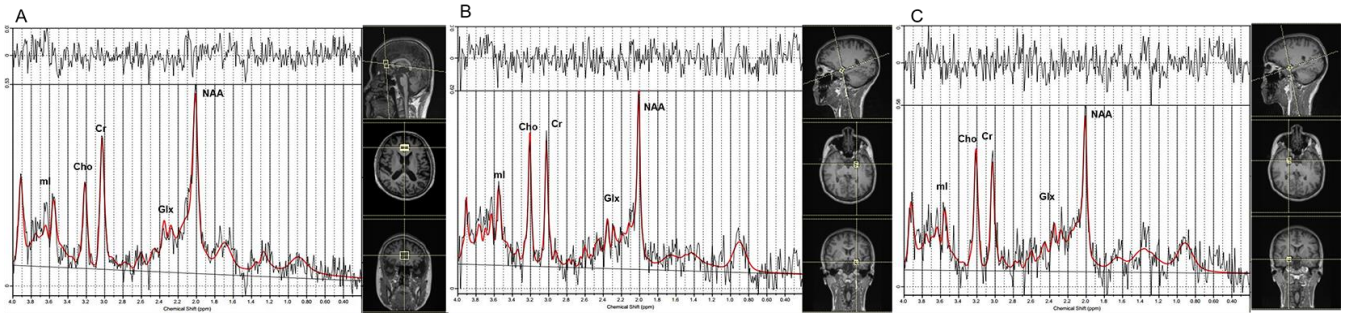

Figure 2. The manual segmentation of bilateral amygdalae on 3-dimensional T1-weighted MR images. A, cross section; B, sagittal section; C, coronal section.

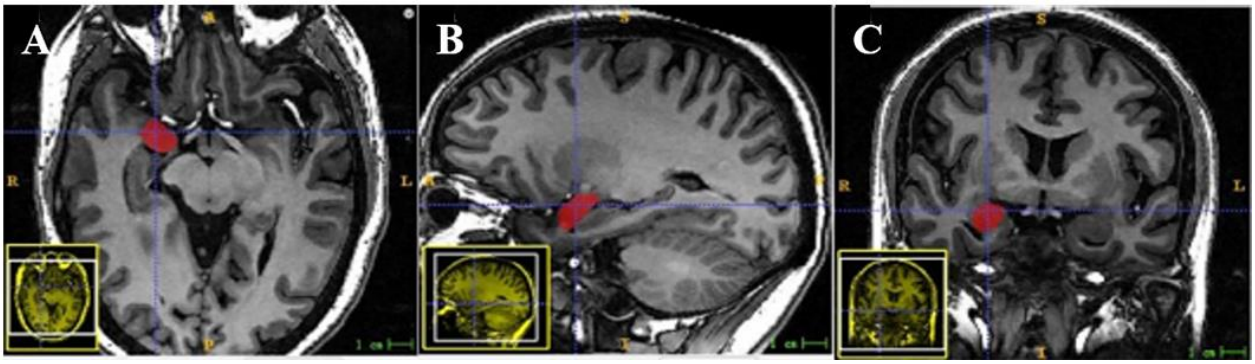

Figure 3. Metabolite levels in the ACC (A) and bilateral amygdalae (B, C) in PTSD patients and control subjects. Error bars indicate standard deviation. The PTSD group showed significantly increased NAA levels in the ACC, increased Cr levels in the left amygdala, and increased mI levels in the right amygdala.

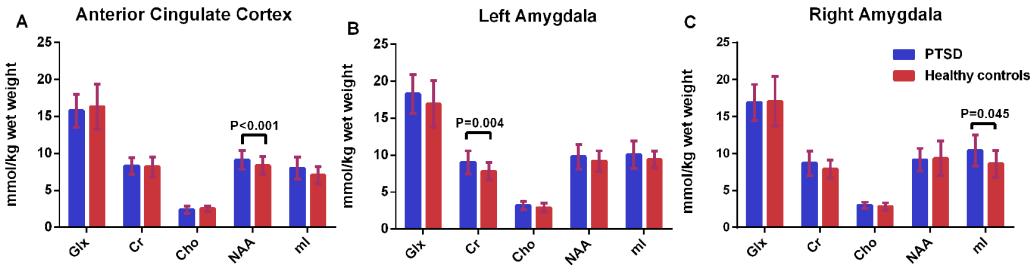

Figure 4. The correlation of NAA concentrations in the ACC of PTSD patients with the time since trauma (r= -0.30, P=0.02)