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A pilot spectroscopy study of adversity in adolescents

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Abstract

Background: Childhood adversity is a global health problem affecting 25–50% of children worldwide. Few prior studies have examined the underlying neurochemistry of adversity in adolescents. This cross-sectional study examined spectroscopic markers of trauma in a cohort of adolescents with major depressive disorder (MDD) and healthy controls. We hypothesized that historical adversity would have a negative relationship with spectroscopic measures of glutamate metabolites in anterior cingulate cortex.

Methods: Adolescent participants (aged 13–21) underwent a semi-structured diagnostic interview and clinical assessment, which included the self-report Childhood Trauma Questionnaire (CTQ), a 28-item assessment of childhood adversity. Proton magnetic resonance spectroscopy (^{1}H-MRS) scans at 3 Tesla of an anterior cingulate cortex (ACC) voxel (8 cm^3) encompassing both hemispheres were collected using a 2-dimensional *J*-averaged sequence to assess *N*-acetylaspartate (NAA), Glx (glutamate+glutamine) and [NAA]/[Glx] concentrations. Generalized linear models assessed the relationships between CTQ scores and metabolite levels in ACC.

Results: Thirty-nine participants (17 healthy controls, 22 depressed participants) underwent ^{1}H-MRS and completed the CTQ measures. There were decrements in [NAA]/[Glx] ratio in the ACC of participants with childhood adversity while no significant relationship between CTQ total score

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Statement of Interest

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and any of the ACC metabolites was found in the combined sample. Exploratory results revealed a positive association between Glx levels and CTQ scores in depressed participants. Conversely the [NAA]/[Glx] ratio had a negative association with total CTQ scores in the depressed participants. Emotional Abuse Scale showed a significant negative relationship with [NAA]/[Glx] ratio in the combined sample when adjusted for depression severity.

Conclusions: Our findings suggest that childhood adversity may impact brain neurochemical profiles. Further longitudinal studies should examine neurochemical correlates of childhood adversity throughout development and in populations with other psychiatric disorders.

Keywords

Glutamate; NAA; ^{1}H-MRS; Anterior cingulate; ACC; Trauma; Adolescent; Depression

Introduction

Childhood adversity is defined as physical or emotional abuse and/or neglect, or sexual abuse before 18 years old (Nelson et al., 2017). Approximately 85% of adolescents have experienced at least one adverse event during childhood (Soares et al., 2016a, 2016b). Adversity in early life has been linked to a broad range of psychiatric disturbances and ensuing functional impairment in adulthood (Edwards et al., 2003), with increased risk of developing anxiety, affective, and psychotic symptoms across a variety of mental disorders (van Nierop et al., 2015). Other studies have demonstrated that an increased frequency of childhood adversity is associated with more severe psychiatric illness course and outcome (Aas et al., 2016). Childhood adversity is an under-studied contributor to the clinical symptoms and biological underpinnings of depression in adolescents (Sekowski et al., 2020).

The treatment of early onset psychiatric disorders is often challenging due to a poor understanding of the related biologic mechanisms. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive imaging method that is utilized to study metabolic changes in the brain. Extant research suggests that adolescents with mood disorders have dysregulated cortical glutamatergic concentrations. The most frequently replicated ^{1}H-MRS finding in major depressive disorder (MDD) is reduced glutamate and Glx (glutamate+glutamine) levels in prefrontal and limbic regions in patients who are currently experiencing a depressive episode (Maddock and Buonocore, 2011). Specifically, there is converging evidence for reduced *N*-acetylaspartate (NAA) levels and Glx in pregenual ACC (pgACC) (Capizzano et al., 2007; Ende et al., 2006; Yildiz-Yesiloglu and Ankerst, 2006) while *y*-aminobutyric acid (GABA) levels remain unchanged (Hasler et al., 2007). Glutamatergic dysfunction in MDD is further supported by pharmacological benefits of glutamate modulating substances (Zarate Jr et al., 2005).

Both preclinical and clinical evidence demonstrates that the glutamatergic system is also involved in stress responsivity, adaptability, and related psychopathology (Averill et al., 2017). Prior spectroscopy work demonstrated that increased early stress was associated with reduction in Glx in the hippocampus of depressed patients (Poletti et al., 2016). CTQ trauma index was negatively related to glutamatergic transmission (Glu/Naa) in the

medial prefrontal cortex in the adult healthy sample (Duncan et al., 2015). In a study of post-traumatic stress disorder (PTSD), ACC glutamate level was negatively correlated with increased arousal (Meyerhoff et al., 2014). One study showed that children and adolescents with PTSD resulting from maltreatment had lower NAA in the anterior cingulate compared to healthy controls (De Bellis et al, 2000). In adolescents with PTSD, significantly lower Glx levels in the rostral ACC were reported in PTSD relative to healthy controls, as well as in those with remitted symptoms relative to healthy controls (Yang et al., 2015).

Emerging research also suggests that the [NAA]/[Glx] ratio may be a sensitive and dynamic measure of glutamate metabolism (Lewis et al., 2020). NAA is highly abundant in the brain and frequently described as a marker of neuronal viability (Maddock and Buonocore, 2011). NAA and Glx are both involved in the same metabolic pathways. NAA and Glu are linked mainly through the tricarboxylic acid (TCA) and glutamate-glutamine cycles (Moffett et al., 2007). It is proposed that NAA serves as a reservoir for glutamate synthesis (Clark et al., 2006). The correlation between NAA and Glx has been shown in various parts of the brain in healthy individuals (Maddock and Buonocore, 2011; Moreno et al., 2001; Waddell et al., 2011). In individuals with psychiatric disorders the equilibrium of NAA and Glx appears to provide a better correlation with severity of pathology than the individual metabolite levels due to variability in methodology (Coughlin et al., 2015; Kraguljac et al., 2013; Lewis et al., 2020; Martens et al., 2021; Rosso et al., 2017; Walter et al., 2009). The ratio of [Glx]/[NAA] has been suggested as a more sensitive marker of dysregulation in both genetic and clinical studies (Lewis et al., 2020; Martens et al., 2021). Therefore, we also explored the ratio of in the present study.

Exposure to stress appears to change glutamatergic transmission. On one hand this change may invoke plasticity and on the other hand it may exert toxic effects. The neurochemical correlates of trauma and in adolescent mood disorders have been incompletely studied and understood. In this pilot study, we aimed to assess neurometabolic correlates of trauma in a cohort of adolescents using ^{1}H-MRS. Participants had varying degrees of childhood adversity without a PTSD diagnosis except for one adolescent. In this cross-sectional study, our goal was to characterize the association between various types of historical adversity and metabolite levels in an adolescent sample.

The primary hypothesis was that metabolite levels would be significantly different among adolescents with lower CTQ scores and elevated CTQ scores. The secondary hypothesis was that in a sample of healthy and depressed adolescents, measures of adversity (CTQ total score and subscale scores) would demonstrate negative relationships with Glx and NAA in the anterior cingulate cortex (ACC).

Methods

Overview

All study procedures were approved by the Mayo Clinic institutional review board (Rochester, MN, USA) prior to any participant recruitment or research activities. This was an exploratory study of participants who had CTQ scores and reliable spectra data.

Eligibility criteria

Adolescents in the healthy control group had no prior psychiatric diagnosis, no prior psychopharmacologic or psychotherapeutic treatment, and had depression severity raw scores less than 30 on the Children's Depression Rating Scale, Revised (CDRS-R; [(Poznanski et al., 1984)]). Participants in the depressed group had active diagnoses of unipolar depressive disorders on the (the Schedule for Affective Disorders and Schizophrenia for School Aged Children, K-SADS-PL [(Kaufman et al., 1997)]) diagnostic interview and had CDRS-R raw scores of 30 or greater. Exclusion criteria for all participants consisted of lifetime history of mania or psychosis, the presence of any active substance use disorder except nicotine, orthodontic hardware that would cause artifact in magnetic resonance images, and any contraindication to MRI/MRS as determined by the MRI safety screen and MRI safety codes, such as implanted ferromagnetic material.

Participants and clinical assessments

Adolescents with depressive symptoms between ages 13–21 years were recruited from a psychopharmacology clinic. Control participants (who had no history of psychiatric disorder or treatment) were recruited from pediatric primary care clinics and through community advertising. Written informed consent was obtained from the parents or guardians of participants under 18 years of age, and from participants of 18 years of age and older. Written assent was obtained from participants younger than 18 years. All participants underwent clinical assessment by a board-certified child and adolescent psychiatrist, including semi-structured diagnostic interview K-SADS-PL (Kaufman et al., 1997). Severity of depressive symptoms was rated using the CDRS-R (Poznanski et al., 1984), and the Quick Inventory of Depressive Symptomatology (17-item) Adolescent and Parent Self Report (QIDS-A17-SR; [(Bernstein et al., 2010)]). Healthy control participants (HC) had no prior psychiatric diagnoses based on the K-SADS-PL interview.

Trauma was assessed by Childhood Trauma Questionnaire (CTQ; [(Bernstein et al., 1998)]) total score and subscale scores. CTQ is a 28-item self-reported instrument measuring childhood adversity (Bernstein et al., 1997). Items are rated on a five-point Likert scale ranging from 1 (never true) to 5 (very often true). Scores are generated for five subscales: emotional abuse (EAS), physical abuse (PAS), sexual abuse (SAS), physical neglect (PNS) and emotional neglect (ENS), in addition to a total trauma score. Higher scores indicate greater adversity and trauma. The cut-off scores for each subscale according to CTQ Manual were summarized in Table 1 (Bernstein et al., 1998). Full list of participants, medications, exposure to adversity and comorbities provided in the supplementary materials (Appendix A1). Stimulant medications were held on the day of the scan.

Proton magnetic resonance spectroscopy

Eligible participants (both healthy and depressed adolescents with no contraindication to magnetic resonance imaging) underwent ^{1}H-MRS scans at 3T to assess glutamatergic metabolite concentrations in anterior cingulate cortex (ACC). Measurements were collected from an 8-cm^{3} voxel corresponding to the pregenual ACC positioned according to previously published methods (Croarkin et al., 2016; Lewis et al., 2016; Port, Unal, Mrazek, and Marcus, 2008). The voxel was encompassing the pregenual anterior cingulate

cortex of both hemispheres (Broadman areas 24a, 24b, and 32) (Fig. 1A). A FAST 3D SPGR sequence was utilized to acquire volumetric data for voxel positioning and tissue segmentation. Spectroscopic data were acquired using a 2-dimensional J-averaged PRESS (2DJ) sequence (TR = 2000 ms, TE = 35–195 ms in 16 steps, TR = 2000 ms, 8 averages, 3-way phase cycling) designed specifically for improved glutamate measurement (Hurd et al., 2004) (Fig. 1B). 2D acquisition methods can examine chemical shift and spin-spin coupling in different dimensions, allowing quantification of J-coupled metabolites with overlapping spectra. J-resolved sequences have the capacity to enhance accuracy of glutamate measurement but this costs diminished repeatability (Maddock and Buonocore, 2011). At the time of the data collection for our study, 2DJ-averaged PRESS for glutamate offered a better focused and improved quantification of glutamate and related metabolites in 3T. The protocol described by Hurd et al., comprises 16 steps with a TE increment of 10 ms per step, starting from a TE of 30–35 ms. This allows adequate coverage of the J-dimension and produces spectra with an effective TE of 105 ms, keeping the loss of signal due to T2 relaxation moderate.

Following the scan, quantitative metabolite concentrations were estimated using LCModel software version 6.3–1 K and a vendor-provided basis set. Scans were reviewed by a neuroradiologist (JDP) to exclude scans with visible artifacts and verify integrity of spectra (Provencher, 2001). Metabolites measured included creatine (Cr), choline (Cho), glutamate (Glu), NAA and Glx. Metabolite concentration measurements were corrected to the cerebrospinal fluid (CSF) fraction according to previously published methods (Lewis et al., 2016). Cramér-Rao lower bounds (Cavassila et al., 2001) is an estimate of the fitting error used as a quality criterion to exclude data sets with unreliable quantification results. Only the measurements with a Cramér-Rao standard deviation less than 20% were included for analysis. Scans with signal-to-noise ratio <10 were excluded. Hence the analyses were restricted to subjects who met strict quality criteria to indicate reliable spectra for each metabolite (Schulte and Boesiger, 2006).

Statistical analyses

Statistical tests were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA) and JMP Pro 14.1.0 (SAS Institute, Inc., Cary, NC, USA) software. The significance level was set at $\alpha = 0.05$. The Benjamini-Hochberg False Discovery Rate (FDR) method was used to correct for multiple comparisons (Benjamini and Hochberg, 1995). We listed the individual P values in order, from smallest to largest. The smallest P value has a rank of $i = 1$, then next smallest has $i = 2$, etc. We compared the each individual P value to its Benjamini-Hochberg critical value, $(i/m)Q$, where i is the rank, m is the total number of tests, and Q is the false discovery rate we chose. The largest P value that had $P <$ (i/m) Q was significant, and *all* of the P values smaller than it were also significant, even the ones that were not less than their Benjamini-Hochberg critical value. We chose a relatively high false discovery rate of 0.3, as this is a pilot study for hypothesis generation and planning for future studies.

Demographic and clinical characteristics of the overall sample and each group (healthy controls and depressed participants) were described with the mean and standard deviation for continuous variables and with counts and percentages for categorical variables. Group

differences were assessed with independent samples *t*-tests or Mann-Whitney *U* tests for continuous variables and Pearson's chi-squared test for categorical variables. Shapiro-Wilk normality tests revealed that distribution of age and clinical measure were not normal across trauma levels. Therefore, group comparisons across trauma levels were all run with Mann-Whitney U.

The first aim of this study was to compare metabolite levels in two groups of participants based on their report of CTQ: no (or minimal) trauma versus elevated trauma (low, moderate and severe trauma). The "No Trauma" group included participants who did not report any trauma in any of the subscales according to cut-off scores provided in Table 1 (emotional abuse, emotional neglect, physical abuse, physical neglect and sexual abuse). Shapiro-Wilk test revealed that all distributions were normal. Group comparisons were run with student's *t*-test.

The secondary aim was relationships between ACC NAA, Glx, and [NAA]/[Glx] and a dimensional measure of trauma (CTQ total scores) and 5 subscales (Emotional Abuse Scale, Emotional Neglect Scale, Physical Abuse Scale, Physical Neglect Scale and Sexual Abuse Scale) were examined using generalized linear models to test the hypothesis in the combined sample as well as subgroups. Dependent variable was the ACC metabolite measure (NAA, Glx, and [NAA]/[Glx] ratio). Separate models were constructed for each metabolite and subscale.

Exploratory analyses included assessment of age, sex, depression severity, and medication status as potential covariates.

Results

Total number of participants were 42. Three participants were excluded because the scans did not meet the quality measures of reliable spectra. All three were in the depressed group with elevated trauma. Two had a comorbid diagnosis of ADHD. 39 patients with both spectroscopy scans and CTQ scores were included in analyses. All participants had valid measurements for both NAA and Glx. Demographic and clinical characteristics are reported in Table 2. Groups did not differ significantly by age or sex. CTQ, CDRS-R and QIDS-A17-SR scores were significantly higher in the depressed group than in healthy controls ($p < 0.001$). Twelve participants were prescribed psychotropic medication. The most common comorbidities were cannabis use disorder ($n = 7$, 17.95%) and ADHD ($n = 4$, 10.26%). There were no differences in metabolite levels in healthy participants versus depressed.

Primary outcome: ^{1}H-MRS measured ACC metabolite comparisons between trauma groups

Metabolite levels were compared in two groups of participants based on trauma level. Means and standard errors of [Cr], [Cho], [NAA], [Glx], and [NAA]/[Glx] are reported in Table 3. There was a significant difference in mean [NAA]/[Glx] between "No Trauma" and "Elevated Trauma" ($p = 0.047$, $p_{FDR} = 0.282$). The ratio was greater in the "No Trauma" group. We also calculated a Hedges g (due to difference in sample sizes) to have a

standardized effect size for mean differences across trauma levels. The effect sizes for NAA, Glx, and [NAA]/[Glx] were respectively g = 2.04, g = 1.25, and g = 0.66.

Secondary outcome: relationship between CTQ Total score and subscales and ^{1}H-MRS measured ACC metabolites

Table 4 summarized the results of the models with independent variable CTQ total trauma score in the combined sample. There were no significant associations of the total score in the combined sample. In Table 4, unstandardized parameter estimates and p-values of models assessing the relationship between ACC metabolites and all 5 CTQ subscales were reported in healthy and depressed subgroups.

In the combined sample physical neglect showed significant negative association with NAA levels. In the depressed sample, emotional abuse score was significant for both Glx (β = 2.014, p = 0.034) and [NAA]/ [Glx] ratio (β = −0.017, p = 0.004). In this sample, CTQ total score showed a significant negative association with [NAA]/[Glx] ratio (β = −0.006, p = 0.012). In the healthy group, emotional neglect showed a significant negative relationship to NAA levels (β = −2.016, p = 0.029). We calculated the standardized the regression coefficients by multiplying them by the standard deviation of the predictor (independent variable, X, CTQ scores) and dividing them by the standard deviation of the response (dependent variable, Y, metabolites levels). These are reported in the footnote of Table 4. All significant estimates were in the small to medium range.

Exploratory analyses: exploring age, sex, depression severity and medication use

We explored age, sex, depression severity and medication use as potential covariates in the significant models. Psychotropic medications use was not a significant covariate in any of the analyses. Results are summarized in the supplementary materials (Appendix A.2).

In the healthy control group for emotional neglect was not significantly associated with NAA after adjusting for age. Sex and depression severity were not confounding.

In the depressed group, CTQ total score showed a significant positive relationship with [Glx] when adjusted for for depression severity (β = 0.828, p = 0.046). The negative significant relationship with total CTQ score and [NAA]/[Glx] ratio remained significant after adjusting for age, sex and depression severity. Emotional abuse showed significant negative associations with [Glx] and [NAA]/[Glx] ratio. These results remained significant after adjusting for age, sex and depression severity.

In the combined sample, the association of physical neglect and NAA was rendered nonsignificant after adjusting for depression severity. Emotional abuse showed significant association with [NAA]/[Glx] in the combined sample only after adjusting for depression severity.

Discussion

This preliminary analysis explored potential correlations between CTQ measures of childhood adversity and ACC metabolite levels in an adolescent cohort of depressed and

healthy individuals. In terms of metabolite levels across trauma levels, [NAA]/ [Glx] ratio was the only measure that was significantly smaller in the elevated trauma group. This is in line with prior evidence suggesting a relationship in the same direction with worse mental health outcomes (Averill et al., 2017; Lewis et al., 2020; Martens et al., 2021). Medium to large effect sizes in metabolite levels across trauma levels are reassuring and emphasizing that sample size may be a limiting factor in our results.

In the secondary and exploratory analyses, the most robust findings were the associations between Glx or [NAA]/ [Glx] ratio and overall trauma severity or emotional abuse in the depressed group. Depression in this sample was notable confounding factor. This is supported by the significant positive association between overall childhood adversity and ACC [Glx] in the depressed group, although only when adjusted for depression severity. Among different subtypes of trauma, emotional abuse was the adversity driving the association to metabolites which were significant after adjusting for age, sex or CDRS-R independently. Small to medium standardized parameter estimates may suggest that there is a role of glutamate in pathophysiology of adversity and it warrants further exploration from different aspects. Our findings were comparable with another recent study (Averill et al., 2020) investigating the relationship of trauma and neurometabolites in adults with MDD. Exploratory findings in this study suggested a significant positive correlation between early life stress and occipital glutamine but not glutamate. Post-hoc analyses showed that the association with glutamine was driven by the emotional abuse subscale. Authors found that in a smaller subset (n = 11), those with childhood emotional abuse appeared to have increased occipital glutamate neurotransmission as reflected by increased glutamate/ glutamine cycling and glutamine level. This is also supportive of the positive directions of associations with Glx and emotional abuse in our sample despite the differences in age (13–21 age range versus 18–65 age range) and medication status of samples. Averill et al. included 36 non-medicated adult MDD patients, whereas our sample included 22 depressed adolescents, more than half were on psychotropic medication. However, our exploratory analyses did not support the idea of consequences of psychotropic use in neurochemical levels. Another difference is that the spectroscopy voxel was selected from occipital cortex versus ACC scans in our study. However, it is noteworthy that emotional abuse was related to Glx expression in both studies as a prominent part of overall trauma.

We also acknowledge that comorbidities in our sample introduced heterogeneity which may contributed to some of the nonsignificant results. Cannabis use disorder was comorbid in seven of the depressed participants and one healthy control. Cannabis use was suggested to lower NAA in DLPFC in adolescents (Sneider et al., 2013). ADHD was comorbid in four of the depressed participants. Frontal/striatal glutamatergic resonances (Glx) were elevated in the children with ADHD compared with healthy control subjects, but no differences were noted in NAA, Cho, or Cr metabolite ratios (MacMaster et al., 2003). Although not specifically in a childhood trauma cohort, another study in patients with PTSD reported that within dorsal ACC, there was a positive linear relationship between Glx concentrations and current stress disorder symptoms (Harnett et al., 2017). In our study, the direction between Glx levels and trauma severity was also consistently positive.

Limitations

First, the present data for this exploratory study comes from a cross-sectional study. Cross-sectional analyses cannot causally link trauma and depressive symptoms. Additionally, we suspect our small sample size is limiting power in analyses of sub-sets with respect to specific comorbidities, sex, age, or race. Longitudinal studies are needed to explore the development of depression in youth with history of trauma and to portray brain-based differences between early manifestations of psychiatric disorders. Replication with a larger sample is needed. Validity of self-report in adversity may be questioned as it is prone to recall bias and may depend on participant's wellbeing at the time of measurement. When comparing ^{1}H-MRS results across studies, participant related factors (i.e. age, sex, medication status) and methodological factors such as ^{1}H-MRS sequences and acquisition parameters, anatomic region sampled, processing techniques and quantification strategies must be considered. We acknowledge that 2D-J averaged PRESS is not a widely adopted method. To this date, even though there are multiple ^{1}H-MRS acquisition sequences that are capable of measuring glutamate and related metabolites, newer techniques are still being developed (Al-Ie-dani et al., 2018; Jensen et al., 2017; Lally et al., 2016; Liu et al., 2017). These attempts are driven by the fact that Glutamate and Glutamine both give rise to a complex proton MR spectrum, characterized by the coupled spins of the C2–C4 hydrogen nuclei and J-modulated peak phases. The reality is that regardless of technique, precision for glutamatergic metabolites is less than NAA or Cho (Mullins et al., 2008). Comparisons of these methods in large clinical human populations are lacking. It is not yet possible to definitively say that one method is superior.

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Appendix A1.: Participant's psychotropic medications and comorbidities at the time of the ^{1}H-MRS scan

Participant	Group	Trauma Exposure	Medication (s) and Total Daily Dose	Comorbidities
1	Dep/Med −	EAS, PAS, ENS	none	cannabis use
2	Dep/Med +	No Trauma	Fluoxetine, methylphenidate hydrochloride ER	ADHD
3	HC	No Trauma	none	none
4	HC	No Trauma	none	none
5	HC	EAS	none	none
6	HC	No Trauma	none	none
7	Dep/Med −	EAS, SAS,PNS	none	panic disorder
8	HC	No Trauma	none	none

Participant	Group	Trauma Exposure	Medication (s) and Total Daily Dose	Comorbidities
9	Dep/Med +	EAS, SAS, ENS	sertraline, aripiprazole	none
10	Dep/Med +	SAS	fluoxetine	alcohol use disorder in remission 8 months, anxiety disorder NOS
11	HC	No Trauma	none	none
12	HC	No Trauma	none	none
13	HC	ENS	none	subthreshold depressive symptoms
14	Dep/Med −	EAS, ENS	none	none
15	Dep/Med −	EAS, PAS, ENS, PNS	none	cannabis use disorder
16	Dep/Med +	EAS, SAS	sertraline	none
17	HC	No Trauma	none	none
18	Dep/Med −	EAS, SAS, ENS, PNS	none	alcohol cannabis nicotine (all since 2014)
19	Dep/Med −	EAS, ENS, PNS	none	none
20	Dep/Med −	EAS, SAS	none	ADHD, Osgood Schlatter
21	Dep/Med +	EAS, SAS, ENS	Fluoxetine, dextroamphetamine saccharate	ADHD, GAD, Motor Tic Disorder
22	Dep/Med +	EAS, ENS, PNS	fluoxetine	cannabis use disorder
23	Dep/Med −	EAS, ENS	none	persistent dysthymic disorder (8 years)
24	Dep/Med +	No Trauma	fluoxetine	restless leg syndrome, migraines
25	Dep/Med −	EAS, PAS, ENS, PNS	none	persistent dysthymic disorder, cannabis use disorder
26	HC	No Trauma	none	none
27	HC	PNS	none	none
28	Dep/Med +	No Trauma	duloxetine	ADHD, minor depressive disorder
29	Dep/Med +	No Trauma	bupropion hydrochloride xl	none
30	HC	PNS	none	cannabis use disorder
31	HC	No Trauma	none	none
32	Dep/Med −	EAS, PAS, SAS, ENS	none	PTSD
33	HC	No Trauma	none	mild concussion history
34	Dep/Med +	EAS, PAS, SAS	fluoxetine	cannabis use disorder
35	Dep/Med +	No Trauma	amitriptyline	learning disability, chronic headaches
36	Dep/Med +	EAS, PAS, SAS, ENS	fluoxetine	none
37	HC	No Trauma	none	none
38	HC	No Trauma	none	none
39	HC	No Trauma	none	none

All stimulants were held on the day of MRS scan for the parent study. “No Trauma” group reported none or minimal trauma based on cut-off scores provided in CTQ Manual. See Table 1 in the manuscript. Emotional abuse (EAS), physical abuse (PAS), sexual abuse (SAS), physical neglect (PNS) and emotional neglect (ENS). PTSD: post-traumatic

stress disorder. ADHD: attention deficit hyperactivity disorder. GAD: generalized anxiety disorder. NOS: not otherwise specified. Healthy Control (HC), Dep/Med+(Depressed Subject treated with an antidepressant), Dep/Med− (Depressed Subject unmedicated)

Appendix A.2.: Exploratory analyses and multivariate models

ACC 2DJ	Independent Variable	CTQ Total Score		CTQ EAS			
NAA/Glx		Depressed Group, n = 22		Depressed Group, n = 22		Combined Sample, n = 39	
	Co-vary	β	p-value	β	p-value	β	p-value
	None	−0.006	**0.012**	−0.017	**0.004**	−0.009	0.063
	Age	−0.006	**0.012**	−0.017	**0.004**	−0.009	0.072
	Sex	−0.006	**0.012**	−0.017	**0.003**	−0.009	0.058
	CDRS-R	−0.006	**0.021**	−0.018	**0.007**	−0.014	**0.027**

ACC 2DJ	Independent Variable	CTQ Total Score		CTQ EAS	
Glx		Depressed Group, n = 22		Depressed Group, n = 22	
	Co-vary	β	p-value	β	p-value
	None	0.738	0.05	2.014	**0.034**
	Age	0.732	0.053	2.061	**0.036**
	Sex	0.712	0.051	2.222	**0.017**
	CDRS-R	0.828	**0.046**	2.352	**0.026**

ACC 2DJ	Independent Variable	CTQ ENS		CTQ PNS	
NAA		Healthy Group, n = 17		Combined Sample, n = 39	
	Co-vary	β	p-value	β	p-value
	None	−2.016	**0.029**	−1.092	**0.03**
	Age	−1.814	0.064	−1.17	**0.019**
	Sex	−2.016	**0.028**	−0.973	0.05
	CDRS-R	−2.544	**0.014**	−0.683	0.196

Note: Each β and *p*-value pair represents the coefficient and p-value of CTQ score from a separate generalized linear model. EAS, Emotional Abuse Subscale; ENS, Emotional Neglect Subscale; PAS, Physical Abuse Subscale; PNS, Physical Neglect Subscale; SAS, Sexual Abuse Subscale.

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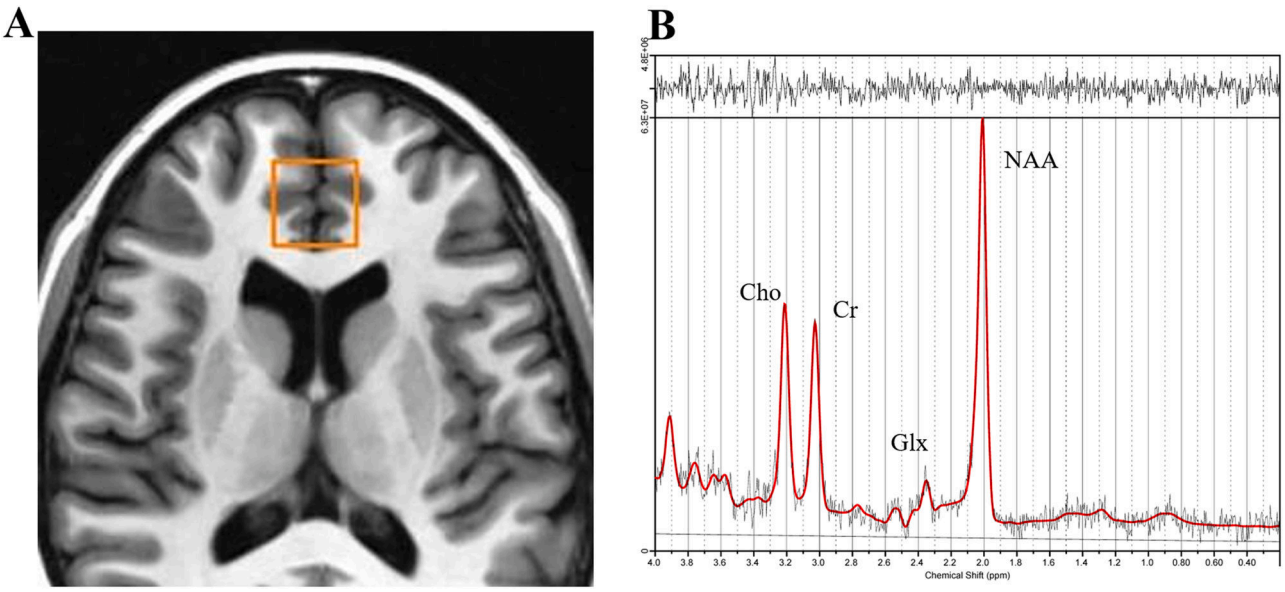

Fig. 1.

A) Pregenual ACC voxel. Axial view. This voxel encompasses the pregenual ACC of both cerebral hemispheres. B) Spectroscopic data were acquired via a 2D-J TE-averaged PRESS sequence at 3T. Quantitative analysis was performed by LCModel (Provencher, 1993). Also shown are signal peaks of Glx (glutamate + glutamine), choline (Cho), creatine (Cr), and *n*-acetylaspartate (NAA).

Table 1

Childhood trauma questionnaire cutoff scores.

Level of abuse	Emotional abuse	Physical abuse	Sexual abuse	Emotional neglect	Physical neglect
None	8	7	5	9	7
Low	12	9	7	14	9
Moderate	15	12	12	17	12
Severe	16+	13+	13+	18+	13+

Note: CTQ (Childhood Trauma Questionnaire) Manual cutoff scores and categories for each subscale. Example: Those who scored 8 or less in emotional abuse reported minimal or no trauma. Those who scored 9 or higher in emotional abuse reported some level of emotional abuse.

Table 2

Demographics and clinical characteristics.

Mean (SD)	Combined sample	Healthy controls	Depressed	*p*	No trauma	Elevated trauma	*p*
n	39	17	22		18	21	
Age	16.36 (2.08)	16.65 (2.21)	16.14 (2.01)	0.46	16.39 (1.94)	16.33 (2.24)	0.4
Female, %	24 (62)	12 (70.6)	12 (54.5)	0.3	11 (61.1)	13 (61.9)	0.9
CDRS-R	35.03 (17.9)	19.12 (2.89)	47.32 (14.49)	<0.001	24.33 (11.89)	44.19 (17.24)	<0.001
QIDS-SR-A17	7.9 (6.28)	2.71 (2.14)	11.9 (5.4)	<0.001	3.72 (3.78)	11.48 (6.28)	<0.001
CTQ Total Score	36.54 (11.01)	28.35 (3.28)	42.86 (10.72)	<0.001	27.67 (3.14)	44.14 (9.45)	<0.001
CTQ EAS	8.82 (4.08)	6.06 (1.39)	10.95 (4.2)	<0.001	5.83 (0.99)	11.38 (3.98)	<0.001
CTQ ENS	8.54 (3.95)	6.41 (1.91)	10.18 (4.36)	0.002	6.06 (1.66)	10.67 (4.13)	<0.001
CTQ PAS	6.21 (1.96)	5.18 (0.53)	7 (2.29)	0.006	5.28 (0.67)	7 (2.35)	0.006
CTQ PNS	6.41 (2.21)	5.71 (1.36)	6.95 (2.59)	0.124	5.33 (0.6)	7.33 (2.65)	0.012
CTQ SAS	6.49 (4.12)	5 (0)	7.64 (5.24)	0.015	5 (0)	7.76 (5.34)	0.001

Note: Trauma level is based on Childhood Trauma Questionnaire (CTQ) scoring guide. No trauma group reported none or minimal exposure in any of the subscales. Sex differences across groups were assessed with Chi-Square. Age across healthy vs depressed student's *t*-test. Shapiro-Wilk normality test revealed that distribution of age and clinical measure were not normal. Therefore group comparisons across trauma levels were all run with Mann-Whitney U. (EAS, Emotional Abuse Subscale; ENS, Emotional Neglect Subscale; PAS, Physical Abuse Subscale; PNS, Physical Neglect Subscale; SAS, Sexual Abuse Subscale).

Table 3

^{1}H-MRS anterior cingulate cortex metabolites across trauma levels.

ACC (2DJ) metabolites mean (SE)	Combined sample n = 39	No trauma n = 18	Elevated trauma n= 21	*p*	p_{FDR}
Glutamate	87.93 (1.96)	87.32 (2.87)	88.45 (2.75)	0.779	0.779
[Glx]	113.22 (3.27)	110.05 (5.58)	115.94 (3.78)	0.377	0.566
N-acetyl aspartate	84.72 (1.17)	86.55 (2.15)	83.15 (1.11)	0.15	0.414
[NAA]/[Glx]	0.77 (0.02)	0.81 (0.03)	0.73 (0.02)	0.047*	0.282*
Creatine	59.7 (0.87)	60.9 (1.5)	58.67 (0.97)	0.207	0.414
Choline	21.06 (0.43)	21.21 (0.63)	20.94 (0.61)	0.762	0.779

Cut-off score was based on CTQ scoring guidelines. See Table 1. Glutamine was not measured. Glx: glutamate+glutamine. Shapiro-Wilk test revealed that all distributions were normal. Group comparisons run with student's *t*-test. False discovery rate was chosen as 0.3.

*statistically significant.

Table 4

Relationships between CTQ scores and 1H-MRS measured ACC metabolites.

Metabolites	Scores	CTQ TOTAL		CTQ EAS		CTQ ENS		CTQ SAS		CTQ PAS		CTQ PNS	
		β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
NAA	Healthy	−1.015	0.067	0.058	0.968	−2.016	**0.029** *	0	–	−1.23	0.743	−1.813	0.194
	Depressed	−0.012	0.918	−0.152	0.618	0.104	0.724	0.086	0.728	0.095	0.866	−0.672	0.16
	Combined	−0.181	0.077	−0.424	0.13	−0.103	0.164	−0.101	0.722	−0.508	0.392	−1.092[d]	**0.03** *
Glx	Healthy	−0.7	0.636	−0.189	0.957	−1.7	0.501	0	–	4.317	0.638	−1.184	0.741
	Depressed	0.738	0.05	2.014[a]	**0.034** *	1.013	0.302	0.826	0.312	1.949	0.297	0.184	0.913
	Combined	0.134	0.652	0.616	0.44	0.058	0.944	0.446	0.574	0.701	0.674	−0.533	0.719
NAA/Glx	Healthy	−0.002	0.797	0.000008	1	−0.003	0.858	0	–	−0.035	0.482	−0.002	0.921
	Depressed	−0.006[c]	**0.012** *	−0.017[b]	**0.004** *	−0.007	0.316	−0.006	0.235	−0.015	0.233	−0.009	0.393
	Combined	−0.003	0.12	−0.009	0.063	−0.004	0.468	−0.005	0.288	−0.01	0.313	−0.006	0.475

Note: Each β and *p-value* pair was generated via separate generalized linear models. Scores were the only independent variables in these models. The results of models with added covariates such as age, sex, depression severity, current psychotropic medication use are available in Appendix A2. Number of healthy participants n = 17, number of depressed participants n = 22, combined sample n = 39.

*statistically significant.

[a]standardized estimate = 0.412.

[b]standardized estimate = −0.523.

[c]standardized estimate = −0.471.

[d]standardized estimate = −0.328.