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A Longitudinal ^{1}H-MRS Study of the Anterior Cingulate Gyrus in Child and Adolescent Victims of Multiple Forms of Violence

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Abstract

Background: The anterior cingulate gyrus is involved in the extinction of conditioned fear responses and is implicated in the pathophysiology of posttraumatic stress disorder. The expression of N-acetylaspartate and choline may be altered in the anterior cingulate gyri of children and adolescents with posttraumatic stress disorder.

Methods: We conducted a proton magnetic resonance spectroscopy study, longitudinally investigating N-acetylaspartate/creatine and choline/creatine ratios in the anterior cingulate gyri of children and adolescents, aged from 8 to 12 years, who had been exposed to various forms of violence or were non-trauma control. Based on baseline posttraumatic stress symptoms ("sub-clinical"), participants were divided into two groups: posttraumatic stress ($n = 19$) and control ($n = 19$). Proton magnetic resonance spectroscopy scans were repeated a year later in trauma exposed participants. Trauma assessments included the Childhood Trauma Questionnaire.

Results: Exploratory analyses revealed a significant negative correlation between follow-up anterior cingulate gyrus N-acetylaspartate/creatine and Childhood Trauma Questionnaire scores in posttraumatic stress ($r = -0.62$, $p = 0.01$) but not control group ($r = 0.16$, $p = 0.66$). However, we found no significant differences in anterior cingulate gyrus N-acetylaspartate/creatine or choline/creatine between posttraumatic stress and control. In addition, there were no significant effects of time, group, or time-by-group interactions.

Conclusions: In this pediatric population, anterior cingulate gyrus N-acetylaspartate/creatine and choline/creatine were not affected by posttraumatic stress and on average these metabolites remained stable over time. However, the study provided intriguing preliminary evidence revealing that participants suffering from posttraumatic stress at baseline have shown, a year later, reduced anterior cingulate gyrus N-acetylaspartate/creatine among those with high trauma severity. This pilot evidence warrants replication in future studies to confirm these findings and to determine the longitudinal effects and interactions between childhood posttraumatic stress and trauma.

Keywords

Child and adolescent, proton magnetic resonance spectroscopy, anterior cingulate gyrus, posttraumatic stress disorder, violence

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Introduction

Child abuse is a global problem with serious life-long consequences[1] including mental disorders such as posttraumatic stress disorder (PTSD). Children and adolescents with posttraumatic stress symptoms (PTSS)

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frequently suffer from functional impairments[2] and cognitive, social, and emotional deficits.[3–5] The prevalence of PTSS among children who have experienced abuse varies from 25% to 76.5% and, on average, 30% of children who experience PTSS develop PTSD.[6]

Proton magnetic resonance spectroscopy (^{1}H-MRS) and magnetic resonance imaging (MRI) are non-invasive and highly useful methods for investigating the most vulnerable patient groups. Like MRI, ^{1}H-MRS measures the signals emitted by the hydrogen atom protons present in almost all biomolecules, including neurometabolites. It is useful for obtaining information about psychological processes in vivo.[7]

The neurometabolites most commonly studied with ^{1}H-MRS are choline (Cho), creatine (Cr), and N-acetylaspartate (NAA). It can also be used to study the glutamate-glutamine complex, myoinositol, γ-aminobutyric acid (GABA), taurine, and lactate.[8] NAA is an amino acid located in neuronal cell bodies, dendrites, and axons. It is a neuronal integrity marker that is associated with nerve cell myelin sheaths and is mostly localized in mature neurons and neuronal processes. It is normally not encountered in glia.[8,9] Ross and Bluml[10] noted that several human studies have shown that NAA is absent or reduced in inborn metabolic errors, trauma, ischemia, degenerative diseases, and brain tumors. This is consistent with NAA being present in neurons and axons and absent from mature glial cells.[10] Changes in NAA levels are the most important indicator of neuronal function and development during the first two years of life,[11,12] after which variations in normal subjects become less pronounced and severe changes are usually interpreted as indicating various pathological conditions that adversely affect neuronal function. Since NAA is synthesized in mitochondria, it may be pathologically altered by mitochondrial malfunctions. Cr is involved in cellular energy metabolism. In many ^{1}H-MRS applications, the Cr level is considered constant and a reliable measure of overall brain metabolism.[13] It is therefore used as an internal reference.[14,15] Cho is a precursor of cell membrane formation and is frequently treated as a marker of malignant cell multiplication, but it can also be involved in development and recovery processes.[16]

The ACC forms part of the limbic-cortical network involved in altered mood states. It is located on the medial surface of the brain's frontal lobes and is believed to be involved in the regulation of attention, the extinction of conditioned fear responses, and the pathophysiology of PTSD.[17] Adults with PTSD express altered metabolites in the ACC, but different studies have reported discrepant results. One study reported that adults with PTSD had lower NAA/Cr ratios than healthy controls (HCs) did,[18] but another study found no such difference.[19] However, both studies found that the adults with PTSD had significantly higher Cho/Cr ratios. In contrast, De Bellis et al.[20] found no significant difference between the Cho/Cr ratios of maltreated children and adolescents with PTSD and those of age-matched HCs.

We aimed to conduct a cross-sectional and longitudinal investigation of anterior cingulate gyrus (ACG) NAA/Cr and Cho/Cr ratios in children and adolescents who were exposed to violence.

Materials and Methods

Subjects

The current report included 38 children (19 with post-traumatic stress (PTS) and 19 control) whose demographic and clinical characteristics are displayed in Table 1 (additional clinical assessment and diagnostic classification details are provided in the Online Supplementary Material). Of the 19 PTS subjects, 6 subjects met DSM-IV criteria for PTSD. Of the 19 controls, 10 subjects have been victims of violence (which included witnessing domestic violence, physical and/or emotional violence, neglect and sexual abuse). All trauma-exposed participants (i.e., 19 PTS and 10 control) were scheduled for a one-year follow-up; however, 4 withdrew from the study prior to the follow-up assessments and scans.

The exclusion criteria were (1) histories of mental retardation; (2) histories of schizophrenia or autism; (3) counter-indications to MRI, such as the presence of any metal or electrically conductive implants or foreign bodies; (4) histories of substance dependence; and (5) histories of clinically notable head traumas, epilepsy, or other neurological disorders.

The nine HCs were examined only once. The other 29 subjects were scheduled for a one-year follow-up, but 4 withdrew from the study, leaving 25 who completed both assessments.

This study was approved by the Institutional Review Board of the Federal University of São Paulo. The children's primary caregivers gave written informed consent, and the children gave written assent. The research was conducted in accordance with the principles of the Declaration of Helsinki.

^{1}H-MRS Methods

Before ^{1}H-MRS scanning, all children participated in a ludic desensitization session described in the Online Supplementary Material "Pre-Imaging Preparations" section.

All subjects underwent a complete MRI examination on a 1.5-tesla Siemens Sonata scanner which included a sagittal 3D T1 scan (repetition time (TR) = 2000 ms, echo time (TE) = 3.2 ms, inversion time = 1100 ms, field of view (FOV) = $256 \times 256\,mm^2$, matrix size = 256×256, slice thickness = 5 mm, flip angle = 15°), fluid-attenuated

Table 1. Demographic, clinical data, and statistical between-group (PTS and control) in age and Tanner stages.

			PTS (n = 19)			Control (n = 19)					
Time		n	Mean or %	SD	SEM	n	Mean or %	SD	SEM	MWU	p-value
Baseline	NAA	18	1.55	0.29	0.07	10	1.63	0.52	0.16	67.5	0.280
	Cho	18	0.87	0.28	0.07	9	0.85	0.25	0.08	74.5	0.738
Follow-up	NAA	15	1.68	0.25	0.07	17	1.75	0.25	0.06	111.5	0.545
	Cho	16	0.86	0.24	0.06	17	1.03	0.57	0.14	113.5	0.417
Demographic features	Age	19	10.47	1.26	0.29	11	9.45	1.37	0.14	2.1	0.048
	Female	11	57.89%			7	36.84%				0.194[a]
Puberal stages	Tanner I	2	10.52%			5	26.31%				
	Tanner II	9	47.37%			9	47.37%				
	Tanner III	1	5.26%			2	10.53%				
	Tanner IV	3	15.79%			2	10.53%				
	Tanner V	4	21.05%			1	5.26%				0.516[b]

Note: Cho: Choline; SEM: standard error of the mean; n: number of subjects; MWU: Mann-Whitney U; NAA: N-acetylaspartate; PTS: posttraumatic stress.
[a]p-value obtained from a chi-square test.
[b]Fisher exact test.

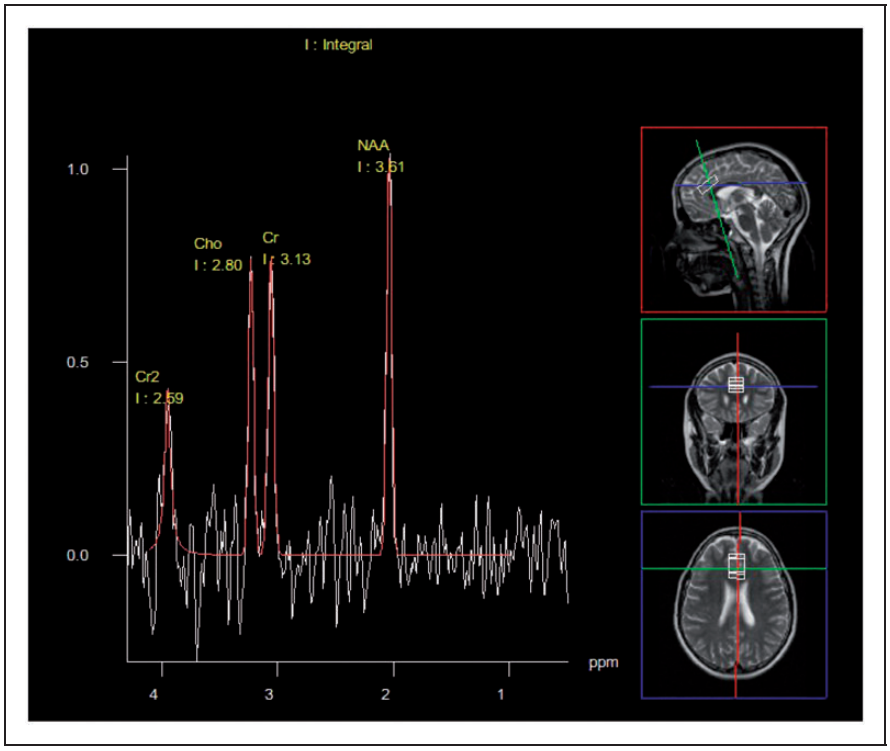

Figure 1. Positioning of the spectroscopy voxel in the anterior cingulate gyrus (ACG) and metabolites rates.

inversion recovery (TR = 9000 ms, TE = 108 ms, FOV = $210 \times 240\,mm^2$, matrix size = 256×256, slice thickness = 5 mm), and single-voxel spectroscopy (point-resolved sequence[21]: TE = 135 ms, TR = 1500 ms, bandwidth = 1000 Hz, samples = 1024, averages = 192, total scan time = 5 min, voxel dimensions = $10 \times 20 \times 30\,mm^3$) targeting the ACG as shown in Figure 1. All images were radiologically evaluated for signs of the exclusion criteria described in the "Subjects" section. In total, 61 scans were performed.

The spectroscopy data were exported from the scanner in DICOM format and processed with open source

Tarquin v 4.3.10 software[22] using a customized basis set consisting of only four independent basis functions for NAA (2.008 ppm), Cho (3.208 ppm), Cr (3.027 ppm), and $CrCH_2$.Cr analogue with an extra $–CH_2$ group (3.913 ppm), though the last was not further analyzed. Our long TE spectra in the ACG position did not show any appreciable lipid or lactate signals. Nevertheless, we enabled Tarquin's lipid filter option as a precaution. To facilitate the determination of full-width at half-maximum, we zero-filled the spectra to 16,000 data points. All spectra were processed in command line batch mode, and valid spectra were required to have a signal-to-noise ratio greater than 5 and a goodness-of-fit level lower than 1.5.[22,23]

From this point onward, "NAA" and "Cho" are used in reference to the NAA/Cr and Cho/Cr ratios, respectively.

Statistical Analysis

Mann–Whitney and Fisher's exact test were first used to compare demographics and brain metabolites between the two study groups. These analyses were followed by linear generalized estimation equations (GEEs) in SPSS version 23 to compare the NAA/Cr and Cho/Cr values in subjects with PTS to controls across timepoints. This balanced design, in our context, offered the optimum statistical power (i.e., 1-beta error). We also conducted exploratory analyses with Childhood Trauma Questionnaire (CTQ) scores as a continuous predictor of both brain metabolite ratios.

As a supplementary post hoc procedure (see Online Supplementary Material), we conducted secondary analyses with the GEE approach to (a) evaluate whether the six subjects with PTSD differed from the remaining 13 subjects with PTS but no PTSD (PTSS), (b) explore whether the 10 trauma exposed controls (EC) differed from the remaining 9 controls with no trauma (HC), and (c) evaluate whether the four subgroups (i.e., HC, EC, PTSS, and PTSD) exhibited significant longitudinal changes. Due to the longitudinal design, the GEEs allowed us to simultaneously analyze within-group across-time changes and perform between-group comparisons. Finally, we used paired t-tests to explore the longitudinal changes in the trauma-exposed subjects.

We evaluated the likely confounding effects of sex and age on the ratios via the Kruskal–Wallis test and bivariate Spearman correlations, respectively. We used Fisher's exact test for between-group comparisons of Tanner stages. We defined statistical significance as $p < 0.05$.

Results

In our combined PTS group, 14 children had comorbidities including enuresis (n = 4), encopresis (n = 1), major depressive disorder (n = 5), dysthymia (n = 1), separation anxiety (n = 2), generalized anxiety (n = 1), attentional deficit hyperactive disorder (n = 4), and simple phobia (n = 2). See the Online Supplementary Material for a description of these evaluations. Such comorbidity of psychiatric disorders is commonly reported in maltreated children and adolescents.[24]

The PTS group children were significantly older than the control group children ($t_{(28)} = 2.066$, $p = 0.048$) by approximately one year. Table 1 reports the means, standard deviations, and standard errors of the NAA and Cho values in the PTS and control groups at baseline and follow-up assessments. We observed no significant between-group differences in NAA or Cho ratios. Fisher's exact test revealed no significant between-group differences in Tanner stages.

The Kruskal–Wallis test revealed no significant effect of sex on either ratio at either assessment. Moreover, bivariate Spearman analysis of the ratios and age revealed no significant correlations. The correlation that came closest to significance was that between age and Cho ratios in the follow-up assessments ($r = 0.265$, $p = 0.190$).

Time and Groups Effects on the NAA and Cho Ratios

Table 2 shows the GEE results for the main comparison of NAA and Cho ratios. We found no significant effects of group (subjects with PTS vs. control subjects) or time (baseline vs. follow-up). Adding age as a covariate in the GEE models did not yield any significant effects (Table 3). Online Supplementary Table 1 shows the effects of time and group on NAA and Cho ratios when different group configurations were used in the GEE approach. No significant effects emerged.

Table 4 shows the effect of time exclusively among the 24 trauma-exposed subjects. Paired t-tests revealed no significant results for either ratio.

GEE Adding Age, Group, and Time as Covariates on NAA and Cho

Table 5 shows group difference and time differences, represented by Δ, on NAA and Cho when controlled for age. For both outcomes, group and time effects were not statistically significant.

Subanalysis of CTQ Scores as a Covariate

For the subjects who answered the CTQ, two additional GEEs were performed. Table 5 shows the GEE results for the effects of CTQ scores and time on the ratios. We found no significant effects. Online Supplementary Table 2 shows a Spearman correlation matrix separated by group (subjects with PTS vs. control subjects). This analysis identified a significant negative correlation

between follow-up NAA ratios and follow-up CTQ scores ($r = -0.623$, $p = 0.013$).

Discussion

We conducted the first spectroscopy study to longitudinally examine NAA and Cho ratios in children exposed to multiple types of violence. We aimed to resolve discrepancies in the literature concerning the effects of PTS on NAA and Cho in the brains of children. We found no significant between-group or across-time differences in the ratios and no significant effects of CTQ scores on the ratios except for a significant negative correlation between follow-up NAA ratios and follow-up CTQ assessment scores. More studies are needed to elucidate the pathophysiology of PTSD and improve available treatments and also should compare the clinical prognoses and manifestations of adult and pediatric PTSD.

The inconclusive tendencies in our Cho measurements, both in the between-group comparisons and the apparent but non-significant recovery in follow-up assessments, contradict our expectations. Based on findings for other severe pathologies, we had expected to observe an increase in Cho concentrations over time. Such increases were reported in studies of adults with PTSD,[15] but the physiological processes in children's developing brains may be substantially different from those in adults' brains. Cho metabolism may be more fragile in the developing brain, such that any perturbation in the developmental process effectively reduces Cho levels. De Bellis et al.[20] found no significant differences in Cho ratios when comparing children with PTSD to age-matched HCs. The source of this difference between children and adults remains undetermined. Other metabolites such as glutamate, myoinositol, and GABA may also provide insights into the specific changes in brain metabolism and development associated with pediatric PTSD.

Previous reports speculated that complex physiological and genetic processes may influence Cho ratios in white matter. The difficulties in reproducing such results in gray matter studies may be at least partly explained by the fact that the majority of glial cells are found in the gray matter, disproportionately reducing the Cho concentrations compared to white matter. For example, Villarreal et al.[25] observed white matter lesions in samples from adults with PTSD, and Kaufer et al.[26] found that stress induces long-lasting changes in cholinergic gene expression. Furthermore, Seedat et al.[19] suggested that increased Cho levels in adult women with PTSD should be evaluated in the context of decreased NAA levels, which indicate neuroglial proliferation due to decreased neuronal densities.

De Bellis et al.[20] conducted a ^{1}H-MRS study with 11 maltreated children and adolescents with PTSD and 11 age-matched HCs and found lower NAA ratios in the patients, which is consistent with findings from studies in adults with PTSD.[18] Schuff et al.[27] also investigated brain volume changes in PTSD but explicitly noted that "NAA appears to be a more sensitive marker for neuronal abnormality in PTSD." Nevertheless, the reduced brain volumes reported by many different PTSD studies[28–30] support the hypothesis that PTSD is associated not only with metabolic changes due to cellular damage, as indicated by reduced NAA levels but also with severe neuronal losses, as already suggested by De Bellis et al.[20] One case report[31] described a recovery of NAA levels associated with pediatric PTSD treatment. However, this case involved pharmacological treatment with clonidine, and our patients received psychiatric treatment without clonidine.

Our observation of a negative correlation between follow-up NAA ratios and follow-up CTQ scores carries

Table 2. GEE results for the NAA and Cho ratios and effect of time between-group (PTS and control).

Outcome	Predictors	Wald chi-square	df	p-value
NAA	(CTRL vs. PTS)	0.488	1	0.485
	Time	2.067	1	0.151
Cho	(CTRL vs. PTS)	0.923	1	0.337
	Time	0.680	1	0.410

CTRL: HC + Control ; PTS: PTSS + PTSD; PTS: posttraumatic stress; PTSD: posttraumatic stress disorder; HC: healthy control; GEE: generalized estimation equation.

Table 3. GEE results between effects of CTQ scores and time on the ratios.

Outcome	Predictors	Wald chi-square	df	p-value
Naa/Cr	CTQ	0.593	1	0.441
	Time	3.459	1	0.063
Cho/Cr	CTQ	0.006	1	0.940
	Time	0.940	1	0.332

CTQ: Childhood Trauma Questionnaire; GEE: generalized estimation equation.

Table 4. The effect of time (baseline vs. follow-up) exclusively among 24 trauma-exposed subjects (this includes all subjects from the PTS group, and the EC, but not the HC) for both ratio in a paired-t test.

	Baseline			Follow-up				
Outcome	Mean	N	SD	Mean	N	SD	t	p-value
NAA	1.66	23	0.27	1.72	23	0.27	−0.923	0.36
Cho	0.84	24	0.25	0.84	24	0.23	−0.007	0.99

SD: standard deviation; NAA: N-acetylaspartate.

Table 5. Generalized estimation equation adding age, group, and time as covariates on NAA and Cho.

Outcome	Covariates	B	Standard error	Hypothesis test Wald chi-square	df	p-value
NAA	Group effect (ΔPTS−Control)	−.081	.1095	.545	1	0.461
	Time (ΔBaseline−Follow-up)	−.136	.0809	2.820	1	0.093
	Age	−.013	.0263	.241	1	0.623
Cho	Group effect (ΔPTS−Control)	−.113	.1498	.569	1	0.451
	Time (ΔBaseline−Follow-up)	−.058	.0813	.502	1	0.479
	Age	.001	.0302	.001	1	0.974

Δ: difference; df: degrees of freedom; PTS: posttraumatic stress.

the intriguing implication that the children who were more exposed to traumatic events, such as emotional, physical, or sexual abuse or emotional or physical negligence, had reduced NAA ratios. In 2016, we reported[32] that our analysis of structural brain changes revealed no significant between-group differences or time effects. In the absence of decreased tissue volumes, reduced NAA ratios could indicate uneven neuronal losses without glial tissue losses. They could also indicate neuronal dysfunction without neuronal losses. In this regard, NAA ratio alterations could provide more sensitive detection of neuronal alterations in PTSD than volumetric measures can.

Kitayama et al.[33] reported that right ACC volumes in adults with abuse-related PTSD were significantly smaller than those in HCs. Moreover, NAA is synthesized in mitochondria. It is therefore possible that some traumas alter mitochondrial function and thereby induce the NAA abnormalities observed in our patients. These alterations may reflect or even trigger the PTSD-associated brain volumetric reductions or atrophy reported in the hippocampus,[34–37] amygdala,[38,39] ACC,[39,40] corpus callosum,[41,42] and temporal and frontal gray matter.[43]

Pediatric neuroimaging studies[44–47] have reported linear increases in white matter volumes between the ages of 4 and 20 years. However, they have found that gray matter volumes exhibit a preadolescent increase and a postadolescent decrease with regionally specific peaks. For example, the peak occurs in the frontal and parietal lobes at the age of 12 years, in the temporal lobe at 16 years, and in the occipital lobe around 20 years.

Our study was limited by the small sample of subjects who were sexually abused, such that no meaningful statistical analyses could be performed on this subgroup. Furthermore, the use of expression ratios relative to Cr, the lack of unsuppressed water ratios, and the lack of a measure of PTS severity are limitations of this study. Due to these limitations, only large between-group and across-time effect sizes were identifiable. Another limitation was that the follow-up period was only one year. Furthermore, we only examined NAA, Cho, and Cr. A longer prospective study with more assessments and more neurometabolite measurements, such as for glutamate, myoinositol, and GABA, could better illuminate developmental neurometabolite alterations. However, glutamate and GABA measurements require greater MRI field strengths. We studied child victims of multiple forms of abuse, so future studies should examine alterations in those exposed to specific types of violence.

Conclusion

Structural MRI may be insufficiently sensitive for detecting subtle or short-lived changes in the ACG in pediatric PTSD, but ^{1}H-MRS can detect such changes. Spectroscopy enables testing of the hypothesis that NAA ratio changes in the ACG in pediatric PTSD are related to metabolic changes and cellular damage, possibly including mitochondrial dysfunctions. These metabolite alterations originate from PTSD's chronic nature and can be observed before brain structure alterations. More studies are needed to understand the pathogenesis of PTSD, develop therapies for the cognitive and social impairments in affected children and adolescents, and clarify the distinctive clinical prognosis and manifestations of pediatric PTSD. Understanding altered brain development, its impact on maltreated children, and its potential reversibility remains an important challenge.

Declaration of Conflicting Interests

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Supplemental Material

Supplementary material for this article is available online.

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