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Evaluation of the hippocampus and the anterior cingulate gyrus by proton MR spectroscopy in patients with post-traumatic stress disorder

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PURPOSE
Previous studies have shown that post-traumatic stress disorder (PTSD) is associated with lymbic system dysfunction. The purpose of this study is to evaluate whether or not the neuronal integrity in hippocampus and anterior cingulate gyrus is affected in PTSD as assessed by proton magnetic resonance spectroscopy.

MATERIALS AND METHODS
Single voxel MRS was performed in 10 PTSD patients and 6 healthy subjects in two cerebral areas highly involved in the pathophysiology of PTSD (the hippocampus and the anterior cingulate gyrus). Spectra were obtained using PRESS sequence. Voxel sizes were 3.7 cm^3 (hippocampus) and 6-7.2 cm^3 (anterior cingulate gyrus). Metabolite ratios of NAA/Cr and Cho/Cr were calculated and compared to the control subjects. The severity of PTSD in the patient group was evaluted by Clinician-Administered PTSD Scale.

RESULTS
Analysis of the proton MR spectra showed reductions in NAA/Cr ratio in bilateral hippocampus of PTSD subjects as compared to normal controls ($p<0.001$), whereas Cho/Cr ratios were increased ($p<0.001$). Reductions in NAA/Cr ratio were found in the the anterior cingulate gyrus of PTSD subjects as compared to normal controls ($p<0.01$), whereas Cho/Cr ratios did not differ significantly ($p>0.05$).

CONCLUSION
Changes in the metabolite ratios provide support for either neuronal dysfunction or neuronal loss both in the hippocampus and the anterior cingulate gyrus and may be associated with reduced neuronal integrity. Further studies with MRS in larger patient populations are needed to clarify the relationship between brain structures and neurobiology of PTSD.

Key words: • *stress disorders, post-traumatic* • *magnetic resonance spectroscopy* • *hippocampus* • *gyrus cinguli*

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Post-traumatic stress disorder (PTSD) is an anxiety disorder observed in people who have been exposed to severe emotional or physically life-threatening traumatic events like war, sexual abuse, and natural disasters. PTSD patients live with psychiatric symptoms, which include reexperiencing the trauma in their thoughts or dreams, avoidance of trauma-related thoughts and events, and numbing for many years (1, 2).

Proton magnetic resonance spectroscopy (MRS) is a noninvasive method of investigation that reveals metabolic changes of the living brain. MRS is capable of showing biochemical evidence of underlying neural processes, even when cerebral anatomic changes are not evident.

In previous MRS studies on PTSD patients, a decrease in the N-acetylaspartate/creatine (NAA/Cr) ratio in the medial temporal lobe and hippocampus, or a decrease in hippocampal NAA concentration have been reported (3-5). In positron emission tomography (PET) studies on these patients, dysfunction of the anterior cingulate gyrus (ACG) was reported (6). However, metabolic changes in ACG among adult PTSD patients have not yet been studied with MRS.

Materials and methods

Among the patients with a possible diagnosis of PTDS that were evaluated by an expert psychiatrist, ten meeting the diagnostic criteria of the disease according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV, American Psychiatric Association) were included in the study (7). Exclusion criteria were alcohol or substance abuse during the last six months, use of drugs of any kind (to exclude any possible psychotropic effect), claustrophobia that would hinder the ability to undergo MR examination, and known contraindications to MR imaging such as pregnancy and the presence of metal prostheses, implants, etc. Symptomatology and symptom severity were assessed with the Clinician-Administered PTSD scale (8).

The Clinician-Administered PTSD scale (CAPS-total) consists of 17 items that are evaluated by interviewers. These items include core symptoms of PTSD determined by DSM-IV criteria. These symptoms consist of the reexperience of traumatic events in the form of irritating thoughts, flashbacks, and distressing dreams that are measured with the CAPS-B subscale; avoidance of trauma-related thoughts and events, and restricted emotions that are measured with the CAPS-C subscale; and signs of arousal such as sleep disorders, uneasiness, and hypervigilance, which are measured with CAPS-D subscale. Items that investigate the frequency and the severity of the symptoms, such as guilt over behaviors that have or have not been expressed, guilt over surviving the traumatic event, depersonalization, and a decrease in the sense of awareness of the environment have been added to the scale under the

heading "accompanying symptoms of PTSD". CAPS also includes five additional scales to evaluate social and vocational functioning, general severity, changes in the severity of the symptoms, and validity of patient feedback. CAPS affords clinicians the opportunity to evaluate PTSD for the last month or for a lifetime.

Seven out of the 10 patients were male, 3 were female. Patients were between 21 to 52 years of age. The control group consisted of 6 people (3 males and 3 females) between 23 and 45 years of age. Patients were exposed to the following events: six patients had been involved in antiterrorist combat, one patient had witnessed the death of his/her friend in a fire, one patient had been sexually abused during childhood, and one patient had been trapped under a landslide in an earthquake. The mean duration of the PTSD was 3.5 years (range, 1.5-15 years). The study was approved by the local ethical committee of Zonguldak Karaelmas University and informed written consents of the patients were obtained.

MR examinations were conducted in a 1.5 T scanner (Gyroscan Intera, Philips, Best, the Netherlands). A standard head coil was used for conventional MR imaging and MRS. Conventional imaging was performed in the transverse plane, using T1 weighted (560/15 ms) and fluid attenuated turbo inversion recovery (FLAIR; 6000/100 ms) sequences, and in the transverse, sagittal, and coronal planes in T2 weighted (3000-3400/110 ms) sequences.

The five point visual scale, originally developed by Scheltens et al., was used to assess hippocampal atrophy. In this method, a score of 0 corresponded to a normal hippocampus, whereas a score of 4 corresponded to severe atrophy (9, 10).

In single-voxel proton MRS examinations, an average of 128 measurements were acquired from 512 data points by using PRESS sequence (2000/136 ms). Two different MRS studies were performed. In one of them, a 30x11x12 mm^3 voxel was placed on the hippocampus using three planar T2 weighted images. In the other study, 30x20x10 mm^3 or 30x15x16 mm^3 voxels were placed on the ACG (Figure 1, 2). Chemical shift selective saturation pulse (CHESS) method was used to suppress the water. Spectral bandwidth was 1000 Hz. Automated shimming option was used to homogenize the local magnetic field. A special shimming method, provided by the manufacturer to be used on small structures, was used for hippocampi. After acquiring the MR spectrum and performing manual baseline correction, 4k zero filling, 3 Hz Gaussian line broadening, and the Fourier transformation, metabolite peaks were determined. Areas under N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) peaks were measured by using the standard software of the manufacturer. NAA/Cr and Cho/Cr rates were used in the statistics.

Mann-Whitney-U test was used to make comparisons of metabolite ratios between the patient and control groups. A p value under 0.05 was accepted as the level of significance.

Table 1. Metabolite ratios of both hippocampi and anterior cingulate gyrus, measured with single voxel MRS method, in patients and controls

	Patients	Controls	p value
Right HP NAA/Cr	1.22	1.45	<0.001
Right HP Cho/Cr	1.20	1.05	<0.01
Left HP NAA/Cr	1.20	1.41	<0.001
Left HP Cho/Cr	1.17	1.01	<0.01
ACG NAA/Cr	1.32	1.50	<0.01
ACG Cho/Cr	1.08	0.99	>0.05

HP: hippocampus, ACG: anterior cingulate gyrus, NAA: N-acetylaspartate, Cho: choline, Cr: creatine

Table 2. CAPS subscale scores and metabolite ratios of the patient group (mean values of the data from both hippocampi were used)

Patient No.	Reexperiencing	Avoidance-numbness	Arousal	Total	HP NAA/Cr	HP Cho/Cr	ACG NAA/Cr	ACG Cho/Cr
1	17	12	12	41	1.16	1.24	1.33	1.04
2	13	16	16	45	1.08	1.32	1.18	1.13
3	12	14	14	40	1.06	1.18	1.16	1.24
4	16	16	8	40	1.11	1.30	1.28	1.12
5	23	24	23	70	1.14	1.20	1.19	1.13
6	36	30	34	100	1.34	1.12	1.42	1.02
7	29	33	30	92	1.29	1.08	1.36	1.00
8	28	26	26	80	1.30	1.09	1.48	0.99
9	21	19	20	60	1.25	1.12	1.34	1.10
10	27	25	24	76	1.24	1.25	1.42	1.04
Mean	22.20	21.50	20.70	64.40	1.21	1.19	1.32	1.08

CAPS: Clinician-Administered PTSD scale, HP: hippocampus, ACG: anterior cingulate gyrus

Results

In conventional MR imaging, we did not observe hippocampal atrophy in any of the subjects. Accordingly, hippocampal atrophy scores were found to be 0 in 7 patients and 1 in 3 patients. When compared with the controls, NAA/Cr ratios in both hippocampi and ACGs were found to be significantly lower in the patient group ($p<0.001$ for each hippocampus, and $p<0.01$ for ACG) (Table 1, Figures 1 and 2). The Cho/Cr ratio of hippocampi was higher in patients than in controls ($p<0.01$), whereas no significant difference was found between the two groups regard-

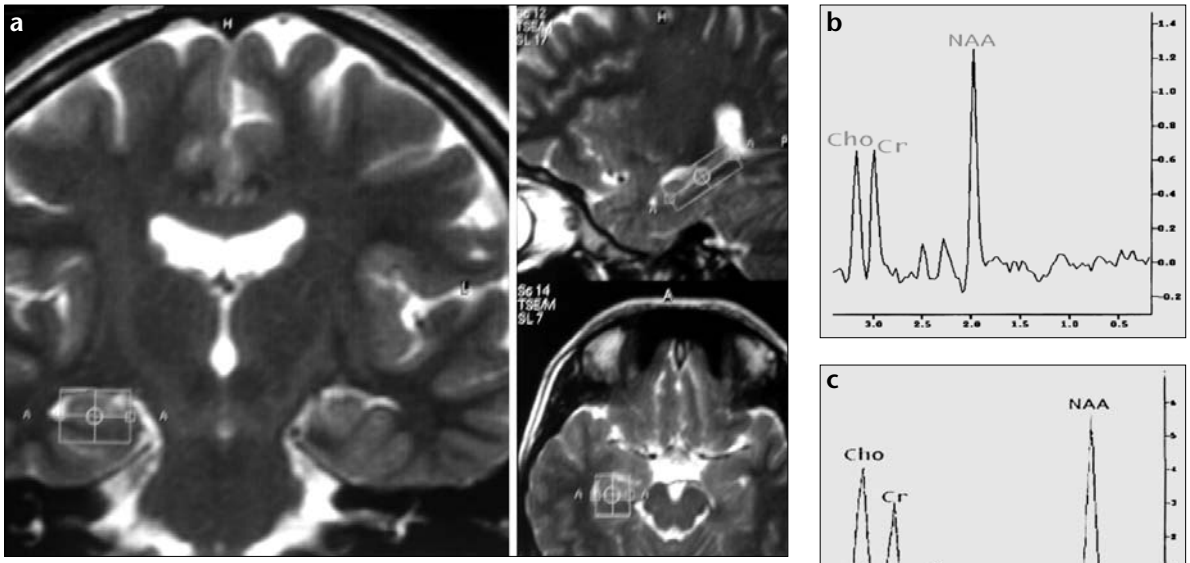

Figure 1. a-c. The localization of the voxel on the hippocampus is shown on coronal, sagittal, and transverse T2 weighted images **(a)**. The corresponding spectral sample of a patient with PTSD **(b)** reveals a decreased NAA/Cr ratio and an increased Cho/Cr ratio in comparison to the spectrum of a healthy subject **(c)**.

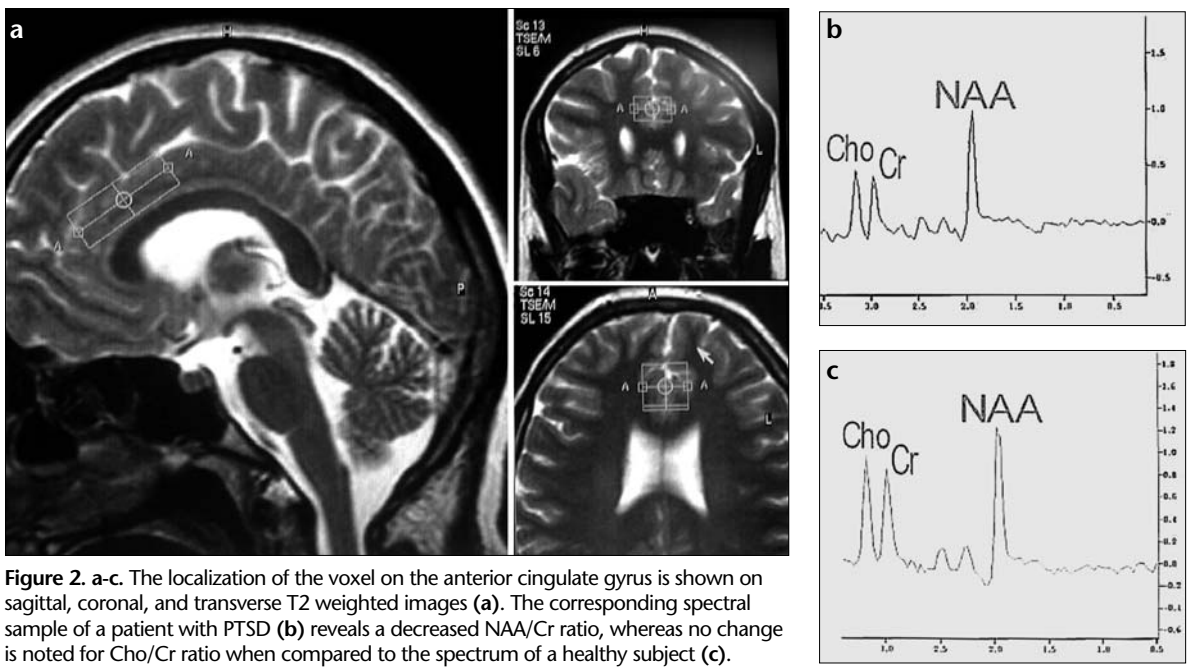

Figure 2. a-c. The localization of the voxel on the anterior cingulate gyrus is shown on sagittal, coronal, and transverse T2 weighted images **(a)**. The corresponding spectral sample of a patient with PTSD **(b)** reveals a decreased NAA/Cr ratio, whereas no change is noted for Cho/Cr ratio when compared to the spectrum of a healthy subject **(c)**.

ing Cho/Cr ratios in ASGs ($p > 0.05$) (Table 1).

Psychiatric symptoms scored with CAPS are shown in Table 2. The mean CAPS score was 64.4 (range, 40-100). The mean CAPS-B subscale (reexperiencing) score was 22.2 (range, 12-36), the mean CAPS-C subscale (avoidance) score was 21.5 (range, 12-32), and the mean CAPS-D subscale (arousal) score was 20.7 (range, 8-34). No significant correlation was found between metabolite ratios and CAPS scores.

Discussion

PTSD, also popularly called "Vietnam syndrome", is an anxiety disorder. It consists of a specific group of symptoms observed in subjects who have been exposed to severe emotional or physically life-threatening traumatic events. The most frequently encountered causes are events such as war combat, childhood sexual abuse, and natural or human disasters. These events generally cause despair that is accompanied by fear and terror. Some people who are exposed to traumatic events of these kinds repeatedly reexperience the original trauma in their dreams or thoughts. In addition to the symptom of reexperience, they show many emotional, cognitive, and behavioral psychiatric symptoms, like refraining from everything that reminds them of the traumatic event, numbing, and being startled or alert, as if the event may reoccur at any time (1, 2).

Electrophysiological and neurochemical studies, including animal models, have provided findings on the neurobiological nature of PTSD.

Cerebral imaging studies to reveal the relationship between the limbic structures of the brain and PTSD have been a focus of research (11). The hippocampus, the most important structure of the limbic system, has three main functions. Of these, the one related to the memory is well known. Sustaining the normal diurnal rhythm and playing a role in stress response via the hypothalamo-pituitary-adrenal axis are other functions. Furthermore, the hippocampus is the structure that processes emotional information. The most active structure in encoding and recalling traumatic memories is the hippocampus. The anterior cingulate plays a role in the modulation of conditional fear responses.

With the advent of cerebral imaging techniques like PET and MR imaging, we now have the opportunity to investigate the pathogenesis and the pathophysiology of PTSD more thoroughly. In prior studies of PTSD, hippocampal volume differences were shown with MR imaging, and increased blood flow in limbic structures was shown with PET (14-16).

Proton MRS is a radiological method that is increasingly used in the investigation of neuropsychiatric disorders. In our study, a decrease in the NAA/Cr ratio of the hippocampus was observed in PTSD patients when compared to healthy controls (Figure 1b, c). This finding was compatible with prior MRS studies (3). In similar MRS studies, in which absolute hippocampal NAA concentrations were measured, NAA concentrations in PTSD patients were found to be decreased (4, 14, 16). Although the function of NAA is not well known, it is known that it exists abundantly in neural tissues, that it is synthesized in neural mitochondria, and that it is transported into cytosoles. In comparative group studies, Cr and its ratio (NAA/Cr) were presented and a decrease in the NAA/Cr ratio was interpreted to be an indicator of neuronal tissue damage (3).

In some studies, a hippocampal volume loss in PTSD was observed; however, several others were in coherence with our study in which no volume loss was observed (4). This may be explained by the proliferation of glial tissue compensating for the neural volume loss. Moreover, some of the prior studies included alcoholic and/or depressive patients. These two situations might partially be responsible for hippocampal atrophy (14).

In PTSD, NAA loss resulting in a decrease in the NAA/Cr ratio may primarily be the result of a neuronal dysfunction. Thus, transitory NAA loss has been reported in patients with amyotrophic lateral sclerosis (17) and multiple sclerosis (19), in patients who underwent epilepsy surgery (18), and in schizophrenic patients who were treated with antipsychotic agents (20). These changes are considered to be related to temporary disorders in oxidative metabolism, upon which NAA production depends (4). Therefore, hippocampal NAA decrease in PTSD may be a sign of neuron loss and gliosis, as well as a sign of neural metabolic damage.

In the context of the relevant literature, the increased hippocampal Cho/Cr ratio we found in PTSD patients may be explained by the decreased Cr level, because an active gliotic reaction to increase the Cho level is not expected in chronic PTSD (4). Moreover, in a study in which metabolite concentrations were measured, no change in Cho level was found, but decreases in NAA and Cr levels were (4). Cho level is known to increase in multiple sclerosis and tumor cells. The reason for this is the increased membrane damage and glial proliferation. In chronic diseases like PTSD, Cho increase is not expected unless there is a continuous and adequate gliotic process that follows the neural damage (4). Unfortunately, we have not measured metabolite concentrations; therefore, we can not eliminate the possibility of Cho increase.

To the best of our knowledge, there is no MRS study in the literature on the ACGs of adult PTSD patients. In an MRS study that was performed on child and adolescent PTSD patients in the post-treatment period, ACG NAA concentrations were reported to have decreased (21). We think that the decreased ACG NAA/Cr ratio in our patients was caused by the loss of the neural tissue, similar to the loss encountered in the hippocampus (Figure 2b, c).

Metabolic changes in the hippocampus and ACG may be related to the cellular damage originating from the chronic nature of PTSD. Acute stress is a physiological response of the hypothalamo-pituitary-adrenal axis and is mediated by monoaminergic neurotransmitters (22). During the chronic period, this results in an increase of glucocorticoid level in the brain and plasma. Sustained levels of glucocorticoids were found to cause neural damage in the hippocampus (23, 24).

The most important limitation of the present study was the small number of patients. However, metabolite ratios in the patients were significantly lower in the hippocampus and ACG. Another limitation of the study was the lack of absolute measurements of metabolite concentrations. However, many studies of this type had only utilized ratio measurement. Metabolite ratios in the hippocampus and ACG were significantly abnormal in patients, a finding that points to the disruption of the neuronal integrity. However, it seems difficult to determine which metabolite(s) play(s) a primary role in neuronal disintegration, and at which rate. The relationship between these abnormalities and PTSD is also not clear. Whether the course of the disease causes these changes or existing intraneuronal changes predispose to disease is open to discussion.

In conclusion, not only the hippocampus, but also the ACG, another part of the limbic system, is affected in PTSD. Metabolites that can be detected with MRS are more accurate pathophysiological indicators of PTSD than volume loss. Significant changes in metabolite ratios may be observed long before structural changes occur. The pathophysiological changes observed in both structures, without accompanying morphological changes, may be responsible for PTSD's clinical picture. It is necessary to repeat similar studies in larger and more homogeneous series, and to investigate the relation between the above-mentioned parameters and symptoms.

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