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Metabolite alterations in the hippocampus of high-functioning adult subjects with autism

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Abstract

The aim of the present study was to investigate metabolite alterations in the hippocampal formation as they relate to aggression in high-functioning adults with autism. We measured concentrations of *N*-acetylaspartate (NAA), choline-containing compounds (Cho), and creatine plus phosphocreatine (Cr + PCr) in the hippocampal formation by proton magnetic resonance spectroscopy in 12 non-medicated male subjects with autism and 12 age- and sex-matched controls. Aggression was scored in the autistic subjects using the Buss–Perry Aggression Questionnaire. The concentrations of Cho and Cr + PCr in the hippocampal formation in autistic subjects were significantly higher than the corresponding values in control subjects, and a significant positive correlation was observed between the concentrations of these metabolites in the hippocampal formation and scores on the Buss–Perry Aggression Questionnaire in autistic subjects. Results suggest that high-functioning adult subjects with autism have abnormal metabolite concentrations in the hippocampal formation, which may in part account for their aggression.

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Introduction

Autism is a neurodevelopmental disorder characterized by qualitative impairment of social interaction and communication, as well as by restricted repetitive and stereotyped patterns of behaviours, interests, and activities. In addition to the core features of autism, there are common comorbid psychiatric symptoms such as anxiety, depression, and aggression (Matson & Nebel-Schwalm, 2007). The presence of aggressive behaviour can reduce the effectiveness of treatment interventions, and many individuals with autism remain significantly impaired. Therefore, from a treatment

perspective, it would be advantageous to clarify the underlying mechanism for the development of aggression in individuals with autism. The brain structures that are involved in the control of aggression include limbic structures such as the amygdala, hippocampus (Gregg & Siegel, 2001), and the cerebellum (Berman, 1997). Interestingly, the most significant findings from post-mortem studies of autism have been confined to regions of the limbic system and the cerebellum, e.g. reduced neuronal cell size and increased cell packing density in the hippocampus (Bauman & Kemper, 2005), shrinkage of nuclei of the amygdala (Schumann & Amaral, 2006), and reduced Purkinje cell density in the cerebellum (Bailey *et al.* 1998; Bauman & Kemper, 2005; Ritvo *et al.* 1986). However, the correlation between structural abnormalities associated with dysfunction in these structures and aggression in autism has not yet been clearly established.

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Proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) is a non-invasive tool for evaluating neurochemical changes related to the clinical characteristics of psychiatric disorders. $^1\text{H-MRS}$ produces spectra that represent concentrations of *N*-acetylaspartate (NAA), a marker of neuronal integrity (Clark, 1998); choline-containing compounds (Cho), markers of cell number and/or membrane turnover (Miller *et al.* 1996); and creatine plus phosphocreatine (Cr+PCr), a marker of overall (i.e. neuronal plus glial) cellular density (Sartorius *et al.* 2008). Of the 35 post-mortem cases of autism reported in the previous studies mentioned above, 26 had died aged ≥ 19 yr. It is expected that $^1\text{H-MRS}$ study of the brain of autistic adults will be a helpful initial step in clarifying the involvement of the limbic system and cerebellum in the expression of aggression by individuals with autism. However, previous $^1\text{H-MRS}$ studies of the limbic system and cerebellum of autistic patients have primarily focused on children (i.e. patients aged < 20 yr) and have been confounded by the inclusion of learning-disabled subjects and subjects with seizure disorders. In this regard, the only exception was a study by Page *et al.* (2006), in which the authors examined high-functioning adults with autism and reported a significant increase in Cr+PCr levels in the amygdala-hippocampal region. However, in that study, the relationship between neurobiological changes and clinical features was not examined. Furthermore, the putative roles of the amygdala and hippocampus in the expression of aggression appeared to differ (Gregg & Siegel, 2001), although these two regions are closely interconnected. To date, there has been no report of a $^1\text{H-MRS}$ evaluation of concentrations of metabolites in the cerebellum of adult subjects with autism.

In the present study, we hypothesized that alterations of metabolites may be present in the hippocampus and cerebellum of high-functioning adults with autism, and such metabolite alterations could be correlated with the aggression exhibited in a subset of these patients. To test these hypotheses, we recruited non-medicated, high-functioning male adults with autism in order to measure metabolite concentrations in the hippocampal formation (not including the amygdala) and cerebellum of these subjects using $^1\text{H-MRS}$. In addition, we examined the correlation between metabolite levels and aggression in autistic adults.

Methods

The Ethics Committee of the Hamamatsu University School of Medicine approved the study. Each

participant gave written informed consent after being given a complete description of the study. We conducted the Wechsler Adult Intelligence Scale – Revised (WAIS-R) to assess the intelligence quotient (IQ) of all participants. The diagnosis of autism was made according to the Japanese version of the Autism Diagnostic Interview – Revised (ADI-R; Lord *et al.* 1994) and the Autism Diagnostic Observation Schedule (ADOS; Lord *et al.* 2000). Control subjects were recruited from the community by advertisement. All autistic and control subjects were screened to exclude psychiatric illnesses (i.e. schizophrenia, affective disorders, mental retardation, and personality or behavioural disorders) by means of the Structured Clinical Interview for DSM-IV (SCID). We excluded individuals with epilepsy, with psychotropic medication, and those with mental retardation as defined by a full-scale IQ of < 70 . Twelve male subjects with autism and 12 age-matched healthy male controls were included in the present study. All participants were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). We evaluated aggression in the participants using the Japanese version of the Aggression Questionnaire (Ando *et al.* 1999). The original version of the Aggression Questionnaire (Buss & Perry, 1992) is a 29-item, self-administered test designed to measure aggression (i.e. physical aggression, verbal aggression, anger, and hostility) as a personality trait. The validity and reliability of the Japanese version of the Aggression Questionnaire has previously been established (Ando *et al.* 1999).

Participants were scanned using a GE 1.5 T magnetic resonance scanner at the Hamamatsu University Hospital. We selected two volumes of interest (VOIs) for each subject, i.e. the left hippocampal region and the right cerebellar hemisphere (Fig. 1*a–c*). The rationale for the selection of two VOIs was as follows: (1) since all the participants were right-handed, the left hippocampus and the right cerebellum were regarded as dominant (Jansen, 2005); (2) although there is no known direct projection from the hippocampus to the cerebellum, most of the efferents from the temporolimbic region send information to the contralateral cerebellar cortex via the cortico-ponto-cerebellar pathway (Schmahmann, 2000); and (3) left-sided abnormalities have been associated with aggression in humans (Tebartz van Elst *et al.* 2000; Zetzsche *et al.* 2007). To establish these VOIs, whole-brain images were acquired with a 3D fast-spoiled gradient-echo imaging protocol. A sagittal scout image was used to select oblique coronal slices perpendicular to the long axis of the hippocampal formation (Fig. 1*a*). A slanted rectangular VOI ($2 \times 2 \times 1.5 \text{ cm}^3$) was selected such

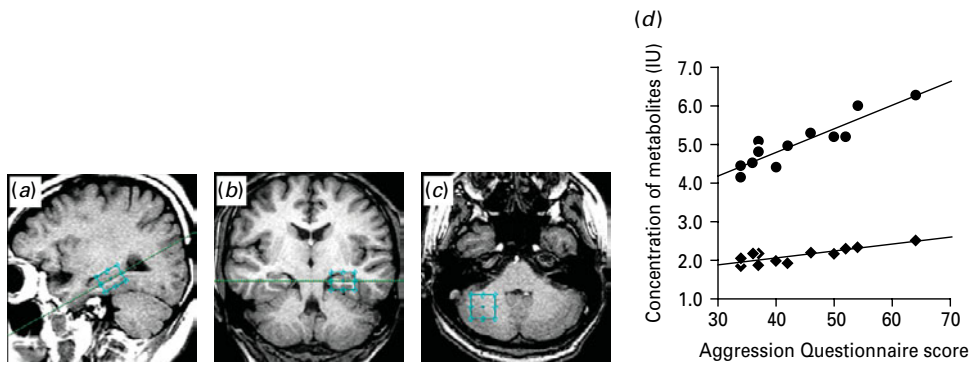


Fig. 1. (a–c) Locations of a 6-ml volume of interest (VOI) in the left hippocampus (a, b) and of an 8-ml VOI in the right cerebellum (c). (d) Choline-containing compounds (\blacklozenge ; Cho) and creatine plus phosphocreatine (\bullet ; Cr+PCr) concentrations in the hippocampus of autistic subjects. Both values showed a significant and positive correlation with the Aggression Questionnaire score (Cho: $\rho=0.719$, $p=0.008$; Cr+PCr: $r=0.884$, $p<0.001$).

that it was aligned along the long axis of the hippocampus, starting just posterior to the amygdala (Fig. 1a, b). The centre of the VOI was at the cornu ammonis of the left hippocampus; as a result, the VOI contained the hippocampus proper and a portion of the parahippocampal gyrus. A cerebellar VOI ($2 \times 2 \times 2 \text{ cm}^3$) was placed on one axial image, which showed the full length of the middle cerebellar peduncle (Fig. 1c). To explore metabolite levels in the cerebellar cortex, we excluded the dentate nucleus from each VOI; the medial side of the VOI was just adjacent to the lateral margin of the dentate nucleus, which was determined by axial T_2 -weighted images. A point-resolved spectroscopy (PRESS) spectrum [repetition time (TR)=1500 ms, echo time (TE)=144 ms, 256 averages] was obtained after chemical shift selective water suppression. To determine the tissue composition of the VOI, fast-spoiled gradient-echo images were segmented into white matter, gray matter, and cerebrospinal fluid (CSF) using the software package Dr View/Linux. We measured the concentrations of NAA, Cho, and Cr+PCr using the LC model algorithm and corrected the concentrations for the proportion of CSF within the VOI.

Statistical analyses were performed using two-tailed Student's t test, Spearman's ρ correlation coefficient, and Bonferroni's correction. The level of significance was set at $p<0.05$.

Results

The demographic characteristics of the autistic and control subjects are shown in Table 1. There was no significant difference in the distribution of age or full-scale IQ between the two groups, indicating that

subject matching was successful. As shown in Table 1, there were no significant inter-group differences in the compositions of gray matter, white matter, and CSF within either of the two VOIs. In the hippocampus, the autistic group had significantly higher concentrations of Cho ($p<0.001$) and Cr+PCr ($p<0.001$) compared to control subjects (Table 1). In the cerebellum, the concentration of NAA in the autistic group was significantly lower than that in the control group ($p<0.001$, Table 1). We performed a Spearman's correlation analysis to determine whether the concentrations of Cho and Cr+PCr in the hippocampus and NAA in the cerebellum were correlated with autistic subjects' scores on the Aggression Questionnaire. Both Cho ($p=0.008$) and Cr+PCr ($p<0.001$) concentrations in the hippocampus, but not the NAA concentration in the cerebellum, were significantly positively correlated with the Aggression Questionnaire scores (Fig. 1d). There was no correlation between metabolite alterations (i.e. hippocampal Cho and Cr+PCr, and cerebellar NAA) and IQ or autistic symptoms as assessed by ADI-R (data not shown).

Discussion

In the present study, the observed increase in the Cr+PCr concentration in high-functioning subjects with autism was compatible with the results of a previous ^1H -MRS study by Page *et al.* (2006), who measured the concentration of this metabolite combination in the right amygdala–hippocampal complex in autistic adults of normal intelligence. We also found an elevation of Cho in the hippocampal formation (not including the amygdala) of high-functioning adults with autism, although it should be noted that the

Table 1. Demographic characteristics and ¹H-MRS measurements of the hippocampus and cerebellum in subjects with autism and controls

	Control (<i>n</i> = 12)	Autism (<i>n</i> = 12)	<i>t</i> value	<i>p</i> value
Age (yr)	22.3 ± 1.8 (19–24)	22.0 ± 2.2 (18–25)	0.384	0.705
Full-scale IQ	105.2 ± 12.6 (85–125)	96.3 ± 14.0 (71–117)	1.645	0.114
ADI-R (Diagnostic algorithm)				
(A) Social	n.a.	20.9 ± 5.3 (12–28)		
(B) Communication				
Verbal	n.a.	14.2 ± 5.4 (4–22)		
Non-verbal	n.a.	8.4 ± 4.0 (2–14)		
(C) Stereotype	n.a.	5.5 ± 2.0 (3–10)		
Aggression Questionnaire	n.a.	45.3 ± 9.4 (34–64)		
Hippocampus				
NAA (IU)	7.79 ± 1.57	8.14 ± 0.84	−0.672	0.509
Cho (IU)	1.87 ± 0.21	2.19 ± 0.17	−4.176	<0.001*
Cr + PCr (IU)	3.80 ± 0.97	5.16 ± 0.59	−4.124	<0.001*
Gray matter (%)	64.5 ± 6.9	65.6 ± 7.3	0.394	0.866
White matter (%)	33.9 ± 8.3	32.2 ± 7.5	0.609	0.769
Cerebrospinal fluid (%)	1.6 ± 2.0	2.2 ± 1.9	0.215	0.880
Cerebellum				
NAA (IU)	9.01 ± 0.50	8.29 ± 0.71	3.949	<0.001*
Cho (IU)	2.12 ± 0.18	2.07 ± 0.22	0.505	0.618
Cr + PCr (IU)	6.08 ± 0.56	5.85 ± 0.63	0.909	0.373
Grey matter (%)	71.6 ± 9.2	71.8 ± 8.0	0.620	0.495
White matter (%)	28.2 ± 9.2	27.7 ± 8.9	0.154	0.448
Cerebrospinal fluid (%)	0.0 ± 0.0	0.1 ± 0.0	0.031	0.084

ADI-R, Autism Diagnostic Interview – Revised; n.a., not applicable. NAA, *N*-acetylaspartate; Cho, choline-containing compounds; Cr + PCr, creatine plus phosphocreatine; IU, institutional unit.

Values are expressed as means ± S.D. (range).

*Statistically significant difference, as determined by two-tailed Student's *t* test.

study by Page *et al.* (2006) did not demonstrate such changes in Cho level. The discrepancy between the two studies presumably may reflect a difference in VOI placement, i.e. the Page *et al.* study included the amygdala, whereas our study did not. The ¹H-MRS Cho peak is known to be caused by free choline, phosphocholine (components of membrane synthesis), and glycerophosphocholine (a product of the degradation of membrane phosphatidylcholine). Therefore, an increase in the Cho concentration has been interpreted as representing increased membrane synthesis and/or membrane disruption in processes including tumour growth, demyelination, and gliosis (Miller *et al.* 1996). An elevation of Cr + PCr on ¹H-MRS reflects high-energy phosphate metabolism (Sartorius *et al.* 2008). Therefore, the elevation of Cho and Cr + PCr levels observed here suggests active, viable neuronal turnover in the hippocampal region of autistic adults. Previous structural MRI studies (Rojas *et al.* 2004; Schumann *et al.* 2004) have demonstrated enlarged

hippocampal volume in autistic adults of normal intelligence. However, since in our sample no changes were observed in the NAA concentration in the hippocampal region, it remains unclear whether or not elevated Cho and Cr + PCr levels are associated with changes in hippocampal volume. Further studies are required to clarify this issue.

Both Cho and Cr + PCr concentrations in the left hippocampal region were significantly and positively correlated with trait aggression, as assessed by the Aggression Questionnaire administered to our adult subjects with autism. To the best of our knowledge, this is the first report to describe a link between metabolite alterations in the hippocampal formation and a clinical feature of autism. There is no available data to account for the mechanism by which the hippocampus affects the trait of aggression in autism. However, the accumulated evidence does suggest that the hippocampal formation modulates aggression in animals. For instance, electrical stimulation of the

dorsal or ventral hippocampus has been shown to inhibit or facilitate, respectively, aggressive behaviour in cats (Gregg & Siegel, 2001). Electrophysiological recordings have revealed that aggressive behaviours are associated with increased frequency in hippocampal discharge patterns in rabbits (Fontani & Vegni, 1990). Furthermore, the present findings agree with the results of previous clinical studies indicating that left hemispheric lesions may be associated with a higher risk for the development of aggression (Tebartz van Elst *et al.* 2000). Zetzsche *et al.* (2007) reported that increased lifetime aggression in patients with borderline personality disorder was significantly correlated with volume of the left, but not the right, hippocampus. Taken together, these findings suggest that the elevations in the Cho and Cr+PCr concentrations observed here reflect altered function in the hippocampal formation, which in turn was found to be associated with aggression in adult subjects with autism. Since it is still unclear whether the functional alterations in the hippocampal formation are causative of aggression in people with autism, further studies are needed.

As assessed by ¹H-MRS, the concentration of NAA in the cerebellum was significantly lower in autistic adults than controls. This finding is comparable with that of previous ¹H-MRS studies of autistic children, who exhibited a similar reduction in metabolite concentrations in the cerebellum (Chugani *et al.* 1999; Otsuka *et al.* 1999). Since post-mortem studies have repeatedly demonstrated decreased Purkinje cell density in the cerebellum (Bailey *et al.* 1998; Bauman & Kemper, 2005; Ritvo *et al.* 1986), and since a decrease in the ¹H-MRS NAA peak is a putative marker of neuronal loss (Clark, 1998), it is possible that the decreased NAA concentration observed here reflects a relative decrease in the number of Purkinje cells in the cerebellum of high-functioning subjects with autism.

There are some limitations of our study. The small sample size renders the data presented here preliminary, and a larger study with more subjects with autism will be necessary. However, recruitment for the present study was limited to a group of high-functioning subjects with autism, all of whom were given no psychotropic drugs, and all were able to complete magnetic resonance spectroscopy without sedation. Therefore, we believe that our data are free from possible confounding factors and thus may reflect a certain common pathology in people with autism. Other limitations of this study include the following: (1) the lack of an assessment of the right hippocampal formation; (2) inclusion of not only the hippocampus proper, but also the parahippocampal gyrus in the hippocampal VOI; and (3) a relatively

short TR acquisition period in the ¹H-MRS method, which could have affected metabolite levels. Furthermore, we did not include autistic adults without aggression as a comparison group, which might have been useful to test our hypothesis. These limitations should be factored into any interpretation of the present results. Nevertheless, in the existing literature, few studies to date have combined results from neuropsychological tests with neurometabolic levels in specific brain areas, which is a main advantage of our study.

In conclusion, although the sample size was small, our findings suggest that high-functioning people with autism have metabolite alterations in the cerebellum and hippocampus, and the latter in particular may play an important role in the expression of aggression in individuals with autism.

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Statement of Interest

None.

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