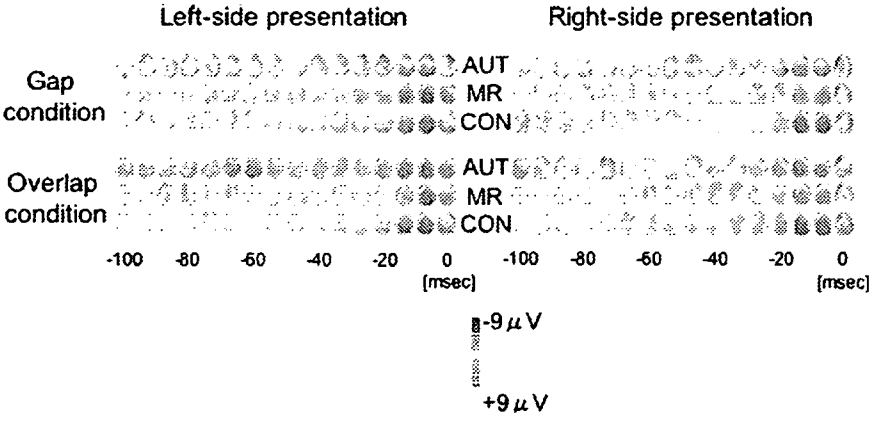

Fig. 4. ERP map series for each condition. AUT, autism ($N = 16$); MR, mental retardation ($N = 17$); CON, control ($N = 14$).

positivity represents inefficiently more resource for attentional disengagement in the autism group. In other words, higher and longer electric activities as indexed by pre-saccadic positivity would be necessary for the autistic patients to reach a threshold for executing the saccade.

Additionally, a clear differentiation between autism and mental retardation groups suggests that this attentional dysfunction is not a mere consequence of general cognitive disability. Since there was no difference in SRT or correct response rate between autism and mental retardation groups, we may argue against the possibility that the observed neurophysiological difference between groups is merely attributable to the difference in task performance. Furthermore, we found that individuals with autism show attention deficits in the overlap condition but not in the gap condition. The attentional system is thought to be comprised of two mechanisms: one is the exogenous component that is thought to be triggered reflexively by external stimuli; and the other is the endogenous component that is thought to be controlled by internal, volitional, or central executive mechanisms (Posner, 1980). Under the gap condition, where attention on the central stimulus is disengaged automatically by the disappearance of that stimulus, exogenous disengagement occurs, while under the overlap condition, where attention is disengaged intentionally as the central stimulus remains, endogenous disengagement occurs (Fischer and Weber, 1993). Developmental studies of attentional disengagement (Hood and Atkinson, 1993; Matsuzawa and Shimojo, 1997) have suggested that the inability of young infants to disengage their attention from a stimulus on which they have fixated is responsible for their difficulty in visual orientation. Different developmental time courses were shown for the gap and overlap disengagement, with early maturation of the gap disengagement, i.e. exogenous disengagement, and later maturation of the overlap disengagement ability, i.e., endogenous disengagement. Taken together with the fact that the exogenous attentional disengagement normally occurred in the gap condition in the autism since the central stimulus to which the participants attended disappeared before the peripheral stimulus appeared, the present study suggests that individuals with autism had specific deficits in endogenous attentional disengagement.

According to Posner's model, the disengagement component of visuospatial attention is associated with function of the parietal cortex. Recent neuroimaging evidence has also suggested that parietal cortex is important in visually guided shift of spatial attention (Kincade et al., 2005; Shomstein and Yantis, 2006). Thus, dysfunction in attentional disengagement could be attributable to parietal abnormality proposed in autism (Courchesne et al., 1993; Minshew et al., 1999; Belmonte and Yurgelun-Todd, 2003; Haist et al., 2005; McAlonan et al., 2005). However, the t-maps for group comparison under the overlap condition showed that pre-saccadic positivity for 100–70 ms occurred significantly higher in the individuals with autism than in the other two groups at broad scalp areas. EEG recording has a limitation in knowing precise anatomical location for the generator due to volume conductance of the intervening tissues. Future studies possibly using simul-

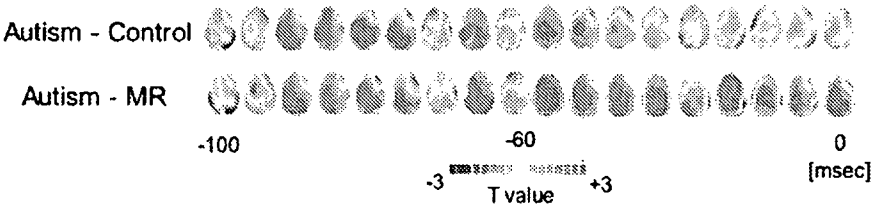

Fig. 5. T-maps in the overlap condition. The upside is the t-map representing comparison between the autism group and the control group; the downside is the t-map between the autism group and the mental retardation group.

taneous recording of EEG and fMRI, or using MEG, should be necessary to determine the issue.

Consistent with prior studies that recorded the saccade-locked ERP in healthy adults during the gap overlap task without discrimination (Gómez et al., 1996; Csibra et al., 1997) the present study demonstrates that the pre-saccadic spike potential immediately precedes the saccade onset and is clear at parietal sites in the three groups. Previous studies reported that 6-month-old infants did not elicit the pre-saccadic spike potential (Csibra et al., 1998) and 12-month-old infants (Csibra et al., 2000) and elder adults (Doig and Boylan, 1989) showed smaller amplitude of pre-saccadic spike potential than younger adults. Csibra and some other researchers have claimed that the pre-saccadic spike potential arises from saccade planning circuits of the parietal cortex (Balaban and Weinstein, 1985). Some fMRI studies suggest that the parietal cortex is involved in saccade execution (Nobre et al., 2000; Perry and Zeki, 2000). In the present study the pre-saccadic spike potential amplitude was not significantly different among the three groups; thus, the results suggest that the saccade execution processing in the individuals with autism and mental retardation was similar to healthy adults.

In the autism group, higher pre-saccadic positivity amplitude in the overlap condition was associated with more severe deficits in "Imitation" and "Near receptor responsiveness" items of the CARS. The "Imitation" item evaluates the level of disability to imitate both verbal and nonverbal acts. The "Near receptor responsiveness" refers to a rating of the individual's response to stimulation of taste, smell, and touch senses (including pain) and whether or not the individuals make appropriate use of these near sense modalities. The sensory responsiveness often plays a part in the overselectivity (Schopler et al., 1980). Significant correlations between the pre-saccadic positivity and these scores suggest that the dysfunction of attentional disengagement may contribute to indifference to action observation of others and preoccupation to non-social stimuli in individuals with autism. However, these interpretations should be regarded as tentative, since our analyses were preliminary in nature and did not make correction for multiple comparisons.

Some limitations of our study should be noted. First, the participants with autism had comorbidity of mental retardation. Although our design including a comparison with a separate mental retardation group has partially resolved the problem, future studies should include high functioning autism participants to further eliminate the influence of mental retardation. Second, future investigations should also include children in order to obtain a more comprehensive view of the developmental course of the attentional system in autism.

In summary, the present study, using the gap overlap task, provides electrophysiological evidence for deficits in attentional disengagement in adults with autism. We have also demonstrated that the physiological substrates underlying deficits in visuospatial attention in autism and mental retardation are different.

Acknowledgements

This study was supported by grants from the Ministry of Health, Labour, and Welfare (Health and Labour Sciences Research Grants, Research on Psychiatric and Neurological Diseases and Mental Health, H17-kokoro-ippan-004 to K.K. and N.K.), and from the MEXT (Grant-in-Aid for Scientific Research for Young Investigators, No. 17790806 to Y.K.), Japan.

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Brain & Development 29 (2007) 491–495

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Imprinting status of paternally imprinted *DLX5* gene in Japanese patients with Rett syndrome

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Received 26 October 2006; received in revised form 24 January 2007; accepted 24 January 2007

Abstract

Rett syndrome (RTT) is an X-linked severe neurodevelopmental disorder mostly affecting female and is mainly caused by mutations of methyl-CpG-binding protein 2 gene (*MECP2*). *MECP2*, which has a crucial role for transcriptional repression and chromatin remodeling, consists of methyl-CpG binding domain (MBD) and transcriptional repression domain (TRD). Paternally imprinted distal-less homeobox gene 5 (*DLX5*), that has an important role for the development of γ-aminobutyric acid (GABA)-ergic neurons, was identified as a target of *MECP2* recently. We selected the 12 samples from the 40 RTT lymphoblast cell lines by a mononucleotide repeat polymorphism within the 3′UTR of *DLX5*. In 12 samples, 5 and 6 samples have the mutations located in MBD and TRD, respectively. No expression and 25–75% expression of the mutated *MECP2* allele were detected in 4 samples with MBD mutation and 4 samples with TRD mutation. In this study, the expression of mutated *MECP2* alleles was low especially in the samples with the MBD mutation suggesting the biased frequency of the cells during the culture. However, a sample with high expression of mutated *MECP2* in TRD mutation showed bialleic expression of *DLX5* suggesting loss of imprinting.

Keywords: Rett syndrome (RTT); Methyl-CpG-binding protein 2 gene (*MECP2*); Imprinted gene; Distal-less homeobox gene 5 (*DLX5*)

1. Introduction

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that almost exclusively affects females and is characterized by mental retardation, severe cognitive impairment, autistic behavior, stereotypic hand wringing, respiratory irregularities, and frequent seizures [1,2]. As the cause of RTT, mutations of Methyl-CpG binding protein 2 gene (*MECP2*) were identified [3]. *MECP2* contains two functional domains of methyl-CpG binding domain (MBD) and transcriptional repressor domain (TRD). MBD is applied to recognize the methylated CpG groups selectively and TRD is the sequence that cooperates with Sin3A and histone deacethylase for transcriptional repression [4].

Although *MECP2* has a crucial role for binding methylated genes and their silencing, microarray-based gene expression studies did not exhibit dramatic changes in gene expression in Mecp2-null mouse brains [5]. As a target gene of MeCP2, brain-derived neurotrophic factor (*BDNF*) was identified, which repressed by binding MeCP2 directly to the *BDNF* promoter [6,7].

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doi:10.1016/j.braindev.2007.01.006

Recently, distal-less homeobox gene 5 (*Dlx5*) that has an important role in regulating the development of γ-aminobutyric acid (GABA)-ergic neurons, was also clarified to be controlled by MeCP2 through MeCP2-mediated histone modification and the formation of silent-chromatin loop structure [8]. Human *DLX5* is paternally imprinted in normal human lymphoblastoid cell lines (LCLs) and brain tissues [9], whereas this gene exhibits loss-of-imprinting (LOI) in LCLs from RTT patients harboring mutations within *MECP2* gene except for mutation in TRD domain (R294X) [8].

Because the genotypes of *MECP2* have been considered to affect the severity of RTT, numerous trials to develop the genotype–phenotype correlations have been conducted. However, these studies provide conflicting results because there is a diverse range of variability in the clinical phenotypes and each study adopts each clinical severity scales and diagnostic criteria [10–12]. Moreover, skewed X chromosome inactivation (XCI) has been assumed to influence the severity of phenotype [13,14]. Consequently, establishing genotype–phenotype correlations would require the classification of mutation type or location being associated with alterations of target genes. To disclose the mutation types which affect the transcription of target gene, the imprinted *DLX5* gene, we have examined the allele specific expression of this gene in LCLs derived from RTT patients.

2. Materials and methods

2.1. Subjects

The RTT samples of EBV-transformed lymphoblasts (LCLs) were previous reported [15]. The study was approved by the ethical committee for the research of human genome and gene analysis in Tottori University School of Medicine.

LCLs were cultured in RPMI 1640 supplemented with 10% fetal calf serum at 37 °C, 5% CO_2. Total RNA was extracted from LCLs using RNeasy Mini Kit (QIAGEN, Bethesda, MD, USA) according to the manufacture's instructions, followed by the treatment with RNase-free DNase I (NIPPON GENE, Tokyo, Japan). First strand cDNA was synthesized with M-MLV reverse transcriptase (Gibco BRL) and Oligo(dt)$_{15}$ primer (Promega, Madison, WI, USA). Integrity of RNA was assessed by PCR amplification of human glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) gene. Genomic DNA samples were extracted from LCLs by standard procedure.

2.2. Expression of DLX5 gene

To clarify the subjects carrying polymorphism, all LCLs were genotyped by the C7/C8 mononucleotide repeat polymorphism within the 3′UTR of *DLX5* (dbSNP ID rs5886002) [9]. For PCR amplification, the following primers were used: DLX5-F (5′-GGAGAA CTCTGCATCCTGGTACACAAGT-3′) and DLX5-R (5′-AGTTGAGGTCATAGATTTCAAGGCACCA-3′). PCRs of genomic DNA and complementary DNA were processed through the following program: initial denaturation at 95 °C for 10 min; 35 cycles consisting of 95 °C for 45 s, 70 °C for 45 s and 72 °C for 45 s; followed by final extension at 72 °C for 5 min. PCR products were purified and sequenced directly by Big-Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencings were performed on ABI 3130xl automated sequencer, and signal intensities for two alleles at a polymorphism site were measured by Sequencing Analysis Software version 5.1. The percentage ratios between two alleles were indicated by the averages of more than three time trials. Because the percentage ratios were calculated from signal intensities in Sequencing Analysis software version 5.1 from direct sequencing analysis, actual counts would have quantity of measurement errors. So we approximately evaluate five grades of expression with mutated allele (0%, 25%, 50%, 75% and 100%).

2.3. Expression of MECP2 gene

Since all samples had been genotyped in previous report [15], we analyzed the expression ratio of wild type to mutated *MECP2* alleles. Each LCL sample harboring mutation assessed by RT-PCR and direct sequencing as described in the previous section. Primer sequences for amplification of *MECP2* are as follows: For samples from R294X, R270X, R255X, G232A, R168X, T158M and D156A mutations located exon 4 of *MECP2*, MECP2ex4-F (5′-AAAGCCTTTCGCTCTAAAGTGG AGTTGA-3′) and MECP2ex4-R (5′- GCTTCACCAC TTCCTTGACCTCGAT-3′); and for samples from R106Q and R106W mutations located exon 3 of *MECP2*, MECP2ex3-F (5′-AAGAAAAGTCAGAAG ACCAGGACCTCCA-3′) and MECP2ex3-R (5′-CAA ATACACATCATACTTCCCAGCAGAGC-3′). The program of PCRs were the same as amplification of *DLX5*, except for 62 °C of annealing temperature in the case of using primer sets of MECP2ex3-F and R.

3. Results

We selected 12 samples out of 40 LCLs established from RTT patients by genotyping by C7/C8 mononucleotide repeat polymorphism and used for the allele specific expression analysis of *MECP2* and *DLX5* genes. We tried to analyze at least three times and confirmed the result. In these informative 12 samples, 5 and 6 samples have the mutations of *MECP2* located in

MBD and TRD domain respectively. We could not distinguish between paternal and maternal allele expression since both parental LCLs were not available. Considering that normal DLX5 gene shows expression derived from only maternal allele, we concluded that the expression ratios between two alleles were more important rather than parental allele expression.

Initially, the allele specific expressions of *MECP2* in these samples were analyzed by RT-PCR followed by direct sequencing of the heterozygous mutated legion (Fig. 1a). We approximately evaluate the amount of expression of mutated allele (0%, 25%, 50%, 75% and 100%) by the sequencing chart. No expression of mutated allele was detected in 7 samples including 4 samples with MBD mutations. In TRD mutations, 4 out of 6 samples expressed mutated MECP2 allele (25–75%) (Table 1).

The allele specific expression of *DLX5* was analyzed using C7/C8 mononucleotide repeat polymorphism (Fig. 1b) with the same grading for *MECP2* expression. The expression of a single parental allele (100%) was shown in 6 samples and suggested to keep imprinting state (Table 1). We found aberrant imprinting in 5 samples. A sample (RTT217), which showed the high expression of mutated allele of *MECP2*, expressed 50% of a single parental *DLX5* allele suggesting aberrant biallelic expression.

4. Discussion

We attempted to disclose the imprinting status of *DLX5* in RTT in the study. Initially, we evaluated the allele specific expression of *MECP2*. Unexpectedly, no mutated allele was expressed in more than half of the samples, especially in 80% of samples with MBD mutations. These would be influenced by skewed X-inactivation, but almost samples from Japanese patient's peripheral blood leukocytes did not show a skewed X-inactivation pattern by an androgen receptor gene polymorphism in the previous research [15]. Our data are different from the previous report by Horike et al. [8]. They reported the abnormal biallelic expression of *DLX5* by the mutations of MBD domain in *MECP2*. In the recent study, selective growth advantage of neurons expressing the wild-type allele had reported in primary cultured neuron derived from *Mecp2*$^{308/Y}$ mice [16]. Moreover, in the analysis of T-lymphocyte single cell cloning, mutated *MECP2* expressing clones have appeared at a lower frequency than wild type clones

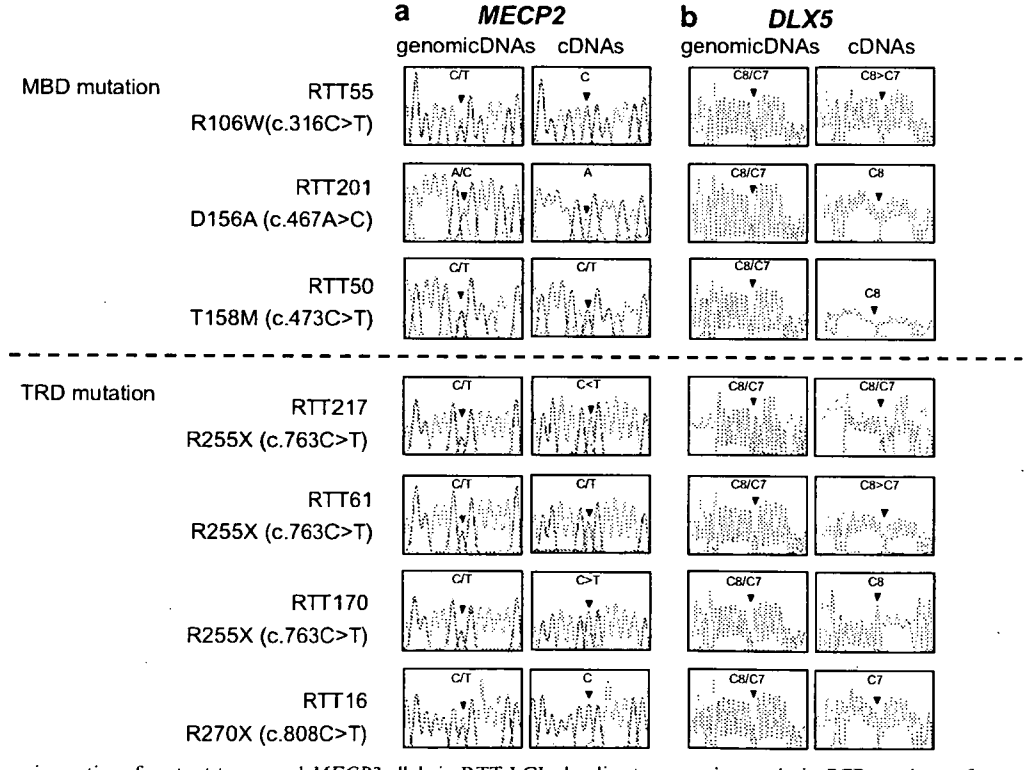

Fig. 1. (a) Expression ratios of mutant to normal *MECP2* allele in RTT LCLs by direct sequencing analysis. PCR products of genomic DNAs and RT-PCR are indicated left panels and right panels, respectively. Arrowheads show the positions of mutation. (b) Allele-specific expression analysis of *DLX5* in RTT LCLs. Allelic expressions were detected by direct sequencing analysis of RT-PCR products (right panels) and PCR products from genomic DNA (left panels) with C7 or C8 mononucleotide repeat polymorphism in the 3'UTR of DLX5. Arrowheads show the porymorphism sites.

Table 1
Allelic expressions of *MECP2* and *DLX5* in 12 patients with Rett syndrome

No.	Subjects	*MECP2*			*DLX5*
		Mutation type		Mutated allele expression (%)	Mono allelic expression (%)
		Genotypes (Nucleotide change)	Involved functional domain		
1.	RTT55	R106W (c.316C > T)	MBD	0	75
2.	RTT184	R106Q (c.317G > A)	MBD	0	100
3.	RTT201	D156A (c.467A > C)	MBD	0	100
4.	RTT136	T158M (c.473C > T)	MBD	0	75
5.	RTT50	T158M (c.473C > T)	MBD	50	100
6.	RTT53	R168X (c.502C > T)	Inter domain	0	75
7.	RTT108	G232A (c.695G > C)	TRD	0	ND
8.	RTT217	R255X (c.763C > T)	TRD-NLS	75	50
9.	RTT61	R255X (c.763C > T)	TRD-NLS	50	75
10.	RTT170	R255X (c.763C > T)	TRD-NLS	25	100
11.	RTT16	R270X (c.808C > T)	TRD-NLS	0	100
12.	RTT42	R294X (c.880C > T)	TRD	25	100

and this frequency have been lower in early mutation than late mutation [17]. Therefore, our data suggest the possibility that early mutation of *MECP2*, especially located in MBD, tends to induce a growth disadvantage in cultured cells. In a sample (RTT50) with T158M mutation in MBD domain, monoalleic expression of *DLX5* was shown and our data were disagreement with the report by Horike et al. [8]. The culture condition may be important, however the difference of the patients or other condition will be suggested.

In the samples with no expression of mutated MBD allele, we cannot evaluate the imprinting status of *DLX5* in RTT accurately. By the reason, we mainly evaluated the samples with TRD mutations. We notably detected the bialleic expression of *DLX5* suggesting loss of imprinting in a sample (RTT217) with high expression of the mutated MBD allele. The mutation of this sample is R255X in TRD domain. The other sample (RTT61) with the same mutation, which showed relatively high expression of the mutated allele of *MECP2*, expressed relatively low *DLX5* expression of single parental allele (Table 1). Our data are also disagreement with the report by Horike et al., in which LOI of *DLX5* was not detected in a samples with R255X mutation [8]. Loss of imprinting of *DLX5* will be influenced maily by the mutated MECP2 expression level, and not by the type of mutation in TRD domain.

We have difficulty in concluding LOI of *DLX5* is specific for involving *MECP2* mutations, but it is likely that TRD mutations tend to be accompanied by loss-of-imprinting of *DLX5* gene. Quantitative PCR could not show the association between biallelic expression and twofold levels of mRNA because of detection limit using LCLs by real-time PCR analysis (data not shown). We could also detect biallelic expression of *DLX5* gene corresponding to imprinting polymorphism in some unaffected LCLs (data not shown). To reveal this phenomenon in RTT, further samples including original lymphocyte will be necessary. Considering the skewed X-inactivation and random X-inactivation, cultured clonal cells from various *MECP2* mutations may give the best information about target of MeCP2 like some imprinted genes were analyzed previously [17].

Recently, mouse *Mecp2* has disclosed the ability of RNA splicing in reporter minigenes. In $Mecp2^{308/Y}$ mice, abnormal RNA splicing of *Dlx5* gene has taken place [18]. Furthermore, *Mecp2* plays a key role in silencing of *Dlx5* gene with chromatin looping in regard to binding DNA [8]. Although these reports support the important mechanism of Mecp2 for regulating the expression of *Dlx5*, we could not conclude that *MECP2* mutations and LOI of *DLX5* have reciprocal relationship in our data. However, *DLX5* is likely to play an important role on the pathogenesis of neurodevelopmental disorder including Rett syndrome. We did not analyze other imprinted genes in same samples. In several imprinted genes, ubiquitin ligase UBE3A/E6-AP (*UBE3A*) is well known to reduce expressions by *MECP2* deficiency [19,20]. But this gene shows imprinting specifically in the brain. So, we could not confirm this gene by the lymphoblast cell lines.

Acknowledgements

This study was supported in part by the Health and Labour Sciences Research Grants for Research on Psychiatric and Neurological Disease and Mental Health form Ministry of Health, Labour and Welfare in Japan.

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Research report

Decreased prefrontal activation during letter fluency task in adults with pervasive developmental disorders: A near-infrared spectroscopy study

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Received 8 January 2006; received in revised form 6 May 2006; accepted 10 May 2006
Available online 23 June 2006

Abstract

Functional neuroimaging studies have suggested that dysfunction of prefrontal cortex (PFC) is present in persons with pervasive developmental disorders (PDD). Recently, the development of near-infrared spectroscopy (NIRS) has enabled noninvasive bedside measurement of regional cerebral blood volume. Although NIRS enables the noninvasive clarification of brain functions in many psychiatric disorders, it has not yet been used to examine subjects with PDD. The aim of our study was to conduct an NIRS cognitive activation study to verify PFC dysfunction in PDD. The subjects were 10 adults with PDD and 10 age- and gender-matched healthy subjects. Hemoglobin concentration changes were measured with a 24-channel NIRS machine during the letter fluency task. While the number of words generated during the letter fluency task did not differ significantly between groups, the analysis of covariance including IQ as a confounding covariate showed that the PDD group was associated with bilateral reduction in oxy-hemoglobin concentration change as compared with the control group. The statistical results did not change when only IQ-matched high-functioning subjects ($N=7$) were included. Moreover, reduced oxy-hemoglobin concentration change for the right PFC was significantly correlated with verbal communication deficits within the PDD group. The present findings are consistent with proposed prefrontal dysfunction in PDD subjects identified by other neuroimaging modalities. The present results may be also potentially useful for applying NIRS to clinical settings of child psychiatry.

Keywords: Pervasive developmental disorders (PDD); Near-infrared spectroscopy (NIRS); Letter fluency task; Prefrontal cortex

1. Introduction

Pervasive developmental disorder (PDD) is defined on the basis of a selected set of behavioral disturbances that more or less map onto specific functional systems of the brain [26]. The difficulties with social reciprocity, communication, and restricted behaviors and interests that occur with PDD suggest that the syndrome affects a functionally diverse and widely distributed set of neural systems [30].

Functional neuroimaging studies have repeatedly suggested that the function of the prefrontal cortex (PFC), critical for working memory and executive functioning [7], is disturbed in persons with PDD. A functional magnetic resonance imaging (fMRI) study using the embedded figure task showed reduced activation of PFC and greater activation of ventral occipitotemporal regions in adults with PDD [25]. An fMRI study showed that autistic adults had significantly less task-related activation in dorsolateral PFC in comparison with healthy adults during a spatial working memory task [16]. An fMRI study reported a variable and scattered representation along the lateral convexity of the frontal and parietal lobes during a visually cued motor sequencing task in adults with autism [22]. Another line of studies have used "theory of mind" tasks to investigate medial PFC abnormalities in PDDs; a pilot positron emission tomography (PET) study of adults with Asperger's disorder showed specific engagement of the medial PFC, except that the center of activation was displaced below and anterior in the patients compared with controls [8], while another PET study showed reduced dorsomedial PFC activation in adults with PDD [4]. In accordance with the latter investigation, a PET study reported

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doi:10.1016/j.bbr.2006.05.020

reduced dopaminergic activity in the dorsomedial PFC of autistic children [5].

While functional brain imaging methodologies such as PET and fMRI have excellent spatial resolution, they are limited in that they require large apparatus which prevents their use in a bedside setting for diagnostic and treatment purposes. Recently, the development of near-infrared spectroscopy (NIRS) has enabled noninvasive and bedside measurement of regional cerebral blood volume (rCBV) in terms of the relative concentrations of oxyhemoglobin [oxyHb] and deoxyhemoglobin [deoxyHb].

NIRS is a neuroimaging modality that is especially suitable for psychiatric patients, due to the following reasons [18]. Because NIRS is relatively insensitive to motion artifact, it can be applied to emotional activation that might cause some motion of the subjects. The subject can be examined in a natural sitting position, without any surrounding noise or feeling of obstruction. In addition the cost is much lower than other neuroimaging modalities and the set-up is very easy.

Although NIRS enables the noninvasive clarification of brain functions in many psychiatric disorders, including schizophrenia, bipolar disorder, depression, dementia of Alzheimer type and post-traumatic stress disorder [6,12,17–20,28], NIRS has not yet been applied to examine subjects with PDD. To evaluate [Hb] change in the prefrontal cortex using NIRS, the verbal fluency test has been often used for cognitive activation, since previous NIRS studies have used letter fluency test for cognitive activations, and showed displayable prefrontal activation during the verbal fluency task in healthy subjects [10,11,13]. Thus, the letter fluency test may be considered a valid cognitive activation task to detect PFC dysfunction by NIRS. Therefore we conducted an NIRS cognitive activation study, using letter fluency test, to verify PFC dysfunction in PDD. Based on previous studies using other neuroimaging techniques that showed lateral prefrontal dysfunction during executive tasks in PDD, we predicted that the subjects with PDD would show lower prefrontal [oxy-Hb] change than healthy subjects. We also predicted that decreased [oxy-Hb] would be associated with severity of symptoms in the PDD group.

2. Materials and methods

2.1. Subjects

The subjects were 10 adults with PDD (six men and four women; 18–37 years old) and age- ($t[18]=0.543$, $p=0.594$) and gender- (χ-square test: $p=0.121$) matched healthy subjects (9 men and 1 woman; 24–34 years old). All individuals with PDD were outpatients of the Department of Neuropsychiatry, Tokyo University Hospital, Japan. Healthy subjects were recruited from college students, hospital staff and their acquaintances. PDD subjects were diagnosed according to DSM-IV criteria [1] by two trained child psychiatrists (H.K. and M.I.). Eight patients were taking psychotropic drugs at the time of examination (Table 1). Their psychopathology was assessed with the childhood autism rating scale (CARS)—Tokyo version [15,27]. We used a CARS score of 27 as the cut-off point for autistic disorder [21], but not for Asperger's disorder nor PDD not otherwise specified. All participants were right-handed as based on the Edinburgh Inventory [24]. IQ scores were obtained using the Wechsler Adult Intelligence Scale-Revised. Although the mean IQ was significantly higher in healthy controls than in PDD, 7 out of 10 PDD subjects were high-functioning with IQ > 85 (Table 1). None of the subjects had a history of substance or alcohol abuse or dependence. This study was approved by the Ethical Committee of Faculty of Medicine, University of Tokyo (No. 630), and written informed consent was obtained from all the subjects prior to their participation in the study.

2.2. Activation task

Hemoglobin concentration changes were measured during the letter fluency task, according to a method similar to that of Suto et al. [28]. The subjects sat on a chair with their eyes open throughout the measurements. The cognitive activation consisted of a 30-s pretask baseline, a 60-s letter fluency task, and a 60-s post-task baseline. In the pre- and post-task baseline periods, the subjects were instructed to simply repeat aloud the syllables /a/, /i/, /u/, /e/, and /o/. In the activation-task period, the subjects were instructed to generate as many words whose initial syllable was /a/, /ka/, or /sa/ as they could. The three initial syllables changed in turn every 20 s during the 60-s task, to reduce the time during which the subjects were silent. The subjects were instructed with an auditory cue at the start and end of the task or baseline period and at the change of the designated

Table 1
Clinical and demographic details of the subjects

Case no.	Age	Sex	IQ	Subtype	CARS	Medication (mg/day)	LFT
Pervasive developmental disorder							
PDD1	19	M	132	Au	35	Risperidone 1	14
PDD2	27	M	122	As	26	Levomepromazine 10	19
PDD3	36	F	75	Au	37	Haloperidol 3, valproate 600, paroxetine 10	12
PDD4	21	F	101	As	25.5	Lithium 400	9
PDD5	21	M	59	PDDNOS	28	Pimozide 9, zotepine 300, carbamazepine 600	16
PDD6	25	F	62	Au	29.5	paroxetine 10	15
PDD7	37	M	96	Au	34.5	No medication	12
PDD8	34	F	122	Au	29.5	Pimozide 1	14
PDD9	18	M	101	As	33.5	Lithium 200, paroxetine 20	10
PDD10	27	M	94	Au	31.5	No medication	14
Mean	26.5	M6/F4	96.4		31		13.5
S.D.	7.1		25.1		3.9		2.9
Control subjects ($n=10$)							
Mean	27.9	M9/F1	118.3				15.4
S.D.	4.1		15.2				2.5

CARS, childhood autism rating scale; LFT, letter fluency task; M, male; F, female; Au, autistic disorder; As, asperger disorder; PDDNOS, pervasive developmental disorder not otherwise specified.

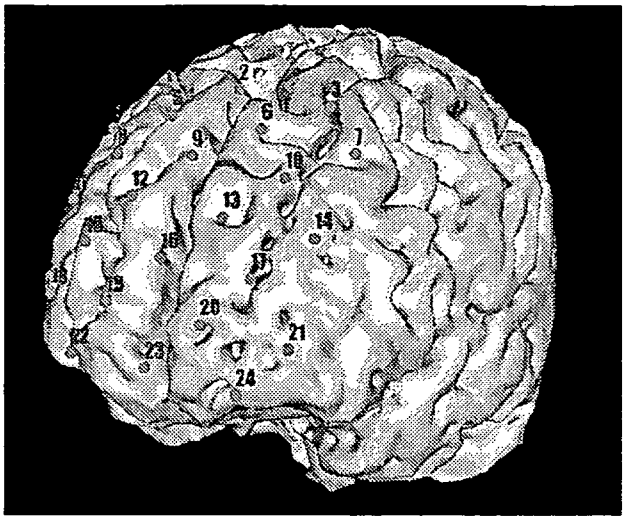

Fig. 1. NIRS measurement points superimposed on a representative subject's 3D-MRI.

syllables. The number of words generated during the letter fluency task was determined as a measure of task performance.

2.3. NIRS measurement

[oxyHb], [deoxyHb], and [totalHb] were measured with a 24-channel NIRS machine (Hitachi ETG-100; Hitachi Medical Corporation, Tokyo, Japan) at two wavelength of near-infrared light (760 and 840 nm). Concentration changes of [oxyHb] and [deoxyHb] were calculated from the difference in the light absorption characteristics of these two hemoglobin species based on Beer–Lambert law. [TotalHb] was calculated as the sum of [oxyHb] and [deoxyHb].

Sixteen probes (eight emitters and eight detectors) were placed over a subject's frontal regions, with the lowest probes positioned along the Fp1–Fp2 line according to the international 10/20 system used in electroencephalography (Fig. 1). The inter-probe distance was 3.0 cm, and the 16 probes can measure [Hb] changes at 24 measurement points in a 9 cm × 9 cm area. The machine measures [Hb] change approximately 2–3 cm beneath the scalp, i.e., the cortical surface area [12].

The absorption of near-infrared light was measured with a time resolution of 0.1 s. Baseline correction was made by using linear fitting based on the two baseline data (the pre-task baseline: the mean across a 10-s period just before the activation period; the post-task baseline: the mean across a 5-s period beginning 50 s after the activation period). This was intended to correct the data during the fluency task for activation due to simple speaking. Moving average methods were used to exclude short-term motion artifacts in the analyzed data (moving average window: 5 s).

2.4. Statistical analysis

[OxyHb] and [deoxyHb] data in each channel were averaged across the two segments (pretask baseline segment and task segment). [OxyHb] or [deoxyHb] at pretask baseline and that at the task period were compared using paired t-test for all channels to test whether [Hb] showed significant changes at the task period relative to the baseline (degree of freedom varied across channels due to a low signal to noise (S/N) ratio at upper prefrontal channels). Low signal to noise ratio was observed at upper frontal channels (channels #1–14) for some subjects, which would substantially reduced the sample size in the repeated measures ANOVA using all 24 channels. Therefore, we adopted repeated measures ANOVA using lower frontal channels (eight channels; left: channels 17, 20, 21, 24; right: 15, 18, 19, 22; approximately Brodmann's areas 10 and 46; see Fig. 1) which had good data for all subjects. More specifically, three-way mixed model repeated measures analysis of covariance (ANCOVA) was performed for [oxyHb] or [deoxyHb] data during the activation-task period, using diagnosis as the between-subject factor, hemisphere and channel as within-subject factors, and IQ as the covariate. To further rule out the effect of IQ, this analysis was repeated for data only including high-functioning PDD subjects ($N=7$; mean IQ = 110 [S.D. = 15], matched with control subjects [$t=1.15$, $p=0.27$]). When the sphericity assumption was violated, Greenhouse–Geisser correction was performed and associated epsilon was reported. We also conducted group comparison of [oxyHb] or [deoxyHb] change using t-tests at each channel.

We performed correlational analysis between task performance and [oxyHb] change at channels #15, 17, 18, 19, 20, 21, 22, and 24 for the control group, PDD group, or for the combined subjects using Pearson's correlation.

In the PDD group, exploratory correlational analyses were performed between the averaged [oxyHb] data during task segment across left 4 (channels 17, 20, 21, 24) or right 4 (channels 15, 18, 19, 22) channels and scores on each item of the CARS using Spearman's rho.

3. Results

The number of words generated during the letter fluency task showed no statistically significant differences between the groups (PDD group: mean 13.5, S.D.: 2.9; control group: mean 15.4, S.D.: 2.5; comparison: $t[18]=1.56$, $p=0.14$).

In the control group, [oxyHb] rapidly increased immediately after the start of the task period, was maintained at the activated level during the task period, and decreased gradually after the task was finished. In the control group, [oxyHb] change showed a significant increase during the activation period relative to the pretask period for 18 of 24 channels, while the [deoxyHb] did not show significant decrease for any channel. These results confirmed that the task employed in this study produced measurable increase in [oxyHb] in healthy subjects. In the PDD group, [oxyHb] showed a very slight increase, and decreased immediately after the end of the task period (Fig. 2). All PDD subjects showed similar waveforms (Fig. 3).

For [oxyHb], the ANCOVA revealed a significant main effect of "diagnosis" ($F[1,17]=6.45$, $p=0.021$). There was no significant diagnosis-by-hemisphere ($F[1,17]=0.005$, $p=0.95$), diagnosis-by-channel ($F[3,51]=0.926$, $p=0.41$; $\varepsilon=0.713$), or diagnosis-by-hemisphere-by-channel ($F[3,51]=0.676$, $p=0.50$; $\varepsilon=0.598$) interaction. There was no significant main effect of hemisphere ($F[1,17]=0.058$ $p=0.81$) or channel ($F[3,51]=0.150$, $p=0.87$; $\varepsilon=0.713$). For subset of data only including high-functioning subjects, the statistical conclusion did not change (diagnosis main effect: $p=0.035$; diagnosis-by-hemisphere interaction: $p=0.99$; diagnosis-by-channel interaction: $p=0.45$; diagnosis-by-hemisphere-by-channel interaction: $p=0.57$; hemisphere main effect: $p=0.15$; channel main effect: $p=0.32$). The t-test at each channel showed that the PDD group had decreased [oxyHb] than the control group at channels no. 4, 10, 11, 14–22, and 24, most of which were lower frontal channels that were included in the repeated measures ANCOVA. The PDD group did not show increased [oxyHb] than the control group at any channel.

For [deoxyHb], the repeated measures ANCOVA did not show a significant main effect or interactions. The t-test at each channel showed that there was no significant difference between groups for any channel except for channel no. 4 (control: mean [deoxyHb], 0.0616; PDD: mean, −0.0452; $p=0.045$). However, this significance was driven by a significant increase of

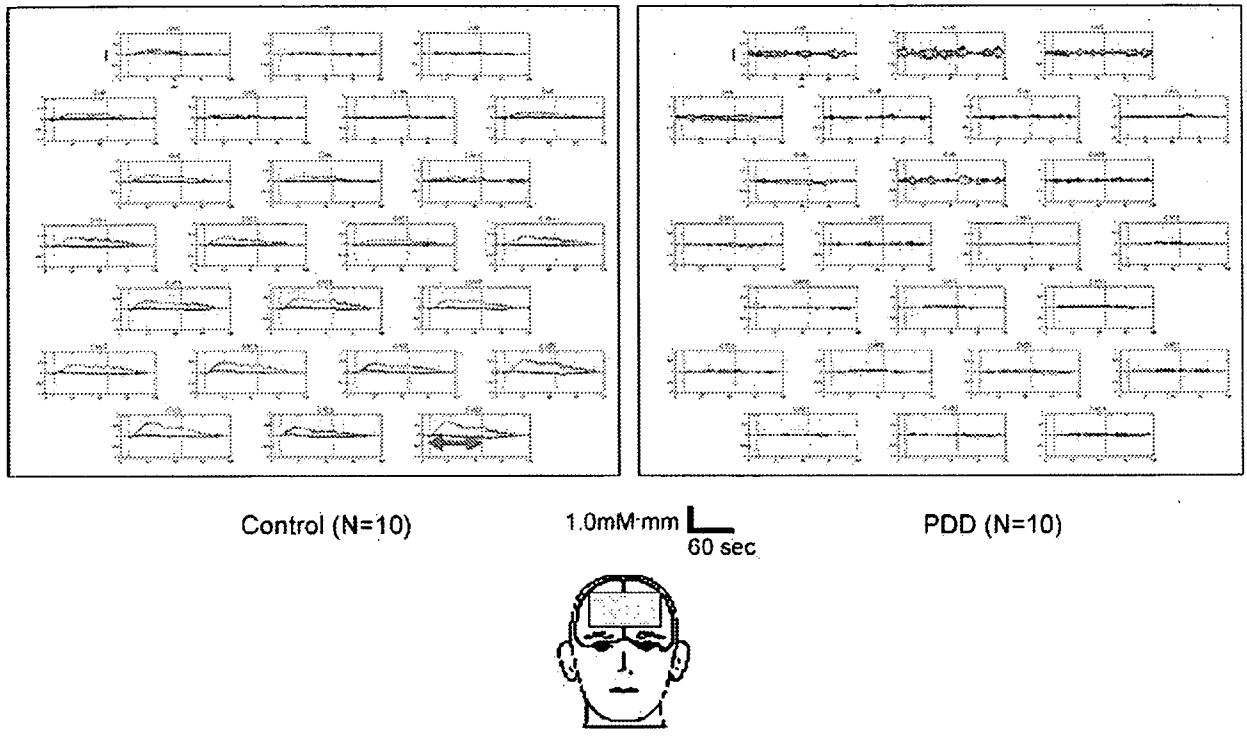

Fig. 2. Grand average waveforms of hemoglobin concentration changes during cognitive activation for controls ($N = 10$) and PDDs ($N = 10$). [OxyHb], red line; [deoxyHb], blue; and [totalHb], green. The red arrow indicates the period of cognitive activation.

[deoxyHb] at this channel in the control group (paired t-test, $p = 0.050$), which is difficult to interpret and is likely to be driven by a type 1 error.

For association between task performance and [oxyHb] change, there was no significant correlation for any channel for the PDD or control subjects. However, there were significant correlation for channels #15, 17, 19, 20, 21, and 24 for the combined subjects (p's = 0.012–0.049) although these correlations did not remain significant after Bonferroni correction. It is likely that these trends in the combined subjects but not in the separate group were spuriously driven by the consistently lower [oxyHb] change in the PDD group.

Correlational analysis between [oxyHb] and CARS showed that [oxyHb] for the right hemisphere was negatively correlated with "verbal communication" score on the CARS (rho = −0.652, $p = 0.041$) in the PDD group, although this correlation did not remain significant after Bonferroni correction for multiple comparisons.

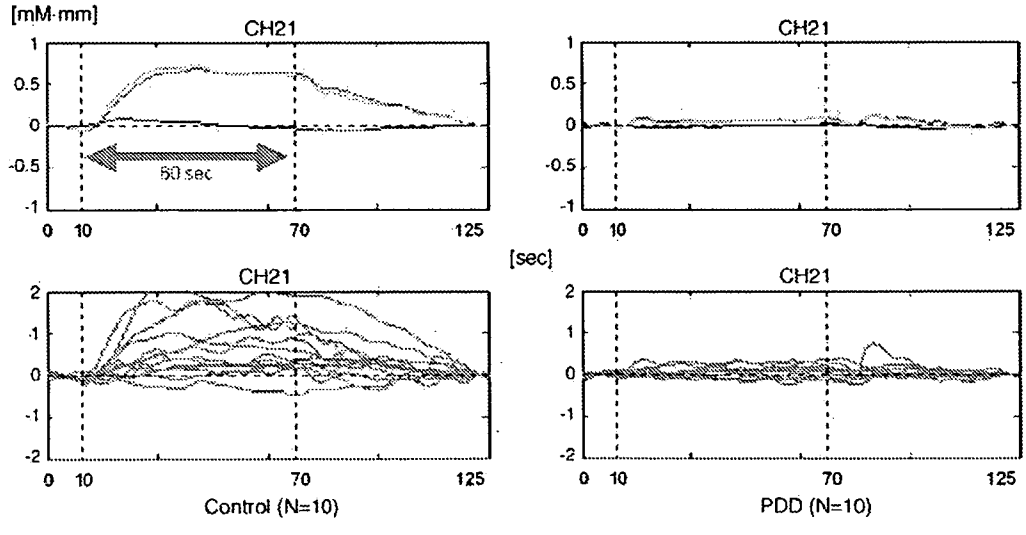

Fig. 3. Upper: grand average waveforms for [oxyHb] (red line), [deoxyHb] (blue), and [totalHb] (green) shown for a representative channel (channel 21; left DLPFC). Lower: [oxyHb] for each subject superimposed. The red arrow indicates the period of cognitive activation.

4. Discussion

To our knowledge, this is the first NIRS study that evaluated prefrontal activation in individuals with PDD. The present findings are consistent with the proposed prefrontal dysfunction in PDD subjects identified by other imaging modalities, such as fMRI and PET. Depressed prefrontal blood volume change in PDD during an executive task is congruent with fMRI studies which reported decreased activation in PFC during executive function tasks [16,25]. However, this result is incongruent with an fMRI study by Müller et al. [22] which reported increased activation in PFC during a visuomotor task, and with an fMRI study by Baron-Cohen et al. [2] who reported decreased activation in the amygdale but not the PFC of adult PDDs during social cognitive tasks. This difference may be partly explained by the difference in the tasks employed, and this difference indicates that the PFC dysfunction of adult PDDs may be task specific.

For clinical implications, our preliminary analysis showed that lower [oxyHb] in the right prefrontal cortex during letter fluency task predicted worse verbal communication scores in individuals with PDDs. However, these results should be considered to be tentative, since the analysis was exploratory in nature.

Possible explanations should be discussed for our observation of significantly depressed prefrontal blood volume change during the letter fluency task in spite of preserved task performance. There may be two possible interpretations. First, the PDD subjects may use alternative cognitive strategies that activate different brain regions, such as lateral and medial temporal lobe, to compensate for the task performance. Consistent with this interpretation, it has been noted that successful performance on the word fluency task, which includes a letter fluency task, may depend on the ability to produce clusters of related responses, and reduced clustering had been reported in PDD [3,29]. Ring et al. [25] showed reduced activation in PFC and increased activation in occipitotemporal regions during the embedded figure task in PDD. They proposed that differences in functional anatomy might indicate that the cognitive strategies adopted by PDD group were different from those adopted by the normal group. Happé et al. [8] employed a "Theory of Mind Task" and found that both a PDD group and a control group showed task-related activation in regions immediately adjacent to the left medial prefrontal cortex, but that only the control group showed significant activity in the left medial prefrontal cortex itself. Their explanation was that PDD subjects were using a more general purpose reasoning mechanism in order to infer mental states. However, because of limited coverage of measurement areas, the present study cannot fully rule out the possibility that other areas such as superior temporal cortex may play a compensatory role thus resulting in the preserved task performance we observed.

Another explanation for significant difference in [oxyHb] change despite preserved task performance may be possible. The underactivation of the prefrontal cortex, compromising verbal communication ability as assessed by CARS in the PDD subjects, may not be sufficient to impair more elementary executive task performance such as an easier version of letter fluency task used in the present study. These possibilities should be further tested in studies manipulating task difficulty and using NIRS with a denser and wider probe array.

NIRS enables measurement of Hb concentration changes not as absolute values but as those relative to pretask baseline. Therefore we cannot empirically rule out the possibility that the present findings may be due to a difference in prefrontal blood volume in the pretask period (i.e., *hyper*perfusion in the pretask period in PDD). However, single photon emission computed tomography studies have found significant *hypo*perfusion during the resting state in the frontal areas of PDD children [9,14,23,31,32] and adults [32] as compared to normal controls. Thus, decreased activation during the cognitive task was not likely to be due to saturated hemodynamic state in the pretask baseline in PDD.

Other methodological considerations of our study need to be commented upon. First, our sample of PDD included some low-functioning subjects. However, we carefully controlled the effect of IQ by using ANCOVA model as the statistical analysis, and by conducting confirmatory analysis with high-functioning subjects only. Thus, IQ may not be a major confounding factor in the present study. Second, the inclusion of medicated subjects may confound the results. However, although the small sample size of the divided PDD subgroups according to medication status (medicated, $N=8$; unmedicated, $N=2$) did not permit strict statistical analysis, the medicated and unmedicated subgroups did not significantly differ in [oxyHb] change for left nor right PFC (averaged values as used in the correlational analysis; Mann–Whitney U-test, p's = 0.60 and 0.60, respectively). Third, because of small sample sizes, the present study could not refer to the difference between Autistic disorder, Asperger's disorder and PDD not otherwise specified. Further research will be necessary to clarify this point. Finally, Hermann et al. [11] found left hemispheric predominance for [oxyHb] change during VFT in healthy subjects. However, Suto et al. [28] and Kameyama et al. [13], which used the similar task procedure to ours and Japanese subjects showed bilateral activation. The discrepancy may be explained by difference in task procedure and language.

In conclusion, to our knowledge this is the first study which evaluated prefrontal activation using NIRS in individuals with PDD. Although the mechanism behind depressed blood volume change during letter fluency task remains unclear, the present results point to be potential for gainful application of NIRS in clinical settings of child psychiatry.

Acknowledgements

This study was supported by Grants from the Ministry of Health, Labour and Welfare, Japan (to KK and NK), and in part by Grant-in-Aid for Scientific Research for Young Investigators (to YK) from the MEXT, Japan. MR was supported by a Postdoctoral fellowship from the Japan Society for the Promotion of Science (P 05234).

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CATATONIA IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDERS IN ADOLESCENCE AND EARLY ADULTHOOD: A LONG-TERM PROSPECTIVE STUDY

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The objective is to cast light on diagnosis and catastasis, course, and comorbidity as concerned with catatonia in patients with autism spectrum disorders (ASDs) with respect to long-term prospective follow-up. Eleven patients (all male) were enrolled. The mean age and the mean follow-up duration were 27.6 years (standard deviation (SD) 5.5) and 18.7 years (SD 8.7), respectively. The mean IQ was 27 (SD 16.4). Information was garnered from medical case records; current examination and observation of patients, interview of parents, and questionnaires completed by parents or other caretakers. Informed consent was obtained from the parents. Criteria for catatonia in this study were: (1) abrupt stop of movements and maintenance of immobility or bizarre posture beginning in adolescence and early adult life, (2) such a cataleptic state had continued for at least several minutes and appeared many times a day to the point of interfering with

INTERNATIONAL REVIEW OF NEUROBIOLOGY, VOL. 72
DOI: 10.1016/S0074-7742(05)72003-1

0074-7742/06 $35.00

daily activities. We described two typical catatonic cases of ASDs. The average onset age was 19 years (SD 6). In all cases, our diagnostic criteria of catatonia evaluating at worse are fully compatible with those of Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV). In 8 out of 11, the onset of catatonia was clearly preceded by the appearance of slowness in movements accompanying the exacerbation of obsessive-compulsive symptoms. Catatonia was also found to have some connection with Tourette syndrome (3 cases), adjustment disorders ($N = 1$), and depressive mood disorders ($N = 1$). In one case, the manifestations of catatonia had to be distinguished from parkinsonism caused by antipsychotics.

Catatonia in ASDs seems to be a chronic condition in most cases. However, there were also a few cases in which catatonia repeatedly aggravated over short spans of time. Catatonia in ASDs may be considered an epiphenomenon of ASDs or a manifestation of comorbidity in adolescence or early adulthood.

I. Introduction

The concept of catatonia has broadened in recent years. It is no more restricted to schizophrenia but is also thought to occur in mood and organic disorders (Fink and Taylor, 2003). Realmuto and August (1991) reported three catatonic adolescents with autism and other psychiatric conditions. Among patients with autism in adolescence and early adulthood, motoric immobility is occasionally observed along with delayed action and repeated conduct. Wing (1996) defined "catatonia" as this sort of immobility. Although catatonia in autism spectrum disorders (ASDs) has been recognized among clinicians and researchers, the nature of catatonia in ASDs is yet to be clarified. The nature and treatment of this psychomotor syndrome as well as its scope and course remain virtually unidentified. Few published studies are available on the long-term course of catatonia in patients with ASDs.

We previously reported about eight ASD patients with catatonia, aged 20 or over, who had regularly visited the outpatient clinic at Department of Neuropsychiatry of Tokyo University Hospital (Ohta *et al.*, 1999). With an elapse of almost 6 years, "*M*," a child psychiatrist, seeing most of these ASD patients with catatonia, moved to the "Z" center. Many of the patients also did so later. In the meantime, there appeared new patients suspected of having catatonia.

This study is primarily designed to cast light on diagnosis and catastasis, onset and course, and association with complications as concerned with catatonia in patients with ASDs in terms of the long term prospective study. A few suggestions for treatment of catatonia in ASDs are made.

II. Subjects

The subjects came up to a total of 11, including 8 cases reported at the 40th Congress of the Japanese Society for Child and Adolescent Psychiatry (Ohta *et al.*, 1999). The three new cases were identified among ASDs patients, aged 20 or over, who regularly visited the "ℤ" Center from June to December in 2003, who fit into the criteria for catatonia, and who had symptoms that considerably hampered their everyday lives or had had them in the past. All the authors of this chapter were seeing at least one of those patients as the attending doctors or the clinical psychologist. The 11 patients fulfilled the criteria of autistic disorder in DSM-IV-Text Revision (TR) (APA, 2000). The average age at the time of initial diagnosis was 8.7 years (standard deviation (SD) 6.4) with the mean follow-up duration at 18.7 years (SD 8.7). All of them were male. The ages at the time of our investigation averaged 27.6 years (SD 5.5, age range: 21–40). Mean IQ was 17 on the Tanaka–Binet scale of intelligence (within the range of 13–70). As evaluated according to the Ohta Staging, which is an evaluation system of cognitive development in autistic children devised and standardized by Ohta *et al.* (Mutoh *et al.*, 2003; Ohta, 1987; Ohta *et al.*, 1989), three came on Stage II, four on III-1, two on III-2, and one on IV.

At the time of investigation, 8 of the 11 patients were visiting the doctor on a regular basis. The whereabouts of two cases reported in 1999 were unknown, and information about conditions of another case could be secured from the mother by telephone.

III. Methods

Regarding the course of the illness, data could be obtained from the statements made by the parents and the descriptions in the medical charts, as well as psychiatric interviews of each visit. The parents consented to our publishing the results of the study.

The term "catatonia," once solely attributed to schizophrenia, is broadly employed today as a behavioral syndrome for other disorders. Psychiatrically, we took catatonia as a failure to manifest spontaneous will and defined it as follows, while referring to descriptions about catatonic disorders in the Catatonic Disorder Due to a General Medical Condition and the Catatonic Features Specifier in the 4th edition of the DSM-IV-TR (APA, 2000), and those both in the Guideline (WHO, 1992) on Organic Catatonic Disorders in International Classification of Diseases, 10th revision (ICD-10) and Diagnostic Criteria for Research (DCR) (WHO, 1993), and those by Wing (Wing, 1996; Wing and Shah, 2000).

A. Criteria For Catatonia in this Study

Catatonia is a behavioral syndrome, and the severity changes during the span of a day and according to the mode of life, so that it would be difficult to come to grips with the loss of voluntary will. Therefore, we focused on movement that comes out and then stops halfway, in situations where the conditions may be accurately grasped by physicians at the outpatient clinic or the parents and other persons in their everyday lives. We picked up only the cases that fell under the category of this condition at the worst time.

1. In adolescence and early adult life they had abruptly stopped their movements and gotten locked into immobility or maintained bizarre posture.
2. Such a cataleptic state had continued for at least several minutes and appeared many times a day.
3. The disturbance caused clinically significant impairment in social, occupational, or other important areas of functioning, and continued for 3 months or more.
4. Clear drug-induced parkinsonism or cases in which the immobility could be explained by an inner state of absorption should be excluded.

B. Severities of Catatonia

The severities of catatonia were classified into "none," "mild," "moderate," and "severe," and the degree of severity was judged depending on the social impairment caused by compulsions referring to "interference due to compulsive behaviors" on the Yale-Brown obsessive-compulsive scale (Y-BOCS) (Goodman *et al.*, 1989a,b). "Mild" represents slight impairment in social and vocational activities without hampering efficiency as a whole; "moderate," some degrees of impairment evidently existent in those activities; and "severe," the degree at which the patients and their families feel it measurably difficult to cope with.

IV. Presentation of Cases

Case 4: 27-year-old male; IQ 40, Ohta Stage III-2

This is a typical case of catatonia as described by Wing (1996), which continued for about 10 years.

There was nothing noteworthy about him in the prenatal, perinatal, and infantile periods. At 12 months of age he started toddling. At about 18 months, he appeared to lag far behind in language development and was markedly

TABLE 1
DESCRIPTION OF 11 CASES WITH ASDs AND CATATONIA

Case	1	2	3	4	5	6
Sex	m	m	m	m	m	m
Outcome	Visiting	Visiting	Dropout	Visiting	Visiting	Dropout
Current age	31	30	40	27	25	31
Ohta Stage[a]	II	III-2	III-1	III-2	I-3	IV
Age of first visit (yy:mm)	9:06	15:03	7:11	3:05	5:06	23:05
Duration of follow-up (yy:mm) (Oct 2003)	18:00	14:08	32:07	23:10	19:11	5:06
IQ	17	30	22	40	13	70
Preceding slowness (age)	15	No	No	14	15	20
Preceding symptoms such as obsessive–compulsive symptoms (OCS)		Bad feeling, negativism, self-injurious, aggression		Excitement, OCS], manifestation of TS[c]	Aggression] ordering]	
History of antipsychotics	Yes			Yes		
Age of manifestation of catatonia	23	15	19	19	18	21
Social situation at onset	Workshop	Special school	Workshop	Competitive job	Workshop	Workshop
Severity at worst	Mild	Moderate	Moderate	Severe	Moderate	Severe
Course and outcome of catatonia (yrs)	Suddenly developed and lasted for 7 months After that no catatonia	1st lasted few months 2nd occurred along with TS	Suddenly occurred with slowness and lasted for less than 1 year. After that time no catatonia	Has lasted in a mild form, but no TS symptoms	Difficulty in initiation has lasted	Subacutely occurred and lasted till the time of dropout
Second phase (yr)		19				
Epilepsy			Yes	Yes		
Psychiatric comorbidity		TS[a]		TS[b]		
Family history						

(*Continued*)

TABLE 1 (*Continued*)

Case	7	8	9	10	11
Sex	m	m	m	m	m
Outcome	Visiting	Dropout	Visiting	Visiting	Visiting
Current age	27	28	23	21	21
Ohta Stage[a]	III-1	III-1	III-2	III-1	II
Age of first visit (yy:mm)	15:11	3:11	3:05	3:08	4:03
Duration of follow-up (yy:mm) (Oct 2003)	11:07	23:03	20:01	17:10	18:01
IQ	19	14	32	27	13
Preceding slowness (age)	No	19	20	17	20
Preceding symptoms such as obsessive–compulsive symptoms (OCS)	Repetitive movement}	Touching compulsion	Ordering	Sleep disturbance, ritual behavior	Touching compulsion, excitement
History of antipsychotics	Yes (2nd time)				Yes
Age of manifestation of catatonia	15	21	21	17	20
Social situation at onset	Special school	Workshop	Workshop	Workshop	Workshop
Severity at worst	Moderate	Severe	Moderate	Severe	Moderate
Course and outcome of catatonia (yrs)	1st suddenly occurred and lasted for 1 years. 2nd (21yr) occurred followed by eyes rolling and lasted for 8 months. After that time no catatonia.	Lasted for 2years, mitigated at the time of dropout	Separating from his sib, he entered a group home. Soon after disappeared	Lasted for less than 2 years	Lasted for more than 1year, but touching compulsion has lasted with the same intensity
Second phase (yr)	21				
Epilepsy				Yes	
Psychiatric comorbidity	Parkinsonism?		Adjustment disorder	Sleep disturbance	TS[b]
Family history			Sib: schizophrenia	Father: depression	

[a]Ohta Stage: Levels of cognitive development in autistic children devised and standardized by Ohta *et al* (1989).
[b]TS: Tourette syndrome.