Evaluation of the GABAergic nervous system in autistic brain: ^{123}I-iomazenil SPECT study

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・Abstract

Purpose:

To evaluate the $GABA_A$ receptor in the autistic brain, we performed ^{123}I-IMZ SPECT in patients with ASD. We compared ^{123}I-IMZ SPECT abnormalities in patients who showed intellectual disturbance or focal epileptic discharge on EEG to those in patients without such findings.

Subjects and methods:

The subjects consisted of 24 patients with ASD (mean age, 7.3±3.5years), including 9 with autistic disorder (mean age, 7.0±3.7years) and 15 with Asperger's disorder (mean age, 7.5±3.2years). We used 10 non-symptomatic partial epilepsy patients (mean age, 7.8±3.6years) without intellectual delay as a control group.

For an objective evaluation of the ^{123}I-IMZ SPECT results, we performed an SEE (Stereotactic Extraction Estimation) analysis to describe the decrease in accumulation in each brain lobule numerically.

Results

In the comparison of the ASD group and the control group, there was a dramatic decrease in the accumulation of ^{123}I-IMZ in the superior and medial frontal cortex. In the group with intellectual impairment and focal epileptic discharge on EEG, the decrease in accumulation in the superior and medial frontal cortex was greater than that in the group without these findings.

Conclusion

The present results suggest that disturbance of the GABAergic nervous system may contribute to the pathophysiology and aggravation of ASD, since the accumulation of ^{123}I-IMZ was decreased in the superior and medial frontal cortex, which is considered to be associated with inference of the thoughts, feelings, and intentions of others (Theory of Mind).

KEY WORDS : autistic spectrum disorders (ASD), ^{123}I-iomazenil SPECT, γ-aminobutyric acid (GABA), epilepsy

1. Introduction

Autistic spectrum disorders (ASD) have various causes, including inherited disease, central nervous system infection, brain malformation, chromosomal aberration, and other. Unknown causes account for 80-90% of cases. The probability that a monozygotic twin is autistic when the other twin is autistic is 60-90%, which is significantly higher than the probability in dizygotic twins (several percent). [1] Therefore, it is thought that genetic factors contribute to the onset of autism even in cases with otherwise unknown causes, and considerable effort is being devoted to identify the causative gene(s).

Since Prader-Willi syndrome and fragile X syndrome, which are associated with Chromosome 15 or X chromosome disorders, complicate autism in many cases, the genes in these chromosomes have recently been considered to be associated with the cause of autism. [2],[3] These chromosomes contain the gene for the γ-aminobutyric acid (GABA) receptor.[4] Cook et al. detected an abnormality in the GABA receptor subunit gene on chromosome 15q11–q13 in autistic disorder. [5] In a genome-wide expression profiling study, Gantois et al. found that the differential expression in neurons of fragile X knockout mice was limited to only three cDNAs, including the δ subunit of the $GABA_A$ receptor.[6]

Over the past few years, in investigations that included only part of the brain, reductions in and denaturation of the $GABA_A$ receptor have been reported neuropathologically in the brains of patients with autism. Fatemi et al. found that the GABA receptor was decreased in the superior frontal cortex (Brodmann's area 9), parietal cortex (Brodmann's area 40), and cerebellum. [7] Oblak et al. found significant reductions in the number of $GABA_A$ receptors and benzodiazepine (BZD) binding sites in the superficial layers of the posterior cingulated cortex and the fusiform gyrus, and in the number of BZD binding sites in the deep layers of the fusiform gyrus. [8]

Therefore, it is considered that disturbance of the GABAergic nervous system may contribute to the onset of ASD and the complication of epilepsy.

ASD is frequently complicated with epilepsy and epileptic discharge on electroencephalogram (EEG). [9] With regard to the pathogenesis of epilepsy, a recent genetic molecular biology study found abnormality of the GABA receptor in addition to abnormality of ion channels involved in membrane permeability, such as those for electrolytes. If an ASD patient has an abnormality of the GABAergic nervous system, we may be able to explain any associated epilepsy or epileptic discharge on EEG by this disturbance.

Recently, ^{123}I-iomazenil (IMZ) single photon emission computed tomography (SPECT) has been increasingly used for noninvasive evaluation of the GABAergic nervous system. ^{123}I-IMZ is a ligand that binds to central benzodiazepine receptors (BZR). BZR and the $GABA_A$ receptor form a complex, and therefore we can obtain information about the GABA receptor indirectly by ^{123}I-IMZ SPECT.

To evaluate the $GABA_A$ receptor in the autistic brain, we performed ^{123}I-IMZ SPECT in patients with ASD. Furthermore, we compared ^{123}I-IMZ SPECT findings in patients with intelligence disturbance or focal epileptic discharge on EEG to those in patients without such findings.

2. Subjects:

The subjects consisted of 24 patients (mean age 7.3±3.5 years, range 2 to 15) with ASD, which includes autistic disorder, Asperger disorder, and related conditions in DSM-Ⅳ. Nine of the 24 had autistic disorder (mean age 7.0±3.7 years, range 2 to 15) and 15 had Asperger disorder (mean age 7.5±3.2 years, range 4 to 11) (Table 1).

An intelligence test (Wechsler intelligence scale for children third edition: WISC-Ⅲ) was performed in all cases; 7 subjects had IQ <70 (IQ 50.9±13.9), 8 had 70≦IQ<85 (IQ 77.5 ±3.6), and 9 had IQ ≧85 (IQ 99.6±7.2).

We classified the 15 patients with IQ <85 as intellectual impairment (mean age 7.5±3.9 years, range 2 to 15 years) and the 9 patients with IQ≧85 as no intellectual impairment (mean age 7.1±2.9 years, range 4 to 11).

EEG was performed in 18 patients with ASD. In 6 of these 18 patients (mean age 6.2 ±2.7 years, range 4 to 11), focal epileptic discharge was noted on EEG; in the frontal region in 4 patients (F4+F8, F3, F3, F3+F7) and in the central region in 2 (C3, C3). The only patient who was taking an anti-epileptic drug was diagnosed as autistic disorder, and the antiepileptic drug was sodium valproate. None of the patients were taking benzodiazepine drugs. Twelve of these 18 patients (mean age 6.4±3.3 years, range 4 to 12) had no epileptic discharge on EEG

We used 10 non-symptomatic partial epilepsy patients (mean age 7.8±3.6 years, range 6 to 13) without intellectual delay (IQ 99.8±2.5) or any developmental disorder as a control group. None of the children in the control group showed epileptic discharge in the frontal lobe. Seven of these 10 children used an anti-epileptic drug (carbamazepine: 4 patients, phenytoin: 1, sodium valproate: 2). Furthermore, none of the children in the control group had abnormal findings in brain MRI or ^{123}I-IMZ-SPECT, as assessed by both a pediatric neurologist and a radiologist.

This study was approved by the Institutional Review Board of our institution, and informed consent was obtained from the family members of all of the children after the purpose and risks of the study had been fully explained.

3. Methods

After the infusion of ^{123}I-IMZ, radioactivity in the brain cortex reaches a peak at 20 to 40 minutes. Therefore, the intracerebral distribution of ^{123}I-IMZ reflects regional cerebral blood flow immediately after administration. Images taken 2-3 hours after administration reflect the benzodiazepine receptor distribution, since ^{123}I-IMZ is accumulated in BZR for a long time. [10] Therefore, in this study, late images were obtained 180 minutes after the administration of ^{123}I-IMZ at a dose of 167 MBq. All patients were treated with triclofos sodium (Tricloryl; 0.5 ml/kg body weight) for sedation one hour before the SPECT measurement. The SPECT measurement was conducted with a two-head gamma camera scanner (E.CAM: Toshiba, Tokyo, Japan) with a fan beam collimator, a Butterworth filter, and filtered back projection (matrix size = 64x64: voxel size = 3.4 mm).

We needed a method for the objective evaluation of ^{123}I-IMZ SPECT results, which would not be influenced by the skill of the reviewer. Therefore, we performed a 3D-Stereotactic Surface Projection (SSP) analysis by iNEUROSTAT® (Nihon Medi+Physics) for the objective evaluation of ^{123}I-IMZ SPECT results. After stereotactic anatomic standardization, ^{123}I-IMZ accumulation in an individual's ^{123}I-IMZ SPECT image set was extracted to a set of predefined surface pixels (three-dimensional stereotactic surface projection, 3D-SSP), which was used in the subsequent analysis.[11]

A normal database was previously created by averaging extracted datasets of normal adult subjects, and is available for use. For adults, patients' datasets are individually compared with the normal database by calculating a Z-score on a pixel-by-pixel basis, and are displayed in 3D-SSP views for visual inspection: Z score= ((average voxel level in the control group－voxel level in a patient) / Control group standard deviation)

However, in children, a comparable open normal database is not available. Therefore, we used 10 non-symptomatic partial epilepsy patients in our hospital (mean age 7.8 years).

In this study, we used iSSP3.5_2tz®(Nihon Medi+Physics) to compare the 3D-SSP results in ^{123}I-IMZ SPECT between patients and control subjects.[12] The extracted cortical activity in the patient group was compared to that in the control group using a two-sample Student's *t*-test on a pixel-by-pixel basis. Calculated *t* values were converted to *Z* values using a probability integral transformation. We also examined whether the presence or absence of intelligence disturbance and focal epileptic discharge on EEG influenced the results of ^{123}I-IMZ SPECT.

After the results in the two groups were compared, we performed SEE (Stereotactic

Extraction Estimation) analysis by medi+SEE version2® (Nihon Medi+Physics) to explain the decrease in accumulation numerically for each brain lobule.[13] We divided the whole brain into segments according to SEE methods (level 3, gyrus level classification) and assessed the extent of the abnormal region in each segment.

Results (Table 2)

First, we compared the ASD group (n=24) and the control group. (Figure 1) There was a dramatic decrease in the accumulation of ^{123}I-IMZ in the superior and medial frontal cortex. By an SEE analysis, 29.4% of the left superior frontal gyrus, 23.9% of the right superior frontal gyrus and 28.7% of the left medial frontal gyrus showed accumulation decreases with Z scores of more than 2.

Next, we divided the 24 patients with ASD into 2 groups: 15 with intellectual impairment (IQ<85) and 9 with no intellectual impairment (IQ≧85). We compared each of these groups with the control group. (Figure 2) In the group with intellectual impairment, there was a dramatic decrease in accumulation in the superior and medial frontal cortex. By an SEE analysis, 39.7% of the left and 25.1% of the right superior frontal gyrus showed accumulation decreases with Z scores of more than 2. In addition, 26.7% of the left medial frontal gyrus also showed an accumulation decrease with a Z score of more than 2.

In the group with no intellectual impairment, there was no dramatic decrease in accumulation in the superior and medial frontal cortex. By an SEE analysis, only 6.8% of the left and 14.8% of the right superior frontal gyrus showed accumulation decreases with Z scores of more than 2.

We directly compared the group with intellectual impairment to the group without intellectual impairment. However, this comparison did not show clear decreases in accumulation with Z scores of more than 2.

EEG was performed in 18 patients with ASD. We divided these 18 patients into 2 groups: 6 (mean age 6.2±2.7 years, range 4 to 11) with focal epileptic discharge and 12 (mean age 6.4±3.3 years, range 2 to 11) with no epileptic discharge. We compared each of these groups with the control group. (Figure 3A, 3B)

In the group with focal epileptic discharge on EEG, there were dramatic decreases in accumulation in the bilateral superior and medial frontal cortex and the left inferior frontal cortex. By an SEE analysis, 47.9% of the left and 26.2% of the right superior frontal gyrus and 38.9% of the left and 23.9% of the right medial frontal gyrus showed accumulation decreases with Z scores of more than 2. In addition, 24.9% of the left middle and 14.6% of the left inferior frontal gyrus showed accumulation decreases with Z scores of more than 2.

The group with no focal epileptic discharge on EEG also showed an accumulation decrease in the superior and medial frontal cortex. By an SEE analysis, 28.8% of the left and 22.9% of the right superior frontal gyrus showed accumulation decreases with ~~a~~ Z scores of more than 2.

We directly compared the group with focal epileptic discharge on EEG to the group without focal epileptic discharge on EEG. (Figure 3C)　In the former group, slight decreases in accumulation were noted in the superior and medial frontal cortex and the left middle and left inferior frontal gyrus.

5. Discussion

In this study we focused on ^{123}I-IMZ SPECT, which can be used to indirectly evaluate intracerebral synaptic cleft $GABA_A$ receptors to gain a deeper understanding of the GABAergic nervous system in ASD.

In 1979, it was reported that BZR was decreased at focal sites of epilepsy, and a method for imaging the distribution of BZR was developed. [14] The intracerebral distribution of BZR in humans has usually been studied by positron emission tomography (PET) with [11C] flumazenil (FMZ) as a tracer. [11C] FMZ is a BZR antagonist. [15] However, the use of PET has been limited because not all institutions have this capability. Therefore, as a simpler and easier alternative to PET, SPECT with ^{123}I-IMZ as a tracer was developed [16]

Central benzodiazepine binds to BZR sites located on $GABA_A$ receptors or forms a conjugated complex with a chloride ion channel, resulting in inhibition of the neural network. Therefore, the BZR status can be used as an index to indicate changes in the inhibitory function of the central nervous system. [17] We think that it may reflect a function of the GABAergic nervous system. However, some limitations must be considered before we can evaluate the GABAergic nervous system by IMZ-SPECT. The benzodiazepine (BZD) site is part of the GABA receptor that is separate from the GABA site. It is possible that it is influenced by various physio-pathological processes, such as neuronal loss leading to a reduced number of GABA receptors (without any actual change in these receptors), changes in the number/density or affinity of GABA receptors, or changes in the BZD site alone.

There are also several potential problems that should be considered when using ^{123}I-IMZ. It has been reported that patients on benzodiazepine medication (Clonazepam) showed an approximately two-fold higher elimination rate and did not show any focal abnormalities of receptor density, and thus were excluded from further analysis. [18] Among the patients in this study, only one was taking an anti-epileptic drug (VPA), and none were taking benzodiazepine drugs. Therefore, anti-epileptic drugs were considered to have had little effect on ^{123}I-IMZ SPECT.

Another problem is the difference in the normal intracerebral distribution of BZR between adults and children. [19] The normal intracerebral distribution of BZR in children changes with growth. There is currently no normal database available for use with children, since the existing normal database was created based on results in normal adults. If we use an adult control, we may misdiagnose findings that are normal for infants and children as abnormal. Therefore, we used 10 non-symptomatic partial epilepsy patients in our hospital (mean age 7.8 years) without intellectual delay

or any developmental disorder as a control group. Therefore, we think that the present decrease in ^{123}I-IMZ accumulation in the superior and medial frontal cortex in ASD compared with a pediatric control group is not simply due to their younger age.

Hashimoto et al. examined sleep EEG findings in autistic children, and reported that 43% showed epileptic discharges, which were observed mainly in the frontal region. In addition, half of the autistic children with epileptic discharges were complicated with epilepsy. [9] In our study, among ASD patients with focal epileptiform activity on EEG, the decrease in ^{123}I-IMZ accumulation in the superior and medial frontal cortex compared to the control group was greater than that in the group without focal epileptiform activity on EEG. The region in which accumulation decreased was similar to the site where there was substantial focal epileptiform activity on EEG. Abnormality of the GABAergic nervous system is considered to be one of several causes of the onset of epilepsy in ASD.

“Theory of Mind” refers to the ability to represent the mental states of others, i.e., their thoughts, desires, beliefs, intentions, and knowledge. Theory of Mind allows a person to attribute mental states to oneself and others to explain and predict behavior. A series of neuroimaging studies have examined the neural systems that are engaged during representation of the mental states of others relative to conditions that do not require such representation. The results have indicated that the medial prefrontal cortex, the temporal-parietal junction, and the temporal poles play important roles in representing the mental states of others. [20]

Wang et al. performed functional MRI in 18 ASD boys to examine the neural circuitry that underlies impairments in interpreting communicative intentions in ASD using irony comprehension as a test case. [21] Reduced activity in the medial prefrontal cortex and right superior temporal gyrus was observed in children with ASD relative to typically developing boys during the perception of potentially ironic vs control scenarios. Furthermore, medial prefrontal cortex activity was inversely related to the severity of symptoms in children with ASD, such that children with greater social impairment showed less activity in this region.

Fletcher et al. reported a functional neuroimaging study with PET in which they examined brain activity in normal volunteers while they performed story comprehension tasks that required the attribution of mental states ("theory of mind"). [22] They used $H_2{}^{15}O$ as a PET tracer. The resultant brain activity was compared with that measured in two control tasks: "physical" stories that did not require this mental attribution, and passages of unlinked sentences. Only the "theory of mind" task produced activation in the medial frontal gyrus on the left (Brodmann's area 8).

Happé et al. used the same paradigm in five patients with Asperger syndrome. [23] In the "theory of mind" task, while no task-related activity was found in the left medial prefrontal region, normal activity was observed in immediately adjacent areas. These findings suggest that a highly circumscribed region of the medial prefrontal cortex is a crucial component of the brain system that underlies the normal understanding of other minds, and this region is affected in ASD patients.

In our study, in the ASD group, the accumulation of ^{123}I-IMZ was decreased in the medial frontal cortex compared to that in the control group, and ASD patients with intellectual impairment showed a greater decrease in the accumulation of ^{123}I-IMZ than those without such impairment, which suggests that disturbance of the GABAergic nervous system in the medial frontal cortex contributes to the pathophysiology and aggravation of ASD.

We directly compared ASD patients with intellectual impairment to those without such impairment. However, the results did not clearly show accumulation decreases with Z scores of more than 2. Although we believed that there was a clear tendency for ASD patients with intellectual impairment to show greater accumulation decreases than those without such impairment, this difference was not statistically significant, perhaps because both groups had already shown some accumulation decrease. This suggests that the IMZ decrease may be more closely related to autistic features or autism per se, rather than intellectual impairment alone.

This is the first report to demonstrate a decrease in BZR in ASD patients by ^{123}I-IMZ SPECT. A disturbance of the GABAergic nervous system may contribute to the onset of ASD because a decrease in the accumulation of ^{123}I-IMZ was observed in the superior and medial frontal cortex, which is where we infer the thoughts, feelings, and intentions of others (Theory of Mind). ASD patients with intellectual impairment and focal epileptic discharge on EEG showed a greater decrease in the accumulation of ^{123}I-IMZ than patients without these findings, which suggests that disturbance of the GABAergic nervous system contributes to the aggravation of ASD.

The present study has some limitations. First, there were fewer subjects in the control group than in the patient group, and this may have influenced the results. Second, we should have evaluated the severity of ASD, such as with the Autism Diagnostic Interview-Revised (ADI-R) or the Childhood Autistic Rating Scale (CARS).

Further studies with a greater number of patients with ASD and control subjects and an evaluation of the correlation between the severity of ASD and the decrease in IMZ binding will be needed to establish ^{123}I-IMZ SPECT as a specific and accurate tool for identifying biological markers of ASD.

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Figure 1

Autism spectrum disorders [n=24] vs control

There was a dramatic decrease in the accumulation of ^{123}I-IMZ in the superior and medial frontal cortex. By an SEE analysis, 29.4% of the left and 23.9% of the right superior frontal gyrus and 28.7% of the left medial frontal gyrus showed accumulation decreases with Z scores of more than 2.

Surface: Brain surface extraction data (Z score) after comparison of the two groups.
Level 3: The level of brain classification. Level 3 is classified in every brain lobule.
Extent: The percentage of each lobule that shows a Z score $\geqq$ 2.

Figure 2

Comparison of the presence and absence of intellectual impairment

A: IQ<85 [n=15] vs control

There was a dramatic decrease in accumulation in the superior and medial frontal cortex. By an SEE analysis, 39.7% of the left and 25.1% of the right superior frontal gyrus showed accumulation decreases with Z scores of more than 2. In addition, 26.7% of the left medial frontal gyrus also showed an accumulation decrease with a Z score of more than 2.

B: IQ$\geqq$85 [n=9] vs control

The accumulation decrease in the superior and medial frontal cortex was not substantial.

Figure 3

Comparison of the presence and absence of focal epileptiform activity on EEG

A: Patients with focal epileptiform activity on EEG [n=6] vs control

There was a dramatic decrease in accumulation in the bilateral superior and medial frontal cortex and the left inferior frontal cortex. By an SEE analysis, 47.9% of the left and 26.2% of the right superior frontal gyrus and 38.9% of the left and 23.9% of the right medial frontal gyrus showed accumulation decreases with Z scores of more than 2.

In addition, 15.4% of the left middle frontal and 19.3% of the left inferior frontal gyrus also showed accumulation decreases with Z scores of more than 2.

B: Patients with no focal epileptiform activity on EEG [n=12]　vs control
A decrease in accumulation was also seen in the superior and medial frontal cortex.　By an SEE analysis, 28.8% of the left and 22.9% of the right superior frontal gyrus showed accumulation decreases with Z scores of more than 2.

C: Patients with focal epileptiform activity on EEG [n=6] vs Patients with no focal epileptiform activity on EEG [n=12]
Slight decreases in accumulation were seen in the superior and medial frontal cortex and the left middle and left inferior frontal gyrus.

Table 1　Clinical data for autism spectrum disorders

	Average age SD (range)	IQ (Patients with epileptic discharge, with no epileptic discharge on interictal EEG)			Epileptic discharge on interictal EEG	AED
		〈70	≧70, <85	≧85		
Autistic disorder [n=9]	7.0 years ±3.7 (2 to 15)	7 (3,3)	2 (0,1)	0 (0,0)	3 (frontal spikes ×2(F4+F8,F3+F7), central spikes(C3))	1 (VPA)
Asperger disorder [n=15]	7.5 years ±3.2 (4 to 11)	0 (0,0)	6 (1,4)	9 (2,4)	3(frontal spikes ×2(F3,F3), central spikes(C3))	0
Total [n=24]	7.3 years ±3.5 (2 to 15)	7 (3, 3)	8 (1,5)	9 (2,4)	6	1

SD: Standard deviation, IQ: intelligence quotient, EEG: electro encephalography, AED: anti-epileptic drug, VPA: sodium valproate

Table 2　SEE analysis in frontal lobe (vs control)

Underlining indicates Z score≧2 in more than 20% of the gyrus.

		ASD [n=24]	IQ<85 [n=15]	IQ≧85 [n=9]	focal epileptic discharge on EEG [n=6]	no focal epileptic discharge on EEG [n=12]
Superior Frontal Gyrus	Lt	29.4%	39.7%	6.8%	47.9%	28.8%
	Rt	23.9%	25.1%	14.8%	26.2%	22.9%
Middle Frontal Gyrus	Lt	3.8%	1.0%	11.5%	24.9%	3.1%
	Rt	1.8%	4.7%	3.1%	8.3%	4.1%
Inferior Frontal Gyrus	Lt	2.7%	0.0%	11.9%	14.6%	1.7%
	Rt	0.0%	0.7%	6.4%	1.4%	7.1%
Medial Frontal Gyrus	Lt	28.7%	26.7%	7.1%	38.9%	18.0%
	Rt	14.0%	9.9%	8.3%	23.9%	11.9%
Orbital Gyrus	Lt	0.0%	0.0%	0.0%	0.0%	0.0%
	Rt	0.0%	0.0%	0.0%	0.0%	0.0%
Rectal Gyrus	Lt	0.0%	0.0%	0.0%	0.0%	0.0%
	Rt	0.0%	0.0%	0.0%	0.0%	0.0%

ASD: autism spectrum disorders, IQ: intelligence quotient,

Fig.1

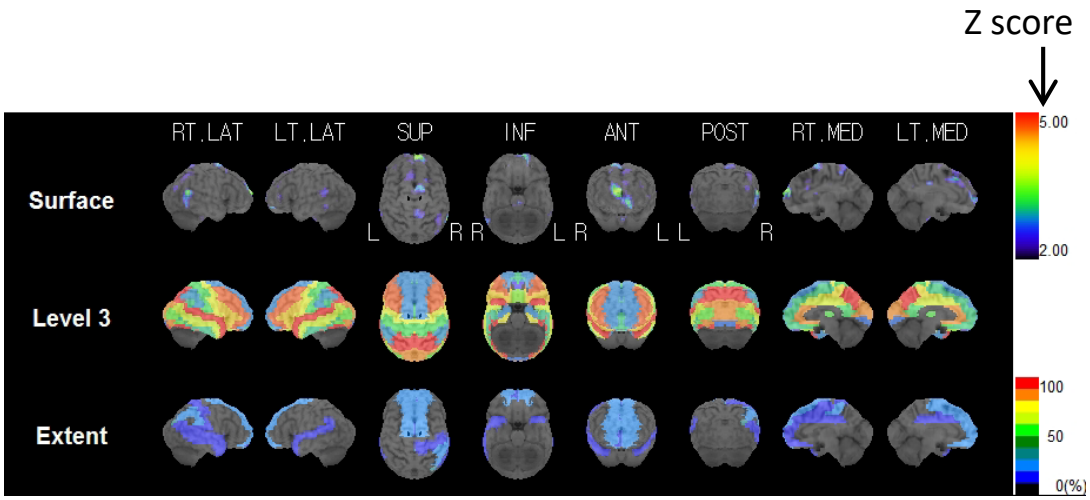
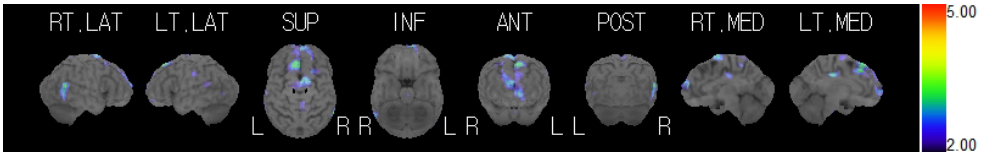

Z score
RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
Surface
Level 3
Extent
L
R R
L R
L L
R
5.00
2.00
100
50
0(%)

Fig.2

A


RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
L
R R
L R
L L
R
5.00
2.00

B

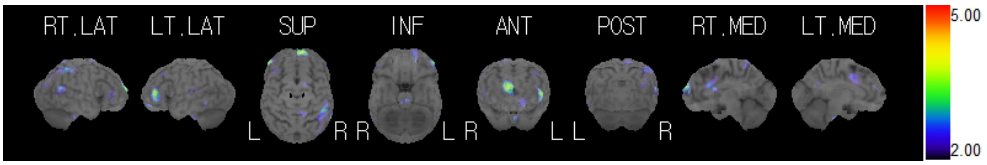
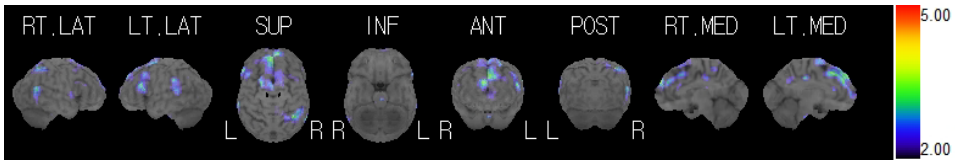
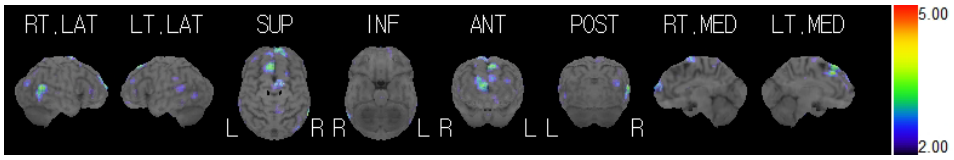

RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
L
R R
L R
L L
R
5.00
2.00

Fig.3

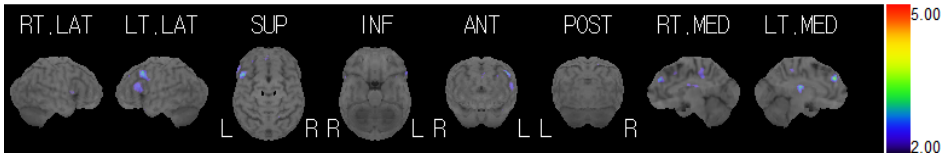
A


RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
L
R R
L R
L L
R
5.00
2.00

B


RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
L
R R
L R
L L
R
5.00
2.00

C


RT.LAT
LT.LAT
SUP
INF
ANT
POST
RT.MED
LT.MED
L
R R
L R
L L
R
5.00
2.00