tion onto projection neurons and increasing inhibition onto interneurons (Bissière et al., 2003).

Although detailed examination of subnuclei of the amygdala is difficult in this imaging method, the dorsal portion of the amygdala roughly corresponds to the central nuclei of amygdala (CeA) and the ventral portion of the amygdala corresponds to the basolateral nuclei of amygdala (BLA) and intercalated cell masses (ICM) (Whalen et al., 2009). The amygdala clusters identified both in fMRI task effect analysis and in correlation analysis between D1 binding and amygdala reactivity were located in the ventral portion of the amygdala. Thus, our findings seem to mainly reflect BLA and ICM properties. It is worth mentioning that the highest density of D1 receptors within the amygdala was found in the ICM, followed by BLA, and the expression of D1 receptors is low in CeA (de la Mora et al., 2009; Muly et al., 2009). In contrast, D2 receptors are mainly distributed in CeA (de la Mora et al., 2009). Both D1 and D2 receptors are expressed both postsynaptically in dendrites and presynaptically in axon terminals (Pinto and Sesack, 2008; Muller et al., 2009; Muly et al., 2009), but D1 receptors in BLA are mainly expressed in the dendrites, indicating that DA directly modulates the excitability of BLA projection neurons and interneurons. At the same time, DA also acts on presynaptic D1 receptors to increase the probability of neurotransmitter release from glutamatergic terminals (Muly et al., 2009). Thus, the net DA effect on D1 receptors in the amygdala is a complex mixture of post- and presynaptic actions at several sites.

Although both DA D1 and D2 receptors contribute to potentiating amygdala response via various mechanisms as described above, our finding suggested that DA D1 receptors play a major role in the overall potentiation of amygdala response. At a behavioral level, previous animal studies repeatedly reported that D1 agonist and antagonist applications into the amygdala potentiated and decreased fear response, respectively. However, the effects of D2 agonist/antagonist on fear response have not been well established (Pezze and Feldon, 2004; de la Mora et al., 2009). Thus, the current finding could be regarded as being consistent with previous behavioral pharmacological studies. The combination of PET molecular imaging and fMRI seems to represent a powerful approach for understanding molecular functions in system neuroscience. However, this study has several limitations. First, current PET techniques for human do not have enough spatial resolution to distinguish subnuclei of the amygdala. Although analysis of parametric images of BP_{ND} has become well established (Gunn et al., 1997) and is used in many [^{11}C]SCH23390 and [^{11}C]FLB457 studies (Cervenka et al., 2006; Takahashi et al., 2008; Karlsson et al., 2009; McNab et al., 2009), a very small region or a single voxel is susceptible to partial volume effect. Thus, it is recommended that parametric image analysis should be used in combination with ROI analysis. At the same time, current results merit further investigation with a higher resolution PET scanner. Second, PET imaging cannot tell us the exact location of DA receptors expressed in projection neurons and interneurons. Future animal studies or *in vitro* studies would complement our findings to determine which D1 receptor-mediated mechanism is most responsible for the overall amygdala response. Third, differences in DA receptor occupancies by endogenous DA might affect BP, leading to different excitabilities of neurons. It is known that BP of [11C]SCH23390 is not sensitive to competitive endogenous dopamine even if massive dopamine is released by amphetamine (Abi-Dargham et al., 1999; Chou et al., 1999). However, it is possible that differences in receptor affinity might contribute to differences in DA receptor occupancies, although Farde et al. (1995) reported that variability in D2 receptor affinity is smaller than that in D2 receptor density. Finally, gender and race effects might also be possible. Any generalization should be approached with caution. Notwithstanding these limitations, we expect our finding to contribute to a broadening of the knowledge of the molecular mechanism of functional abnormalities of the amygdala implicated in neuropsychiatric disorders such as schizophrenia (Takahashi et al., 2004), depression (Drevets, 2000) and Parkinson's disease (Tessitore et al., 2002).

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Cerebral activation associated with speech sound discrimination during the diotic listening task: An fMRI study

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ABSTRACT

Comprehending conversation in a crowd requires appropriate orienting and sustainment of auditory attention to and discrimination of the target speaker. While a multitude of cognitive functions such as voice perception and language processing work in concert to subserve this ability, it is still unclear which cognitive components critically determine successful discrimination of speech sounds under constantly changing auditory conditions. To investigate this, we present a functional magnetic resonance imaging (fMRI) study of changes in cerebral activities associated with varying challenge levels of speech discrimination. Subjects participated in a diotic listening paradigm that presented them with two news stories read simultaneously but independently by a target speaker and a distracting speaker of incongruent or congruent sex. We found that the voice of distracter of congruent rather than incongruent sex made the listening more challenging, resulting in enhanced activities mainly in the left temporal and frontal gyri. Further, the activities at the left inferior, left anterior superior and right superior loci in the temporal gyrus were shown to be significantly correlated with accuracy of the discrimination performance. The present results suggest that the subregions of bilateral temporal gyri play a key role in the successful discrimination of speech under constantly changing auditory conditions as encountered in daily life.

1. Introduction

Selective listening is an auditory process that enables one to attend to a specific speech of interest among a mixture of parallel conversations. Accomplishment of this ability, commonly known as the cocktail party effect, requires not only appropriate orientation and sustainment of auditory attention but also a multitude of concomitant cognitive processes including sound discrimination, human voice recognition, language processing, and so forth. To reveal the underlying neural mechanisms, the so-called dichotic listening paradigm has long been used as an effective measure in combination with neuroimaging studies (Pugh et al., 1996; Beaman et al., 2007). In this paradigm, two different auditory stimuli are presented simultaneously, but with one of the stimuli delivered to one ear and the second to the other ear (Kimura, 1961; Bryden, 1988). The types of auditory stimuli ranged from simple tones (Jäncke et al., 2003; Petkov et al., 2004) to syllables (Lipschutz et al., 2002), to meaningful words (Grady et al., 1997; Jäncke et al., 2001), and to sentences (Hashimoto et al., 2000). Previous imaging studies based on this paradigm and using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have revealed brain regions implicated in selective listening. Robust activity is observed in the bilateral temporal lobes, including the superior temporal gyrus (STG) (Tzourio et al., 1997; Alho et al., 1999; Hugdahl et al., 1999; Zatorre et al., 1999; Hashimoto et al., 2000; Jäncke et al., 2001; van den Noort et al., 2008) during dichotic listening tasks. These areas are well known to be involved in auditory perception (van den Noort et al., 2008). In addition, significant activation is found in the lateral frontal (Hashimoto et al., 2000; Lipschutz et al., 2002; Thomsen et al., 2004) and parietal cortices (Hashimoto et al., 2000; Lipschutz et al., 2002; van den Noort et al., 2008). Mid-ventrolateral (BA 45/47) and mid-dorsolateral areas (BA 9/46) in the lateral frontal cortex are involved in pruning out unwanted information by responding selectively to relevant information (Lipschutz et al., 2002). The parietal cortex, especially the temporoparietal junction extending toward the inferior parietal lobe (IPL), plays a major role in attentional orientation during dichotic listening (Lipschutz et al., 2002). Therefore,

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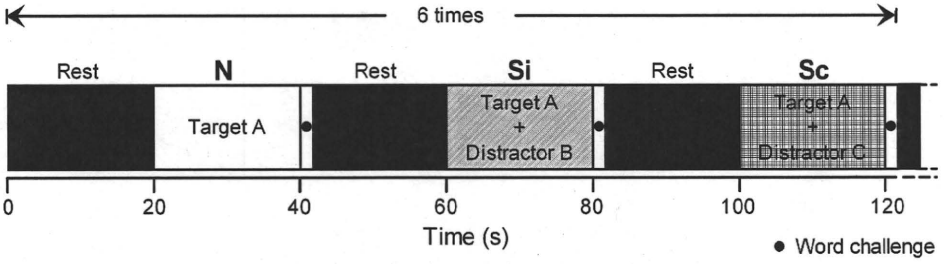

Fig. 1. The experimental block design of the session. Three conditions were sequentially presented to subjects: N, non-selective listening, a news story read out by a target speaker only; Si, selective listening, news stories read out by a target speaker and a distracting speaker of incongruent sex; Sc, selective listening, news stories read out by a target speaker and a distracting speaker of congruent sex. Each condition was presented for 20 s and interleaved with 20-s rest. After each condition, a word was displayed on a screen for 2 s (black circle) for the subjects to answer whether or not it was present in the target news of the preceding block by pressing a button. Each session was repeated six times.

the dichotic listening paradigm has a major advantage in specifying brain areas involved in the selective listening process, although speech discrimination under the constantly changing auditory conditions as encountered in daily life is far from such dichotic listening settings.

While the dichotic listening paradigm is one extreme abstraction of selective listening processes that we encounter in daily life, another experimental paradigm, the diotic listening paradigm, has also been used in previous studies, albeit less frequently, to investigate auditory attention under more natural listening settings. The task consists of binaural presentation of target stimuli superimposed by distracting stimuli (Scott et al., 2004; Shafiro and Gygi, 2007), so that the listening condition is more compatible with that in our daily life where the speech of interest and other conversations are typically mixed together and delivered to both ears. Previously, an fMRI study based on this paradigm reported that discrimination of human speech is associated with blood oxygenation level-dependent (BOLD) activation in Wernicke's area (BA22), Broca's area (BA44/45) and the frontal association cortex (BA6, 9/46, 32, 13/47), suggesting that the neural networks for executing semantic, syntactic, and prosodic processing are implicated in speech discrimination (Nakai et al., 2005). Given that constantly changing auditory environments are encountered in daily life, it is still unclear which brain regions critically work in response to change of challenge level in speech sound discrimination paradigms.

Here we present an fMRI study based on the diotic listening paradigm to evaluate brain activity involved in auditory selective attention. We found that hemodynamic activities in some temporal subregions showed significant correlations with performance accuracy of speech sound discrimination.

2. Materials and methods

2.1. Subjects

Twenty healthy volunteers, 10 males and 10 females, participated in the study (mean age ± SD, 24.9 ± 2.0 years). All subjects were right-handed according to the Edinburgh handedness inventory (mean laterality quotients ± SD, 89.7 ± 15.7) (Oldfield, 1971) and were native speakers of Japanese with normal hearing. None had a previous history of any neurological or psychiatric disorders. All subjects gave written informed consent prior to participation in the experiment. The present study was approved by the Ethics Committees of Nippon Medical School and Asai hospital.

2.2. Experimental design

The diotic listening task consisted of three conditions: (1) a reference condition wherein a single news story was read out by a target speaker (N condition), (2) a diotic listening condition wherein two distinct news stories were read out simultaneously but independently by the target and another speaker (distracter) of incongruent sex (Si condition), and (3) the same as Si condition except the sex of the distracter was congruent (Sc condition). Compared to the Si condition, the congruency of the sex of the speaker in the Sc condition was expected to make speech discrimination more challenging, since male and female reportedly have different phonation frequencies in reading possibly due to anatomical differences in vocal folds (Chen, 2007). The expected different levels of difficulty in speech sound discrimination were defined as 'challenge level' hereafter. The news stories were adopted from television programs and segmented into a 20-s long clip by a digital sound editor (Ulead MediaStudio Pro 6.0, Ulead Systems, Torrance, CA, USA). Each news segment contained 2.5 ± 2.3 sentences in each condition, which were read at a rate of 8.6 ± 0.6 characters/s.

A block design was used for the presentation of stimuli during the fMRI session. An active block of 20-s duration, corresponding to one of three listening conditions, was interleaved with resting periods of the same duration (Fig. 1). The order of conditions was fixed to N–Si–Sc, and this sequence was repeated six times in a single session (i.e., six news stories per condition). The target voice remained identical within a single sequence of conditions (N–Si–Sc), so the subjects could identify during the N condition which voice they should have attended to in the subsequent diotic listening conditions (Si and Sc). To avoid habituation to a particular voice, however, the target voices were altered across the sequences.

To quantify the degree of comprehension of stories read by the target voices, a word challenge was employed at each end of the block. A single word was visually presented for 2 s immediately after each block, and the subjects were required to judge if the word was present or absent in the preceding news segment.

The task presentation during the scan was controlled by SuperLab Pro 2.0.4 (Cedrus Corporation, San Pedro, CA, USA). Auditory stimuli were delivered binaurally through headphones (Resonance Technology Inc., Northridge, CA, USA). Visual stimuli were delivered to a translucent mirror attached to a head coil by a projector located outside the scanner room. During the scan, the subjects were instructed to use their index finger when answering the word challenge.

2.3. fMRI data acquisition

Functional imaging data were acquired with a 1.5 Tesla Signa system with a standard head coil (General Electric, Milwaukee, WI). Functional images of 180 volumes were acquired from each subject with T2*-weighted gradient-echo echo-planar imaging sequences sensitive to BOLD contrast. Each volume consisted of 40 transaxial contiguous sections with a section thickness of 3 mm to cover almost the whole brain (flip angle, 90°; TE, 50 ms; TR, 4 s; matrix, 64 × 64; field of view, 24 cm × 24 cm).

2.4. fMRI data analysis

Data analysis was performed with statistical parametric mapping software 2 (SPM2; Wellcome Department of Cognitive Neurology, University College London, UK) running with MATLAB (Mathworks, Natick, MA). All volumes were realigned to the first volume in each session to correct for head motion and were then spatially normalized to the standard space defined by the Montreal Neurological Institute template. After normalization, all scans had a resolution of 3 mm × 3 mm × 3 mm. Functional images were spatially smoothed with a 3D isotropic Gaussian kernel (full width at half maximum of 8 mm). Low frequency noise was removed by applying a high-pass filter (cutoff period, 80 s) to the fMRI time-series data of each voxel. For subject-level statistical analyses, significant hemodynamic changes in each condition (N, Si, Sc) were examined using the general linear model with boxcar functions convolved with a hemodynamic response function. Statistical parametric maps for each contrast (Si–N, Sc–N, Sc–Si) of the *t*-statistic were calculated on a voxel-by-voxel basis.

For group comparisons, random effect analyses were performed. The contrast images obtained from subject-level statistical analyses were entered into the random effects analyses. One-sample *t*-test was performed to determine group activation for each effect. A height threshold of $P < 0.001$ (uncorrected) and an extent threshold of 10 voxels were considered significant. For anatomical localization, peak voxels were converted from MNI to Talairach coordinates (Talairach and Tournoux, 1988).

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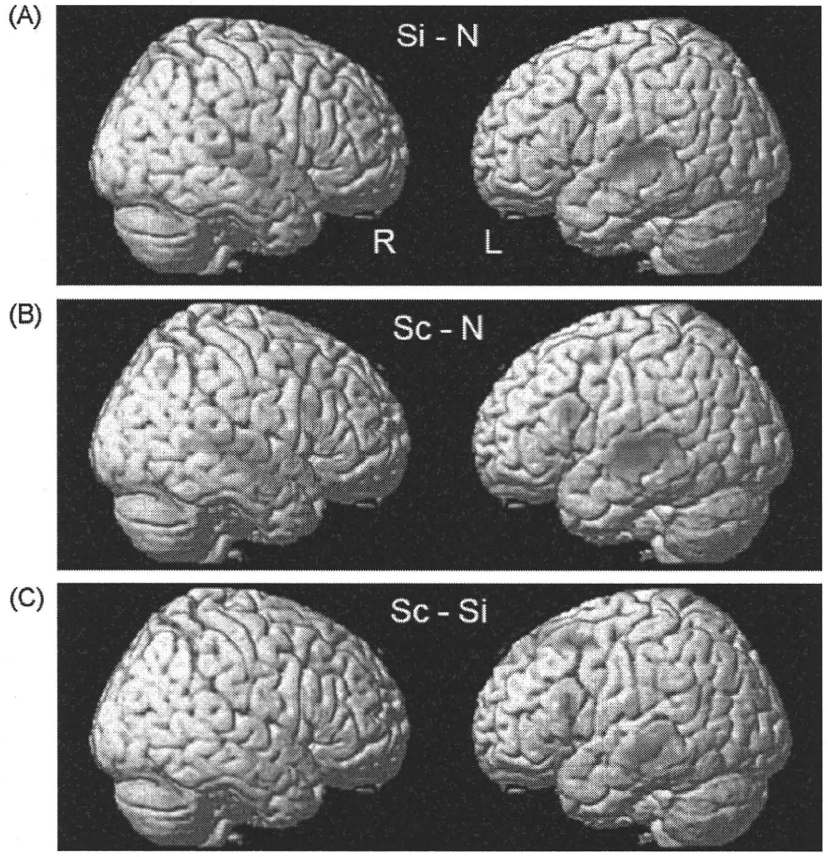

Fig. 2. Cortical rendering of activated areas in selective listening task. (A) Activation in the Si–N constrast. The bilateral temporal, frontal and parietal areas were activated. (B) Activation in the Sc–N contrast. The bilateral temporal, frontal areas, the left insula, cerebellum and the right parietal areas were activated. Compared with Si–N contrast, wider areas were activated in Sc–N contrast. (C) Activation in the Sc–Si contrast. The bilateral temporal, frontal areas and the left parietal areas were activated. All images are shown at the height threshold of $P = 0.001$, uncorrected, and the extent threshold of $k = 10$ voxels.

2.5. Performance analysis of word challenge

Performance score of the word challenge was obtained by counting the number of words that the subjects could answer correctly in the individual conditions with six repetitions. The accuracy rate was expressed as percent of the total number of correct words against all six sequences, and the values were shown as mean ± SD. The accuracy rate was compared among N, Si and Sc conditions using one-way ANOVA, followed by Tukey's multiple comparison test. $P < 0.05$ was considered statistically significant.

Simple regression analyses were performed to examine correlations between task-related BOLD signal changes of Sc–Si contrast and the performance score difference between Si and Sc conditions (Sc–Si). Pearson's correlation coefficient was then calculated with a significance threshold of $P < 0.05$.

3. Results

3.1. fMRI data

To assess the areas activated in selective listening attention under this designed experiment, the BOLD signal changes in both Si–N and Sc–N contrasts were examined. In Si–N contrast, as shown in Fig. 2A, the bilateral STG, middle temporal gyrus (MTG), middle frontal gyrus (MFG), superior parietal lobule (SPL), left precentral gyrus, right inferior frontal gyrus (IFG), precuneus and IPL were significantly activated (Table 1 and Fig. 2A). In Sc–N contrast, there was significant activation in the bilateral STG, MTG, IFG and MFG, the left insula and cerebellum, right medial frontal gyrus, precuneus, supramarginal gyrus (SMG) and inferior parietal gyrus (Table 1 and Fig. 2B). These results suggest that the brain regions activated in Sc condition are more widespread than those in Si condition.

Furthermore, the difference in BOLD signal between Sc and Si conditions was analyzed to reveal how the change of challenge level in speech sound discrimination affects brain hemodynamic activity. In Sc–Si contrast, significant activations were observed in the bilateral STG, MTG, IFG, MFG, medial frontal gyrus, SFG, left inferior temporal gyrus (ITG), SMG and IPL (Table 1 and Fig. 2C).

3.2. Accuracy rate

Next, we investigated whether the difference in challenge level in speech sound discrimination among three conditions reflects the difference in accuracy rate. The subjects judged the presence of the keywords in the preceding narration with 93.3 ± 8.4% (N condition), 92.5 ± 10.1% (Si), and 81.7 ± 17.1% (Sc) of accuracy rates, respectively (ANOVA for group comparison, $P = 0.0065$; Fig. 3). Thus, the subjects answered the target word less correctly in Sc condition than in N and Si conditions ($P < 0.05$), suggesting that they had more difficulty in discriminating the target voice when the distracter was of congruent sex in Sc condition.

3.3. Correlation between fMRI image and performance score

Further, we sought to determine the brain regions responsible for changes of challenge level in speech sound discrimination as measured by BOLD activation. The change in BOLD signal under Sc–Si contrast was positively correlated with the difference in performance score between Si and Sc conditions (Sc–Si) in the left ITG (BA 20, $R = 0.796$, $P < 0.001$), left anterior STG (BA 38, $R = 0.787$,

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Table 1
Regions activated in selective listening in Si–N, Sc–N and Sc–Si contrasts.

Contrast	Hemisphere	Region (Brodmann area)	Talairach			t-Value
			x	y	z	
Si–N	L	Superior temporal gyrus (22)	−59	−25	7	10.59
		Middle temporal gyrus (21)	−59	−6	−5	6.24
		Precentral gyrus (6/4)	−57	7	31	6.92
		Middle frontal gyrus (46)	−48	42	20	4.13
		Superior parietal lobule (7)	−12	−65	55	4.33
	R	Superior temporal gyrus (22/42)	61	−33	7	5.88
		Middle temporal gyrus (21)	67	−14	−4	5.73
		Middle frontal gyrus (10/9/46)	36	47	11	4.67
		Inferior frontal gyrus (44)	53	13	21	4.34
		Superior parietal lobule (7)	18	−69	50	6.65
		Precuneus (7)	16	−54	52	4.36
		Inferior parietal lobule (40)	57	−42	22	4.04
Sc–N	L	Superior temporal gyrus (22/42)	−51	−17	3	12.08
		Middle temporal gyrus (21)	−53	−12	−6	10.28
		Inferior frontal gyrus (45)	−57	20	19	8.01
		Middle frontal gyrus (6)	−46	4	50	6.24
		Insula	−30	19	−1	4.82
		Cerebellum	−14	−79	−21	5.39
	R	Middle temporal gyrus (21)	61	−6	−11	7.85
		Superior temporal gyrus (22/42)	63	−29	5	6.06
		Inferior frontal gyrus (45/44/47)	59	19	21	6.37
		Middle frontal gyrus (6/10/9/8/46)	48	8	46	5.09
		Medial frontal gyrus (8)	2	37	41	4.18
		Precuneus (7)	14	−62	40	4.85
		Supramarginal gyrus (40)	44	−43	37	4.67
		Inferior parietal lobule (39)	48	−58	43	4.45
Sc–Si	L	Middle temporal gyrus (21)	−55	−20	−7	6.66
		Superior temporal gyrus (22/38)	−46	−23	3	5.7
		Inferior temporal gyrus (20)	−46	−7	−25	4.47
		Inferior frontal gyrus (45/47)	−53	18	14	6.5
		Middle frontal gyrus (8/6)	−32	22	47	6.35
		Medial frontal gyrus (8)	−2	31	46	6.21
		Superior frontal gyrus (8/6)	−8	34	52	5.37
		Supramarginal gyrus (40)	−63	−47	28	5.56
		Inferior parietal lobule (39/40)	−50	−59	23	5.02
	R	Middle temporal gyrus (21)	53	1	−25	4.92
		Superior temporal gyrus (22)	46	−21	3	4.6
		Medial frontal gyrus (8/9)	2	39	40	5.08
		Superior frontal gyrus (8)	8	50	36	4.84
		Inferior frontal gyrus (45)	8	36	52	4.75
		Middle frontal gyrus (8/9/6/46)	61	22	21	4.44

$P<0.001$) and right STG (BA 22, $R=0.722$, $P<0.001$), respectively (Table 2 and Fig. 4). The two subregions in the left temporal gyrus (BA 20 and 38) were also included in activated areas under Sc–Si contrast by the overall analysis as shown in Table 1 (superior temporal gyrus and inferior temporal gyrus). At the individual level, 10 subjects showed no difference in the performance score between Sc and Si conditions, while they had increase in BOLD signal in the left ITG and left anterior STG under Sc–Si contrast (Fig. 4A and B). As for the right STG BOLD signal, seven subjects with lower performance score showed lower BOLD activity in this subregion under Sc condition than Si condition (Fig. 4C).

Table 2
Regions of activation correlated with performance score in Sc–Si contrast.

Hemisphere	Region (Brodmann area)	Talairach			t-Value
		x	y	z	
L	Inferior temporal gyrus (20)	−50	−32	−10	5.31
	Superior temporal gyrus (38)	−48	12	−28	4.46
R	Superior temporal gyrus (22)	63	−8	0	4.66

4. Discussion

4.1. Cortical network in auditory selective attention

By means of fMRI with a diotic experimental paradigm based rather on actual human conversation, cortical hemodynamic response was observed robustly in the bilateral STG and MTG and substantially in the bilateral MFG, right IFG and SPL in both Si and Sc conditions. Brain imaging (PET and fMRI) combined with dichotic listening task (O'Leary et al., 1996; Hugdahl et al., 1999, 2000; Hashimoto et al., 2000; Jäncke et al., 2001, 2003; Hund-Georgiadis et al., 2002) has already been extensively used to reveal the regions responsible for attentive listening. These studies have demonstrated widespread activities not only in the temporal cortex including bilateral STG and MTG, but also in the inferior parietal and prefrontal cortices (Jäncke and Shah, 2002; Lipschutz et al., 2002). In addition, an fMRI study (Nakai et al., 2005) using a diotic listening paradigm has also shown similar brain activation in the frontal association cortex as well as STG. Therefore, brain regions activated during the present diotic listening task are consistent with those previously reported, suggesting that the present task mimicking daily life conditions can efficiently activate the auditory attention networks commonly used during selective listening.

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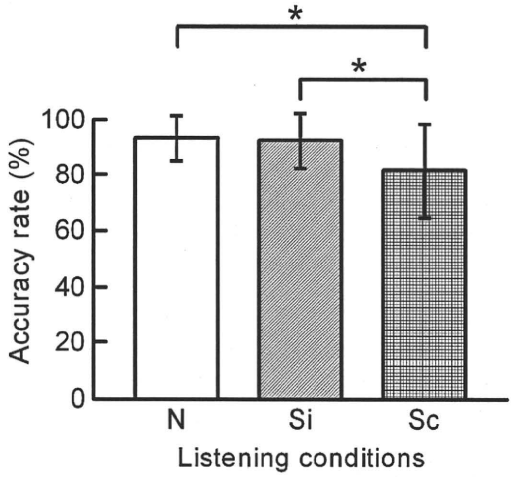

Fig. 3. Accuracy rate of word challenge. The ratio (%) of the number of target words correctly answered by the subjects in the individual conditions in the session was calculated and referred to as accuracy rate. The values were mean ± SD. The accuracy rates were significantly different among the three conditions ($F(2, 57) = 5.5$, $P = 0.0065$). Sc condition has significantly lower accuracy rate compared with N and Si conditions (*$P < 0.05$). N, non-selective listening; Si, selective listening with speakers of incongruent sex; Sc, selective listening with speakers of congruent sex. $n = 20$.

4.2. Diotic selective listening

To accomplish the diotic listening task in the present study, several distinct steps were required to process auditory information: discriminating the voice of the target speaker from that of the distracter, ignoring the distracter's voice, keeping attention focused on the story read by the target speaker, recognizing the words in the story, constructing sentences from the words, and comprehending and memorizing the sentences. In each process, distinct brain regions are thought to be involved. The right anterior part of the superior temporal sulcus and right IFG are reportedly involved in the prosodic component of speech sound processing (Plante et al., 2002; Zatorre et al., 2002) to discriminate the target voice by comparing it with others' prosodic features. The left frontal (BA 6, 8, 9, 44 and 46) and parietal lobes (BA7) have been thought to have a role in ignoring distracter stimuli (Bledowski et al., 2004). Broca's area (BA 44 and 45) is involved in syntactic processing (Stromswold et al., 1996; Caplan et al., 1999). It has been reported that the anterior areas of the left superior temporal gyrus and middle temporal gyrus are involved in speech comprehension (Scott et al., 2000; Davis and Johnsrude, 2003) and the left parietal cortex in working memory retrieval or short-time memory (Majerus et al., 2007; Öztekin et al., 2009). As shown by the results, the present task increased hemodynamic activation in the above-mentioned areas, consistent with the previous observations. Collectively, the present results suggest that the temporal, parietal and frontal regions are extensively involved in overall information processing during selective diotic listening.

However, there are several differences in the activated area between the previous dichotic listening studies and the present results. Activation of the primary auditory area such as BA 41 was not significant at the statistical threshold applied in the present study, while previous dichotic listening task studies found predominant activation in the primary auditory cortex contralateral to the ear of stimulation (Alho et al., 1999; Jäncke et al., 2001). Most of the auditory stimuli adopted in previous dichotic listening task studies were tone and syllables, whereas in the present task we used as stimuli two different contents of sentences read simultaneously but independently by a target and a distracting speaker. Under such condition, the subjects are required to execute neural processes pertaining to lexical access and semantic retrieval for completion of the task. Therefore, the cerebral activation might rather reflect post-sensory linguistic processing. In fact, functional neuroimaging studies using speech as stimuli showed activation of the left-lateralized networks, including the parietal, frontal and temporal cortex (Binder et al., 2009).

In addition, our results showed activation in the bilateral parietal cortices (BA 7) in Si–N contrast, the right parietal cortex (BA 39) in Sc–N contrast, and the left parietal cortex (BA 39 and 40) in Sc–Si contrast. While dichotic listening tasks have been reported to preferentially activate the right parietal cortex in association with spatial auditory processing (Alho and Vorobyev, 2007), left-dominant brain activation has been reported in angular gyrus (BA 39) and its adjacent areas (BA 40 and 7) in the semantic decision task using spoken languages (Binder et al., 1997, 2009). Accordingly, the parietal cortex activation shown in our task may be due to language processing rather than spatial processing.

4.3. Change of challenge level in speech sound discrimination and hemodynamic response in fMRI

As expected from the sex difference in voice properties, Sc condition was more difficult for the subjects to discriminate speech than Si condition when the mean accuracy rate in the performance was compared between Sc and Si conditions. Furthermore, the difference in performance between Si and Sc conditions was positively correlated with the change in BOLD signal under Sc–Si contrast in the left ITG, left anterior and right STG. At the individual level, half of the subjects showed increase in BOLD signal in the left ITG and left anterior STG without apparent difference in performance score between Sc and Si conditions. These results might be interpreted as the subjects making effort to attain good performance, resulting in BOLD increase in these regions. As described above, it has been reported that the left ITG and left anterior STG are involved in lexical–semantic processing (Binder et al., 2009). On the other hand, seven subjects with lower performance score under Sc condition than Si condition showed lower BOLD activity in the right STG. This implies that the activity of this subregion, which reportedly involves sentential prosody processing (Plante et al., 2002; Zatorre et al., 2002), may directly reflect the ability to discriminate performance. The involvement of these regions in successful discrimination may, therefore, indicate that the our present tasks require linguistic processing in addition to phonological one; since targeted words were presented in the news articles, the participants need to comprehend the target speech in the context of the news contents as well as to differentiate the target voice in the context of the phonetic feature. Collectively, these results suggest that these temporal subregions relating to language processing also play important roles in performing speech discrimination under constantly changing auditory conditions intermingled with different speakers' voices.

4.4. Selective auditory attention and psychiatric disorders

Patients with psychiatric disorders are known to often suffer from attention disturbance. For instance, deficit in attention has been thought to be a primary feature of neurocognitive profiles of patients with schizophrenia on the basis of neuropsychological studies (Heinrichs and Zakzanis, 1998; Fioravanti et al., 2005). Intriguingly, functional imaging studies of attention and working memory, however, have reported mixed findings in these patients. Attention task-related activation is attenuated in schizophrenia in DLPFC (Ojeda et al., 2002) and STG (Gallinat et al., 2002) as compared to in healthy subjects. In contrast, enhanced activa-

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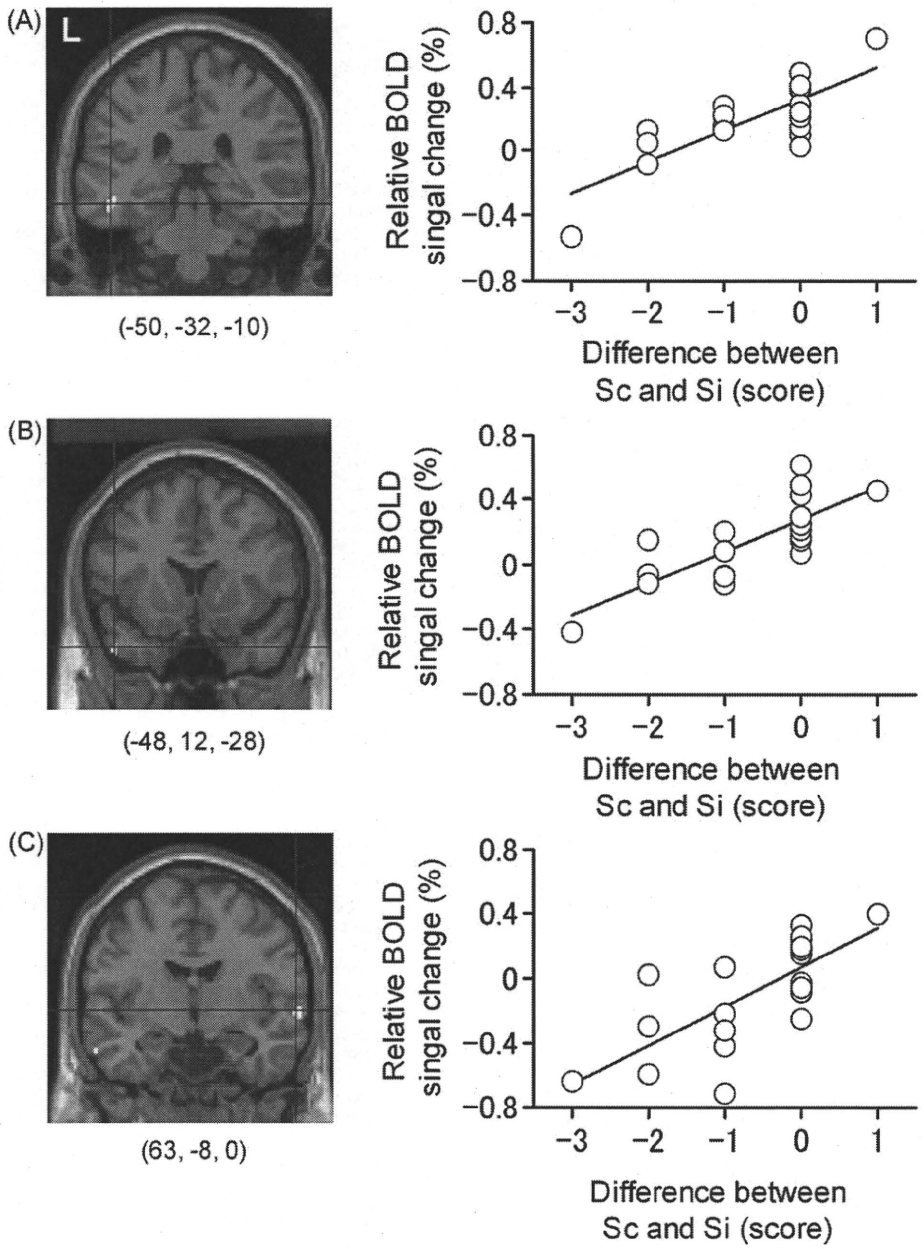

Fig. 4. Correlation between relative BOLD signal change and performance score difference in Sc–Si contrast. In the right panels, the vertical axes represent relative BOLD signal change in Sc–Si contrast, and the horizontal axes indicate the difference in performance score between Si and Sc conditions. Scatter plots illustrate significant positive correlations by simple regression analyses between neural activation of the left inferior temporal gyrus (A), left anterior superior temporal gyrus (B), or right superior temporal gyrus (C) and the performance score difference (Sc–Si). The left panels illustrate the brain regions with corresponding Talairach coordinates showing the correlations as described.

tion in DLPFC (Weiss et al., 2003) and STG (Weiss et al., 2007; Schirmer et al., 2009) has been observed in schizophrenia. Such discrepancy may be derived not only from the wide variety of patient profiles, but also task designs, which focus on different aspects of neurocognitive function of patients. A key feature of the paradigm used in the present study is that it could uniquely depict the brain activity during listening attention encountered in the daily conversational environment. In addition, this task can measure an activity change of the speech sound discrimination in temporal areas, which is reportedly reduced in volume in patients with schizophrenia (Collinson et al., 2009; Sun et al., 2009). Therefore, future studies using the present diotic task may be useful for investigating the cognitive aspect of auditory attention in patients with attention disturbance such as schizophrenia.

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ARTICLE

CINP

Peripheral benzodiazepine receptors in patients with chronic schizophrenia: a PET study with [^{11}C]DAA1106

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Abstract

Inflammatory/immunological process and glial contribution are suggested in the pathophysiology of schizophrenia. We investigated peripheral benzodiazepine receptors in brains of patients with chronic schizophrenia, which were reported to be located on mitochondria of glial cells, using [^{11}C]DAA1106 with positron emission tomography. Fourteen patients and 14 age- and sex-matched normal controls participated in this study. PET data were analysed by two-tissue compartment model with metabolite-corrected plasma input. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale. There was no significant difference between [^{11}C]DAA1106 binding of the cortical regions of normal controls and patients with schizophrenia, whereas the patients showed a positive correlation between cortical [^{11}C]DAA1106 binding and positive symptom scores. There was also a positive correlation between [^{11}C]DAA1106 binding and duration of illness. Although the correlations need to be interpreted very cautiously, involvement of glial reaction process in the pathophysiology of positive symptoms or progressive change of schizophrenia might be suggested.

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Key words: Microglia, peripheral benzodiazepine receptor, positive symptoms, schizophrenia.

Introduction

An accumulating body of evidence has suggested that the pathophysiology of schizophrenia could be related to the dysregulation of the inflammatory response system, such as increased levels of *in vivo* IL-1RA, sIL-2R, and IL-6 (Lin *et al.* 1998; Nawa & Takei, 2006; Potvin *et al.* 2008; Zhang *et al.* 2004). Microglia has been regarded as a mediator of neuroinflammation via the release of pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS) in the central nervous system (CNS). Peripheral benzodiazepine receptor (PBR) was reported to reflect neuronal injury and inflammatory lesions in the brain by increased expression of the number of binding sites in glial cells including activated microglia and reactive astrocytes as visualized *in vivo* using PET with [^{11}C]PK11195 (Shah *et al.* 1994). Recent reports demonstrated that [^{11}C]PK11195 binding was increased in patients with acute-onset schizophrenia (van Berckel *et al.* 2008) and in patients with schizophrenia during psychosis (Doorduin *et al.* 2009). However, the affinity (Chaki *et al.* 1999) and permeability of the blood–brain barrier was low for PK11195, reportedly a substrate of efflux transporter P-glycoprotein (Jakubikova *et al.* 2002; Vaalburg *et al.* 2005). Low uptake of [^{11}C]PK11195 in the brain could hamper stable quantitative analysis.

(*N*-5-fluoro-2-phenoxyphenyl)-*N*-(2,5-dimethoxylbenzyl) acetamide (DAA1106) is a potent and selective ligand for PBR with high affinity (Chaki *et al.* 1999; Okuyama *et al.* 1999). [^{11}C]DAA1106 is accumulated at high levels in the mouse brain (Zhang *et al.* 2003), and the radioactivity of [^{11}C]DAA1106 at 30 min after injection was reported to be four times higher than that of [^{11}C]PK11195 in the monkey brain (Maeda *et al.* 2004). A quantitative analysis method for [^{11}C]DAA1106 binding in the human brain has been well established with the two-tissue compartment model (Ikoma *et al.* 2007). [^{11}C]DAA1106 was

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Table 1. Demographic and clinical characteristics of the patients with schizophrenia

Subject	Age (yr), sex	PANSS				Duration of illness (yr)	Duration of drug treatment (yr)	Haloperidol equivalent (mg)	Main antipsychotics
		Positive	Negative	General	Total				
1	29, F	12	12	25	49	11	9	3	Olanzapine
2	34, F	17	12	33	62	7	5	6	Risperidone
3	37, F	14	23	27	64	0.5	0.5	3	Olanzapine
4	43, F	21	27	49	97	22	19	17	Risperidone
5	46, F	16	15	34	65	33	21	10	Nemonapride
6	49, F	24	20	33	77	23	16	19.4	Haloperidol
7	42, M	15	22	27	64	4	4	4	Olanzapine
8	43, M	15	26	33	74	26	23	9	Haloperidol
9	44, M	22	25	40	87	22	22	8.5	Olanzapine
10	44, M	16	26	37	79	4	4	14	Haloperidol
11	46, M	29	26	56	111	26	26	3.5	Olanzapine
12	46, M	16	16	25	57	24	24	4	Risperidone
13	52, M	24	35	58	117	18	17	16.5	Olanzapine
14	59, M	27	24	47	87	43	39	10.3	Mosapramine
		19.1 ± 5.3	22.1 ± 6.5	37.4 ± 11.1	77.9 ± 20.1	18.8 ± 12.2	16.4 ± 10.8	9.2 ± 5.7	

PANSS, Positive and Negative Syndrome Scale; F, female; M, male.
Haloperidol (1 mg) was equivalent to chlorpromazine (50 mg).

demonstrated to be useful in the study of neurodegerative disorders such as Alzheimer's disease (Yasuno *et al.* 2008).

In this study, we investigated PBR binding in patients with chronic schizophrenia using [^{11}C]DAA1106 to evaluate whether glial reaction was involved in the pathophysiology of schizophrenia.

Materials and methods

Subjects

Fourteen patients with schizophrenia [six females, eight males; 43.9 ± 7.4 yr (mean $\pm$ S.D.)] and 14 normal control subjects (five females, nine males; 42.5 ± 9.0 yr) were enrolled in this study. Patients were recruited from the outpatient and in-patient units of Nippon Medical School Hospital, Asai Hospital and Sobu Hospital, located in Tokyo and Chiba prefecture in Japan. The patients were diagnosed as having schizophrenia and treated by attending physicians at each hospital, and their diagnoses were re-evaluated with structured interviews at our PET centre. All 14 patients were diagnosed with schizophrenia according to DSM-IV criteria. Exclusion criteria were current or past substance, cannabis or alcohol abuse, mood disorders, and organic brain disease. The patients' demographic and clinical data are shown in Table 1. None of the patients had taken benzodiazepines within more than 1 month prior to PET measurements. Psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS; Kay *et al.* 1987). PANSS was completed by three experienced psychiatrists on the same day as the PET measurements. They reviewed the ratings after the interviews, and disagreements were resolved by consensus; the consensus ratings were used in this study. The symptom scores were calculated as total scores, positive symptom, negative symptom, and general symptom subscores of PANSS. The total PANSS score ranged from 49 to 117 (78.6 ± 20.7). The mean positive symptom score was 19.1 ± 5.3, negative symptom score was 22.1 ± 6.5, and general symptom score was 37.4 ± 11.1.

The normal control subjects were recruited from the surrounding community. Based on psychiatric screening interviews, they were free of current and past psychiatric or major medical disease, and had no relatives with neuropsychiatric disorders.

This study complied with the current laws of Japan, and was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from all subjects.

Radiochemistry

[^{11}C]DAA1106 was prepared as described in detail previously (Ikoma *et al.* 2007; Zhang *et al.* 2003). The precursor was supplied by Taisho Pharmaceutical Co. (Japan).

PET data acquisition

PET scans were performed with ECAT EXACT HR+ (CTI-Siemens, USA), which provides 63 planes and a 15.5-cm axial field of view (FOV). A 10-min transmission scan with a ^{68}Ge–^{68}Ga source was followed by a 90-min dynamic scan (20s × 9, 60s × 5, 120s × 4, 240s × 11, and 300s × 6) with a bolus injection of 261–411 (369 ± 27) MBq of [^{11}C]DAA1106. Specific radioactivity was 15.4–220.7 GBq/μmol at the time of the injection. There was no significant difference in injected radioactivity and specific radioactivity between patients and normal controls (373 ± 20 MBq and 60.3 ± 44.4 GBq/μmol for patients, and 366 ± 32 MBq and 98.4 ± 70.7 GBq/μmol for normal controls). Radioactivity was measured in three-dimensional mode, and the data were reconstructed with a Hanning filter with a cut-off frequency of 0.4 (full width half maximum = 7.5 mm).

Arterial blood sampling

To obtain the arterial input function, an automated blood sampling system was used for continuous (counts/s) blood radioactivity measurements during the first 12 min of PET measurement. At the same time, arterial blood samples were taken manually and their radioactivity concentration was measured 13 times during the initial 3 min after the injection, eight times during the next 17 min, and once every 10 min until the end of the scan. To analyse the metabolite fraction in the plasma, arterial blood samples were taken 10 times during PET measurements. The parent ligand, separated from the total radioactive compound, was measured as previously described (Ikoma *et al.* 2007). The mean time-course of the fraction of the parent ligand is shown in Fig. 1. There was a significant group × time interaction using repeated-measures ANOVA with Greenhouse–Geisser correction ($F_{3.4,81.1} = 4.92$, $p = 0.002$), although one subject from each group was excluded for the statistical analysis due to one missing data-point.

MR imaging

T1-weighted magnetic resonance imaging (MRI) of the brain was performed with Philips Intera 1.5 T (Philips Medical Systems, The Netherlands). T1-weighted images of the brain were obtained from all subjects. The scan parameters were 1-mm-thick 3D T1 images with a transverse plane [repetition time (TR)/echo time (TE) 22/9.2 ms, flip angle 30°, matrix 128 × 128, FOV 256 × 256). Voxel size of the magnetic resonance images was 1 mm × 1 mm × 1 mm.

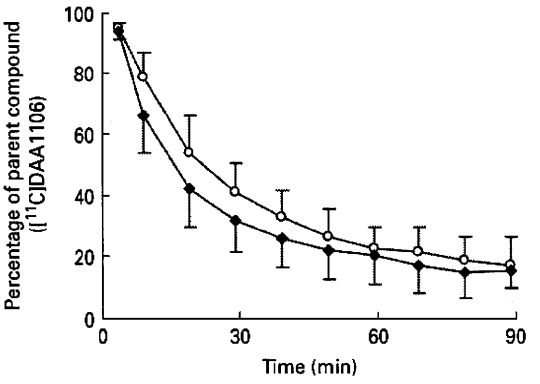

Fig. 1. Mean time-course of the percentage of parent compound ([^{11}C]DAA1106) after venous injection of [^{11}C]DAA1106 between normal controls (–○–) and patients (–◆–) with schizophrenia.

Data analysis

Eleven regions of interest (ROIs) (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, thalamus, striatum, cerebellum, anterior cingulate cortex, and posterior cingulate cortex) were delineated on the co-registered PET/MRI images. In addition to each regional ROI, eight cortical ROIs (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, anterior cingulate cortex, and posterior cingulate cortex) were also summed up as total cortical regions.

Regional time–activity data were analysed with two-tissue compartment model (2-TC) with the metabolite-corrected plasma input function, a model demonstrated to estimate binding potential (BP_{ND}) most reliably for [^{11}C]DAA1106 (Ikoma *et al.* 2007). Rate constants were estimated with weighted least squares and the Marquardt optimizer. For each region, k_1, k_2, k_3, k_4 and blood volume were estimated by 2-TC. BP_{ND} was calculated as k_3/k_4 in this analysis. Data analysis was performed with PMOD 2.65 (PMOD Technologies, Switzerland).

Statistical analysis

Regional ROIs

Statistical analysis of the difference of regional BP_{ND} for each ROI (for total 11 ROIs) between patients and normal controls was performed by repeated-measures ANOVA ($p < 0.05$ was considered significant). When any interaction was found, *post-hoc* Bonferroni correction was used for multiple comparisons.

Table 2. Significant correlation between PANSS scores and regional [^{11}C]DAA1106 binding

PANSS scores	Region	p value
Positive symptom	Medial frontal cortex	0.002*
	Dorsolateral frontal cortex	0.022
	Medial temporal cortex	0.003*
	Lateral temporal cortex	0.013
	Parietal cortex	0.005
	Occipital cortex	0.001*
	Cerebellum	0.022
	Striatum	0.010
Negative symptom	None	
General symptom	Medial frontal cortex	0.018
	Medial temporal cortex	0.027
	Occipital cortex	0.038
Total score	Medial frontal cortex	0.012
	Medial temporal cortex	0.029
	Parietal cortex	0.044
	Occipital cortex	0.017

PANSS, Positive and Negative Syndrome Scale.
* $p < 0.0045$ (0.05/11).

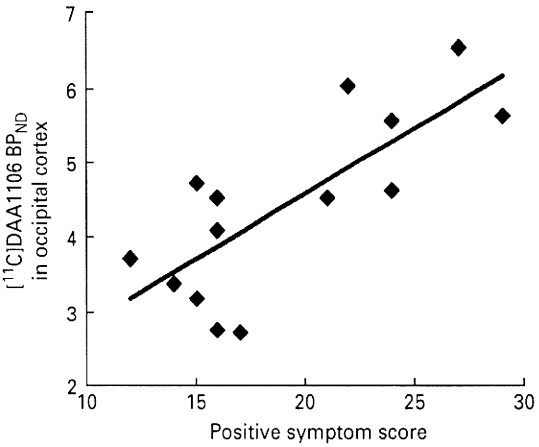

Fig. 2. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the occipital cortex and positive symptom scores in the Positive and Negative Syndrome Scale.

Correlation between regional BP_{ND} values and PANSS scores were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Correlation between regional BP_{ND} values and duration of illness, duration of drug treatment, and chlorpromazine equivalent doses (Inagaki *et al.* 1999) were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Changes in regional BP_{ND} values with age were analysed with Pearson's correlation method for patients and normal controls, respectively ($p < 0.05$ was considered significant).

Total cortical regions

For analysing differences in total cortical regions between patients and normal controls, Student's t test was used ($p < 0.05$ was considered significant).

Correlations between BP_{ND} values in total cortical regions and PANSS scores were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Correlation between BP_{ND} values in total cortical regions and duration of illness, duration of drug treatment, and chlorpromazine-equivalent doses (Inagaki *et al.* 1999) were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Changes in BP_{ND} values in total cortical regions with age were analysed with Pearson's correlation method for patients and normal controls, respectively ($p < 0.05$ was considered significant).

Results

Regional ROIs

Comparison of regional BP_{ND} values for [^{11}C]DAA1106 between the patients with schizophrenia and normal controls by two-way repeated ANOVA with Greenhouse–Geisser correction showed no significant group × region interaction ($F_{1.7,44.4} = 0.542$, $p = 0.558$).

For the correlation analysis between BP_{ND} values in regional ROIs and positive symptom scores in the patient group, significant correlations were found in regions such as the medial frontal cortex, medial temporal cortex and occipital cortex (Table 2) (Fig. 2). No correlation was found between BP_{ND} values of each region and negative symptoms. Those three regions showed trends of positive correlation with general symptoms and total score (Table 2). There was no significant correlation between regional BP_{ND} and the duration of illness.

There was no significant change of regional BP_{ND} values with age in normal controls, whereas significant changes in BP_{ND} values with age in the patients with schizophrenia were observed in the occipital cortex ($p = 0.014$), lateral temporal cortex ($p = 0.023$), parietal cortex ($p = 0.023$), medial temporal cortex ($p = 0.031$), and medial frontal cortex ($p = 0.036$).

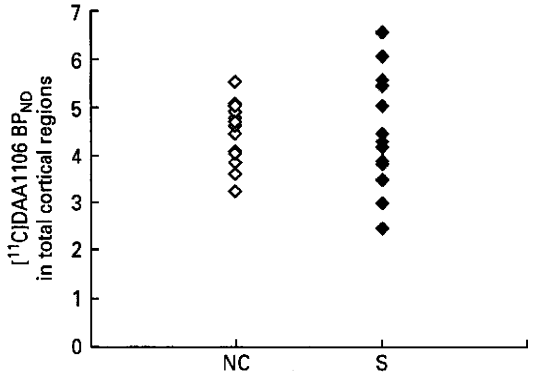

Fig. 3. Comparison of [^{11}C]DAA1106 BP_{ND} of total cortical regions between normal controls (NC) and patients with schizophrenia (S).

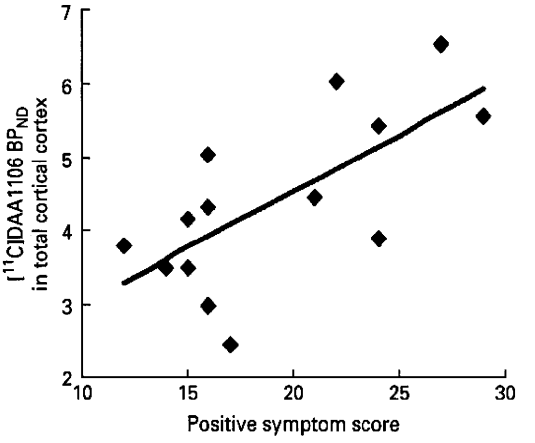

Fig. 4. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the total cortical region and positive symptom scores in the Positive and Negative Syndrome Scale.

Total cortical regions

There was no significant difference of BP_{ND} values in total cortical regions between patients with schizophrenia and normal controls (Fig. 3). Significant correlation was found with the positive symptom scores ($p=0.006$) (Fig. 4). There was no significant correlation with other symptom scores (negative, general, and total symptom scores). Total cortical regions were correlated with duration of illness ($p=0.020$) (Fig. 5) and duration of drug treatment ($p=0.023$). BP_{ND} of total cortical regions was not correlated with chlorpromazine-equivalent doses.

There was no significant change of BP_{ND} values in total cortical regions with age in normal controls, but significant changes of BP_{ND} values with age were observed in total cortical regions of the patients with schizophrenia ($p=0.018$).

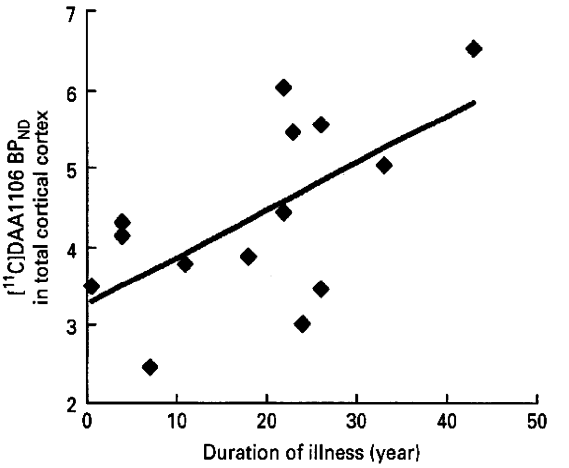

Fig. 5. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the total cortical region and duration of illness.

Discussion

In this study, [^{11}C]DAA1106 binding, which was considered to correspond to the density of PBR, was not different between the patients with chronic schizophrenia and normal controls. A recent study demonstrated that [^{11}C]PK11195 binding increased in total grey matter in patients with acute-onset schizophrenia (van Berckel *et al.* 2008). Another recent study reported that [^{11}C]PK11195 binding in the hippocampus was significantly increased in patients with schizophrenia during acute psychosis, while there was no significant difference in other regions compared with normal controls (Doorduin *et al.* 2009). To understand the difference in the results between the present study and the two [^{11}C]PK11195 studies, several factors, such as the use of different radioligands and different patient groups, should be taken into consideration. Although PK11195 fully displaced the [^{3}H]DAA1106 binding (Chaki *et al.* 1999), a high concentration of PK11195 was required for this displacement. This suggested that the binding domain for DAA1106 contains an extra component that does not interact efficiently with PK11195 (Chaki *et al.* 1999). The mean age of patients with schizophrenia enrolled in the present study was higher (44 yr in 14 patients) than those in the two [^{11}C]PK11195 studies (24 yr in 10 patients, and 31 yr in seven patients). Most of the patients in the present study were at the chronic stage.

Within the patient group, [^{11}C]DAA1106 binding had a significant correlation with the positive symptom score of PANSS, a finding that might be in line

with those recent findings with [^{11}C]PK11195. The present results might indicate that the activated neuroimmune system was related to the pathophysiology of schizophrenia at the chronic stage.

In previous MRI volumetric research in schizophrenia, volume reduction in the brain has been reported in patients with chronic schizophrenia (Shenton *et al.* 2001). However, in the present study, there was no significant difference in the volume of ROIs by ANOVA, and total cortical ROI by Student's *t* test between the patients and normal controls (data not shown). Thus, the insignificance of the difference of [^{11}C]DAA1106 binding between the patients and normal controls is not related to the partial volume effect due to brain atrophy.

In this study, normal controls showed no age effects on [^{11}C]DAA1106 binding in any region. This is in line with the report with [^{11}C]PK11195 binding except the thalamus, where [^{11}C]PK11195 binding was reported to increase with age (Cagnin *et al.* 2001). This might be due to different radioligands or different age ranges between the two studies (24–55 yr in this study and 32–80 yr in the [^{11}C]PK11195 study). On the other hand, [^{11}C]DAA1106 binding was found to increase with age in patients with schizophrenia. Schizophrenia has been considered to be progressive in functional disability and morphological changes (Lieberman *et al.* 2001; Mathalon *et al.* 2001; Saijo *et al.* 2001). The present results of the positive correlation among [^{11}C]DAA1106 binding, duration of illness, and age might suggest that the progressive change occurs at the glial reaction level.

A recent meta-analysis showed that some cytokines such as IL-1RA, sIL-2R, and IL-6 are increased in schizophrenia (Potvin *et al.* 2008). PBR has been considered to modulate the release of pro-inflammatory cytokines in the CNS. PBR was reported to modulate the release of the inflammatory molecules NO and tumour necrosis factor-alpha (TNF-α) (Wilms *et al.* 2003). A PBR ligand, PK11195, has been reported to inhibit lipopolysaccharide-induced expressions of COX-2 and TNF-α in human microglia (Choi *et al.* 2002). Immunomodulatory drugs such as cyclooxygenase-2 (COX-2) inihibitors have been reported to show beneficial effects in schizophrenia (Muller & Schwarz, 2008). The combination of risperidone and COX-2 inhibitor has been reported to show superiority over risperidone alone in positive symptoms and PANSS total scores (Akhondzadeh *et al.* 2007). On the other hand, cytokines such as IL-2 and IL-6 are reported to increase after olanzapine and clozapine treatment (Kluge *et al.* 2009). The present results of PBR binding in the patients with schizophrenia might be in accord with the previous reports of cytokines.

A recent report demonstrated that PBR expression was not confined to microglia but was inducible in nervous tissue cells of neuroepithelial origin (Ji *et al.* 2008). Thus, PBR binding might also arise from astrocytes and other non-microglial elements. Schizophrenia patients with high S100B serum concentration, considered to indicate astrocyte activation, were reported to have cognitive dysfunction compared with patients with low S100B serum concentration (Pedersen *et al.* 2008). DAA1106 binding in patients with schizophrenia might also be related to the change in PBR on astrocytes.

In a post-mortem study, a subgroup of the patients with schizophrenia who committed suicide had increased microglial densities, although microglial HLA-DR expression in the patients with schizophrenia was not different from normal controls (Steiner *et al.* 2008). Microglial activation has been suggested to be interpretable as a consequence of presuicidal stress (Avital *et al.* 2001; Lehmann *et al.* 2002).

Although BP_{ND} of total cortical regions was not correlated with chlorpromazine-equivalent doses in the present study, some antipsychotics were reported to have anti-inflammatory effects (Kato *et al.* 2007; Kowalski *et al.* 2003, 2004; Labuzek *et al.* 2005; Zheng *et al.* 2008). The effect of antipsychotics on DAA1106 binding remains to be studied.

There are several confounding factors in the present study. First, the number of subjects was relatively small. Further larger-scale studies will be needed to confirm the present results. Second, all the patients were under different kinds of antipsychotic treatment. Further study is needed with drug-naive patients and patients under well-controlled drug treatment. Third, the PANSS scores of patients were higher as the duration of the illness was longer and age increased. This might reflect a possible subgroup of treatment-resistant patients.

In conclusion, we found no significant differences in PBR binding between the brains of patients with schizophrenia and those of normal control subjects, unlike recent reports with [^{11}C]PK11195 (van Berckel *et al.* 2008; Doorduin *et al.* 2009). Nevertheless, PBR binding in the patients with schizophrenia was correlated with positive symptoms, disease duration and age. The present results suggest that the glial reaction process might be involved in the pathophysiology of schizophrenia. Although the correlations should be interpreted with caution, these results at least suggest that additional studies are warranted in order to determine whether baseline

differences exist between patients with schizophrenia and healthy subjects, as well as to reveal the biological meanings of the correlations with disease parameters.

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Statement of Interest

None.

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Decreased binding of [^{11}C]NNC112 and [^{11}C]SCH23390 in patients with chronic schizophrenia

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ABSTRACT

Aims: Abnormality of cognitive function in schizophrenia has been suggested to be related to dopamine D_1 receptor. However, the results of previous positron emission tomography (PET) studies of dopamine D_1 receptor in schizophrenia were not consistent.
Main methods: In this study, six patients with schizophrenia in severe residual phase with chronic antipsychotic treatment and twelve healthy age-matched controls participated. Two different radioligands, [^{11}C]NNC112 and [^{11}C]SCH23390, for dopamine D_1 receptor were used on the same subjects. Binding of the ligands was measured by PET, and statistical analysis was performed using one-way analysis of covariate (ANCOVA) with age as covariate.
Key findings: Good correlations between binding potential values (BP_{ND}) and age were observed in all regions of interest (ROIs) with both ligands. ANCOVA with age as covariate of BP_{ND} values of all ROIs revealed that the patient group showed significantly lower BP_{ND} value compared with the control group in both ligands.
Significance: In patients with chronic schizophrenia in severe residual phase with chronic antipsychotic treatment, the binding potential values of both ligands were significantly lower in the striatum and cortical regions than those of healthy controls.

Introduction

Schizophrenia is a chronic illness characterized by positive, negative, cognitive and affective symptoms (Schultz and Andreasen 1999). Although a positive symptom is characteristic of schizophrenia in the acute phase, the characteristic symptoms in the severe residual phase are negative symptom and cognitive dysfunction. The dopamine hypothesis is widely accepted for the pathophysiology of schizophrenia. Regarding dopamine receptors, the density of dopamine D_1 receptor in the cortical region is several times higher than that of dopamine D_2 receptor (Lidow et al. 1998). Abnormality of cognitive function in schizophrenia has been suggested to be related to dopamine function in the prefrontal cortex (Sawaguchi and Goldman-Rakic 1991). Dopamine D_1 receptor plays important roles in cognitive function such as working memory (Goldman-Rakic, 2000). One postmortem study has reported low dopamine D_1 receptors in the striatum in patients with schizophrenia (Hess et al. 1987), but no significant change has been reported in other studies (Seeman et al. 1987; Czudek and Reynolds 1988; Knable et al. 1994). In vivo PET studies reported decreased (Okubo et al. 1997), unaltered (Karlsson et al. 2002), and increased (Abi-Dargham et al. 2002) binding of D_1 receptor in patients with schizophrenia compared with control subjects. Those results were possibly influenced by parameters of the particular patient populations including duration of illness, symptoms and medications. In addition, differences in radioligand [^{11}C]SCH23390 (Okubo et al. 1997; Karlsson et al. 2002) and [^{11}C]NNC112 (Abi-Dargham et al. 2002) were suggested to account for inconsistent PET findings. Furthermore, subjects were medication-free or -naïve patients with schizophrenia in the prodromal, acute or active phase, and the duration of untreated illness may have influenced the difference in dopamine D_1 receptor binding in previous human PET studies.

The purpose of the present study was to compare the dopamine D_1 receptor binding of chronic patients with schizophrenia in severe residual phase with chronic antipsychotic treatment to that of healthy controls in the striatum and extrastriatal regions using both [^{11}C]SCH23390 and [^{11}C]NNC112 in the same subjects.

Materials and methods

Subjects

Six patients with schizophrenia, 1 female and 5 males aged 46.5 ± 8.2 years (mean ± SD), participated in this study (Table 1). All patients

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Table 1
Clinical characteristics of patients.

Patient no.	Age (years)	Gender	Diagnosis (DSM-IV)	Dose of sulpiride (mg)	Duration of illness (years)	PANSS			
						Positive	Negative	General	Total
1	32	M	295.30	1200	15	16	21	30	67
2	45	F	295.30	600	28	25	26	49	100
3	47	M	295.30	1200	8	18	23	44	85
4	47	M	295.30	1000	25	23	26	47	96
5	52	M	295.10	1200	5	13	33	46	92
6	56	M	295.60	600	29	20	43	59	122
Mean ± SD	46.5 ± 8.2			966.7 ± 294.4	18.3 ± 10.5	19.2 ± 4.4	28.7 ± 8.1	45.8 ± 9.4	93.7 ± 18.1

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; PANSS, Positive and Negative Scale for Schizophrenia; M, Male; F, Female.

met the criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) for diagnosis of schizophrenia. The diagnosis was assessed by Structured Clinical Interview for DSM-IV by three psychiatrists. The patients underwent general medical and laboratory evaluation. Organic brain disease was ruled out by CT, T1-weighted magnetic resonance (MR) images, and electroencephalogram.

Prior to this study, they had been prescribed antipsychotics during the periods indicated as 'duration of illness' in Table 1. In chlorpromazine equivalents, daily doses ranged from 200 mg to 606 mg and mean dose was 384 ± 139 mg/day (Inagaki and Inada 2006).

In all patients, the previously used antipsychotic drugs were changed to sulpiride, a selective dopamine D_2/D_3 receptor antagonist without affinity to dopamine D_1 receptor. PET scans were performed after a washout period of at least three weeks after changing to sulpiride. Sulpiride was maintained at the same dosage during the washout period. Because of extrapyramidal side effects, two patients were administered a relatively low dose of sulpiride (600 mg), although there had been no exacerbation of their psychic symptoms. All patients underwent clinical ratings of their psychopathology using the positive and negative syndrome scale (PANSS; Kay et al. 1987), and the following cognitive function tests: Wisconsin Card Sorting Test (Heaton 1981) to evaluate executive function, Stroop test (Cohen and Servan-Schreiber 1992) and *n*-back tasks (2-back minus 0-back using letters as stimulus; Cohen et al. 1994; Owen et al. 2005) to evaluate working memory.

The healthy control sample consisted of 6 females and 6 males, age-matched at 42.8 ± 8.5 years. Based on unstructured psychiatric screening interviews, none had a history of neurological or psychiatric illness. Organic brain disease was ruled out by T1-weighted MRI.

The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. After providing a complete explanation of the study, written informed consent was obtained from all subjects.

PET and MRI procedures

All patients except patient #6 (Table 1) underwent both PET scans using [^{11}C]NNC112 and [^{11}C]SCH23390 on the same day. Patient #6 and twelve healthy controls underwent each of the PET scans with [^{11}C]NNC112 and [^{11}C]SCH23390 within several days. The PET system ECAT EXACT HR+ (CT1-Siemens, Knoxville, TN) was used for all PET studies. The system provides 63 planes with a 15.5 cm axial field of view. After a transmission scan with a ^{68}Ge–^{68}Ga source, a bolus of [^{11}C]NNC112 or [^{11}C]SCH23390 was rapidly injected into the antecubital vein with a 20-ml saline flush. Injected radioactivity and specific radioactivity were 220.5 ± 9.25 MBq and 140.0 ± 64.1 GBq/μmol for patients in the [^{11}C]NNC112 studies, 215.0 ± 14.1 MBq and 152.5 ± 50.6 GBq/μmol for controls in the [^{11}C]NNC112 studies, 200.2 ± 15.9 MBq and 59.7 ± 15.5 GBq/μmol for patients in the [^{11}C]SCH23390 studies, and 220.5 ± 18.1 MBq and 68.6 ± 11.0 GBq/μmol for controls in the [^{11}C]SCH23390 studies, respectively.

Radioactivity in the brain was measured by a series of scans for 90 min for [^{11}C]NNC112 or 60 min for [^{11}C]SCH23390, starting immediately after the injection. During image acquisition, the subjects were instructed to lie quietly with their eyes closed and earplugs in place. Image reconstruction was performed with a Hanning filter with a cut-off frequency of 0.4, a value experientially determined for the purpose of noise reduction, resulting in a final spatial resolution of 7.5 mm FWHM (full width at half maximum).

T1-weighted MR images were acquired on Philips Gyroscan NT, 1.5 T (Philips Medical Systems, Best, The Netherlands). Scan parameters were 1-mm-thick 3D images with a transverse plane (repetition time, TR/echo time, TE 21/9.2 ms, flip angle 30°, matrix 256 × 256, field of view (FOV) 256 × 256), yielding 196 contiguous slices of the head.

PET data analysis

Regions of interest (ROIs) were manually drawn on the transverse slices from each subject's PET summation images referred from MRI images coregistered to the reconstructed PET images. ROIs were set to cover 3 adjacent slices for the striatum including both the caudate nucleus and the putamen, anterior cingulate, cerebellum, temporal cortex and frontal cortex including the superior frontal gyrus, middle frontal gyrus, and inferior frontal gyrus, which roughly corresponds to dorsolateral prefrontal cortex. The sets of ROIs for each section were transferred to the corresponding PET images, and time–activity curves (TACs) were obtained. The TACs of each region were analyzed using a simplified reference tissue model in a least-squares manner, in which the cerebellum was used as reference tissue (Lammertsma and Hume 1996). This procedure produced the binding potential (BP_{ND}; Innis et al. 2007) value.

Statistical analysis

Statistical analysis of the regional BP_{ND} obtained from patients with schizophrenia and healthy control subjects was performed using one-way analysis of covariance (one-way ANCOVA) with age as covariate using SPSS for Windows 16.0.2J (SPSS Inc, Chicago, Illinois, USA 2008), and post hoc Bonferroni correction was used for multiple comparisons. p value $< 0.05/4 = 0.0125$ was considered significant.

Results

Table 1 lists the clinical profiles of the patients. The average duration of illness after schizophrenia diagnosis was 18.3 years. Scores of the two cognitive functional tests are shown in Table 2, and significant group effects were found in each cognitive function test. Because four patients, #2, #3, #5 and #6, were not able to do *n*-back task (2 back), results were not shown in Table 2.

Significant correlations between BP_{ND} and age were observed in patients with [^{11}C]NNC112 (frontal cortex, $r = -0.924$, $p = 0.004$; striatum, $r = -0.981$, $p = 0.001$), controls with [^{11}C]NNC112 (striatum, $r = -0.886$, $p < 0.001$) and controls with [^{11}C]SCH23390 (frontal cortex, $r = -0.757$, $p = 0.004$; striatum, $r = -0.700$, $p = 0.011$). Trend

Table 2
Cognitive task scores of patients.

Patient no.	W-CST			Stroop test	
	Category	PEN	DMS	Error	Time score
1	6	0	0	0	17.4
2	2	9	5	11	46.6
3	1	7	3	1	7.4
4	5	1	1	0	5.4
5	2	14	0	2	68
6	Incapable	Incapable	Incapable	2	75
Mean ± SD	3.2 ± 2.2	6.2 ± 5.8	1.8 ± 2.2	2.7 ± 4.2	36.6 ± 30.8
Controls					
Mean ± SD	4.7 ± 1.6	1.4 ± 2.0	0.8 ± 1.4	0.8 ± 1.2	5.6 ± 4.0

W-CST, Wisconsin card sorting test; PEN, errors of nelson; DMS, difficulty in maintaining set.

level correlations were observed in other regions and patients with [^{11}C]SCH23390.

All BP_{ND} values of both ligands are shown in Fig. 1 and summarized in Table 3. ANCOVA with age as covariate ($df = 1,15$) of BP_{ND} values of all ROIs revealed that the patient group showed significantly lower BP_{ND} value compared with the control group in both ligands ([^{11}C]NNC112: temporal cortex, $F = 26.24$, $p < 0.001$; striatum, $F = 60.08$, $p < 0.001$; anterior cingulate cortex, $F = 9.14$, $p = 0.009$; frontal cortex, $F = 42.96$, $p < 0.001$, [^{11}C]SCH23390: temporal cortex, $F = 34.68$, $p < 0.001$; striatum, $F = 25.46$, $p < 0.001$; anterior cingulate cortex, $F = 8.91$, $p = 0.009$; frontal cortex, $F = 37.60$, $p < 0.001$). There was significant correlation between average BP values of [^{11}C]NNC112 weighted by ROI size and that of [^{11}C]SCH23390 ($r = 0.859$; $BP_{NNC} = 0.613\ BP_{SCH} + 0.0414$).

There was no significant correlation between BP_{ND} values and doses of antipsychotic drugs and between BP_{ND} values and PANSS scores for positive symptom, negative symptom, general symptom and total score in any of the brain regions.

Discussion

Both [^{11}C]NNC112 and [^{11}C]SCH23390 bindings in the striatum and cortical regions of patients with schizophrenia in severe residual phase were significantly lower compared with healthy controls. In previous PET studies of patients with schizophrenia who were antipsychotics-naïve or -free, BP of [^{11}C]SCH23390 was decreased (Okubo et al. 1997) or unchanged (Karlsson et al. 2002), and was increased when measured by [^{11}C]NNC112 (Abi-Dargham et al. 2002). Several differences in those studies have been discussed, including those regarding duration of illness, medications, race, severity of symptoms and radioligands. Guo et al. (2003) reported different characteristics of in vivo binding of the two radioligands in rat brain, increased [^{11}C]NNC112 binding and decreased [^{3}H]SCH23390 binding, following subchronic dopamine depletion with reserpine. But the inconsistent results cannot be explained solely by the difference of radiotracers, and demographics of patients might have been contributing factors.

Although [^{11}C]SCH23390 and [^{11}C]NNC112 are selective radioligands for dopamine D_1 receptor, both ligands have some affinity for 5-

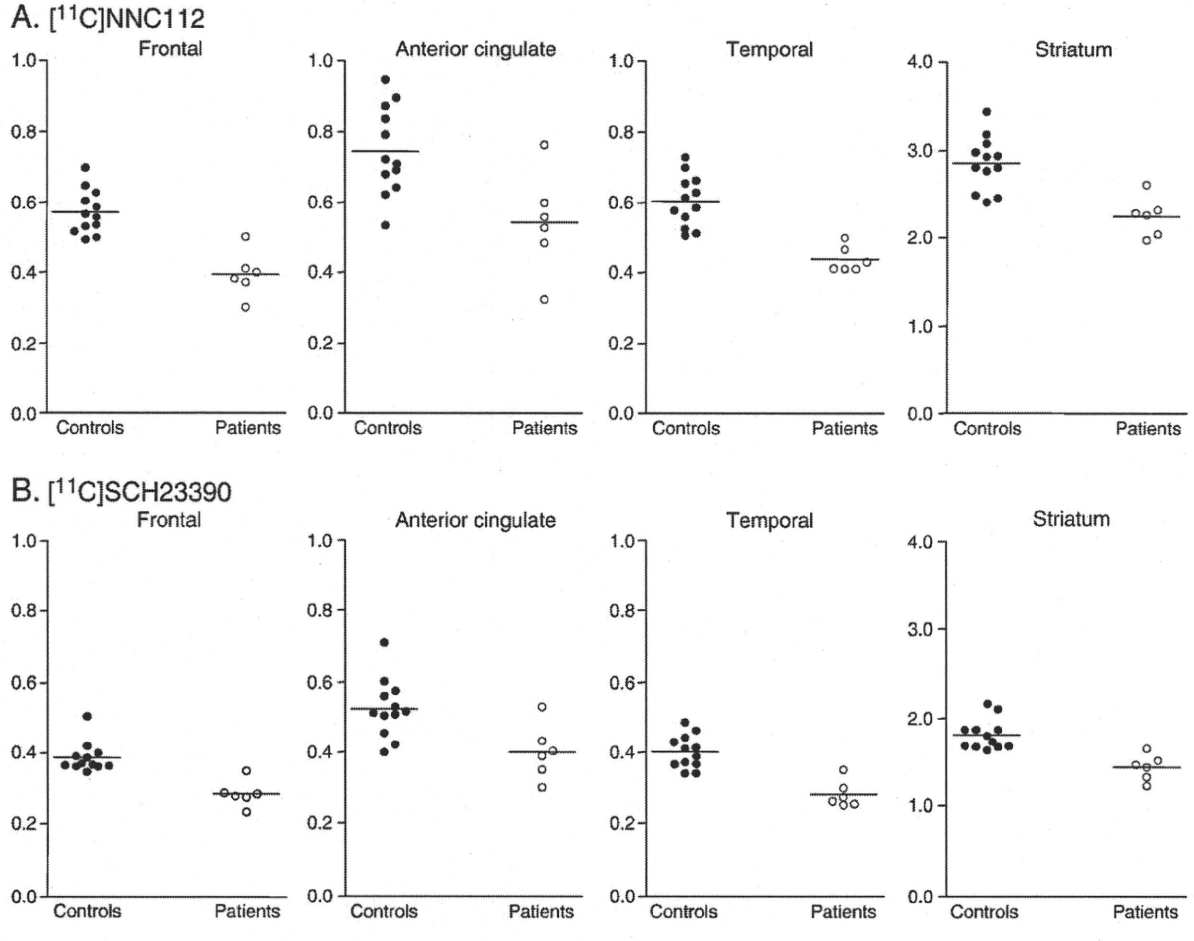

Fig. 1. BP_{ND} values of all subjects in both ligands [^{11}C] NNC112 and [^{11}C]SCH23390. Filled circles represent controls and open circles represent patients. A. BP_{ND} measured by [^{11}C] NNC112; B. BP_{ND} measured by [^{11}C]SCH23390. The horizontal line represents the group mean. In all ROIs, statistically significant differences were observed between patients with schizophrenia and healthy controls (one-way ANCOVA with age as covariate, $p < 0.0125 = 0.05/4$).