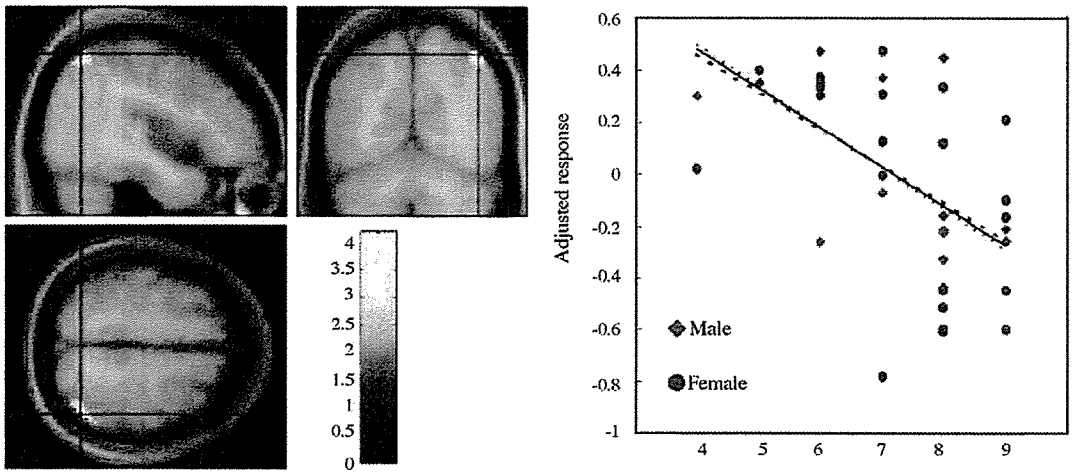

Fig. 5. Results of correlational analysis (negative correlation). Left: the area where activation responds linearly to the task performance. There was a significantly negative correlation between task performance and the degree of activation in the right superior parietal lobule (BA7). Right: the correlation between the task performance and the degree of activation at the voxel of peak activation in the right BA7. A significantly negative correlation was noted (whole group: $y = -0.1512X + 1.087$, $r = 0.5956$, $p < 0.001$; men: $y = -0.1444X + 1.0411$, $r = 0.651$, $p < 0.001$; women: $y = -0.1584X + 1.1346$, $r = 0.58$, $p < 0.001$). Blue diamonds: male subjects; red circles: female subjects; black solid line: the regression line for all subjects; blue dashed line: regression line for male subjects; red dashed line: female subjects.

navigation in a daily life. The mean total scores of five items (S.D.) in each group were 6.9 (1.38) in male poor navigators, 6.4 (1.16) in female poor navigators, 16.7 (1.85) in male good navigators, and 17.2 (1.54) in female good navigators, respectively. The ANOVA, navigation ability (good and poor) by sex, with the score of SDQ-S, revealed only a significant main effect of navigation ability ($F = 606.7, p < 0.001$). There is no main effect of sex or interaction effect. The main effect of navigation ability showed that good navigators showed higher scores of SDQ-S than poor navigators did ($p < 0.001$).

3.2. fMRI measurements

This one sample t-test for all the subjects revealed significant bilateral activity of the middle occipital gyri, lingual gyri, fusiform gyri, parahippocampal gyri, hippocampus, posterior cingulate cortex (PCC), precuneus, cuneus, parietal association areas and right dorsolateral prefrontal cortex (DLPFC, Broadman area 9), (Table 1 and Fig. 2). Additionally, significant activations in the bilateral superior colliculus were noted (Fig. 2, left).

In the post hoc group comparison for ANOVA (main effect of navigation ability), good navigators showed a significantly stronger activation in the bilateral parahippocampal gyri and the precuneus than poor navigators (Table 2 and Fig. 3, left). On the other hand, poor navigators showed a significantly stronger activation in the right inferior parietal lobule (Table 2 and Fig. 3, right). There was no interaction effect and main effect of sex in any region even at a lenient statistical threshold (uncorrected $p < 0.005$).

In the correlational analysis, there was a significant positive linear correlation between the number of correct answers and

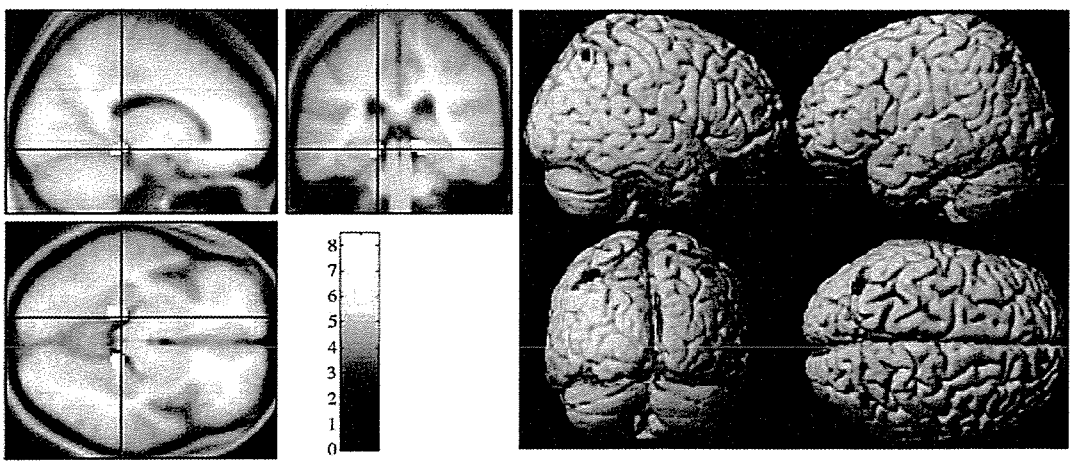

Fig. 6. Brain activity correlated with SDQ-S scores. Left: there was a significantly positive correlation between scores of five items of the SDQ-S and the degree of activation in the bilateral hippocampi and parahippocampal gyri. Right: a significantly negative correlation with scores of five items of SDQ-S was noted in the bilateral superior parietal lobules (BA7).

Table 3
Results of regions of interest analysis

Regions	Correlation with task performance		Correlation with scores of five items of the SDQ	
	Correlation coefficient	p-Value	Correlation coefficient	p-Value
L: hippocampus	0.45	0.0048	0.54	0.0036
L: parahippocampal gyrus	0.48	0.0046	0.58	0.0034
R: hippocampus	0.34	0.03	0.58	0.0041
R: parahippocampal gyrus	0.38	0.03	0.56	0.0045

the degree of activation in the left hippocampus and parahippocampal gyrus (Table 2 and Fig. 4, left). Fig. 4(right) demonstrates the correlation between the task performance and the degree of activation at the voxel of peak activation in the left parahippocampus. A significantly positive correlation was noted (whole subjects: $y = 0.195X - 1.4$, $r = 0.72$, $p < 0.001$; men: $y = 0.1793X - 1.239$, $r = 0.723$, $p < 0.001$; women: $y = 0.218X - 1.572$, $r = 0.719$, $p < 0.001$). On the other hand, a significant negative linear correlation between the number of correct answers and the degree of activation was noted in the right superior parietal lobule (BA7) (Table 2 and Fig. 5, left). Fig. 5(right) demonstrates the correlation between the task performance and the degree of activation at the voxel of peak activation in the right BA7. A negative correlation was noted (whole group: $y = -0.1512X + 1.087$, $r = 0.5956$, $p < 0.001$; men: $y = -0.1444X + 1.0411$, $r = 0.651$, $p < 0.001$; women: $y = -0.1584X + 1.1346$, $r = 0.58$, $p < 0.001$) (Fig. 5, left). There was no interaction effect between sex and task performance on the degree of activation in the left medial temporal region and right superior parietal lobule. Additional correlational analysis; i.e. correlation between brain activity and scores of five items of the SDQ-S, demonstrated significant positive correlation between scores of SDQ and degree of activation in the bilateral hippocampus and parahippocampal gyri (Fig. 6, left and Table 2). On the other hand, a significantly negative correlation between scores of five items of the SDQ-S correlated and brain activity was noted in the bilateral superior parietal lobules was noted (Fig. 6, right and Table 2).

Table 3 demonstrates results of ROI analysis. A significantly positive correlation between the task performance and degree of activation in the bilateral hippocampi (L: $r = 0.45$, $p = 0.0048$; R: $r = 0.34$, $p = 0.03$) and bilateral parahippocampal gyri (L: $r = 0.48$, $p = 0.0046$; R: $r = 0.38$, $p = 0.03$). There was no interaction effect between region (hippocampus or parahippocampal gyrus) and task performance on the degree of activation. There was a significant positive correlation between scores of five items of the SDQ and degree of activation in the bilateral hippocampi (L: $r = 0.54$, $p = 0.0036$; R: $r = 0.58$, $p = 0.0041$) and bilateral parahippocampal gyri (L: $r = 0.58$, $p = 0.0034$; R: $r = 0.56$, $p = 0.0045$). There was no interaction effect between region and scores of five items of the SDQ on the degree of activation.

4. Discussion

In the present study, several regions were robustly activated by our passive maze task. Here, we first discuss the general pattern of brain activity observed. The issue whether different brain activity was related to sex or individual navigation skill and/or strategies, is then discussed.

4.1. Activation during the maze navigation in a passive manner

We found activity in the hippocampus, parahippocampal gyrus, retrosplenial area, parietal association areas, and the visual association areas during our virtual maze task. Although the task was presented in a passive manner, activated areas are consistent with findings of previous activation studies of visual-spatial navigation in an active manner and demonstrate neural activity for allocentric and egocentric spatial-information processing (Aguirre et al., 1996, 1998; Maguire et al., 1997a; Maguire, 1997b; Maguire et al., 1998; Gron et al., 2000). The retrosplenial areas, such as the posterior cingulate cortex (PCC), precuneus and the cuneus, are thought to be related to spatial-information processing and episodic memory (Shallice et al., 1994; Fletcher et al., 1995, 1996; Aguirre et al., 1996, 1998; Maguire et al., 1997a; Maguire, 1997b). The lingual and fusiform gyri are parts of the occipitotemporal pathway engaged in object discrimination and recognition (Mishkin et al., 1983). The superior colliculus activity could be explained by the oculomotor response required for the shift of attention within the visual field during the task condition (Foreman and Stevens, 1987). The activity in the right prefrontal area (Broadmann's area 9) may reflect the working memory demand to hold spatial information 'on-line' keeping track of navigation. One would be skeptical about our task, because they are very simple without landmarks and offered no alternative routes to a destination; therefore, they could be only represented as sequences of right/left turns. Because we used forced choice to the behavioral measurement, it could be possible some of correct answers may be only a fluke. However, to obtain higher score, the subjects have to construct a 'cognitive map' during the task period. At the level of the whole group, our task replicated activation in the hippocampus and parahippocampal gyrus considered to be essential neural components for allocentric topographic representation. We considered that observed brain activities in the study should be associated with the cognitive map-type navigation system.

4.2. Navigation skill, strategies and brain activity

Importantly, good navigators demonstrated significantly stronger activations in the bilateral hippocampi, parahippo-

campal gyri and precuneus than poor navigators. These results were associated with a similar difference in the task performance. As expected, there was no significant interaction effect between sex and navigation ability on brain activity. The results strongly indicate that the hippocampus and parahippocampal gyrus should be preferentially activated in good navigators. The correlational analysis also revealed that the activity in the left medial temporal area positively correlated with the task performance during fMRI measurements. There was no sex effect on task performance dependent activity in the left medial temporal area. These results suggest that the task performance of men and women might rely on the medial temporal region in the same manner. However, our fMRI measurements were only performed in deviated groups obtained by self-administrated questionnaire and SDQ-S in whole sample demonstrated that men were better than women in the navigation ability in daily life. To reach the conclusion that men and women have the same neural mechanisms for navigation, we have to investigate a larger sample including average people.

Several behavioral studies have demonstrated sex-specific different performance in navigation, i.e. males better at navigation than females (Galea and Kimura, 1993; Astur et al., 1998; Moffat et al., 1998). An environmental research theory distinguishes between two different strategies for route navigation; a route strategy (reference to landmarks and route directions) and an orientation strategy (reference to metric distances and cardinal directions) (Evans, 1980; Lawton, 1994, 1996). As well as navigation performance, Lawton et al. reported sex differences in navigation strategies. In general, men used the orientation strategy, whereas women preferred to rely on the route strategy. The preferred use of the orientation strategy related to a male advantage in pointing accuracy and to better results in a task of spatial perception (Lawton, 1994, 1996). Taken together, the different performance in the e navigation can be explained by the preferred navigation strategy. O'Keefe (1991) suggested that the use of cardinal direction (polar coordinated system) may be most effective for translating egocentric coordinates into an allocentric reference frame. Similar results were obtained in our study. The scores of five items of SDQ-S revealed that poor navigators were not good at the allocentric orientation strategy, particularly the use of cardinal direction, and relied on the egocentric route strategy. On the other hand, good navigators were good at the orientation strategy and obtained a good score on the maze task. Furthermore, activity in the bilateral hippocamal regions was positively correlated with scores of five items of SDQ-S that reflect the use of allocentric orientation strategy in the daily life. A previous fMRI study demonstrated different brain activation for individuals using allocentric or egocentric strategies in a virtual navigation task (Jordan et al., 2004). According to the study, individuals using an allocentric strategy demonstrated stronger activation in the hippocampus, parahippocampal gyrus, and the thalamus (Jordan et al., 2004). Although a recent human single neuron recording study revealed functional difference associated with navigation between cells in the hippocampus and those of the parahippocampal gyrus (Ekstrom et al., 2003), we could not find functional difference between hippocampus and parahippocampal gyrus even with ROI analysis. The discrepancy could be caused by relatively lower spatial and/or temporal resolution of the present study. The event-related fMRI study with higher spatial resolution will clarify this issue.

Taken together, we consider that the medial temporal area is tightly related to navigation skill and allocentric topographic representation, which are essential for the orientation strategy. In this context, our results are not inconsistent with those of Gron's report (2000) and extend it in a straightforward way.

On the other hand, poor navigators showed significantly increased activity in the right inferior parietal lobule (right BA40). The regression analysis revealed that activity in the right superior parietal lobules (BA7) negatively correlated with the task performance. The results suggest that brain activity in poor navigators can be characterized by dominant activity in the right parietal association area and less activity in the medial temporal areas. In line with previous reports, parietal activity reflected the egocentric space-representation frame that subjects constructed during navigation (Colby, 1999). Because of the first-person view of the maze, the parietal activity was generated during the translation of retinal coordinates to body-centered coordinates. The parietal association area has strong neural connections to hippocampus/parahippocampal areas (Seltzer and Pandya, 1984; Suzuki and Amaral, 1994). We assume that poor navigators mainly rely on egocentric space representation during navigation mediated by the parietal association areas, and have difficulty in recognition of world-centered allocentric coordinates mediated by hippocampus and hippocampal gyri that is essential for navigation.

Although our fMRI data demonstrated a possibility that individual differences of navigation ability may account for some aspects of sex differences, it is still true that across many behavioral studies, men perform consistently better than women in navigation tasks based on the orientation strategy. In fact, our screening of SDQ-S in 246 individuals demonstrated sex different navigation ability; i.e. men had higher scores of SDQ-S than women had. This still leaves open the question of why such differences exist; namely, are such differences inherent, acquired, or associated with gender role? We speculate that navigation skill and strategies might be an acquired ability affected by the subject's background and a gender role. A morphological MR study demonstrated that London taxi drivers have a larger posterior hippocampal region than that of control subjects (Maguire et al., 2000). Furthermore, the volume of hippocampus positively correlated with the duration of work as a taxi driver (Maguire et al., 2000). Being a London taxi driver requires intensive training (2 years to acquire the license on average) for route navigation as well as knowledge of many landmarks. The data suggest that navigation related structural changes in the hippocampus are acquired through experience.

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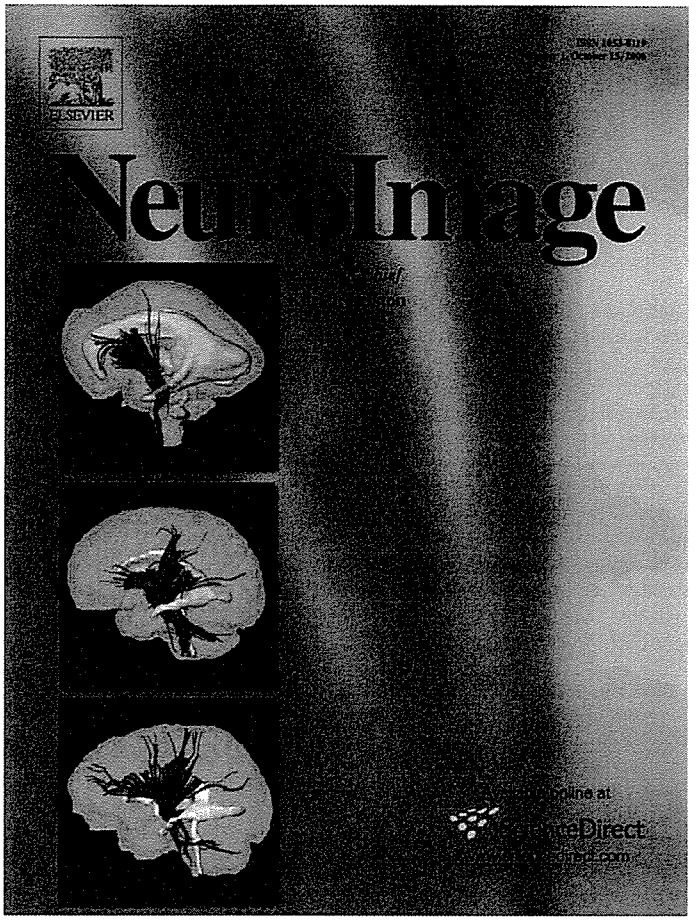

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Changes in cerebral glucose utilization in patients with panic disorder treated with cognitive–behavioral therapy

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Several neuroanatomical hypotheses of panic disorder have been proposed focusing on the significant role of the amygdala and PAG-related "panic neurocircuitry." Although cognitive–behavioral therapy is effective in patients with panic disorder, its therapeutic mechanism of action in the brain remains unclear. The present study was performed to investigate regional brain glucose metabolic changes associated with successful completion of cognitive–behavioral therapy in panic disorder patients. The regional glucose utilization in patients with panic disorder was compared before and after cognitive–behavioral therapy using positron emission tomography with ^{18}F-fluorodeoxyglucose. In 11 of 12 patients who showed improvement after cognitive–behavioral therapy, decreased glucose utilization was detected in the right hippocampus, left anterior cingulate, left cerebellum, and pons, whereas increased glucose utilization was seen in the bilateral medial prefrontal cortices. Significant correlations were found between the percent change relative to the pretreatment value of glucose utilization in the left medial prefrontal cortex and those of anxiety and agoraphobia-related subscale of the Panic Disorder Severity Scale, and between that of the midbrain and that of the number of panic attacks during the 4 weeks before each scan in all 12 patients. The completion of successful cognitive–behavioral therapy involved not only reduction of the baseline hyperactivity in several brain areas but also adaptive metabolic changes of the bilateral medial prefrontal cortices in panic disorder patients.

Introduction

Panic disorder (PD) is a common and debilitating anxiety disorder in which a "fear network" that is the assumed pathways subserving conditioned fear in animals and other related neurocircuitry has been suggested to play a significant role (Coplan and Lydiard, 1998; Gorman et al., 2000; Uys et al., 2003). Using information from preclinical studies, Gorman et al. reported that the fear network "has at its center the central nucleus of the amygdala and includes the hippocampus, thalamus, and hypothalamus, as well as the periaqueductal gray (PAG) region, locus ceruleus and other brainstem sites" interacting with higher cortical sites, such as medial prefrontal cortex (mPFC) and insula. On the other hand, Coplan and Lydiard stressed the role of the PAG in unconditioned fear as well as the role of the amygdala in conditioned fear, and they included both in their model of the "panic neurocircuitry." The PAG has been suggested to be activated by numerous aversive stimuli, such as pain, asphyxia, and species-specific danger cues, and activation of the PAG by either viscerosensory or visuospatial/auditory/cognitive stimuli is assumed to produce a fear-like response that is not linked with a conditioned stimulus. In addition, they noted the role of the dysfunctional rostral serotonergic systems, including the dorsal (DRN) and median raphe nuclei (MRN), in perturbing the panic circuitry. These hypotheses have prompted functional neuroimaging investigations to elucidate the neurocircuitry of PD.

The first functional neuroimaging study in PD was reported by Reiman et al. (1984), who examined PD patients and control subjects by $H_2^{15}O$ positron emission tomography (PET). Patients who were vulnerable to lactate-induced anxiety attacks showed abnormal hemispheric asymmetries (left less than right) of parahippocampal blood flow, blood volume, and oxygen metabolism, and abnormally high whole-brain metabolism of oxygen in the resting, non-panic state. Nordahl et al. studied PD patients at rest by ^{18}F-fluorodeoxyglucose (FDG) PET. They found no differences in global gray matter glucose metabolism between patients and normal controls, but found hippocampal region asymmetry (left less than right) and metabolic decreases in the left

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inferior parietal lobule and in the anterior cingulate (trend), as well as an increase in the metabolic rate of the medial orbitofrontal cortex (trend) (Nordahl et al., 1990). Bisaga et al. found similar asymmetries but dominance in the opposite side by FDG-PET in six women with PD. They observed hyperactivity of the left hippocampal and parahippocampal regions and hypoactivity of the right inferior parietal and the right superior temporal brain regions in PD patients as compared to control subjects (Bisaga et al., 1998). Therefore, although there is theoretical appeal of a role of the amygdala and PAG-based panic neurocircuitry, functional neuroimaging studies revealed no abnormalities in this region. Recently, using three-dimensional PET and voxel-based analysis on a sufficient number of subjects, we demonstrated higher regional brain glucose utilization in pretreatment PD patients with heightened state anxiety relative to that in normal controls in the bilateral amygdala, hippocampus, and thalamus, and in the midbrain around the PAG, caudal pons, medulla, and cerebellum, which supported the presence of an activated "panic neurocircuitry" including a fear network (Sakai et al., 2005).

PD can be treated successfully with anti-depressants, such as selective serotonin reuptake inhibitors (SSRIs) and tricyclics, but psychosocial treatments, such as cognitive behavioral therapies (CBT), have also been sufficiently effective and appeared durable in a follow-up phase reported in one large-scale randomized clinical trial (Barlow et al., 2000). These findings suggest that a psychotherapeutic approach, such as CBT, has the potential to modify the dysfunctional neural circuitry with PD, as reported in several previous studies on the effectiveness of CBT with spider phobia (Paquette et al., 2003), social anxiety disorder (Furmark et al., 2002), and major depressive disorder (Goldapple et al., 2004), of interpersonal psychotherapy with major depression (Martin et al., 2001; Brody et al., 2001), and of behavior therapy with obsessive-compulsive disorder (Baxter et al., 1992; Schwartz et al., 1996). It was argued that treatment involves not only normalization of disease-related baseline dysfunction but also other adaptive metabolic changes in the brain (Paquette et al., 2003; Furmark et al., 2002; Brody et al., 2001; Sackeim, 2001), and common sites of action for medication and psychosocial treatments that were specific for the treated disease were generally observed in those studies that investigated the effects of both kinds of treatment (Furmark et al., 2002; Martin et al., 2001; Brody et al., 2001; Baxter et al., 1992; Schwartz et al., 1996). On the other hand, Goldapple et al. (2004) reported treatment-specific patterns of change in CBT and paroxetine responders in major depression although they performed a post hoc comparison of two independent studies, which supported their initial hypothesis that each treatment targets different primary sites with differential top–down and bottom–up effects. Similarly, Gorman et al. (2000) suggested a specific hypothesis regarding different treatment modalities of PD as follows. Medications such as SSRIs may reduce panic attacks by decreasing the activity of the amygdala and interfering with its ability to stimulate projection sites in the hypothalamus and brainstem. Cognitive behavioral and other effective psychotherapies most likely operate upstream from the amygdala, reducing phobic avoidance by deconditioning contextual fear learned at the level of the hippocampus and decreasing cognitive misattribution and abnormal emotional reactions by strengthening the ability to inhibit the amygdala. In fact, Prasko et al. (2004) investigated the effects of CBT and SSRI on six PD patients each speculating that CBT improve processing of top–down effects and SSRIs of bottom–up effects. However, the results of their study indicated similar changes in brain metabolism (^{18}FDG uptake) after treatment with either modality. As they used only six subjects in each condition and did not detect changes in FDG uptake in the limbic region, including the hippocampus, parahippocampal gyrus, and amygdala, their findings may not be regarded conclusive.

The present study was performed to examine changes in regional brain glucose utilization associated with anxiety alleviation by successful completion of CBT in the same PD patients examined in our previous study by baseline comparison to normal controls (Sakai et al., 2005). We hypothesized that upstream from the amygdala, including the mPFC and anterior cingulate cortex (ACC), as well as the hippocampus may be modulated adaptively in CBT responders. In addition, with increased glucose uptake in the bilateral amygdala, hippocampus, and thalamus, and in the midbrain, caudal pons, medulla, and cerebellum in pretreatment baseline and as all of these areas can be located in the "panic neurocircuitry" (Sakai et al., 2005), it was hypothesized that any of these areas may be soothed during recovery. First, we compared baseline regional glucose utilization to that in a follow-up study in patients with PD treated successfully, as judged by a reduction in the severity score of more than 50%, with 6 months of individual treatment with CBT. Second, we examined the correlation between the percent change relative to the pretreatment value of glucose utilization in several relevant brain areas set as regions of interest (ROIs) and the scores of symptomatic measures in all PD patients. Finally, we picked up two ROIs based on the above results and correlational analyses between regional glucose utilization in each ROI and those of other brain areas were conducted to elucidate functional neural connections and the results were compared before and after CBT treatment.

Materials and methods

Subjects

Outpatients visiting the Research Center of Panic Disorder and meeting the DSM-IV criteria for PD, who had taken no medication for at least 2 weeks, had never taken fluoxetine before scanning, and had never undergone any type of cognitive–behavioral therapy were recruited for this study. Patients were excluded if they had comorbid current major depression, bipolar disorder, schizophrenia, social phobia, obsessive-compulsive disorder, posttraumatic stress disorder, generalized anxiety disorder, risk of suicide, substance abuse, or drug abuse as assessed by the Mini-International Neuropsychiatric Interview, personality disorder according to the Structured Clinical Interview for DSM-III-R, or any physical illness. Twelve patients (mean age=29.8 years, SD=6.2; 3 men and 9 women; all right-handed) who had participated in our previous study (Sakai et al., 2005) participated in the present study. Demographic and baseline symptomatic characteristics of these patients are shown in Table 1. All patients were Japanese. The severity of PD was measured by the total, panic-attack-related first subscale, and anticipatory anxiety and agoraphobia-related second subscale score on the clinician-rated Panic Disorder Severity Scale (PDSS) (Shear et al., 1997, 2001) and the frequency of panic attacks during the previous 4 weeks. DSM-III-R criteria were used to assess the severity of agoraphobia; the results indicated that eleven patients had mild and one had moderate agoraphobia. In addition, the State Trait Anxiety Inventory (STAI-T/S) (Spielberger et al., 1970) and the 17-item Hamilton Depression Rating Scale

Table 1
Characteristics of 12 panic disorder patients receiving cognitive–behavioral therapy

Subject[a]	Age (years)	Sex	Duration of PD (years)	PA/4w before CBT[b]
1	21	M	0.21	2
2	26	M	0.21	8
3	25	F	6.5	20
4	44	F	10.5	2
5	27	F	2.8	4
6	25	F	1.0	2
7	28	F	2.6	0
8	37	F	0.59	42
9	30	F	1.6	14
10	28	F	4.0	7
11	32	F	12.7	10
12	34	M	12.0	4

CBT, cognitive–behavioral therapy; M, male; F, female; PD, panic disorder; PA, panic attack.
[a] Subjects 1–11 were analyzed before and after CBT and Subject 12 showed aggravation of symptoms after CBT.
[b] Number of panic attacks experienced during the 4-week period before the first positron emission tomography (PET) procedure.

(HAM-D) (Hamilton, 1967) were used for assessment of general clinical severity.

This study was performed in accordance with the Declaration of Helsinki and was approved by the Human Ethics Committees of the National Center of Neurology and Psychiatry and of the Warakukai Incorporated Medical Institution. All subjects gave their written informed consent to participate in the study after the procedure had been fully explained.

Cognitive–behavioral therapy

The subjects were treated individually with 10 sessions of cognitive–behavioral therapy (CBT) over a period of about 6 months without medication. The schedule of the sessions was once in 2 weeks in the first 4 months and once a month in the last 2 months, which varied according to each patient's convenience. Each session lasted approximately 50 min. The CBT program for PD, consisting of psycho-education on symptoms and treatment plan of PD, relaxation training (muscle relaxation, breathing control, reciprocal body movement), in vivo exposure homework, attention control technique, self-instruction, self-reinforcement, thought stopping, and cognitive restructuring, had been developed by a clinical psychologist with considerable experience in CBT for PD (Y. Sakano) (Sakano and Kaiya, 1999; Sakano, 2001), referring to commonly used programs (e.g., Barlow and Craske, 1994; Clark, 1989). The main ingredient of this program was in vivo exposure homework for agoraphobia and/or panic attacks. The patients were told to observe the ebb and flow of their symptoms while performing exposure tasks for their agoraphobia or experiencing panic attacks until their anxiety levels decreased substantially. A physician (Y. Sakai) with more than 5 years of clinical experience in the field of anxiety disorders provided these treatments to the PD patients, supervised by Y. Sakano.

PET procedure

Investigations were performed using a PET scanner (Siemens ECAT 47 EXACT HR; Siemens, Munich, Germany), in three-dimensional mode at the National Center Hospital for Mental, Nervous, and Muscular Disorders. Images had a normal resolution of 3.6×3.6×4.8 mm as defined by the manufacturer and were reconstructed using backprojection and filtering (Hanning filter, cutoff frequency FWHM 0.5 cm). The reconstructed images were resliced to 47 slices about 3 mm thick and displayed in a matrix of 256×256×47 voxels (voxel size 0.881×0.881×3.125 mm). PD patients answered the State Trait Anxiety Inventory (STAI) before entering the examination room (Spielberger et al., 1970; Nakanishi, 2002). A transmission scan using a retractable, rotating $^{68}Ga/^{68}Ge$ source with three rods was performed to correct for tissue attenuation and background activity before acquisition of the emission data. For the PET scan, FDG was injected intravenously at a dose of 55 MBq over a period of 60 s. Emission data were acquired for 10 min after a 50-minute uptake period. During the FDG distribution phase, all subjects were placed in the camera gantry with a blindfold and a standard head holder to minimize head movement and were asked to remain in a wakeful and resting state. We monitored EEG throughout the PET scan and checked visually that the subjects were not falling asleep. In cases in which EEG showed signs of drowsiness, the examiner roused the subject by gently patting their leg. All PD patients had fasted for more than 3 h, and their blood glucose levels were 70–110 mg/dl before PET acquisition.

Table 2
Clinical rating scale results before and after successful cognitive–behavioral therapy

Rating scale	Pre-CBT (n=11)			S12	Post-CBT (n=11)			S12	Z score	Prob>|Z|
	Median	QD	Range		Median	QD	Range			
PDSS	16	5	11–20	15	5	4	1–8	19	−2.95	0.003
PDSS 1st	5	3	0–7	3	0	3	0–4	6	−2.84	0.004
PDSS 2nd	11	3	8–13	12	5	2	1–7	13	−2.95	0.003
PA/4w	7	12	0–42	4	0	4	0–5	10	−2.36	0.018
STAI-T	46	13	28–59	62	44	12	29–65	67	−1.01	0.313
STAI-S	56	13	36–62	50	41	11	31–64	58	−2.68	0.007
HAM-D	8	8	1–21	12	3	6	1–12	17	−2.63	0.009

PDSS, Panic Disorder Severity Scale. PDSS 1st, panic-attack-related first subscale of the PDSS. PDSS 2nd, anticipatory anxiety and agoraphobia-related second subscale of the PDSS. PA/4w, number of panic attacks in the 4 weeks before the PET procedure. STAI-T, trait subscale of State Trait Anxiety Inventory. STAI-S, state subscale of State Trait Anxiety Inventory. HAM-D, 17-item Hamilton Depression Rating Scale. CBT, cognitive–behavioral therapy. QD, quartile deviation. S12, Subject 12 in Table 1, who became worse after CBT.

Table 3
Changes in normalized regional brain glucose uptake in panic disorder patients ($n=11$) after successful cognitive–behavioral therapy

Brain region		Brodmann's area	Talairach coordinates			Z value
			x	y	z	
Areas of increase						
Medial prefrontal	Left	9	−8	56	30	3.68
	Right	10	14	59	14	3.39
Areas of decrease						
Hippocampus	Right		32	−33	−2	4.10
Anterior cingulate	Left	32	−6	35	−5	3.96
Cerebellum, uvula	Left		−10	−74	−33	3.49
Cerebellum, pyramis			−16	−79	−26	2.75
Pons			16	−22	−19	3.45
			2	−15	−23	3.21

The first PET scan for patients with PD was conducted before CBT. The second PET scan was completed after 10 sessions during approximately 6 months of CBT.

Data analysis

One of the 12 PD patients (Subject 12) was excluded from investigation of the changes in regional brain FDG uptake because he had panic attacks shortly before completion of the CBT program and the PD symptoms became worse at the time of the second assessment. The remaining 11 PD subjects showed at least a 50% reduction in the total PDSS score, and the significance of improvement assessed by not only the PDSS but also other PD symptomatic and general clinical measures was confirmed by Wilcoxon's signed rank sum test (Table 2) using SPSS for Windows, version 10.0.5J (SPSS Japan Inc., Tokyo, Japan). Changes in the regional brain FDG uptake were analyzed within the 11 PD subjects before and after successful CBT using Statistical Parametric Mapping 99 (SPM99: The Wellcome Department of Cognitive Neurology, London, UK). Prior to SPM analysis, the images of each subject were realigned and then transferred into 3D standardized coordinate space (Talairach and Tournoux, 1988). All FDG images were co-registered to the MRI image templates generated at the Institute from 24 normal subjects in whom both FDG and MRI were performed (unpublished data set). Co-registered FDG images were smoothed with a Gaussian filter of $10\times10\times6$ mm. This Gaussian filter smoothed images to a similar final resolution in all three axes to remove possible noise voxels to avoid spurious clusters that could reach statistical significance. The paired t test was used for statistical analysis of FDG uptake values between before and after successful CBT at each voxel, using proportional scaling with $P<0.005$ (uncorrected) at the voxel level and cluster extent $k>50$ voxels (degrees of freedom=[1.0,10.0]). In proportional scaling, the images were scaled to a value of 50 using the mean global values obtained in each image. These were converted to T scores for graphical display as parametric maps. In addition, t test was used for statistical analysis of FDG uptake values after CBT treatment between 11 PD patients and the 1 excluded patient at each voxel to confirm the deviation of this patient's FDG uptake pattern. Proportional scaling with $P<0.005$ (uncorrected) at the voxel level was used as in the

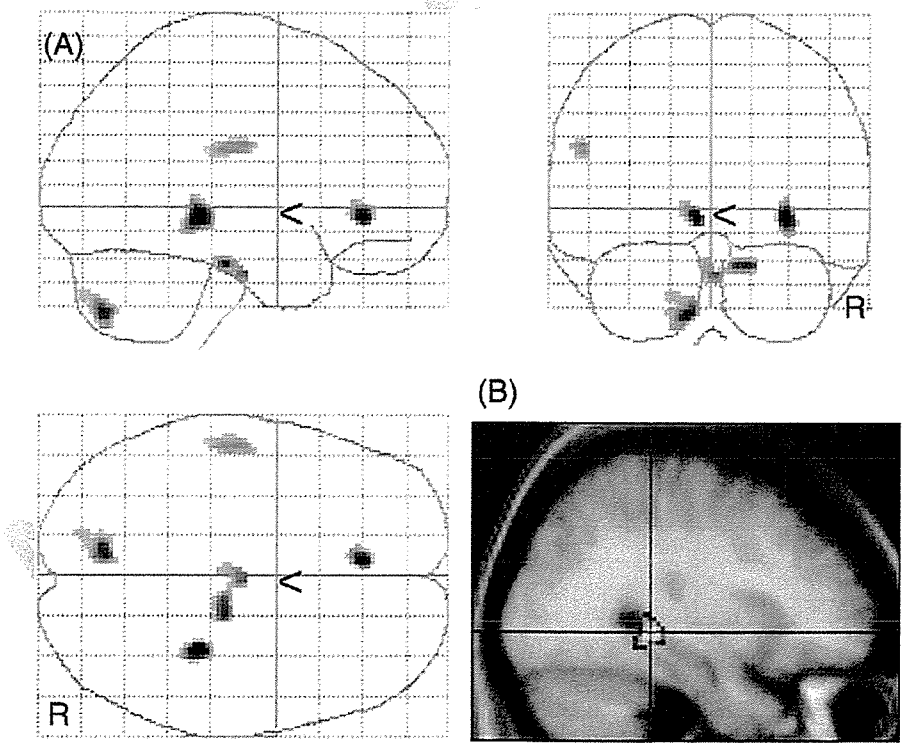

Fig. 1. Statistical parametric map of decreased FDG uptake in patients with panic disorder ($n=11$) after successful completion of cognitive behavior therapy. (A) The whole brain viewed in a transparent fashion from the side, from the back, and from the top. (B) Sagittal view of the right hippocampus.

analyses between before and after CBT, but cluster extent was set at $k>280$ voxels ($P<0.05$, corrected; degrees of freedom= [1.0,10.0]) because this was a preliminary analysis with only one subject in one group.

ROI analysis was applied to assess the correlation between the percent change relative to the pretreatment value ([(posttreatment value−pretreatment value)/pretreatment value]×100) of glucose utilization and those of PD symptomatic measures, including the total, first, and second subscale scores of the PDSS and panic attack frequency during the previous 4 weeks in all 12 PD patients. Normalized glucose utilization rates of each ROI were calculated for each image using MarsBaR software (Brett et al., 2002) with proportional scaling in which the images were scaled to a value of 50 using the mean global values obtained in each image. We set ROIs at all significantly changed areas as indicated in Table 3, including the bilateral mPFC, right hippocampus, left ACC, left cerebellum, and pons, and in spherical regions 5 mm in radius centered in the bilateral amygdala (Talairach coordinates: $x=-24$, $y=-8$, $z=-10$ (left); $x=22$, $y=-10$, $z=-8$ (right)) and the midbrain around the PAG region ($x=2$, $y=-21$, $z=-1$) referring to our previous study (Sakai et al., 2005), in which the above areas showed significantly increased glucose utilization in pretreatment PD patients as compared to normal controls. We analyzed the correlation between the percent changes in the normalized glucose utilization of each ROI and those of each symptomatic measure with Kendall's rank correlation coefficient using SPSS for Windows, version 10.0.5J (SPSS Japan Inc., Tokyo, Japan).

Finally, we picked up the ROIs at the bilateral mPFC because these were the only brain areas in which glucose utilization was increased after CBT treatment, and correlational analyses were conducted at each voxel within the 11 PD patients using normalized glucose utilization rates of each ROI calculated using MarsBaR as covariates of interest in SPM99. Proportional scaling with $P<0.005$ (uncorrected) at the voxel level was used as in the analyses between before and after CBT, but cluster extent was set at $k>250$ voxels and $P<0.05$, corrected (degrees of freedom= [1.0,9.0]) because this was an exploratory analysis without a clear hypothesis.

Results

Clinical findings

The PD symptomatic and general clinical measures of 1 aggravated (Subject 12) and 11 successfully treated patients are presented in Table 1. The PDSS, its first and second subscales, the number of panic attacks during the previous 4 weeks, the state anxiety subscale of STAI, and the HAM-D were significantly improved by the treatment in 11 patients (Table 2). Only the trait anxiety subscale of STAI did not change in 11 patients, while all of the above measures became worse in Subject 12. Based on the pretreatment HAM-D scores, half the successfully treated patients showed at least mild depressive states. However, major depression, which covers most of the comorbid depressive disorders in panic disorder (American Psychiatric Association, 2000) or bipolar disorder, was diagnosed in none of the subjects, and because the correlation of the scores of anxiety and depression is notoriously high (Gotlib and Cane, 1989), which was also the case in the present study ($r=0.71$ with STAI-S before CBT), we assume that

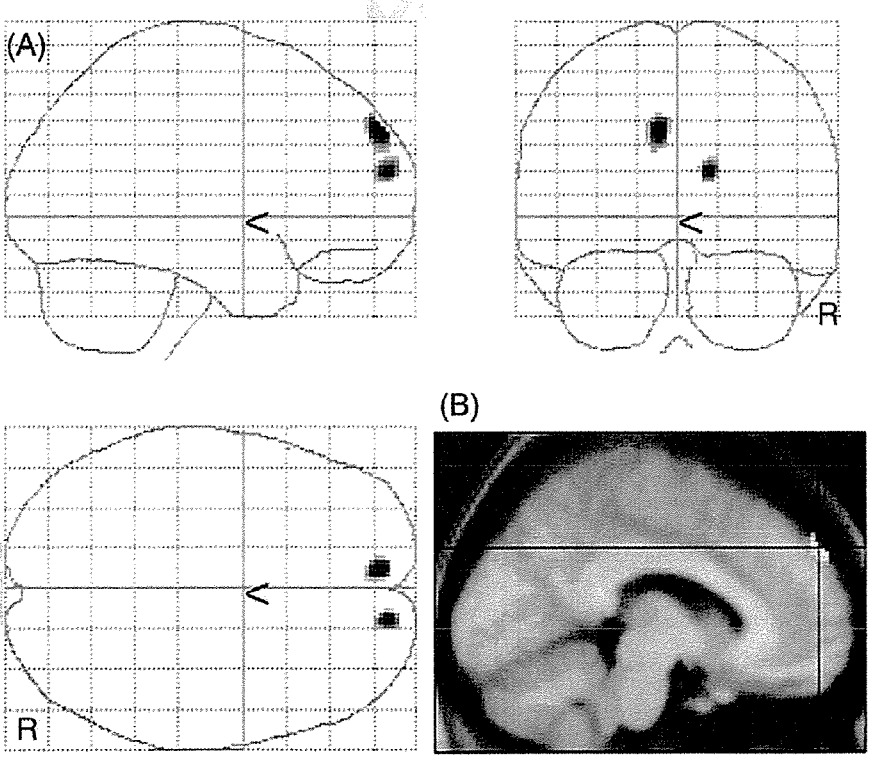

Fig. 2. Statistical parametric map of increased FDG uptake in patients with panic disorder ($n=11$) after successful completion of cognitive behavior therapy. (A) The whole brain viewed in a transparent fashion from the side, from the back, and from the top. (B) Sagittal view of the left medial prefrontal region.

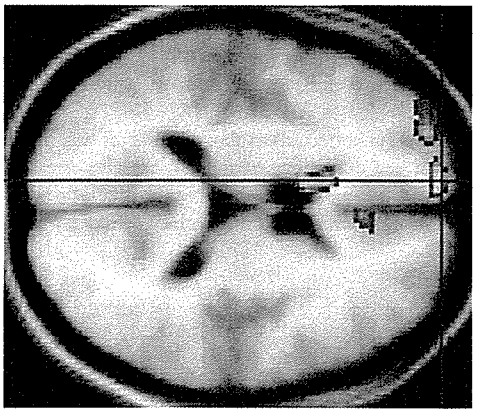

Fig. 3. Statistical parametric map of areas of positive correlation with the left mPFC (BA 9) in the left caudate head and bilateral ventral ACC (BA 24) and left medial and lateral prefrontal cortex (BA 10) before CBT treatment.

the heightened HAM-D scores mainly reflected the heightened state of anxiety.

Changes in regional brain glucose metabolism after completion of CBT

After successful completion of CBT, areas of decreased metabolism were detected in the right hippocampus, left ventral ACC (Brodmann's area [BA] 32), left cerebellum uvula and pyramis, and pons (Fig. 1, Table 3), whereas areas of increased regional brain glucose metabolism were detected in the bilateral medial prefrontal regions (left: BA 9; right: BA 10) (Fig. 2, Table 3). Although an area of decreased metabolism was also found in the contiguous region of the left precentral and postcentral gyri (Fig. 1), it was not regarded as meaningful because the area had not been included in the hypothesis.

Table 4
Correlated brain areas with the left or right mPFC in panic disorder patients ($n=11$) before and after cognitive–behavioral therapy

Brain region		Brodmann's area	Talairach coordinates			Z value
			x	y	z	
Areas of positive correlation with the left mPFC (BA 9) before CBT						
Caudate nucleus	Left		−8	20	6	4.43
			−8	10	11	3.77
Ventral anterior cingulate cortex	Bilateral	24	−4	29	−5	3.62
Medial and lateral prefrontal cortex	Left	10	−10	63	10	3.96
			−28	57	10	3.50
			−40	55	14	2.95
Areas of positive correlation with the left mPFC (BA 9) after CBT						
Rostral pons			−4	−28	−19	3.43
			−6	−31	−29	3.31
Caudal midbrain			2	−29	−2	3.25
Areas of positive correlation with the right mPFC (BA 10) before CBT						
Dorsal anterior cingulate cortex	Left	32, 24	−4	6	46	4.62
Medial frontal gyrus	Left	6	−4	5	53	4.52

mPFC, medial prefrontal cortex. CBT, cognitive–behavioral therapy. BA, Brodmann's area.

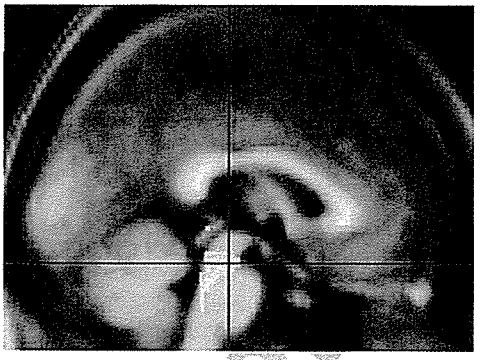

Fig. 4. Statistical parametric map of areas of positive correlation with the left mPFC (BA 9) in the contiguous region of midline caudal midbrain and rostral pons after CBT treatment.

When we compared FDG uptake values after CBT treatment between 11 improved PD patients and 1 aggravated patient, areas of lower glucose metabolism were detected in the contiguous bilateral mPFC (BA10) mainly in the right hemisphere in the one patient showing aggravation of symptoms (Talairach coordinate of local maximum: $x=2$, $y=62$, $z=1$; $k=451$).

Correlational analyses between the changes in brain glucose utilization and clinical measures

Significant correlations were observed between the percent changes in glucose utilization in the left mPFC and those of the second subscale of the PDSS ($\tau=-0.473$, $P=0.033$), and between those of the midbrain around PAG and those of the number of panic attacks during the 4-week period before each scan ($\tau=0.500$, $P=0.034$).

Correlational analyses between brain glucose utilization of the mPFC and of other brain areas

With the left mPFC (BA 9), areas showing a positive correlation were detected in the left caudate head and bilateral ventral ACC (BA 24) and left medial and lateral prefrontal cortex (BA 10)

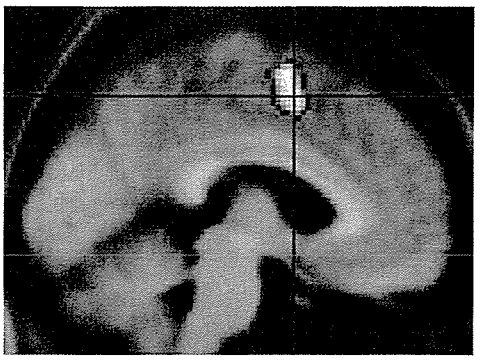

Fig. 5. Statistical parametric map of areas of positive correlation with the right mPFC (BA 10) in the contiguous region of left dorsal ACC (BA 32, BA 24) and medial frontal cortex (BA 6) before CBT treatment.

before CBT treatment (Fig. 3, Table 4) and in the contiguous region of the midline of the caudal midbrain and rostral pons after CBT treatment (Fig. 4, Table 4), whereas no areas showing negative correlations were detected. With the right mPFC (BA 10), an area showing a positive correlation was found in the contiguous region of left dorsal ACC (BA 32, BA 24) and medial frontal cortex (BA 6) before CBT treatment, whereas no other areas showing positive or negative correlations were detected before or after CBT treatment (Fig. 5, Table 4).

Discussion

PD symptoms measured by the PDSS total score were reduced by more than 50% in 11 of 12 right-handed PD patients who completed the CBT program. Improvement of PD symptoms was associated with attenuated normalized glucose utilization in the right hippocampus, left ventral ACC, left cerebellum, and pons and with increased normalized glucose utilization in the bilateral medial prefrontal regions. Significant negative and positive correlations were observed between the percent change in glucose utilization in the left mPFC and that in the second (anticipatory anxiety and agoraphobia-related) subscale of the PDSS, and between that of the midbrain around PAG and the frequency of panic attacks during the previous 4 weeks, respectively. Significant positive correlations were found between glucose utilization in the left mPFC and those in the left caudate head and bilateral ventral ACC and in the left medial and lateral prefrontal cortex before CBT treatment and those in the contiguous region of midbrain and pons after CBT treatment, whereas only one brain region showing a positive correlation was found with glucose utilization in the right mPFC, which was a contiguous region of left dorsal ACC and medial frontal cortex before CBT treatment.

This study had certain limitations. The generality of the present results is insufficient due to the small sample size. There is also a possibility of Type I errors in the correlation analyses between the changes in brain glucose utilization and in clinical measures because multiple correlations were carried out at a significance level of $P<0.05$. Although supervised by an experienced clinical psychologist, only one therapist conducted CBT, and the efficacy of the present program may not be generalized to other settings. Moreover, no waiting list or attention-placebo control groups were included in this study, and thus the results obtained could not be confirmed to be specific to CBT. However, they should be due to the present intervention and not due simply to the passage of time because the median duration of PD was 2.6 years, during which none of the patients recovered from the disease. In addition, as the aim of this study was to investigate the changes in cerebral glucose utilization with a CBT program rather than to confirm the effectiveness of this program in treating PD, the present results are sufficient to conclude that the PET changes reported were attributable to the improvement of symptoms by a CBT intervention. With these limitations in mind, we discuss the findings of this study below.

The observations that the levels of glucose utilization of the right hippocampus, bilateral mPFC, and left ventral ACC were modulated by CBT were compatible with our first hypothesis that upstream from the amygdala may be modulated adaptively in CBT responders. The decrease in glucose utilization in the right hippocampus was compatible with the assumption that deconditioning of contextual fear is necessary (Gorman et al., 2000), but the changes in glucose utilization in the bilateral mPFC and the left ventral ACC were more complex, which requires further discussion. The glucose utilization in the bilateral mPFC and in the left ventral ACC changed in the opposite direction, which may be related to the contrasting functions of these two areas for working on emotional responses mediated by the amygdala and hippocampus. The lesions in the ventral mPFC including mainly the ventral ACC inhibited the extinction of fear reactivity to only the conditioned stimulus, whereas the lesions in the dorsal mPFC composed of the dorsomedial PFC around BA 9 and dorsal ACC generally enhanced fear reactivity to both the conditioned stimulus and contextual stimuli during both acquisition and extinction phases of fear conditioning (Morgan and LeDoux, 1995). In addition, the ventral mPFC is particularly sensitive to stressful or anxiety-inducing stimuli and lesions in the ventral mPFC attenuated anxiety and stress responses to unconditioned stimuli (Sullivan and Gratton, 1999, 2002). Furthermore, in healthy humans scanned during anxious anticipation of an electric shock, cerebral blood flow increased in the dorsomedial PFC correlated inversely with changes in anxiety ratings and heart rate, suggesting that this region functions to attenuate emotional expression (Drevets et al., 1994). In summary, it may be the dorsal mPFC that attenuates innate emotional expression and conditioned emotional responses not only to the conditioned stimulus but also to contextual stimuli such as agoraphobia and related anticipatory anxiety in PD patients. The increases in glucose utilization in the bilateral mPFC seen in the present study are compatible with this hypothesis, especially for the left side because a significant negative correlation was found between the percent change of glucose utilization in the left mPFC and that in the anticipatory anxiety and agoraphobia-related subscale of the PDSS. The significant difference in bilateral mPFC between the 1 aggravated patient and 11 patients who showed improvement in the present study also supports the clinical relevance of the changes in these areas.

However, there were some inconsistent findings, such as the lack of a decrease in glucose utilization in the amygdala, and although the glucose utilization in the hippocampus did decrease after CBT, it was not correlated with either that in the mPFC or with the percent changes in any symptomatic measures. The reason why CBT increased the glucose utilization of the mPFC is also not yet clear. As the present CBT program stressed the importance of monitoring in vivo anxiety levels of the subjects, the activity of mPFC could be increased through enhancement of the capacity of reflective awareness of emotion correlated with the activity of dorsomedial PFC (Lane, 2000), although there were no behavioral data supporting this interpretation. On the other hand, the decrease in glucose utilization in the left ventral ACC may be a consequence of alleviated sensitivity to the environmental or viscerosensory stressors or to anxiety itself because of the clinical improvement, although there were also no supporting behavioral data in the present study. Relevant behavioral data, such as the Levels of Emotional Awareness Scale (Lane et al., 1990) and the Anxiety Sensitivity Index (Reiss et al., 1986), should be collected, and the mechanism of action of the mPFC and ACC related to the improvement of PD symptomatology should be clarified in future studies.

With regard to the neurocircuitry working with the mPFC before and after CBT treatment, more brain areas were correlated with the left mPFC, which may be related to the above-mentioned clinical relevance of the left mPFC. This may be because the left side was more dorsal (BA 9) and larger than the activated area in

the right side (BA 10). The BA 9 cortex sends efferent projections to the lateral PAG and the dorsal hypothalamus in primates (Ongur and Price, 2000) through which it may attenuate cardiovascular responses associated with emotional behavior (Drevets, 2004), which may be important because a significant correlation was observed between the percent change in glucose utilization of the midbrain around PAG and the frequency of panic attacks during the previous 4 weeks in the present study. The correlated brain areas with the left BA 9 were the left caudate head and bilateral ventral ACC (BA 24) and left medial and lateral prefrontal cortex (BA 10) before CBT treatment and the contiguous region of the midbrain and pons after CBT treatment. These observations suggest that conflicting activities of the dorsomedial PFC and ventral ACC as well as caudate head were present before CBT, whereas cooperative activities of the dorsomedial PFC and midbrain, including the DRN and MRN, both of which inhibit the amygdala and PAG (although no significant decreases in glucose utilization were found in either areas in the present study), were brought about by CBT treatment.

Interestingly, Goldapple et al. (2004) showed hippocampal and mid and anterior cingulate increases coupled with decreases in dorsolateral, medial frontal, and ventrolateral prefrontal activity with CBT treatment of major depressive disorder, most of which were contrary to the present results. The discrepancy is most likely due to the patients' diseases, which probably affect different parts of the brain. They interpreted these changes as correlates of CBT-conditioned increases in attention to personally relevant emotional and environmental stimuli associated with a learned ability to reduce online cortical processes at the level of encoding and retrieval of maladaptive associative memories, as well as reductions in both the overprocessing of irrelevant information and ruminations. Panic and depressive disorders have opposite symptomatology in many areas, such as arousal state and self-referential processing, which may explain the unique directional changes seen in the two studies. Depressive disorder patients are inclined to attend to the self and explore the signs of their own ineffectiveness, while panic disorder patients attend to outside the self, including environmental and bodily stimuli, and explore the signs of impending danger.

The decreases in glucose utilization in the right pons and left cerebellum as well as the right hippocampus suggested that the functional abnormalities in these regions were normalized by CBT. On the other hand, the amygdala, thalamus, and medulla in which glucose utilization was not changed may be associated more with trait disturbance or could be affected to a greater extent by medication, as suggested by Gorman et al. (2000). Another possibility is that, even if the effects of increased and decreased glucose metabolism in the mPFC and hippocampus, respectively, on amygdala function cannot be seen in the resting state, they could still inhibit amygdala function under conditions of heightened anxiety, as shown in a previous ^{15}O-labeled H_2O PET study of social anxiety disorder (Furmark et al., 2002). To confirm this possibility, anxiety-provoking stimuli or panicogens, such as lactate or CO_2, should be used, and the regional cerebral blood flow of the brain sites mentioned above should be investigated before and after successful completion of CBT.

Our results indicated that the improvement of symptoms by the present psychological intervention could produce effects in the brain summarized by Paquette et al. (2003) as "change the mind and you change the brain" upstream of the amygdala, such as the hippocampus and mPFC, as well as normalization of glucose utilization of the pons and cerebellum. An activation study with challenge tests to provoke panic attacks is required and may reveal whether the efferent pathway of the fear network other than the PAG and caudal pons shows elevated neural activity before treatment and whether the activity of that pathway including the amygdala is suppressed after CBT. In addition, further studies are required to investigate the effects of drug treatment using the same procedures to determine whether medication can suppress abnormal activity of the amygdala as well as its downstream sites, such as the PAG, pons, and hypothalamus.

Acknowledgments

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The prediction of rapid conversion to Alzheimer's disease in mild cognitive impairment using regional cerebral blood flow SPECT

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Mild cognitive impairment (MCI) comprises a heterogeneous group with a variety of clinical outcomes and they are at risk for developing Alzheimer's disease (AD). The prediction of conversion from MCI to AD using the initial neuroimaging studies is an important research topic. We investigated the initial regional cerebral blood flow (rCBF) measurements using single photon emission computed tomography (SPECT) in individuals with 76 amnesic MCI (52 subjects converted to AD and 24 subjects did not convert to AD at 3-year follow-up) and 57 age- and gender-matched controls. We sought functional profiles associated with conversion to AD, then evaluated the predictive value of the initial rCBF SPECT. As compared with controls, AD converters demonstrated reduced blood flow in the bilateral parahippocampal gyri, precunei, posterior cingulate cortices, bilateral parietal association areas, and the right middle temporal gyrus. Non-converters also demonstrated significant reduction of rCBF in the posterior cingulated cortices and the right caudate nucleus when compared to controls. As compared with non-converters, converters showed reductions of rCBF in the bilateral temporo-parietal areas and the precunei. The logistic regression model revealed that reduced rCBF in the inferior parietal lobule, angular gyrus, and precunei has high predictive value and discriminative ability. Although a cross-validation study is needed to conclude the usefulness of rCBF SPECT for the prediction of AD conversion in individuals with MCI, our data suggest that the initial rCBF SPECT studies of individuals with MCI may be useful in predicting who will convert to AD in the near future.

Keywords: Mild cognitive impairment (MCI); Alzheimer's disease (AD); Regional cerebral blood flow (rCBF); Single photon emission computed tomography (SPECT)

Introduction

Mild cognitive impairment (MCI) is an operational diagnostic term developed to describe the preclinical stage of Alzheimer's disease (AD) (Petersen et al., 2001a). The risk for conversion to AD is higher in individuals with MCI than in the general aged population, as annual conversion rate of 6%–25% from MCI to AD (Petersen et al., 2001b). Furthermore, a recent study suggested that progression from MCI to AD is time-dependent. According to Palmer's study, people with MCI have a high risk of progressing to dementia over the next 3 years, and the risk starts to decrease after this point (Palmer et al., 2003). The early detection of MCI individuals who will later convert to AD is an important issue for both clinical and research interests.

The recent advance of computer-assisted statistical image analyses revealed that subjects with very mild AD typically show abnormal metabolic and regional cerebral blood flow (rCBF) patterns, even at the preclinical stage. Using glucose metabolism positron emission tomography (PET) with a voxel-by-voxel statistical analysis, Minoshima et al. reported that the earliest changes observed in very mild AD were in the posterior cingulate cortex (PCC) (Minoshima et al., 1997). This unexpected finding has

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been replicated by other groups using both glucose metabolism measurements with PET and even less sophisticated measurement techniques such as regional cerebral blood flow (rCBF) measurements with single photon emission computed tomography (rCBF SPECT). Our previous rCBF SPECT study demonstrated significantly decreased rCBF in the posterior cingulate gyri and precunei bilaterally in MCI subjects as compared with controls at least 2 years before they met a clinical diagnosis of AD (Kogure et al., 2000). We also reported a diagnostic value of reduced rCBF in the posterior cingulate cortex (PCC) to assist in discriminating between normal subjects and MCI subjects who later developed AD (Imabayashi et al., 2004). Furthermore, a PET study demonstrated hypometabolism of the PCC in young subjects with a high genetic risk of developing AD (Reiman et al., 2004). These results suggest that functional neuroimaging techniques such as PET and SPECT may be promising techniques for the preclinical diagnosis of AD.

However, MCI is a heterogeneous diagnostic category comprised of individuals with a variety of clinical outcomes (Petersen et al., 2001). As such, only a longitudinal study comparing MCI subjects who convert to AD at follow-up (converters) with MCI subjects who do not convert at follow-up (non-converters) is appropriate to determine the predictive value of initial neuroimaging for progression of MCI to AD. Only a few longitudinal studies have been published so far (Celsis et al., 1997; Arnaiz et al., 2001; Huang et al., 2002; Chetelat et al., 2003; Drzezga et al., 2003; Mosconi et al., 2004). These studies have suggested that reduced glucose metabolism in the right temporo-parietal cortex or reduced rCBF and glucose metabolism in the PCC might be good predictors of progression to AD.

On the other hand, morphological magnetic resonance imaging (MRI) studies have demonstrated that higher atrophy rates in the medial temporal regions such as the entorhinal cortex and hippocampus may be good predictors of conversion to AD (Jack et al., 1999; Mungas et al., 2002; Nestor et al., 2004). However, such serial MR studies require at least a 1-year follow-up period to predict AD conversion. As with functional imaging studies, the predictive value of morphological MR studies has not been yet clarified.

The present retrospective cohort study assessed initial rCBF SPECT images in a group of amnesic MCI subjects consisting of AD converters and non-converters who were followed clinically for 3 years. The aim of the present study was to find highly specific and sensitive rCBF changes capable of discriminating between MCI subjects who eventually develop AD from those who do not convert to AD, as early as possible. We also demonstrated the predictive value of the initial rCBF SPECT studies in MCI subjects.

Methods

Subjects

The characteristics of the subjects who participated in this study are summarized in Table 1. We retrospectively studied 82 individuals (40 men and 42 women) with MCI who visited our memory clinic with a chief complaint of memory disturbance. Six MCI subjects (3 men and 3 women) dropped out and therefore their outcomes were unknown. Analyses therefore included 76 MCI subjects (37 men and 39 women) and 57 age- and gender-matched control subjects. All subjects were free of depression as operationalized as a score less than 8 on the Hamilton Depression Scale (Hamilton, 1960). MCI was diagnosed using the criteria proposed by Mayo Clinic Alzheimer's Disease Research Center. Recently, the criteria of MCI was modified (Pertersen, 2004), but when our study was conducted, the criteria required: (1) memory complaint by patient, family, or physician; (2) normal activities of daily living; (3) normal global cognitive function; (4) objective impairment in memory or in one other area of cognitive function as evident by scores >1.5 SD below the age appropriate mean; (5) CDR score (Hughes et al., 1982) of 0.5; and (6) absence of dementia.

MCI subjects ranged in age from 48 to 86 years with a mean ± standard deviation (SD) of 69.0 ± 8.6 years. The Mini-Mental State Examination (MMSE) (Folstein et al., 1975) score ranged from 24 to 29 (mean ± SD 26.5 ± 1.6) at the initial visit. During the subsequent follow-up period of 3 years, 52 patients showed progressive cognitive decline and eventually fulfilled the diagnosis

Table 1
The characteristics of subjects

	MCI (M:F = 37:39)			Results of ANOVA	
	Controls (M:F = 30:27)	Non-converters (M:F = 12:12)	Converters (M:F = 25:27)	*F* value	*P* value
Age	70.4 ± 7.3	68.7 ± 7.6	69.2 ± 9.1	0.5	0.6
Education in years	12.2 ± 2.9	12.2 ± 3.1	12.0 ± 3.1	0.1	0.9
MMSE	28.8 ± 1.5	27.0 ± 1.3*	26.2 ± 1.7*	38.7	<0.001
MMSE (about after 3 years)		26.1 ± 1.4*	19.1 ± 4.3***,****	126.1	<0.001
Digit span					
Forward	5.3 ± 1.0	5.6 ± 1.0	5.4 ± 1.0	0.5	0.6
Backward	4.1 ± 0.8	4.2 ± 0.8	4.1 ± 1.0	0.2	0.8
List learning (10 words)					
Delayed recall (30 min)	7.9 ± 1.2	3.7 ± 3.6*	0.9 ± 2.0***,****	117	<0.001
Story recall (15 elements)					
Delayed (30 min)	7.9 ± 2.5	0.87 ± 1.72*	0.9 ± 1.72*	101.8	<0.001
Rey–Osterrieth complex figure test					
Delayed recall (30 min)	14.47 ± 6.31	4.28 ± 3.76***	2.9 ± 6.93***	85.3	<0.001

Note. Data are mean ± SD in controls (n = 57) or MCI (n = 76).

* Scores of MCI are significantly lower than those of controls, $P < 0.05$ (Bonferroni correction for multiple comparison).

** Scores of the converters are significantly lower than those of non-converters, $P < 0.05$ (Bonferroni correction for multiple comparison).

*** Scores of the converters are significantly lower than those of non-converters, $P < 0.001$ (Bonferroni correction for multiple independent comparisons).

**** Scores of the follow up MMSE are significantly lower than those of the initial MMSE, $P < 0.05$ (paired t test).

of probable AD according to the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ADRDA) (McKhann et al., 1984). Twenty-four of the 76 MCI subjects still did not fulfill the criteria for dementia according to DSM-IV (American Psychiatric Association, 1994) during the follow-up period. Of these participants, 40 converters and 12 non-converters completed follow-up rCBF SPECT studies at the end of the 3-year follow-up period.

Fifty-seven individuals (30 men and 27 women; age 56–86 years, mean ± SD 70.4 ± 7.3 years) did not have memory impairment or cognitive disorders and were assigned to the normal control group. Specifically, their performances were within normal limits (<1 SD) both on the Wechsler Memory Scale-Revised and on the Wechsler Adult Intelligence Scale-Revised, and their MMSE score ranged from 25 to 30 (mean ± SD 28.8 ± 1.5). None of these control subjects manifested cognitive changes during the follow-up period of more than 3 years. The control group did not differ significantly in age or education from the MCI group.

The local ethics committee approved this study for both healthy volunteers and MCI subjects, all of whom gave their informed consent to participate. All subjects were right-handed and screened by questionnaire regarding medical history and excluded if they had neurological, psychiatric, or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2-weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus.

SPECT imaging

Before the SPECT scan was performed, all subjects had an intravenous line established. They were injected while lying supine with eyes closed in a dimly lit quiet room. Each subject received an intravenous injection of 600 MBq of technetium-99 m ethyl cysteinate dimer (99 mTc-ECD). Ten minutes after the injection of 99 mTc-ECD, brain SPECT was performed using three-head rotating gamma cameras (Multispect3; Siemens Medical Systems, Inc., Hoffman Estates, IL) equipped with high-resolution fan-beam collimators. For each camera, projection data were obtained in a 128 × 128 format for 24 angles at 50 s per angle. A Shepp and Logan Hanning filter was used for SPECT image reconstruction at 0.7 cycle/cm. Attenuation correction was performed using Chang's method.

Statistical parametric mapping

Images were analyzed with the statistical parametric mapping software SPM99 (Wellcome Department of Cognitive Neurology, UK). Using a template for Tc-99 m ECD template, the SPECT data were transformed into a standard stereotaxic space (MNI). The spatial normalization algorithm of SPM99 was used for linear and non-linear transformation (basis function: 8 × 9 × 8: iteration: 16). A Gaussian filter (12 mm full width at half maximum) was used to smooth each image. The effect of global differences in CBF between scans was removed by proportional scaling with the threshold at 60% of whole brain activity. Using MRIcro (www.mricro.com), we checked the mask image for statistical analysis and verified that medial temporal regions including the parahippocampal gyrus and hippocampus were encompassed in the analysis. To test hypotheses about regional population effects, data were analyzed by analysis of variance (ANOVA) using the full monthly option. For this F test, we used an alpha value of 0.001 as our level of significance to correct for multiple comparisons. Group comparisons were also done using t tests within the ANOVA design matrix (uncorrected $P < 0.001$ and cluster extent $K > 100$ voxels, small volume correction (SVC) for correction of multiple comparisons). There were twice as many converters as non-converters raising the concern that the SPECT abnormalities in the former might be influenced by statistical power. Therefore, we randomly subdivided AD converters into 2 groups where the group size was matched to that of non-converters. Then, two-sample t tests between non-converters and each group of AD converters were done (uncorrected $P < 0.001$). The resulting sets of t values constituted the statistical parametric maps {SPM (t)}. Anatomic localization was identified using both MNI coordinates and Talairach coordinates obtained from M. Brett's transformations (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) and were presented as Talairach coordinates (Talairach and Tournoux, 1988).

Logistic regression model

To evaluate the predictive value of rCBF change observed in the initial rCBF SPECT, we used as independent variables (X1) Z scores for the mean adjusted rCBF value at the significant clusters obtained from the SPM (t) map in the group comparison (AD converter vs. Non-converter) for the logistic regression model:

$$Y = b0 + b1{*}X1$$

where Y is the logit transformation of the probability P. The logit transformation of the probability of a value is defined as:

$$Y = \log(P/(1-P))$$

where P is the probability of conversion from MCI to AD.

The mean value of the adjusted rCBF in each cluster of each subject was extracted using the Marsbar program (http// www.marsbar.sourceforge.net/), then the Z score was calculated using the following formula: Z score = (mean adjusted rCBF value in the control group minus individual value of adjusted rCBF value)/SD of rCBF value in the control group. The logistic regression model analysis was performed using Statistical Package for the Social Sciences (SPSS, Japan Co., Tokyo, Japan). Because the neuropsychological test scores of converters were significantly lower than those of non-converters (especially on delayed recall of list learning and delayed recall of Rey–Osterrieth Complex Figure Test), we also evaluated the predictive value of those scores at the initial visit using logistic regression analysis.

Results

Conversion rate

In our study, 52 of 82 individuals with MCI converted to AD during the 3-year follow-up period. The annual conversion rate of MCI to AD was approximately 21.14%.

Group comparisons

The ANOVA analysis [SPM (F), $P < 0.001$, corrected for multiple comparisons with family-wise alpha < 0.05] revealed a significant difference among groups in the bilateral precunei (Brodmann area [BA] 7), the posterior cingulate cortices (PCC, BA31, peak $x, y, z = 0, -47, 32$, F value = 35.93), the right inferior parietal lobule (BA40, peak $x, y, z = 46, -64, 44$, F value = 25.23) and the left angular gyrus (BA39, peak $x, y, z = -42, -60, 38$, F value = 16.77) (Fig. 1a). In comparison with controls, AD converters demonstrated reduced blood flow in the bilateral parahippocampal gyri, precunei, PCC, bilateral parietal association areas, and the right middle temporal gyrus (Fig. 1b, Table 2). Non-converters also demonstrated significant reduction of rCBF in the PCC and the right caudate nucleus when compared to controls (Fig. 1c, Table 2). Importantly, significant differences in the bilateral precunei and parietal association areas were found between converters and non-converters (Fig. 1d, Table 2).

Group comparisons of subdivided groups of converters and the non-converters

As compared to non-converters, the first group of 26 converters showed significantly decreased rCBF in the right inferior parietal lobule (Talairach coordinate: 46, −64, 47, t value: 3.82, cluster size: 115) and the left angular gyrus (Talairach coordinate: −40, −58, 36, t value: 4.45, cluster size: 127) (Fig. 2 left). The essentially same result was found in the comparison between non-converters and the second group of 26 converters (Right IPL: Talairach coordinate: 53, −58, 42, t value: 3.65, cluster size: 44; Left angular gyrus: Talairach coordinate: −40, −57, 34, t value: 4.81, cluster size: 180) (Fig. 2, right). We could not find reduced rCBF in the precunei at $P < 0.001$; however, reduction in the precunei was detected at a lenient statistical threshold ($P < 0.005$ without multiple comparisons) in each group comparison (data were not shown).

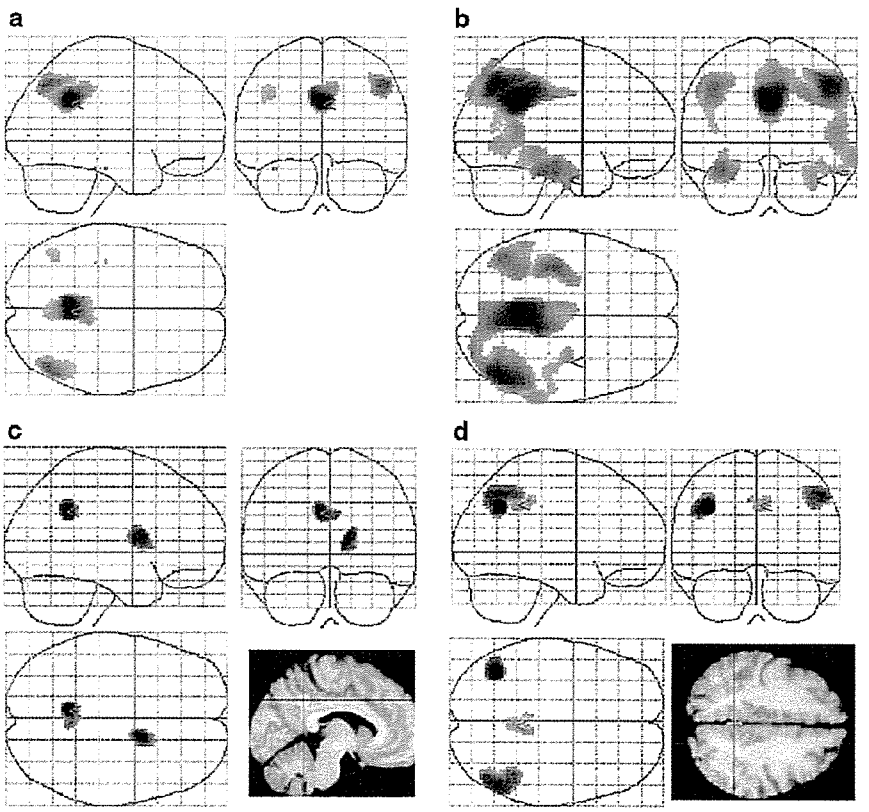

Fig. 1. Results of group comparisons. (a) The SPM {F} is displayed in a standard format as a maximum-intensity projection viewed from the right, the back, and the top of the brain. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of regions with significantly differences between groups (corrected $P < 0.05$) was demonstrated. The ANOVA demonstrated a significant difference among groups in the bilateral precunei, the posterior cingulate cortices, the right inferior parietal lobule, and the left angular gyrus. (b) The SPM is displayed in a standard format as a maximum-intensity projection of regions with significantly decreased rCBF in converters compared with the control group [$P < 0.001$, corrected by small volume correction (SVC)]. The converters demonstrated reduced blood flow in the bilateral parahippocampal gyri, precunei, PCC, bilateral parietal association areas, and the right middle temporal gyrus. (c) The SPM is displayed in a standard format as a maximum-intensity projection of regions with significantly decreased rCBF in non-converters compared with the control group ($P < 0.001$, corrected by SVC). Non-converters demonstrated significant reduction of rCBF in the PCC and the right caudate nucleus. (d) The SPM is displayed in a standard format as a maximum-intensity projection of regions with significantly decreased rCBF in converters compared with non-converters ($P < 0.001$, corrected by SVC). The converters showed a significant reduction of rCBF in the bilateral precunei and parietal association areas.

Table 2
Results of group comparisons and paired *t* tests

Region	BA	Coordinates			*K*	Corrected *P* value (with small volume correction)	*t* value
		x	*y*	*z*			
Controls > AD converters							
Bilateral precuneus, and PCC	BA31						
	B7	2	−45	32	6794	<0.001	8.46
R IPL	BA40	46	−64	44	6794	<0.001	6.95
L Angular gyrus, IPL	BA39	−42	−60	38	1301	<0.001	5.24
L PHG	BA20,36	−38	−22	−17	771	<0.001	5.36
R PHG	BA20,36	34	−15	−19	535	0.005	3.97
R Middle temporal gyrus	BA21	63	−37	−8	535	0.008	4.25
Controls > MCI non-converters							
L PCC	BA31	−8	−49	34	231	<0.001	4.83
R PCC	BA31	4	−47	30	231	0.003	3.81
R Caudate nucleus		14	6	11	158	<0.001	4.63
MCI non-converters > AD converters							
R IPL	BA40	51	−58	45	653	0.001	4.49
L Angular gyrus	BA39	−38	−58	36	368	<0.001	5.21
L Precuneus	BA7	−6	−35	42	140	0.014	3.34
R Precuneus	BA7	2	−45	43	140	0.009	3.51

BA: Brodmann area, IPL: inferior parietal lobule, PCC: posterior cingulate cortex, PHG: Parahippocampal gyrus.

The predictive value of rCBF changes observed at initial SPECT and scores of neuropsychological tests

Given the results of the group comparisons, we hypothesized that rCBF changes in the precuneus and the parietal association areas would be good predictors of progression from MCI to AD in individuals with MCI. Using the *Z* score of each region (Fig. 3) for each MCI subject, we determined the predictive value of the initial rCBF SPECT using a logistic regression analysis. Table 3 shows the results of the logistic regression analysis. We found that higher *Z* scores in the left angular area (Wald $\chi^2 = 11.1$, $df = 1$, $P = 0.001$, odds ratio [OR] 2.174, 95% confidence interval [CI] = 1.38–3.43), right inferior parietal lobule (Wald $\chi^2 = 10.7$, $df = 1$, $P = 0.001$, OR 2.13, 95% CI = 1.35–3.35), and the precuneus (Wald $\chi^2 = 10.13$, $df = 1$, $P = 0.001$, OR 2.417, 95% CI = 1.4–4.16) were good predictors of progression from MCI to AD (Table 3). A cutoff value of 0.5, which best divided the converter and non-converters, provided high sensitivity (82–90%) and adequate overall accuracy (68–73%) in each region (Table 3).

In contrast, lower scores on delayed recall of list learning (Wald $\chi^2 = 8.369$, $df = 1$, $P = 0.004$, odds ratio [OR] 1.413, 95% confidence interval [CI] = 1.118–1.786) and lower scores on delayed recall of the Rey–Osterrieth Complex Figure Test (ROCFT) (Wald $\chi^2 = 7.092$, $df = 1$, $P = 0.008$, OR 1.167, 95% CI = 1.042–1.308) had lower predictive values than those of the rCBF changes observed in SPECT studies. A cutoff value of 0.5, which best divided the converters and non-converters, revealed similar sensitivity (90.3% for word leaning and 86.2% for ROCFT,

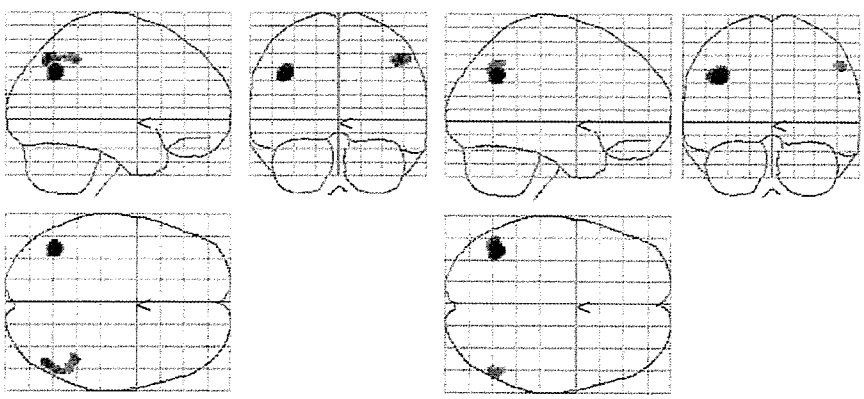

Fig. 2. Results of group comparisons of subdivided groups of converters and the non-converters. The SPM is displayed in a standard format as MIP of regions with significantly decreased in rCBF in converters compared with non-converters (uncorrected $P < 0.001$). Fifty-two converters were randomly divided two groups. Then, two-sample *t* tests between non-converters and each group of AD converters were done. Left: The first group of converters showed a significantly decreased rCBF in the left angular gyrus and the right inferior parietal lobule. Right: The second group of converters also showed essentially the same result.

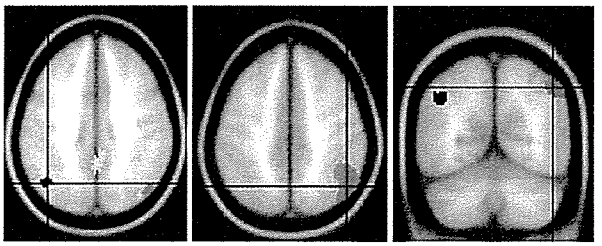

Fig. 3. Regions of interest (ROIs) for the logistic regression model. Red: The ROI for the Z score in the left angular gyrus. Yellow: The ROI for the Z score in the bilateral precunei. Green: The ROI for the Z score in the right inferior parietal lobule.

respectively) and overall accuracy (69.8% for word leaning and 78% for ROCFT respectively) to the sensitivity and accuracy associated with the rCBF changes observed in the initial SPECT (Table 3).

Discussion

The conversion rate

The annual conversion rate of MCI to AD in the current study was 21.14%, which is higher than that observed in other cohorts of MCI subjects (Bruscoli and Lovestone, 2004). A recent review of conversion studies reported that the overall rate of conversion was 10%, but that large differences existed between studies (Bruscoli and Lovestone, 2004). The single most important variable accounting for between-study heterogeneity was the source of subjects, with self-selected clinic attendees having the highest conversion rate (Bruscoli and Lovestone, 2004). In our study, all individuals with MCI were outpatients that attended a memory clinic, and therefore the high conversion rate in the study is not surprising.

Different rCBF changes between converters and non-converters and the predictive value of initial SPECT study

In this study, converters displayed a significant reduction of rCBF in the precuneus and bilateral parietal association areas when compared to non-converters. Although the sample size of converters was larger than that of non-converters, the results of group comparisons with randomly re-sampling the converters in cohorts where the sample sizes were matched to that of non-converters demonstrated essentially the same results. The fact indicated that the greater extent of rCBF abnormalities in converters was not influenced by statistical power. Importantly, we also found that reduction of rCBF in these areas is a good predictor of conversion from MCI to AD. Performance on measures of delayed recall of word learning and ROCFT also showed relatively high discriminative ability, although these scores had lower odds ratios than those associated with reduction of rCBF. These results demonstrate the utility of rCBF SPECT for the prediction of AD conversion. Previous functional neuroimaging studies in very early AD and MCI have consistently demonstrated dysfunction in the PCC and cinguloparietal transitional area or precuneus (Minoshima et al., 1997; Kogure et al., 2000; Imabayashi et al., 2004). A recent PET study showed that the retrosplenial PCC was the only abnormality common to all MCI individuals (Nestor et al., 2003a,b). However, our data suggest that reduced rCBF in the parietal association areas and precuneus are better predictors than PCC hypoperfusion. Indeed, the comparison between the controls and non-converters also demonstrated a significant reduction of rCBF in the PCC. We consider that hypoperfusion in the temporo-parietal regions could be more advanced signs of AD pathology and may precede manifestation of clinical symptoms of AD, and therefore they were better predictors of early conversion. A recent longitudinal FDG-PET study reported similar results to those of the present study: a high predictive value of reduced FDG uptake in the parietal association areas and a lower predictive value of that in the PCC (Chetelat et al., 2003). Mosconi et al. also reported that converters demonstrated reduced glucose metabolism in the inferior parietal cortex as compared with non-converters (Mosconi et al., 2004). Although Nestor's study emphasized the importance of functional abnormality of the retrosplenial cortex in MCI subjects, they also reported that MCI subjects with additional hypometabolism in the parietal association areas converted to AD during the follow-up period (Nestor et al., 2003). These results in conjunction with the current results strongly demonstrate the high predictive value of functional abnormality in the parietal association areas. Furthermore, these results are consistent with the results of a postmortem study of tau pathology in aging and AD (Delacourte et al., 1999). According to Delacourte's study, neurofibrillary degeneration (NFD) with paired helical filaments (PHF)-tau was systematically present in varying amounts in the hippocampal region of non-demented aged subjects, whereas tau pathology in the angular gyrus (BA39) and dorsolateral prefrontal cortex (BA9) was found in all AD patients

Table 3
Results of logistic regression model

	Odds ratio	95% CI	*P* value	Sensitivity (%)	Overall accuracy (%)
SPECT imaging test					
Regions					
L Angular gyrus	2.174	1.38–3.43	<0.001	82	68
R IPL	2.130	1.35–3.35	<0.001	90	73.3
Precuneus	2.417	1.40–4.161	<0.001	88	73.3
Neuropsychological test					
List learning (delayed recall)	1.413	1.118–1.786	0.004	90.3	69.8
Rey–Osterrieth complex figure test (delayed recall)	1.167	1.042–1.308	0.008	86.2	78

CI: confidence interval, IPL: inferior parietal lobule.