



Fig. 1. The scene of PET measurements during the supine and sitting conditions.

one CVD patient. Angiographic studies revealed the cross circulation through the circle of Willis, leptomeningeal collateralization, and the border zone shift according to the definition of the St Louis group (Derdeyn et al., 1999) in all 16 patients, and coronary artery branch stenosis or occlusion in all six CVDC patients (see Table 1). The border zone shift includes the asymmetry filling of anterior or posterior cerebral artery that feeds the middle cerebral artery on the compromised side. The leptomeningeal flow in the parietal region was occasionally seen in the case of retrograde filling from the posterior cerebral artery circulation. Specifically, all the CVDC patients revealed a degree of leptomeningeal connections at the level of posterior watershed regions (middle cerebral artery and posterior cerebral artery border zone). In the two patient groups, none of the patients reported orthostatic symptoms during PET measurement. All of the patients were receiving treatment with antihypertensives, anticoagulants, and other conventional drugs for complications (see Table 1), treatment that could not be suspended before the PET scans were conducted. However, all of these medications were temporarily suspended on the day of the PET examinations. All of the patients underwent magnetic resonance imaging (MRI), which showed mild-to-moderate subcortical abnormalities (high

intensities on the T2-weighted images) in the territory of the ICA on the occluded side and no involvement of the lobe infarction. The study protocol was approved by the Ethics Committee of the Hamamatsu Medical Center, and all participants gave their written informed consent after the nature and possible risks of the experiment were explained.

#### Postural condition and physiology

First, each participant lay calmly with his/her eyes closed on the scanner couch for approximately 40 min. After the supine condition examination was completed, the subjects were instructed to sit back against a 65° reclined (chair-like) couch with their eyes closed for about 25 min (Fig. 1). In both conditions, we recorded systemic arterial blood pressure (ABP) via a catheter placed in the brachial artery and the cardiac rhythm by electrocardiogram. Other physiological parameters, such as PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH, were measured periodically. In our preliminary study, a very short-term elevation of systemic ABP after a postural shift from the supine to the sitting position was found, followed by a gradual decline to the plateau level within about 3 min. These findings were in agreement with results from

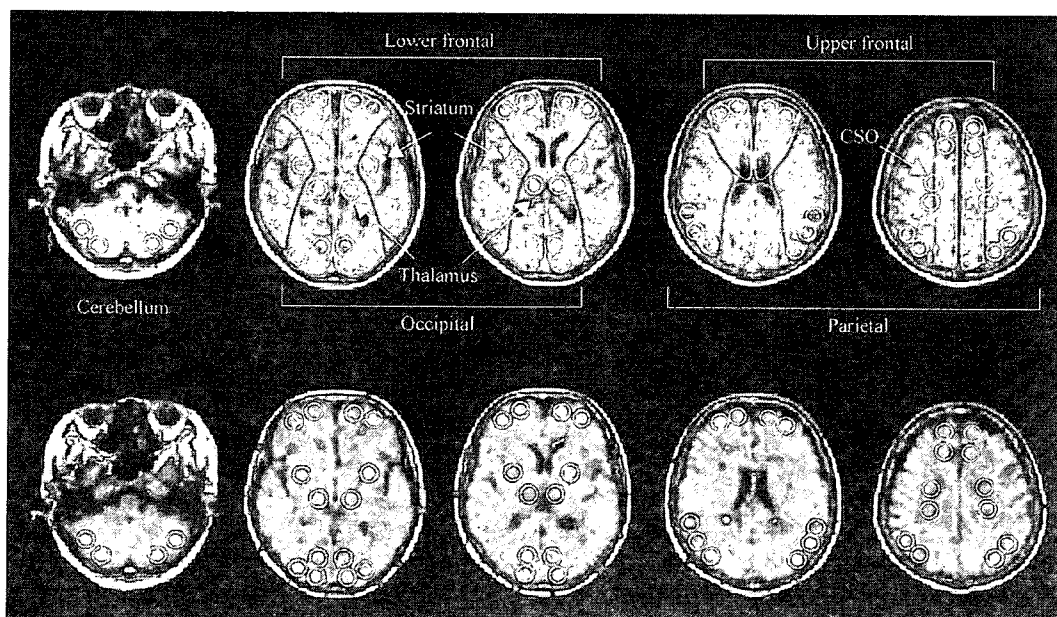


Fig. 2. ROI setting. Multiple circular ROIs consisting of 96 pixels (1.58 cm<sup>2</sup>) were placed on the brain regions on the reformatted MR images (upper row) with the same pixel size as that of PET, which were automatically transferred onto the PET images (lower row). CSO, centrum semiovale.

a previous ultrasonic study (Novak et al., 1998). Thus, the orthostatic PET measurements in this study were performed during the static period of ABP under the head-up condition.

#### PET and MRI procedures

We used a state-of-the-art high-resolution brain PET scanner (SHR12000, Hamamatsu Photonics, Hamakita, Japan), the details of which have been reported elsewhere (Watanabe et al., 2002). In brief, the scanner has 24 detector rings yielding 47 slices simultaneously with a spatial resolution of 2.9 mm (full-width at half-maximum) transaxially and 3.0 mm axially and 163 mm axial field of view. After the backprojection and filtering (Hanning filter), the reconstructed image resolution became  $6.0 \times 6.0 \times 3.2$  mm full-width at half-maximum. The voxel of each reconstructed image measured  $1.3 \times 1.3 \times 3.2$  mm. This PET system has a mobile gantry system in which the gantry can move vertically 180 cm above the floor and tilt from  $-20^\circ$  to  $+90^\circ$  like a mechanical chair. Just prior to the PET measurements, each participant underwent an MRI for determination of the intercommissural (AC-PC) line using a static magnet with three-dimensional mode acquisition (0.3 T MRP7000AD, Hitachi, Japan) (Ouchi et al., 1998). Because the same face mask was used between the MRI and PET studies, and because the center of scan field was beforehand calibrated between the two modalities, the PET gantry was moved, and the brain was scanned parallel to the intercommissural plane according to the MRI information received.

After fixation of the head to the head holder using a radiosurgery-purpose thermoplastic face mask, a 10-min transmission scan was first performed for attenuation correction with a  $^{68}\text{Ge}/^{68}\text{Ga}$  source in the supine position. Then the serial emission scans were started based on  $^{15}\text{O}$ -oxygen bolus inhalation (Mintun et al., 1984) and  $^{15}\text{O}$ -water bolus injection method (Herscovitch et al., 1983) and short-period  $\text{C}^{15}\text{O}$  inhalation for the cerebral blood volume (CBV) data (Lammertsma and Jones, 1983; Lammertsma et al., 1987). During the scan session, the subjects inhaled 2.0 GBq  $^{15}\text{O}_2$  and, for a short period of time, 1500 MBq  $\text{C}^{15}\text{O}$  flowing through a tightly fitted plastic oxygen face mask attached to the subject's face. They also received 300 MBq  $\text{H}_2^{15}\text{O}$  via a catheter in the right cubital vein. Data of arterial oxygen saturation ( $\text{SaO}_2$ ), arterial hemoglobin (Hb), and the hematocrit values yielded the arterial oxygen content ( $\text{CaO}_2$ ) by the following formula:  $\text{CaO}_2 = 1.39 \times \text{SaO}_2 \times \text{Hb}$ . The rCBF- and

CBV-corrected OEF values were estimated on a voxel-by-voxel basis. The voxel-based  $\text{CMRO}_2$  image was finally generated using the following equation:  $\text{CMRO}_2 = \text{CBF} \times \text{OEF} \times \text{CaO}_2$ . The arterial blood radioactivity was determined using the automated arterial blood  $\gamma$ -ray coincidence counter (BACC-2: Hamamatsu Photonics K.K.), which could measure arterial input data per second (Ouchi et al., 2001b). After the first session of PET scans under the supine condition was completed, the subject's head holder was temporarily removed from the receiver of the scanner's gantry, and the head was fixated again in the sitting position. Emission scans with the same protocol were performed under the sitting condition.

#### Data analysis and statistics

As seen in Fig. 2, we first placed multiple circular regions of interest (ROIs) with 96 pixels ( $1.62 \text{ cm}^2$ ) bilaterally over the cerebellar hemisphere, lower frontal area (the orbitofrontal and inferior frontal cortices [Brodmann area or BA: 10/11]), upper frontal area (middle and superior frontal cortices [BA: 6/8]), temporal [BA: 21/22], parietal [BA: 7], occipital [BA: 17/18] cortices, the striatum, the thalamus, and the centrum semiovale (CSO) on the MR images according to the human brain atlas (Mai et al., 1997; Ouchi et al., 2001b). After completion of the ROI placement, the PET images were displayed side-by-side with the MR images using an image processing system (DrView, Asahi Kasei Co, Tokyo, Japan) (Ouchi et al., 2001a,b) on a SUN workstation (Hypersparc ss-20, SUN Microsystems, CA, USA), which enabled the automatic placement of ROIs on the same area on both the MR and corresponding PET images (CBF,  $\text{CMRO}_2$ , OEF, CBV under both supine and sitting conditions). Quantitative data in the normal group were calculated by averaging the bilateral values in each region. The ROIs on the upper frontal and parietal region as well as the CSO were regarded as the loci for the distal part of the affected ICA territory.

For statistical analysis, baseline data collected in the supine posture were compared among the three groups by two-way analysis of variance (ANOVA) with respect to the type of group (CVD, CVDC, and Normal) and type of location, that is, occluded or non-occluded side. Since no cross-interactions were observed in the ANOVA between the two factors ( $P = 0.4384$ ), repeated measure ANOVA was then performed to compare the parameters among groups on each hemispheric side separately. Since the perfusion territorial changes in hemodynamic param-

Table 2  
Physiological data in patients with cerebrovascular disease and normal controls

Group	Condition	MABP	Pulse rate	PaCO <sub>2</sub>	pH
CVD	Supine	103.2 ± 11.3*	72.3 ± 14.3	40.3 ± 3.4	7.4 ± 0.0
	Sitting	99.5 ± 14.7*	74.3 ± 14.9	40.2 ± 3.4	7.4 ± 0.0
	Change (%)	-3.36	3.10	-0.72	0.0
CVDC	Supine	95.7 ± 8.9	73.2 ± 11.6	41.1 ± 2.0	7.4 ± 0.0
	Sitting	90.8 ± 8.2	74.5 ± 8.5	40.6 ± 1.9	7.4 ± 0.0
	Change (%)	-5.1	2.5	-1.1	-0.1
NC	Supine	94.0 ± 8.0	62.4 ± 7.3	41.7 ± 2.9	7.4 ± 0.0
	Sitting	91.2 ± 8.1	65.9 ± 6.8	41.8 ± 3.5	7.4 ± 0.0
	Change (%)	-2.93	3.49	0.01	0.0

Values are expressed as mean ± SD. CVD, cerebrovascular disease (internal carotid artery occlusion or severe stenosis); CVDC, CVD with coronary artery disease; NC, normal controls; MABP, mean arterial blood pressure; Change (%), [(sitting - supine)/supine × 100] expressed as a mean value.

\*  $P < 0.05$  ( $\chi^2$  test) vs. normal control.

Postural shift in patients with cerebrovascular disease and normal controls

Rate	Cerebellum		Lower frontal		Upper frontal		Parietal		Occipital		Striatum		Thalamus		CSO
	Intact	Affected	Intact	Affected	Intact	Affected	Intact	Affected	Intact	Affected	Intact	Affected	Intact	Affected	Intact
supine	42.6 ± 8.8	47.9 ± 9.7	40.2 ± 4.7	35.3*** ± 8.4	43.8 ± 7.0	39.3*** ± 8.7	41.5 ± 8.3	38.7*** ± 8.7	41.7 ± 9.2	41.8 ± 9.9	51.8 ± 9.9	43.8 ± 10.9	52.5 ± 10.4	48.2 ± 9.8	24.0 ±
sitting	41.0 ± 7.9	46.8 ± 8.1	39.2 ± 6.0	34.0 ± 8.9	42.2 ± 5.6	36.6 ± 8.0	40.2 ± 8.3	35.6 ± 8.0	40.0 ± 8.7	40.1 ± 6.2	45.7 ± 9.1	36.2 ± 9.3	49.0 ± 10.1	44.8 ± 9.3	23.6 ±
Δ	-3.28	-3.04	-3.1	-3.6	-3.48	-5.89	-2.75	-6.59***	-1.76	-1.62	-2.50	-5.12	-4.85	-6.39	-2.67
supine	3.1 ± 0.8	3.4 ± 0.9	2.7 ± 0.7	2.6 ± 0.8	3.0 ± 0.6	3.0 ± 0.7	2.7 ± 0.8	2.9 ± 0.8	2.8 ± 1.2	2.8 ± 1.1	3.3 ± 1.0	3.1 ± 1.1	3.2 ± 0.7	3.0 ± 1.2	1.1 ±
sitting	3.3 ± 1.0	3.3 ± 0.8	2.7 ± 0.9	2.6 ± 0.8	2.6 ± 0.8	2.7 ± 0.8	2.8 ± 0.9	2.8 ± 1.0	3.1 ± 0.9	2.9 ± 1.0	2.7 ± 1.1	2.6 ± 1.0	3.0 ± 1.0	3.8 ± 1.2	1.4 ±
Δ	3.45	2.71	2.02	2.2	3.94	3.18	5.22	2.88	3.33	4.32	-0.20	-0.26	1.12	-0.72	2.18
supine	39.9 ± 7.0	40.4 ± 7.8	42.0 ± 7.0	42.8*** ± 8.2	40.3 ± 5.1	40.9 ± 5.2	40.9 ± 6.1	42.4*** ± 5.6	42.6 ± 8.0	42.8 ± 8.8	40.0 ± 8.4	39.1 ± 8.4	37.2 ± 7.7	37.8 ± 8.1	32.4 ±
sitting	43.0 ± 10.2	41.4 ± 8.4	43.1 ± 9.5	44.4 ± 9.0	41.5 ± 7.8	44.7 ± 7.2	44.1 ± 6.5	48.6 ± 7.8	43.8 ± 9.0	45.1 ± 8.7	40.3 ± 9.3	40.5 ± 9.3	38.5 ± 10.4	40.0 ± 9.8	33.0 ±
Δ	5.06	4.74	4.10	6.22	6.97	10.81***	8.9	16.09**	4.10	6.22	4.53	5.06	4.74	6.98	7.90
supine	3.8 ± 0.5	4.3 ± 1.0	2.9 ± 0.5	3.0 ± 0.7	2.7 ± 0.6	2.6 ± 0.3	3.2 ± 0.7	3.6 ± 0.9	3.6 ± 0.5	4.3 ± 1.0	2.7 ± 0.4	2.6 ± 0.3	2.4 ± 0.6	3.0 ± 0.6	1.9 ±
sitting	3.8 ± 0.8	4.7 ± 1.8	3.0 ± 0.5	3.1 ± 0.6	3.0 ± 0.3	2.8 ± 0.4	3.2 ± 0.7	3.6 ± 0.6	3.7 ± 0.4	4.1 ± 0.6	2.7 ± 0.3	2.6 ± 0.3	2.3 ± 0.3	3.9 ± 0.7	2.0 ±
Δ	-0.55	3.01	4.78	2.39	5.8	5.08	3.52	0.78	4.52	4.13	-2.86	2.17	-0.54	3.12	0.87
supine	38.2 ± 0.8	42.3 ± 12.3	34.0 ± 11.0	32.5*** ± 6.1	35.5 ± 10.8	34.6 ± 9.7	35.9 ± 8.5	31.4*** ± 8.2	33.3 ± 8.4	29.9*** ± 10.4	44.9 ± 16.6	45.1 ± 12.1	43.5 ± 16.2	40.2 ± 13.5	18.3 ±
sitting	35.4 ± 12.2	37.0 ± 11.5	33.0 ± 10.3	31.5 ± 7.3	32.2 ± 9.9	29.2 ± 7.2	31.9 ± 7.9	26.8 ± 7.7	34.0 ± 9.2	29.5 ± 9.0	39.3 ± 15.1	37.4 ± 12.7	43.6 ± 11.4	40.1 ± 10.4	18.0 ±
Δ	-8.6	-10.5	-4.3	-3.4	-10.4	-14.7***	-12.2	-14.1***	-9.2	-10.3	-12.5	-15.7***	-1.6	-0.3	-1.9
supine	2.6 ± 0.6	2.6 ± 0.7	2.4 ± 0.8	2.3 ± 0.5	2.4 ± 0.6	2.3 ± 0.5	2.4 ± 0.6	1.9** ± 0.8	2.4 ± 0.5	2.1 ± 0.9	3.2 ± 1.2	3.0 ± 0.6	2.7 ± 0.7	2.8 ± 1.1	1.1 ±
sitting	2.8 ± 0.9	2.6 ± 0.8	2.4 ± 1.1	2.3 ± 0.5	2.3 ± 0.9	2.2 ± 0.6	2.5 ± 0.7	1.9 ± 0.9	2.3 ± 0.7	2.1 ± 1.0	2.9 ± 1.2	3.0 ± 0.5	2.7 ± 1.0	2.8 ± 1.3	0.9 ±
Δ	7.2	1.2	-1.2	0.6	-4.4	-5.2	5.2	-7.8***	-5.7	-2.1	-9.3	0.1	-2.2	-1.0	-9.8
supine	43.1 ± 5.1	38.6 ± 5.8	42.0 ± 7.9	42.9 ± 8.1	39.3 ± 8.5	40.7 ± 9.2	40.8 ± 6.3	40.3 ± 3.5	41.6 ± 9.3	38.3 ± 10.2	43.5 ± 5.6	38.2 ± 6.1	35.4 ± 9.3	36.3 ± 7.8	28.3 ±
sitting	46.4 ± 7.7	41.8 ± 4.0	44.4 ± 2.8	43.8 ± 6.7	42.0 ± 8.1	46.0 ± 8.6	43.5 ±	45.2 ± 3.7	46.5 ± 9.6	38.0 ± 11.2	41.4 ± 5.9	44.6 ± 8.7	31.9 ± 8.4	37.3 ± 6.9	28.0 ±
Δ	7.4	9.2	6.6	3.5	7.7	14.1***	6.0	12.8***	11.7	6.4	-4.8	16.6	-9.0	3.5	0.1
supine	4.0 ± 0.8	4.5 ± 0.7	3.9 ± 4.0	3.6 ± 1.9	3.1 ± 0.6	3.1 ± 0.7	3.4 ± 0.5	3.9 ± 0.8	3.8 ± 0.6	4.5 ± 2.1	3.3 ± 1.4	3.4 ± 1.2	3.6 ± 0.8	3.0 ± 0.3	2.2 ±
sitting	4.1 ± 0.7	4.5 ± 0.8	3.5 ± 0.7	3.5 ± 0.8	2.9 ± 0.3	3.0 ± 0.5	3.5 ± 0.6	3.5 ± 0.6	4.0 ± 0.7	4.2 ± 1.9	2.9 ± 0.7	3.2 ± 1.2	3.6 ± 0.7	3.2 ± 0.2	2.3 ±
Δ	5.8	4.8	-10.6	-3.5	-2.2	-2.7	3.8	-4.8	5.2	-2.1	-5.9	-7.0	0.2	6.4	-1.3
Δ	-2.6		-4.43		-4.87		-4.92		-4.59		-5.43		-5.41		-2.58
Δ	1.51		-2.11		2.05		0.45		0.01		-0.21		-0.38		1.61
Δ	3.7		5.36		5.9		5.61		5.8		5.38		5.2		5.49
Δ	-1.34		3.29		1.14		4.87		4.23		-1.64		4.51		1.25

% CVD, cerebrovascular patients (internal carotid artery occlusive disease); CVDC, internal carotid artery occlusive disease with coronary artery disease; NC, normal controls; CSO, centrum semiovale; CBF, cerebral blood flow; CMRO<sub>2</sub>, oxygen extraction fraction; CBV, cerebral blood volume; %Δ: ((sitting - supine)/supine × 100).

1 controls.  
 (ificant) vs. normal controls.

eters (i.e., the ICA territory) were of interest, a Bonferroni post hoc test was applied within the same territory, and a  $P$  value less than 0.05 was regarded as statistically significant. Simple linear regression analyses were performed to analyze the relation between postural changes of metabolic parameters (CMRO<sub>2</sub> and OEF) and the changes of the perfusion pressure index (CBF/CBV) in the CVD and CVDC groups, separately. In addition, the postural changes in these hemodynamic parameters were compared against the changes in mean ABP in the distal ICA regions. The level of significance was also assumed as  $P < 0.05$ .

In addition, we added a voxel-based analysis to evaluate postural changes in rCBF more objectively between disease and healthy conditions using statistical parametric mapping (SPM) software (SPM99; Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995). The detail procedure was reported elsewhere (Ouchi et al., 2001a). In brief, normalized data were smoothed with an isotropic Gaussian kernel of 8 mm, resulting in the smoothed images with voxel sized of  $2 \times 2 \times 2$  mm. In the present study, however, we used quantitative CBF data for the voxel-wise analysis without analysis of covariance with global normalization. The regions with clusters of voxels over 50 and a peak height  $P < 0.001$  uncorrected for multiple comparison were regarded as significant. In addition, a single-subject analysis was performed to examine individual variability. The significant level of this analysis was assumed to be  $P < 0.003$  uncorrected for multiple comparison for the peak height with voxels over 50.

## Results

### Physiological changes

There was a tendency for postural decrease of the mean arterial blood pressure (MABP) in the CVDC group to occur, but the physiological parameters (MABP, pulse rate, PaCO<sub>2</sub>, and arterial pH) were not significantly different between the supine and upright conditions among the three groups ( $P > 0.05$ , repeated-measures ANOVA) (Table 2). No clinical symptoms, such as hyperventilation, faintness, and syncope, were observed during the upright posture in any of the participants. Each electrocardiogram showed no significant ST-segment depression during the upright condition in the CVDC group.

### ANOVA for absolute postural changes in hemodynamic and metabolic parameters

At baseline, one-way ANOVA showed significant reductions in rCBF in the parietal, frontal, and occipital cortices on the affected side and in the rCMRO<sub>2</sub> in the parietal counterpart in the CVDC group compared with the normal group ( $P < 0.05$ , Table 3). Repeated measures ANOVA showed significant absolute reductions in rCBF and rCMRO<sub>2</sub> in the affected-side parietal cortex in the CVDC group during upright posture compared with those in the CVD and normal groups, while rCBF (with a decrease tendency) and the rCMRO<sub>2</sub> in the area failed to show significant changes in the CVD group. The level of OEF in the affected-sided parietal cortex during sitting was significantly higher in the disease groups than that in the normal group (similar to the hemodynamic change “misery perfusion”) (Baron et al., 1981). Table 4 showed mean levels of each parameter estimated for values of the territory of ICA, hemispheric, and global regions.

### Relations between postural changes in oxygen metabolic parameters (CMRO<sub>2</sub>, OEF) and changes in perfusion pressure index (CBF/CBV)

There were significant negative correlations of the postural changes in % $\Delta$ CBF/CBV with % $\Delta$ OEF in the upper frontal ( $y = -1.38x - 6.12$ ,  $r = 0.70$ ,  $P < 0.05$ ) and parietal ( $y = -1.03x - 1.73$ ,  $r = 0.79$ ,  $P < 0.05$ ) cortices and with % $\Delta$ CMRO<sub>2</sub> in the upper frontal ( $y = -1.59x - 28.5$ ,  $r = 0.68$ ,  $P < 0.05$ ) and parietal ( $y = -1.97x - 32.3$ ,  $r = 0.73$ ,  $P < 0.05$ ) cortices on the affected side in the CVDC group (Fig. 3), indicating that metabolic demand was raised as perfusion pressure decreased. In the CVD group, the postural changes in the parietal OEF tended to correlate negatively with the CBF/CBV changes ( $r = 0.59$ , dotted line) on the affected side, while the two parameters showed positive correlation ( $r = 0.57$ , dotted line) on the non-occluded side (Fig. 3D).

### Relations between postural changes in hemodynamic and metabolic parameters and mean arterial blood pressure

Because the parameters varied significantly in the parietal cortex, we focused on changes in this region. In other brain regions, there was a similar tendency in the upper frontal cortex (not shown).

Table 4  
Mean levels of territorial cerebral blood flow during the postural conditions (supine/sitting) in three groups

Group	Parameter	Global	Hemisphere		ICA territory	
			Intact	Affected	Intact	Affected
CVD	CBF	42.9/41.4	46.6/45.2	42.6/40.4	45.7/44.1	41.2/39.1
	CMRO	2.8/2.9	3.1/3.1	2.9/2.9	3.0/3.1	2.8/2.9
	OEF	38.9/41.0	39.4/41.3	39.8/42.5	39.6/41.8	40.2/43.3
	CBF/CBV	13.6/13.0	14.9/14.4	13.8/12.8	15.0/14.3	13.4/12.4
CVDC	CBF	35.9/33.5	37.9/36.2	35.8/33.1	35.9/34.4	34.3/31.7
	CMRO	2.33/2.41	2.6/2.6	2.4/2.4	2.4/2.5	2.3/2.2
	OEF	37.4/39.9	39.2/40.7	38.5/41.5	38.6/41.2	41.1/43.4
	CBF/CBV	10.7/10.1	11.5/10.9	10.8/10.0	10.5/10.4	10.4/9.9
NC	CBF	43.8/42.1	46.9/44.4	46.4/44.8	43.4/42.0	43.5/41.7
	CMRO	2.7/2.7	2.9/2.9	2.8/2.8	2.7/2.7	2.7/2.7
	OEF	43.8/42.2	38.6/40.4	37.0/38.3	38.7/40.0	38.0/39.4
	CBF/CBV	12.8/12.0	13.6/12.4	13.1/12.4	12.8/11.9	13.0/12.3

CBF, cerebral blood flow (ml/100 g/min); CMRO<sub>2</sub>, cerebral metabolic rate of oxygen (ml/100 g/min); OEF, oxygen extraction fraction (%); CBV, cerebral blood volume (ml/100 g), CBF/CBV (%).

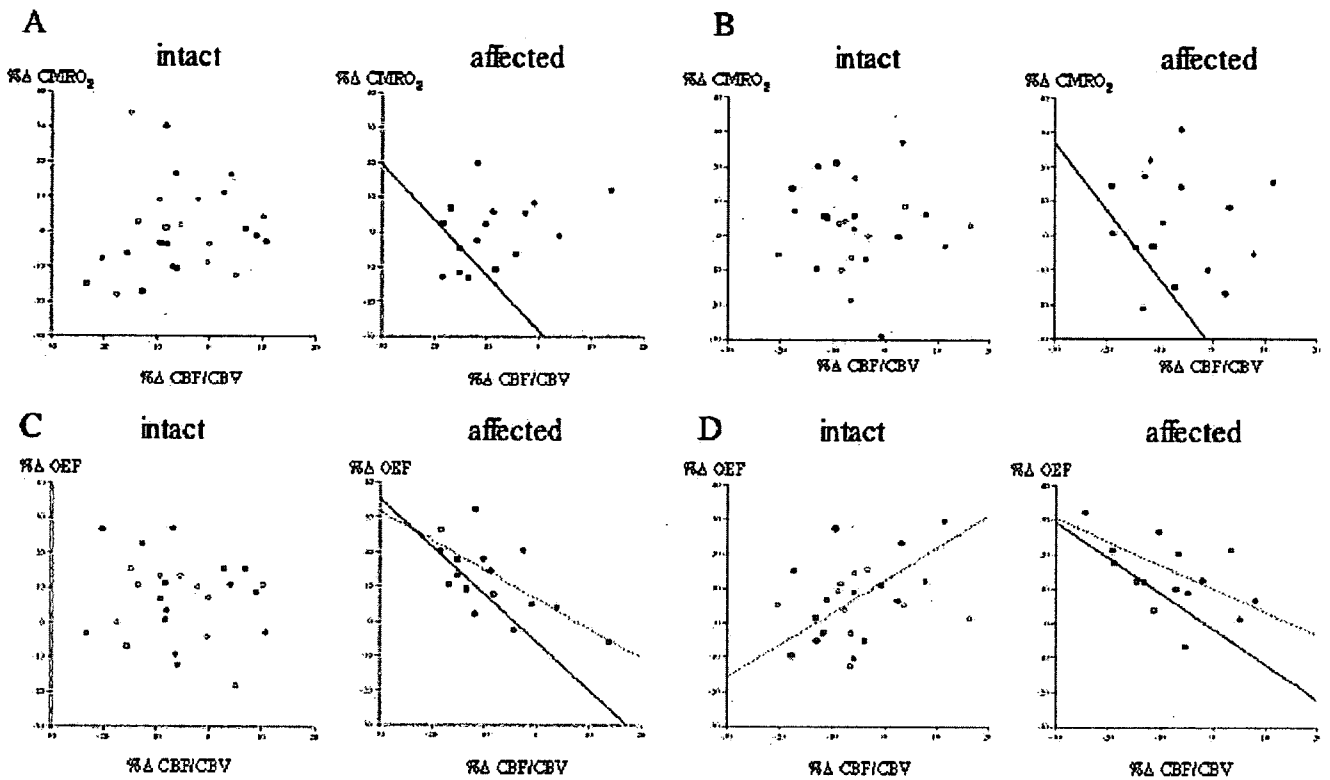


Fig. 3. Correlations between changes in perfusion pressure index (CBF/CBV) and changes in oxygen metabolism (A and B) and oxygen extraction fraction (C and D) in susceptible regions (A and C, upper frontal cortex; B and D, parietal cortex) in the normal (white circle), CVD (black circle, dotted line), and CVDC (marked square, straight line) groups. Straight lines denote significant correlations ( $P < 0.05$ ), and dotted lines show subsignificant correlations ( $P < 0.1$ ).

In the normal group, the values of CBF, CMRO<sub>2</sub>, and OEF in the parietal cortex were almost constant with the MABP change (Figs. 4A–C, intact side, dashes). However, postural reduction in rCBF became significantly greater with MABP reduction ( $y = 2.82x + 0.22$ ,  $r = 0.80$ ,  $P < 0.05$ ) in the CVDC group (Fig. 4A, affected side). Postural changes in metabolic parameters (OEF) significantly correlated negatively with MABP change ( $y = -4.03x - 7.63$ ,  $r = 0.88$ ,  $P < 0.05$ ) (Fig. 4C, affected side), and so did the CMRO<sub>2</sub> change ( $y = -7.07x - 40.5$ ,  $r = 0.74$ ,  $P < 0.05$ ) (Fig. 4B, affected side). In the CVD group, the rCBF change tended to correlate positively ( $r = 0.60$ , Fig. 4A, affected side) and the OEF change negatively ( $r = 0.50$ , Fig. 4C, affected side) with the MABP change.

## Discussion

The present study is the first to show an absolute reduction in CBF and an increase in OEF along with a minor increase in oxygen metabolism in the hemodynamically compromised parietal region (i.e., the region more distal to the occlusion and comparable to the watershed area) in CVD patients during upright posture, suggesting the occurrence of posture-induced local neural tissue deactivation, and a further reduction in rCBF and a moderate increase in OEF along with reduced oxygen metabolism (severer deactivation) in the CVDC patients during posture (Figs. 5 and 6). Although the levels of CBV were not significantly different between postural conditions in each group, the averaged CBV values in the CVDC group tended to decrease during upright posture. The major difference between the CVD and CVDC groups was the opposite deviation in CMRO<sub>2</sub> changes in the ischemic region by a postural

shift. A postural increase in OEF was commonly found in both groups, but the magnitude of the OEF increase was smaller in the CVDC group, which might cause postural reduction in CMRO<sub>2</sub>. The lack of this capacity to maintain CMRO<sub>2</sub> in the CVDC group may be attributable to neuronal impairment or the scantiness of the available vascular density. It has been hypothesized that in patients with coronary artery disease, reduced flow, microemboli, and small necrosis are likely to develop due to a physical and mental stress-induced increase in blood viscosity and coagulability (Muller et al., 1989). This insidious damage in the susceptible cerebrovasculature would prevent augmentation of oxygen metabolism in the CVDC group. A greater reduction in perfusion pressure (CBF/CBV) in CVDC patients than in CVD patients might have been another explanation for the loss of metabolic increase. Taken together, upright posture may generate not only local neural deactivation in the distal part of the brain in the occluded ICA territory, but also impairment of local metabolic regulation in the area, if concomitant with cardiac ischemia. The latter problem can be a consecutive setback in the recovery of neuronal function during rehabilitation.

The postural effect on cerebral hemodynamics has been widely examined with transcranial Doppler sonography that enables the assessment of orthostatically rapid hemodynamic changes. The greater advantage of our study over sonography was the ability to quantitatively measure not only vascular, but also metabolic states in cerebrovascular regulations during assumption of the upright posture. An absolute reduction in rCBF in the hemodynamically vulnerable region under our orthostatic conditions was in line with the sonographic result showing orthostatic reduction in the perfusion velocity of intracranial large vessels (Fig. 7; Daffertshofer et al., 1991; Novak et al., 1998). As for methodological

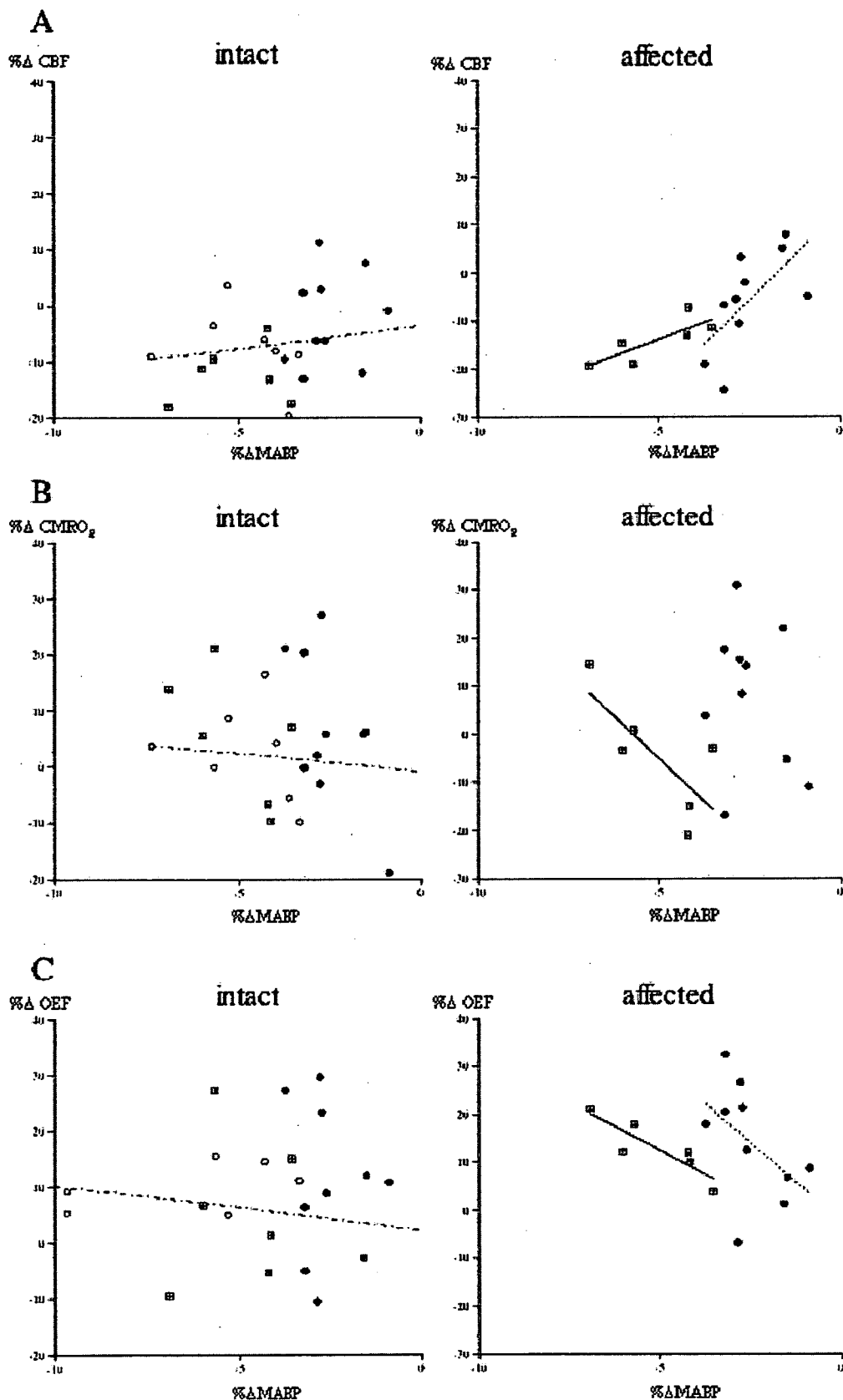


Fig. 4. Correlations of %reduction in mean arterial blood pressure (% $\Delta$ MABP) with %changes in perfusion (% $\Delta$ CBF, A), oxygen metabolism (% $\Delta$ CMRO<sub>2</sub>, B), and oxygen demand index (% $\Delta$ OEF, C) in the hemodynamically vulnerable parietal region in the normal (white circle, dashes), CVD (black circle, dotted line) and CVDC (marked square, straight line) groups. While the cerebrovascular and metabolic parameters reached a plateau with the MABP change in the normal group, significant correlations were observed in the disease groups (see the Results), although the width of the MABP change was relatively small.

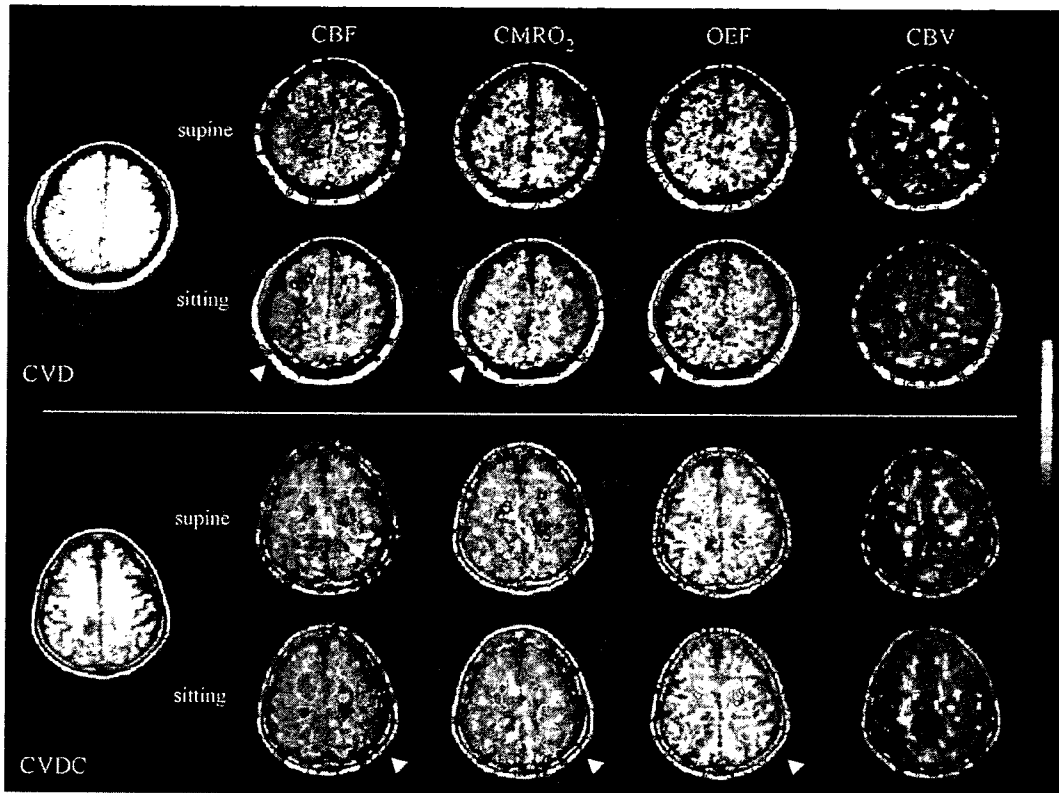


Fig. 5. PET images of a patient with right internal carotid occlusive disease (CVD) and one with left CVD plus coronary artery disease (CVDC) during supine and sitting postures. There were no abnormalities in the magnetic resonance images, but postural alterations in the hemodynamic and metabolic parameters were detected in the susceptible regions (arrowheads) in each disease group: reductions in CBF and  $CMRO_2$  with slight increase in OEF in the CVDC group, and reduction in CBF with stable  $CMRO_2$  and increase in OEF in the CVD group under the sitting condition.

caveats that must be taken, the timing of  $CMRO_2$  acquisition was theoretically different from that of the CBF measurement in our study, and the PET data were acquired at least 15 min after the postural shift from the supine to sitting condition. These methodological limitations did not permit depiction of the dynamic phase of ongoing postural behavior and did not allow oxygen

metabolism to be regarded as an independent measure from CBF, because  $CMRO_2$  was quantified with the value of CBF. No significant changes in physiological parameters during the upright condition, however, could indicate the adequacy of handling CBF as a stable parameter during the upright condition, because the oxy/deoxy hemoglobin concentration reached a plateau 3 min after the

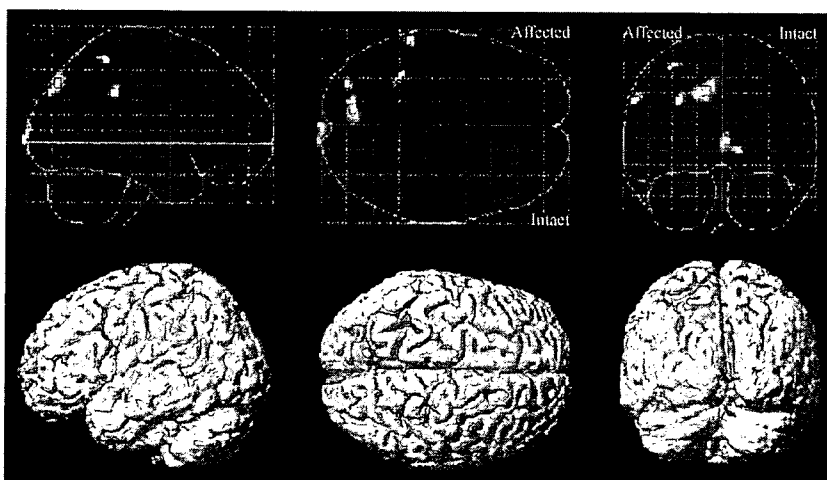


Fig. 6. SPM showed significant reduction in relative rCBF in the superior ( $x y z = -34 -46 60$ ,  $z$  score = 4.02) and inferior ( $x y z = -58 -40 36$ ,  $z$  score = 3.98) parietal lobules and precuneus ( $x y z = -10 -78 40$ ,  $z$  score = 4.38) on the occlusion side during sitting vs. lying ( $P < 0.001$ , uncorrected) in the disease group. The present result was similar to, but topographically different from, the previous result (Ouchi et al., 2001a,b), showing parietotemporal and frontal reductions in CBF. This discordance might be ascribed to differences in PET scanners used and in diseases analyzed (middle cerebral artery occlusion vs. ICAO).

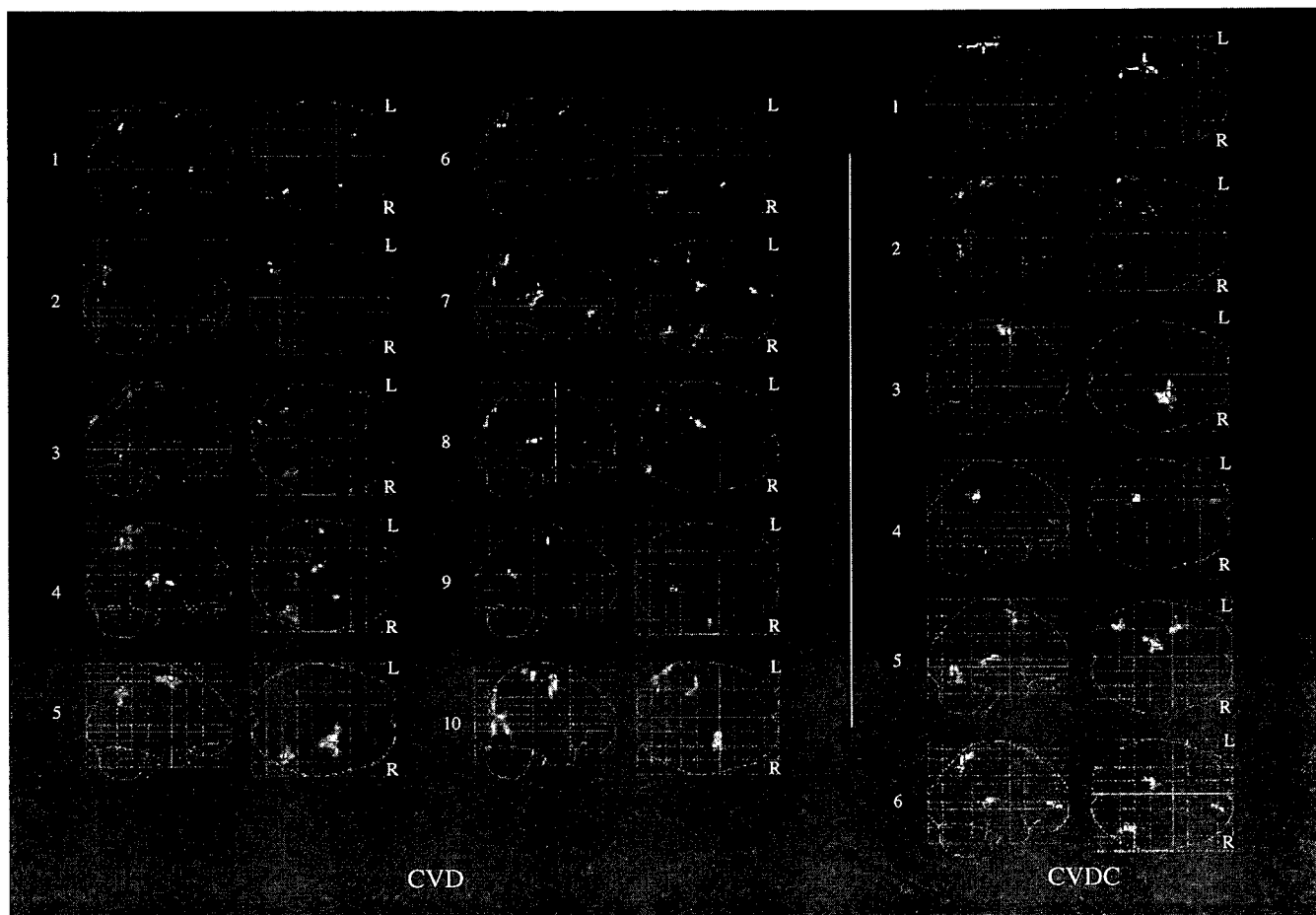


Fig. 7. Single-subject analysis for reduction in rCBF during sitting. The numbers of each glass brain correspond to the patient numbers seen in the Table 1. R, right; L, left.

postural change (Krakow et al., 2000). Although there were no significant differences in physiology among the three groups in the present study, it was not possible to determine the autonomic effect on the vasa neuronal and vasomuscular tones in the cerebral hemodynamics. Therefore, it is possible that there was a greater degree of implicit sympathetic effect that triggered a hemodynamic change in the coronary circulation (Muller, 1999) in the CVDC group, resulting in a reduction in cardiac output followed by a decrease in the cerebrovascular perfusion pressure.

The level of rCBF in the healthy group remained rather unchanged during the upright posture, which means that postural rCBF reduction might be ascribed to the impairment of local autoregulation in hemodynamically vulnerable regions in disease groups. This ABP-related CBF reduction was more significant in the affected-side parietal cortex in the CVDC group, and the relative metabolic demand was inversely augmented with MABP reduction (Fig. 4), suggesting that the susceptible ischemic region would require more energy to survive or maintain normal vasoreactivity for a further vascular dilatation event in CVD patients with cardiac ischemia. This speculation might be further supported by the present finding that a reduction in CBF/CBV correlated negatively with an increase in OEF and CMRO<sub>2</sub> in the frontal and parietal cortex distal to the affected region in the CVDC group (Fig. 3). The tendency of this negative correlation was also seen in the CVD group. In ischemic areas distal to an arterial occlusion, the arteriolar pressure has been considered to be much lower than that in the

normal tissue and below the lower limit of autoregulation (Paulson, 1970; Shima et al., 1983; Symon et al., 1976). In the ischemic situation, the vascular (capillary-to-vein) pool or CBV has been considered to increase due to autoregulatory dilatation in the affected blood vessel to compensate for perfusion pressure reduction (Powers, 1991). Although a significant difference was not detected in the quantitative CBV level, due chiefly to large standard deviations (not shown) in the present study, the CBV tended to decrease in the vulnerable region during the upright posture, particularly in the CVDC group (Table 3). A recent PET study showed that acute administration of acetazolamide, a vasodilatory effect agent, resulted in an increase in the arteriolar blood volume coupled with a rCBF reduction in those CVD patients with good collateral circulation (Okazawa et al., 2003). Thus, it was assumed that energy production might not be enough to make the arterioles respond to impending ischemic events when CVDC patients assume an upright posture. The lack of a decrease tendency of CBV in the CVD group might indicate that the vasodilatory capacity of arterioles in the ischemic region was sufficient.

From a clinical point of view, the incidence of ischemic stroke is at its maximum from eight to noon, the period of day during which blood pressure is at its highest in awake patients with stroke (Marler et al., 1989). The incidence of transient episodes of myocardial ischemia is also at a high point during this period (Muller, 1999). Thus, adequately controlling hypertension may be crucial for preventing the further development of stroke events for



patients when they are in an upright position. This issue is indeed important, because early introduction of active physical exercise after stroke is important for minimizing the long-term burdens of stroke (Hankey et al., 2002). During positive rehabilitation at an early stage, however, a fluctuation in neurological recovery has been reported to be occasionally encountered in patients with cardiovascular problems (Dromerick and Reding, 1994). In addition, acute recovery from cerebral ischemic attack was not considered a good sign, but, rather, an impending omen of subsequent neurological deterioration (Johnston et al., 2003). The present orthostatic PET measurement could provide one insight into the mechanism by which neurological deterioration on a molecular level develops in the susceptible brain region. However, the small sample size in the CVDC group would require a more extensive study for a number of patients with cardiac problems (symptomatic or asymptomatic), and hence the present result should be considered preliminary at this stage.

In conclusion, the current study demonstrated that upright posture caused local neural deactivation in the hemodynamically vulnerable brain region in patients with CVD. When the cardiac problem is accompanied by a CVD condition, the tissue oxygen demand would be increased with a decrease in perfusion pressure in the hemodynamically susceptible region, but a sufficient degree of net energy production might not be expected during the upright posture. The proper control of hypertension or drinking adequate amounts of water (Schroeder et al., 2002; Shannon et al., 2002) can reduce this insidious menace in CVD patients in general.

#### Acknowledgments

We would like to thank Dr. Teiji Nakayama, Dr. Masanobu Sakamoto, Dr. Kenichi Yano, Mr. Fumitoshi Nakamura, Ms. Tomomi Ogasu, and other staff in the Positron Medical Center for their advice and support on PET measurements. This study was supported by a grant from the Takeda Science Foundation.

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# Microglial Activation and Dopamine Terminal Loss in Early Parkinson's Disease

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Neuroinflammatory glial response may contribute to degenerative processes in Parkinson's disease (PD). To investigate changes in microglial activity associated with changes in the presynaptic dopamine transporter density in the PD brain *in vivo*, we studied 10 early-stage drug-naïve PD patients twice using positron emission tomography with a radiotracer for activated microglia [<sup>11</sup>C](R)-PK11195 and a dopamine transporter marker [<sup>11</sup>C]CFT. Quantitative levels of binding potentials (BPs) of [<sup>11</sup>C](R)-PK11195 and [<sup>11</sup>C]CFT in the nigrostriatal pathway were estimated by compartment analyses. The levels of [<sup>11</sup>C](R)-PK11195 BP in the midbrain contralateral to the clinically affected side were significantly higher in PD than that in 10 age-matched healthy subjects. The midbrain [<sup>11</sup>C](R)-PK11195 BP levels significantly correlated inversely with [<sup>11</sup>C]CFT BP in the putamen and correlated positively with the motor severity assessed by the Unified Parkinson's Disease Rating Scale in PD. In healthy subjects, the [<sup>11</sup>C](R)-PK11195 BP in the thalamus and midbrain showed an age-dependent increase. *In vivo* demonstration of parallel changes in microglial activation and corresponding dopaminergic terminal loss in the affected nigrostriatal pathway in early PD supports that neuroinflammatory responses by intrinsic microglia contribute significantly to the progressive degeneration process of the disease and suggests the importance of early therapeutic intervention with neuroprotective drugs.

Ann Neurol 2005;57:168–175

The histopathological hallmarks of Parkinson's disease (PD) are the progressive degeneration of the nigral dopamine neurons and the ensuing loss of dopamine nerve terminals in the striatum.<sup>1</sup> Previous *ex vivo* and animal studies suggest that the loss of dopamine neurons is linked with an activation of microglia in the substantia nigra.<sup>2–9</sup> Neuropathological studies with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine indicated that microglia might contribute to later and ongoing degeneration of nigral neurons.<sup>10,11</sup> Although these post-mortem and animal studies have strongly suggested the relevance of microglial activation to the nigral cell death seen in PD patients, the antemortem relation between microglial expression and progressive loss of dopamine terminals in PD patients at an early stage remains unclear.

Microglia are involved in immune surveillance in the intact brain and become activated in response to inflammation, trauma, ischemia, tumor, and neurodegeneration.<sup>12</sup> A PD rat model with striatal lesions by 6-hydroxydopamine, secondarily causing retrograde damage to the nigral dopamine neurons, showed an increase in the binding of a specific marker for activated microglia, (1-(2-chlorophenyl)-*N*-methylpropyl)-3 iso-

quinoline carboxamide ([<sup>11</sup>C]PK11195)<sup>13,14</sup> in the striatum and substantia nigra, which indicated an inflammatory response resulting from neural injury and/or neuronal death.<sup>15</sup> Based on use of the [<sup>11</sup>C](R)-PK11195 enantiomer and positron emission tomography (PET), more detailed measurement of microglial activation *in vivo* recently has been reported in neurodegenerative disorders.<sup>16,17</sup> Furthermore, one of the dopamine transporter (DAT) markers, [<sup>11</sup>C]2-B-carbomethoxy-3B-(4-fluorophenyl) tropane ([<sup>11</sup>C]CFT), which allows us to depict the density of the dopaminergic terminals in PD patients *in vivo*, has shown a marked reduction in [<sup>11</sup>C]CFT binding in the striatum in parallel with the severity of parkinsonism.<sup>18–20</sup> These *in vivo* imaging methods are advantageous in monitoring double aspects of progressively degenerated dopamine neurons, namely, alterations in neuroinflammatory reactions on the cell-body side and the resulting deletion of nerve terminals in the striatum.

For this purpose, we quantitatively measured the binding potentials (BPs) of [<sup>11</sup>C](R)-PK11195 and [<sup>11</sup>C]CFT in drug-naïve patients with PD at an early stage on the same day because any delay in the measurement of the two tracers could obscure the accurate

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Received May 17, 2004, and in revised form Aug 13 and Oct 7. Accepted for publication Oct 7, 2004.

Published online Jan 26, 2005, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20338

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relation of ongoing neuroinflammation with neurodegeneration in the living brain.

## Subjects and Methods

### Participants

Ten drug-naive patients with PD rated at stage 1 to 2 on the Hoehn and Yahr scale (PD group, four men, six women; mean age, 59.6 years  $\pm$  9.9 standard deviation [SD]; range, 43–72) and 10 age-matched healthy subjects (N group, four men, six women; mean age, 53.1 years  $\pm$  12.5 SD; range, 39–70) participated in the present study. Patients who had been suffering from limb tremor, rigidity, and bradykinesia with a tentative clinical diagnosis as PD were recruited from our head hospital or the neighboring clinics, and the controls who were healthy physically and neurologically without any medication or complications were recruited by in-house advertisement. All participants underwent magnetic resonance imaging (MRI) and neurological and blood tests to exclude the possibility of any accompanying diseases. Diagnostic L-dopa treatment (L-dopa 100mg/day for 2–5 days) for all PD patients after PET examination markedly ameliorated their parkinsonian symptoms (reduction in frequency of tremor or disappearance of rigidity and bradykinesia). Clinical characteristics of PD patient are shown in Table 1. This study was approved by the ethics committee of the Hamamatsu Medical Center, and written informed consent was obtained from all participants after full explanation of the nature of the study.

### Magnetic Resonance Imaging and Positron Emission Tomography Imaging

First, all participants underwent three-dimensional MRI just before the PET measurement. Here, we used a static magnet (0.3 T MRP7000AD; Hitachi, Tokyo, Japan) with three-dimensional mode sampling to determine the areas of the midbrain and the striatal nuclei required for setting the regions of interest (ROIs). The MRI measures and a mobile PET gantry allowed us to reconstruct PET images parallel to the intercommisural (anterior commissure–posterior commissure) line without reslicing; using this approach, we were

able to allocate ROIs on the target regions of original PET images.<sup>20</sup>

PET scans were conducted with a high-resolution brain-purpose SHR12000 (Hamamatsu Photonics K.K., Hamamatsu, Japan) tomograph (intrinsic resolution,  $2.9 \times 2.9 \times 3.4$  full-width half-maximum [FWHM], 47 slices, 163mm axial field of view).<sup>21</sup> After the head fixation by a thermoplastic facemask and a 10-minute transmission scan for attenuation correction, serial scans (time frames:  $4 \times 30$  seconds,  $20 \times 60$  seconds,  $14 \times 300$  seconds) and periodical arterial blood sampling were performed for 92 minutes after a slow bolus injection (taking 1 minute) of a 350MBq dose of [<sup>11</sup>C]CFT.<sup>20,22</sup> The [<sup>11</sup>C](R)-PK11195 PET measurement then was performed after a 3-hour interval to allow for the decay of radioactivity. After the head was fixated at the same position as in the [<sup>11</sup>C]CFT study, dynamic PET scans ( $4 \times 30$  seconds,  $20 \times 60$  seconds,  $8 \times 300$  seconds) were performed for 62 minutes without arterial blood sampling after intravenous injection of a 300MBq dose of [<sup>11</sup>C](R)-PK11195.<sup>14</sup> The chronological order of the two PET studies was counterbalanced among the groups.

### Image Data Analysis

Because gliosis is associated with changes in tissue volume,<sup>16</sup> a morphological study was first performed based on the previous volumetric MRI studies for midbrain morphology by measuring the maximum anteroposterior diameter of the midbrain through the substantia nigra and the maximum interpeduncular distance at the level of the substantia nigra and red nucleus on the MRIs.<sup>23</sup> Then, multiple semicircular ROIs (36–120mm<sup>2</sup>) were drawn bilaterally over the midbrain (covering the substantia nigra, ventral tegmental area, and red nucleus), nucleus accumbens, ventromedial striatum (head of the caudate), the inferolateral (ventral putamen) and superodorsal parts (dorsal putamen) of the striatum, the thalamus, and the cerebellum on the MRIs.<sup>24</sup> These ROIs then were transferred onto the corresponding dynamic [<sup>11</sup>C]CFT images and parametric images of [<sup>11</sup>C](R)-PK11195 BP with 6.8mm slice-thickness data generated after adding two consecutive slices using image-processing software (Dr View;

Table 1. Clinical Characteristics of Parkinson's Disease Patients

Patient No.	Age (yr)	Sex	DD (yr)	H&Y	MMSE	UPDRS (me/act/mo)	Medication
1	56	M	1.6	2	29	4/4/10	Naive
2	43	M	0.9	1	29	2/4/5	Naive
3	65	M	0.8	2	29	3/6/9	Hypnotic/naive
4	72	M	1.8	2	29	4/6/11	Hypnotic/naive
5	58	F	2.5	1	30	2/4/6	Naive
6	70	F	1.2	1	27	3/8/15	Naive
7	57	F	1.9	2	27	3/6/20	Naive
8	71	F	0.9	1	29	3/4/8	Hypnotic/naive
9	48	F	0.4	1	28	2/5/9	Naive
10	56	F	1.9	2	26	4/12/11	Naive

Hypnotic medicines were temporarily used and stopped at least 1 week before position omission tomography measurement.

DD = disease duration; H&Y = modified Hoehn & Yahr disability score (1–5); MMSE = Mini-Mental State Examination (maximum = 30); UPDRS = Unified Parkinson's Disease Rating Scale (me = mentation, behavior, and mood; act = activities of daily living; mo = motor examination).

Asahi Kasei, Tokyo, Japan) on a SUN workstation (Hypersparc ss-20; SUN Microsystems, San Diego, CA).<sup>25</sup> The values of bilateral ROIs in the midbrain and cerebellum were averaged for further analysis.

[<sup>11</sup>C](R)-PK11195 BP parametric images were generated according to the basis-function theory based on a simplified reference tissue model.<sup>14,26</sup> In this procedure, a normalized input curve was first created by averaging the ROIs placed over the cerebral cortical regions including the frontal, parietal, and occipital cortices in the healthy group. Then, we used this normalized mean tissue activity curve as the reference input function according to the cluster analysis theory<sup>27</sup> because a desirable reference region free of specific binding is not present in degenerative disorders, including PD. The population reference input curve (Fig 1, open circle) was used as the time activity curve (TAC) for the reference region. When applying this curve to the individual PD subjects, the normalized curve was calibrated by adjusting the cerebellar TAC peak from each PD patient. Instead of using the cluster segregation method,<sup>14</sup> the extracerebral structures were masked by demarcating cerebral regions on MRIs for further ROI analyses of PET images. Because, as described above, participants were scanned in the same position between the two PET measurements, the same ROIs could be placed on both [<sup>11</sup>C]CFT and [<sup>11</sup>C](R)-PK11195 parametric images. This approach allowed us to examine microglial activation in parallel with the degeneration of the presynaptic terminals *in vivo*. The binding potential (a ratio of binding and dissociation rate constants,  $k_3/k_4$ ) for [<sup>11</sup>C]CFT was analyzed based on the three-compartment model, as described elsewhere.<sup>20,22</sup>

### Statistics

For between-group comparisons of [<sup>11</sup>C]CFT and [<sup>11</sup>C](R)-PK11195 binding levels, two-way analysis of variance (ANOVA) first was performed to assess BP levels with respect to one intersubject factor and the intrasubject factor (hemispheric side of the striatum) for evaluating the inter-hemispheric effect due to clinical manifestation of laterality in early PD. Because there was no significant interaction in the two-way ANOVA between the hemispheric side and types of groups ( $p = 0.082$ ), all estimates were separately evaluated by one-way ANOVA in either region with Bonferroni's test for the correction of multiple comparisons. Statistical significance was given as  $p$  value less than 0.05 because the post hoc multiple comparisons were performed in these analyses. We previously have reported declines in [<sup>11</sup>C]CFT binding associated with increases in age and in the severity of motor functions<sup>20</sup>; as such, simple linear regression analysis also was performed between age and [<sup>11</sup>C](R)-PK11195 binding. In addition, Spearman's rank correlation analysis was performed to compare clinical motor scores of the Unified Parkinson's Disease Rating Scale with [<sup>11</sup>C](R)-PK11195 binding levels in each region. The multiple regression analyses between regional [<sup>11</sup>C]CFT binding and [<sup>11</sup>C](R)-PK11195 binding were performed within each group. The level of significance was assumed to be  $p$  value less than 0.05.

### Results

#### The Levels of [<sup>11</sup>C]CFT and [<sup>11</sup>C](R)-PK11195 Binding

The tissue TACs of [<sup>11</sup>C](R)-PK11195 are shown in Figure 1. After administration of [<sup>11</sup>C](R)-PK11195, the radioactivity in the midbrain and striatum of a healthy subject declined with time in the same fashion as the normalized mean TAC (population input curve) (see Fig 1A). In contrast, TAC of the midbrain in a

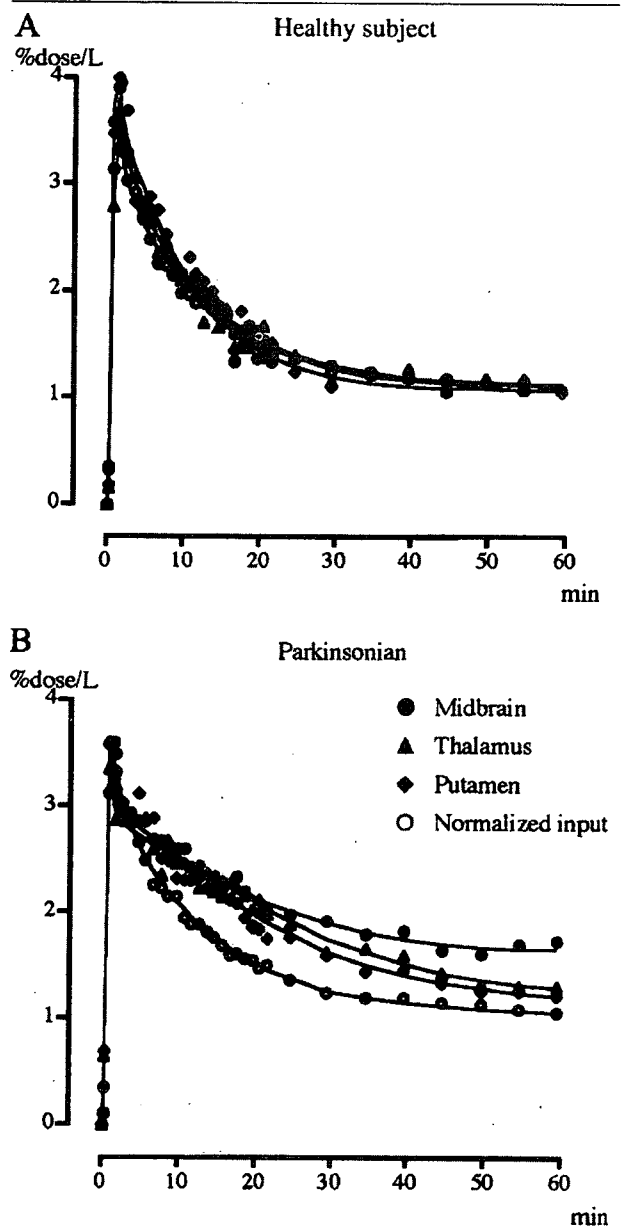


Fig 1. Regional tissue time-activity curves of [<sup>11</sup>C](R)-PK11195 in a healthy subject (A) and a patient with Parkinson's disease (B). The normalized curve was generated as a reference input curve averaged from all the regions of interest values in the cerebral cortical regions without the midbrain, thalamus, and putamen in the healthy group.

Table 2. Levels of Binding Potential ( $k_3/k_4$ ) for [ $^{11}\text{C}$ ]CFT and [ $^{11}\text{C}$ ]PK11195, Mean (SD)

Group	Tracer	DV <sub>CFT</sub> R1 <sub>PK</sub> (Bilateral)	Midbrain Bilateral	$k_3/k_4$									
				Nucleus Accumbens		Caudate		Ventral Putamen		Dorsal Putamen		Thalamus	
				Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
PD	CFT	7.86 (1.01)	ND	2.52 (0.97)	1.93*(0.58)	2.37 (0.86)	2.04*(0.85)	1.34*(0.33)	1.18*(0.27)	1.21*(0.35)	0.83*(0.24)	ND	ND
	PK	0.79 (0.06)	0.35*(0.06)	<0.01	<0.01	0.08 (0.05)	0.01 (0.01)	0.04 (0.03)	0.03 (0.02)	<0.01	<0.01	0.12 (0.06)	0.13 (0.07)
N	CFT	7.95 (0.78)	—	2.95 (0.47)		3.92 (1.13)		4.44 (1.24)		3.64 (0.82)		ND	
	PK	0.74 (0.29)	0.11 (0.06)	0.06 (0.05)		0.04 (0.04)		0.07 (0.05)		0.06 (0.04)		0.10 (0.04)	

DV<sub>CFT</sub> = distribution volume for the cerebellum in the [ $^{11}\text{C}$ ]CFT measurement; R1<sub>PK</sub> = delivery index for the midbrain in the [ $^{11}\text{C}$ ]PK11195 measurement; ND denotes not determined because of the negligible accumulation of [ $^{11}\text{C}$ ]CFT.

\* $p < 0.05$  vs normal group.

PD patient declined less sharply, indicating a time-course accumulation of [ $^{11}\text{C}$ ](R)-PK11195 in the midbrain (see Fig 1B, closed circle). The statistics showed that midbrain values were significantly different from those of the thalamus and putamen after 40 minutes.

One-way ANOVA showed that the levels of  $k_3/k_4$  for [ $^{11}\text{C}$ ]CFT were significantly lower in all parts of the striatum ( $p < 0.01$ ) in the PD group than those in the healthy group. The magnitude of  $k_3/k_4$  in the PD group was lower in the dorsal putamen contralateral to the clinically more affected side (Table 2). No differences in distribution volume (DV) in the cerebellum of [ $^{11}\text{C}$ ]CFT was found between the two groups. In the [ $^{11}\text{C}$ ](R)-PK11195 study, the BP of the tracer was greater in the midbrain of the PD group than in the healthy counterparts (see Table 2), whereas there was no difference in the ratio of delivery ( $R_1$ ) of the tracer between the target (midbrain) and reference tissue between the groups. The thalamus or striatum in PD failed to show any significant difference in binding. Morphometric measurement showed no significant differences in midbrain size (anteroposterior and transverse distances) between groups (Table 3), indicating no significant partial volume effect in further tracer analyses.

#### Correlations of [ $^{11}\text{C}$ ](R)-PK11195 Binding with Age and Motor Severity in Parkinson's Disease Patients

Levels of [ $^{11}\text{C}$ ](R)-PK11195 binding in the midbrain of the healthy group significantly correlated positively with age ( $r = 0.68$ ;  $p < 0.05$ ,  $f[x] = 0.0035*x - 0.073$ ), whereas no such correlation was observed in the PD group (Fig 2A). This midbrain [ $^{11}\text{C}$ ](R)-PK11195 binding in the PD group significantly correlated positively with the motor severity of the UPRDS assessment ( $r = 0.74$ ;  $p < 0.05$ ,  $f[x] = 0.012*x + 0.19$ ; see Fig 2B) but failed to show a significant correlation against the disease duration ( $r = 0.36$ ,  $p > 0.1$ ; see Fig 2C). The examined brain regions other than the midbrain showed no significant correlation between [ $^{11}\text{C}$ ](R)-PK11195 binding and motor scores in either group (not shown).

#### Comparison between Binding Levels of Striatal [ $^{11}\text{C}$ ]CFT and Midbrain [ $^{11}\text{C}$ ](R)-PK11195 in Parkinson's Disease Patients

Regression analyses showed that estimates for [ $^{11}\text{C}$ ](R)-PK11195 binding in the midbrain significantly correlated negatively with those for [ $^{11}\text{C}$ ]CFT binding in the dorsal putamen contralateral to the affected side ( $r = 0.894$ ;  $p < 0.01$ ,  $f[x] = -4.18*x + 2.30$ ) and its ipsilateral counterpart ( $r = 0.72$ ,  $p < 0.05$ ,  $f(x) = -3.94*x + 2.59$ ; Fig 3C). There was a tendency toward a negative correlation in the caudate ( $p > 0.05$ ; see Fig 3A).

#### Discussion

These results showed to our knowledge for the first time with PET markers a higher accumulation of [ $^{11}\text{C}$ ](R)-PK11195 in the midbrain in drug-naive early PD patients than in age-matched healthy subjects in vivo. In addition, the levels of midbrain [ $^{11}\text{C}$ ](R)-PK11195 binding correlated negatively with the levels of [ $^{11}\text{C}$ ]CFT binding in the putamen in the PD group. These results suggest that microglial activation was more significant in patients with more severe damage in the nigrostriatal pathway. This contention was supported by the results from the PD rat model showing the chronological decrease in [ $^{11}\text{C}$ ]CFT accumulation in the lesioned striatum along with a parallel increase in [ $^{11}\text{C}$ ](R)-PK11195 in the substantia nigra,<sup>15</sup> and from the postmortem analysis of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-intoxicated human sub-

Table 3. Magnetic Resonance Imaging-based Midbrain Linear Measurements (cm), Mean (SD)<sup>a</sup>

Group	Anteroposterior Diameter	Interpeduncular Distance
Parkinson's disease	26.5 (1.9)	36.8 (4.1)
Normal	27.6 (2.7)	37.4 (1.7)

There were no significant differences in measures between groups ( $p > 0.05$ , one-way analysis of variance).

<sup>a</sup>Results are maximum values of each distance.

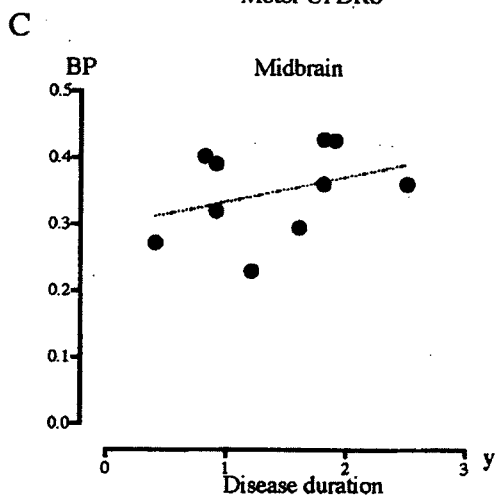
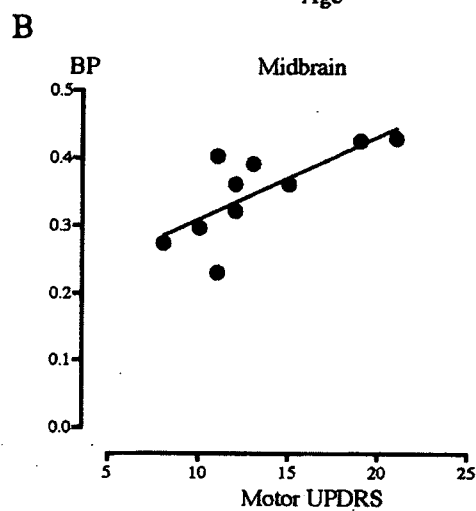
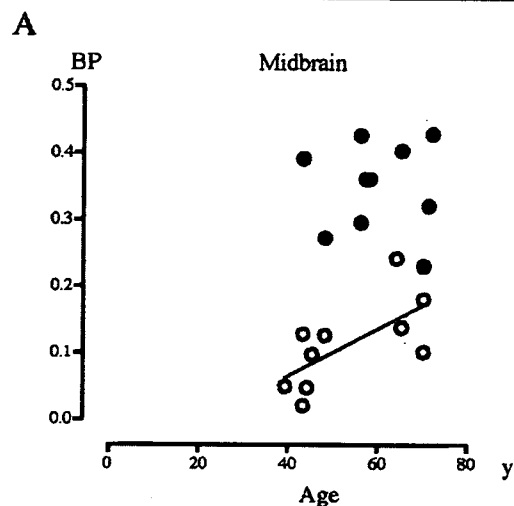


Fig 2. Correlations between levels of [ $^{11}$ C](R)-PK11195 binding in the midbrain and age (A) in the healthy group (open circles) and Parkinson's disease group (filled circles), and motor scores of Unified Parkinson's Disease Rating Scale (UPDRS) (B), and disease duration (year) (C) in the Parkinson's disease group. BP = binding potential.

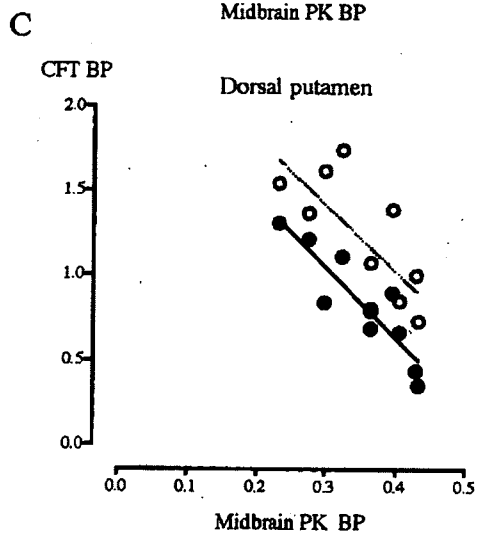
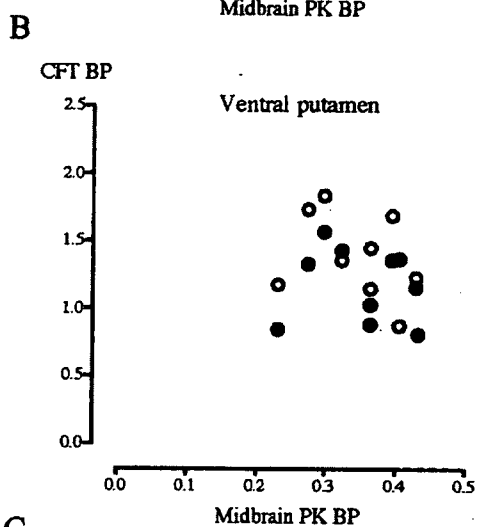
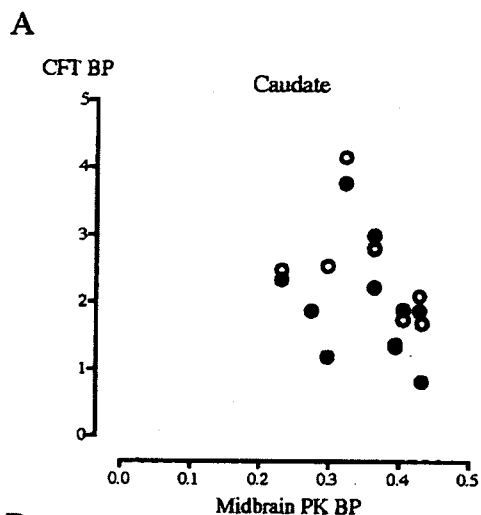


Fig 3. Correlations between levels of [ $^{11}$ C](R)-PK11195 binding potential (BP) in the midbrain and those of [ $^{11}$ C]CFT BP in the caudate (A), ventral putamen (B), and dorsal putamen (C) ipsilateral (open circles) and contralateral (filled circles) to the clinically more affected side in the Parkinson's disease group.

jects showing both elevated glial reaction in the substantia nigra and the dopaminergic neuronal loss.<sup>10</sup> Thus, our in vivo observation is compatible with the contention that microglial activation plays a key role in the initiation and progression of PD.

With respect to the role of microglia, there are lines of evidence that the majority of factors produced by activated microglia such as the cytokines tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and free radicals are proinflammatory or neurotoxic,<sup>28-30</sup> which suggests that activation of microglia would trigger the onset of a cascade of events resulting in a progressive degeneration of dopamine neurons. Although the age-related increase in [<sup>11</sup>C](R)-PK11195 binding seen in healthy subjects in this study was not present in the PD group (see Fig 2A), it is likely that the degree of microglial activation associated with the disease is so great that it simply dwarfs the effect of aging. An age-related change in [<sup>11</sup>C](R)-PK11195 binding in the thalamus in healthy subjects (data not shown) was also observed, as reported elsewhere.<sup>16,31</sup> These age-associated increases in microglial expression in the thalamus and midbrain may be related to the steady decline in higher cognitive and motor functions in normal aging that would jeopardize functional integration of the basal ganglia-cerebral cortical network. A lack of correlation

of microglial activation with the disease duration (see Fig 2C) in this study suggested that the microglial reactive process might not be in parallel to the disease deterioration. To further illuminate this issue, this type of in vivo imaging on a chronological basis would be necessary.

There are a few methodological considerations to be noted in this study. First, PET has a critical limitation regarding image resolution for tiny structures such as the substantia nigra in the midbrain. A higher image resolution would enable us to elucidate the relation between microglial activation and dopaminergic neuronal loss in both nigrostriatal and mesolimbic (via the nucleus accumbens) systems by placing ROIs in those subregions of the midbrain. However, as shown in Figure 4, a greater degree of [<sup>11</sup>C](R)-PK11195 binding tended to be observed in the midbrain contralateral to the clinically more affected side. Second, our tracer kinetic modeling was based on the reference tissue model,<sup>26</sup> and determination of normal reference tissue input was derived from the averaged value from the cerebral cortical regions in the 12 healthy subjects. Slightly lower levels of [<sup>11</sup>C](R)-PK11195 binding in each of the brain regions examined in our study than the reported values<sup>14,16</sup> might be ascribed to the different methodological approach. However, the ratios of

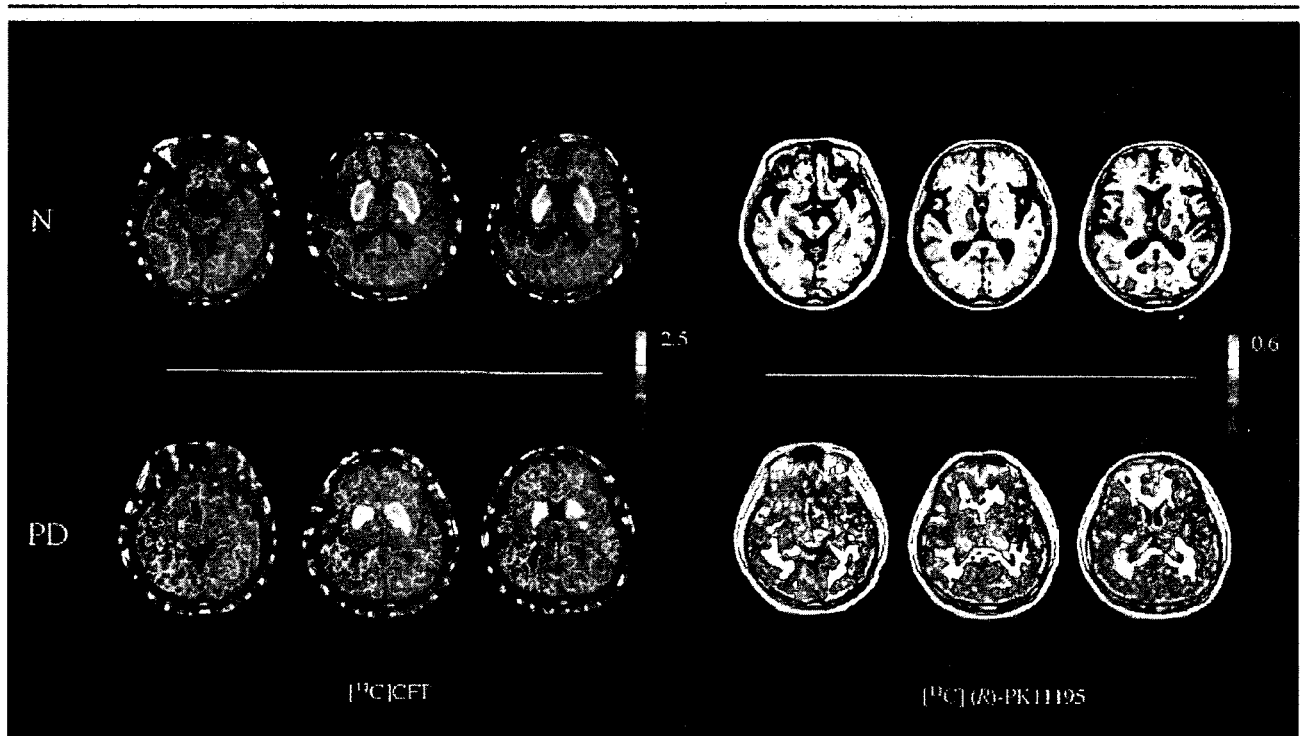


Fig 4. Magnetic resonance imaging positron emission tomography fusion parametric images of [<sup>11</sup>C]CFT accumulation normalized to the cerebellum and [<sup>11</sup>C](R)-PK11195 binding potential in a healthy subject (N) and a Parkinson's disease patient (PD) rated as Hoehn & Yahr stage 2. A marked increase in [<sup>11</sup>C](R)-PK11195 binding was observed in the midbrain contralateral to the more disabled side along with a reduction in [<sup>11</sup>C]CFT uptake in the putamen in the PD patient. For each tracer, red represents the highest value and dark violet represents the lowest value.



disease levels to control levels were fairly similar to those previously reported.

It has been reported that in PD, neuronal loss starts in the lateral ventral tier of the substantia nigra that projects to the dorsal putamen, and hence dopamine in the dorsal zone was more depleted than in the ventral zone in early PD.<sup>1</sup> This topological evidence supports our finding of a correlation between the increased [<sup>11</sup>C](R)-PK11195 accumulation in the midbrain and the decreased accumulation of [<sup>11</sup>C]CFT in the dorsal putamen (see Fig 3C). A more robust negative correlation was found clinically in the dorsal putamen contralateral to the more affected side. Interestingly, this nigral [<sup>11</sup>C](R)-PK11195 accumulation was significantly correlated positively with motor severity in the present PD group (see Fig 2B). In contrast with the tegmental area or the medial tier of the substantia nigra projecting to the limbocortical region or the caudate (linking with mental and cognitive activities), because this dorsal putamen is considered to engage in motor execution,<sup>32,33</sup> this finding would reinforce the idea that the occurrence of microglial activation in the midbrain of PD at an early stage reflects dopamine loss sufficient to cause clinical parkinsonism.

From a therapeutic perspective, it was reported that antiinflammatory steroids,<sup>34</sup> nonsteroidal cyclooxygenase inhibitors,<sup>35</sup> and opioid receptor antagonist naloxone<sup>36</sup> might be promising as antiinflammatory agents for degenerative diseases such as PD. One epidemiological study agreed with the idea that use of nonsteroidal antiinflammatory drugs can delay the onset of PD.<sup>37</sup> In contrast, other clinical studies showed lesser effects of antiinflammatory drugs on a deceleration of the prevalence of PD than that in Alzheimer's disease<sup>38,39</sup> and therapeutic inconsistencies was suspected between laboratory data and clinical outcomes.<sup>40</sup> This result showing microglial activation along with dopaminergic terminal loss in vivo in early PD might afford a scope of discussion for use of the antiinflammatory and neuroprotective agents.

In conclusion, this study supports the contention that microglial activation develops in the midbrain of PD patients at an early stage, and that this appearance may be associated with likely ensuing apoptotic events. The latter issue must be clarified by a chronological study, but the present, one-time, double-tracer study for targeting both neuroinflammation and neuronal loss in the nigrostriatal system provides essential information about the degeneration process in PD.

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We thank all patients and healthy subjects who participated in this study, Dr K. Tanaka and Dr M. Sakamoto for patients' selection, H. Okada for his constant technical support, Dr Y. Magata for his valuable advice on radiocompound syntheses, and our staff for support of the present PET study.

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Research report

## Neural substrates in judgment process while playing go: a comparison of amateurs with professionals

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Accepted 15 October 2004

Available online 10 November 2004

### Abstract

A professional go player shows incomparable ability in judgment during go game. Positron emission tomography (PET) was used to investigate the neural substrates of professional go player's judgment process. Eight professional go players and six amateur players were instructed to think over silently in the opening-stage game (*fuseki*, territorial planning) problems and the life-or-death (*tsume*, checkmate judgment) problems presented on the monitor in front of them for 60 s of  $H_2^{15}O$  PET scans and to state the answer afterwards. We found that in the territorial planning problems the parietal activation was equally observed in both groups with the additional prefrontal activation in the amateur group, and in the checkmate-decision problems the precuneus and cerebellum were activated in professionals while the premotor and parietooccipital cortices (visuospatial processing region) were extensively activated in amateurs. The comparison of the two groups showed stronger activations in the precuneus and cerebellum in the professionals in contrast to the premotor activation in amateurs during checkmate judgment. In addition, the cerebellum was remarkably activated in the higher ranking professional players. These findings suggested the cerebellum and precuneus play important roles in processing of accurate judgment by visual imagery and nonmotor learning memory processes in professional go players.

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*Theme:* Neural basis of behavior

*Topic:* Cognition

*Keywords:* Go; Professional; Judgment; Precuneus; Cerebellum; Positron emission tomography

### 1. Introduction

There are several neuroimaging studies concerning the neural substrates of cognitive processes involved in professional thinking [13,20,25]. In these studies, the contribution of the prefrontal and parietal cortices was reported to be of great importance. Specifically, the precuneus was remarkably activated during the execution of precise topographical memory recall for professional taxi drivers [20] and in visuospatial processing for abacus

experts [13]. In addition to these cerebral activations in cognitive processes, recent findings on the cerebellum in nonmotor cognitive processes are worth noticing because the cerebellum is activated in many other cognitive tasks such as memory, error detection, attention, sensory discrimination and timing [1,2,10,15,18]. Furthermore, since the cerebellum also functions under the condition of the theory of mind [5], it is easy to speculate that an expert would exploit this "cognitive organ" more efficiently in cognitive tasks. However, this issue remains to be investigated.

The game go involves similar thought processes as the Western-style board game of chess. A previous activation study during a chess game revealed neural substrates of

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problem-solving processes in the brain, except for the cerebellum, in nonprofessional players [21]. Playing go is different in nature from the chess game. Chessmen have different roles in chess, but go stones all have the same value. Thus, brain activity highly depends on the contemplation of the position of the stones, not on the selection of chessman. This may result because the perception of the objects determines the actions that can be made toward them [12]. The go game, which does not involve selection of objects, may provide simpler but likely more profound cognitive processing in terms of mental imagination [7]. Obtaining a professional title depends exclusively on the period of experience, knowledge, and ability and the ability to perform mental operations that amateurs cannot attain [7]. Visuospatial processing may be greater in go experts than in novices [14]. Thus, the professional rank may be used as an index of player's ability and utilized as a covariate for examining the neural correlates of the professional go player's judgment process. One activation study of the go game was recently reported that used fMRI [6], but the study design was different from ours in subjects (amateur vs. professional and amateur) and tasks (determining next move vs. judging in different situations the correct or incorrect responses).

The purpose of the current study was to investigate neural substrates of professional strategic thinking by comparing brain activations of certificated professional go players recruited from the Japan Go Association with the neural substrates of amateurs during deliberate life-or-death (checkmate judgment) and territorial planning situations of the game using positron emission tomography (PET) with  $H_2^{15}O$ .

## 2. Materials and methods

### 2.1. Subjects

Eight male right-handed professional go players (mean age  $\pm$  S.D.  $41.1 \pm 11.3$  years) from Japan Go Association and six amateur go players (four male and two female,  $59.5 \pm 5.4$  years) gave their informed consent to participate in the present experiment, which was approved by a local ethics committee at Hamamatsu Medical Center. Professionals had experienced for more than 15 years and practiced go almost everyday for various competitions; in contrast, amateurs had experienced for more than 10 years but practiced go on a recreational basis. Each participant underwent magnetic resonance imaging (MRI) using a 0.3 T static magnet system (MRP7000AD, Hitachi, Tokyo, Japan) before PET measurement, showing no morphological abnormality. All professional players were classified by the certificated professional rank ("dan" or grade ranging from first to ninth grade), consisting of one professional player with 1-dan, one with 3-dan, one with 4-dan, one with 7-dan, one with 8-dan, and three with 9-dan.

### 2.2. Tasks

In the real situation of a go game, two players, sitting face to face and holding black or white stones, take turns placing stones onto one of many vacant line intersections on the board. The goal is to occupy as large an area as possible with given stones. In the present study, three situations were given to all participants. In the first task, each subject performed one baseline task; (Base) staring at the center of the go board without any thought. Then each subject performed two nonmotor cognitive tasks; (Task 1) thinking about moving stones to expand their territory in the given situation on the screen (territorial planning), (Task 2) determining the final move in a life-or-death situation in order to occupy the territory in competition (checkmate judgment) (Fig. 1A). In the two nonmotor tasks, no motor performance was allowed during the scans. In Task 1, placing stones during this stage of the go game was unlimited and there was no correct place to put the stones.

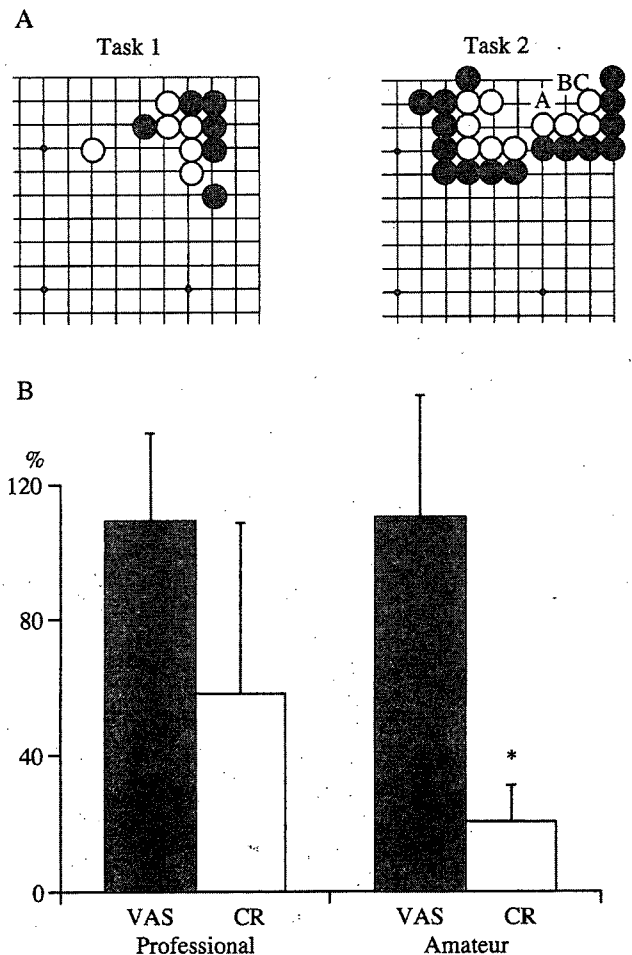


Fig. 1. Go task and task performance. (A) Examples of stones arrangement for Task 1 (territorial planning) and Task 2 (checkmate judgment), (B) visual analog scales (VAS) for task difficulty (black bar) and rates of correct answer (CR, white bar) in professional and amateur groups, which was the result of Task 2. The asterisk indicates the presence of significant difference ( $p < 0.05$ ,  $\chi^2$  test).