Behavioral/Systems/Cognitive

Differential Contributions of Prefrontal and Hippocampal Dopamine D_1 and D_2 Receptors in Human Cognitive Functions

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Dopamine D_1 receptors in the prefrontal cortex (PFC) are important for prefrontal functions, and it is suggested that stimulation of prefrontal D_1 receptors induces an inverted U-shaped response, such that too little or too much D_1 receptor stimulation impairs prefrontal functions. Less is known of the role of D_2 receptors in cognition, but previous studies showed that D_2 receptors in the hippocampus (HPC) might play some roles via HPC–PFC interactions. We measured both D_1 and D_2 receptors in PFC and HPC using positron emission tomography in healthy subjects, with the aim of elucidating how regional D_1 and D_2 receptors are differentially involved in frontal lobe functions and memory. We found an inverted U-shaped relation between prefrontal D_1 receptor binding and Wisconsin Card Sorting Test performance. However, prefrontal D_2 binding has no relation with any neuropsychological measures. Hippocampal D_2 receptor binding showed positive linear correlations not only with memory function but also with frontal lobe functions, but hippocampal D_1 receptor binding had no association with any memory and prefrontal functions. Hippocampal D_2 receptors seem to contribute to local hippocampal functions (long-term memory) and to modulation of brain functions outside HPC ("frontal lobe functions"), which are mainly subserved by PFC, via the HPC–PFC pathway. Our findings suggest that orchestration of prefrontal D_1 receptors and hippocampal D_2 receptors might be necessary for human executive function including working memory.

Key words: **dopamine; D_1 receptors; D_2 receptors; prefrontal cortex; hippocampus; positron emission tomography**

Introduction

Because dopamine D_1 receptors in the prefrontal cortex (PFC) are several times more abundant than D_2 receptors (Hall et al., 1994), the relationship between D_1 receptors and PFC functions have been widely investigated. Sawaguchi and Goldman-Rakic (1994) demonstrated that local administration of D_1 receptor antagonists into PFC induced impairment in working memory task in nonhuman primate. In human, Müller et al. (1998) reported that systemic administration of a mixed D_1/D_2 agonist facilitated working memory, whereas the selective D_2 agonist had no effect, indicating that the dopaminergic modulation of working memory processes is mediated primarily via D_1 receptors. The use of positron emission tomography (PET) allows us to quantify dopamine receptors *in vivo*, and previous studies reported that altered prefrontal D_1 receptors in schizophrenia were associated with working memory deficits (Okubo et al., 1997; Abi-Dargham et al., 2002).

In contrast to D_1 receptors, relatively less attention has been paid to the role of prefrontal D_2 receptors in cognitive functions. It was reported that blockade of D_2 receptors in PFC did not impair working memory in nonhuman primate (Sawaguchi and Goldman-Rakic, 1994), but some human studies reported that systemic administration of D_2 agonist or antagonist modulated cognitive functions that are subserved by the prefrontal cortex (McDowell et al., 1998; Mehta et al., 1999). Because the density of D_2 receptors in extrastriatal regions is very low (Suhara et al., 1999), PET studies investigating the involvement of extrastriatal D_2 receptors in cognition have been limited. With the introduction of high-affinity PET radioligands such as [^{11}C]FLB457, it has become possible to quantify extrastriatal D_2 receptors by PET (Halldin et al., 1995). Using [^{11}C]FLB457, Kemppainen et al. (2003) reported that a reduction of D_2 receptors in the hippocampus (HPC) in Alzheimer's disease patients was correlated with memory impairments. Our recent PET study also showed that D_2 receptors in HPC were associated not only with memory function but also with frontal lobe functions (Takahashi et al., 2007), suggesting dopaminergic modulation on HPC–PFC inter-

Received July 23, 2008; revised Sept. 2, 2008; accepted Oct. 2, 2008.

This work was supported by a consignment expense for Molecular Imaging Program on "Research Base for PET Diagnosis" from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), the Japanese Government, and a Grant-in-Aid for Scientific Research from MEXT (18790858). We thank Katsuyuki Tanimoto, Takahiro Shiraishi, and Toshio Miyamoto for their assistance in performing the PET experiments at the National Institute of Radiological Sciences. We also thank Yoshiko Fukushima of the National Institute of Radiological Sciences for her help as clinical research coordinator.

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DOI:10.1523/JNEUROSCI.3446-08.2008

 0270-6474/08/2812032-07$15.00/0

actions during the cognitive process (Laroche et al., 2000; Thierry et al., 2000; Goto and Grace, 2008).

In this study, we measured both D_1 and D_2 receptors in PFC and HPC using PET in normal healthy subjects, and aimed to elucidate how regional D_1 and D_2 receptors are differentially involved in neurocognitive performance including memory and frontal lobe functions. A body of animal studies has indicated that stimulation of D_1 receptors in PFC produces an inverted U-shaped dose–response curve, such that too little or too much D_1 receptor stimulation impairs PFC functions (Goldman-Rakic et al., 2000; Williams and Castner, 2006; Vijayraghavan et al., 2007). We hypothesized that prefrontal D_1 receptors would be more related to frontal lobe functions than prefrontal D_2 receptors, and that, specifically, an inverted U-shaped relation between prefrontal D_1 receptor binding and prefrontal functions would be observed in the normal physiological condition in healthy volunteers. In addition, we predicted that D_2 receptors in HPC would be more related to memory than D_1 receptors in HPC.

Materials and Methods

Subjects. Twenty-three healthy male volunteers [mean age 25.7 ± (SD) 4.3 years] were studied. Seven of the 23 subjects had participated in our earlier study (Takahashi et al., 2007). They did not meet the criteria for any psychiatric disorder based on unstructured psychiatric screening interviews. None of the controls were using alcohol at the time, nor did they have a history of psychiatric disorder, significant physical illness, head injury, neurological disorder, or alcohol or drug dependence. All subjects were right-handed according to the Edinburgh Handedness Inventory. All subjects underwent magnetic resonance imaging (MRI) to rule out cerebral anatomic abnormalities. After complete explanation of the study, written informed consent was obtained from all subjects, and the study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba Japan.

PET scanning. PET studies were performed on ECAT EXACT HR+ (CTI; Siemens). The system provides 63 planes and a 15.5 cm field of view. To minimize head movement, a head fixation device (Fixster) was used. A transmission scan for attenuation correction was performed using a germanium 68–gallium 68 source. Acquisitions were done in three-dimensional mode with the interplane septa retracted. For evaluation of D_1 receptors, a bolus of 213.9 ± 20.5 MBq of [^{11}C]SCH23390 with specific radioactivities (52.1 ± 28.9 GBq/μmol) was injected intravenously from the antecubital vein with a 20 ml saline flush. For evaluation of extrastriatal D_2 receptors, a bolus of 215.4 ± 24.5 MBq of [^{11}C]FLB457 with high specific radioactivities (171.0 ± 58.0 GBq/μmol) was injected in the same way. The mean injected amounts of [^{11}C]SCH23390 and [^{11}C]FLB457 were 1.18 ± 0.20 μg and 0.47 ± 0.17 μg, respectively. Dynamic scans were performed for 60 min for [^{11}C]SCH23390 and 90 min for [^{11}C]FLB 457 immediately after the injection. All emission scans were reconstructed with a Hanning filter cutoff frequency of 0.4 (full width at half maximum, 7.5 mm). MRI was performed on Gyroscan NT (Philips Medical Systems) (1.5 T). T1-weighted images of the brain were obtained for all subjects. The scan parameters were 1-mm-thick, three-dimensional T1 images with a transverse plane (repetition time/echo time, 19/10 milliseconds; flip angle, 30°; scan matrix, 256 × 256 pixels; field of view, 256 × 256 mm; number of excitations, 1).

Quantification of D_1 and D_2 receptors in PFC and HPC. The tissue concentrations of the radioactivities of [^{11}C]SCH23390 and [^{11}C]FLB457 were obtained from regions of interest (ROIs) defined on the PET images of summated activity for 60 and 90 min, respectively, with reference to the individual MRIs that were coregistered on summated PET images and the brain atlas. The regions were PFC, HPC and cerebellar cortex. Each ROI consisted of three axial slices. ROI of PFC occupies the middle third of the middle frontal gyrus and the rostral portion of the inferior frontal gyrus (approximately corresponding to the dorsolateral prefrontal cortex or Brodmann area 46). ROI of HPC was set at the level of the midbrain. The anterior boundary was identified at the level of the inferior horn of the lateral ventricle. The posterior boundary was identified at the level of the collateral sulcus. Although [^{11}C]FLB457 accumulates to a high degree in the striatum, striatal data were not evaluated because the duration of the [^{11}C]FLB457 PET study was not sufficient to obtain equilibrium in the striatum (Olsson et al., 1999; Suhara et al., 1999). Quantitative analysis was performed using the three-parameter simplified reference tissue model (Lammertsma and Hume, 1996). The cerebellum was used as reference region because it has been shown to be almost devoid of D_1 and D_2 receptors (Farde et al., 1987; Olsson et al., 1999; Suhara et al., 1999). The model provides an estimation of the binding potential ($BP_{ND\ (nondisplaceable)}$) (Innis et al., 2007), which is defined by the following equation: $BP_{ND} = k3/k4 = f2\ Bmax/\{Kd\ [1 + \sum i\ Fi/Kdi]\}$, where k3 and k4 describe the bidirectional exchange of tracer between the free compartment and the compartment representing specific binding, f2 is the "free fraction" of nonspecifically bound radioligand in brain, Bmax is the receptor density, Kd is the equilibrium dissociation constant for the radioligand, and Fi and Kdi are the free concentration and the dissociation constant of competing ligands, respectively (Lammertsma and Hume, 1996).

Neuropsychological tests. A battery of cognitive tests was given by an experienced clinical neuropsychologist. The neuropsychological tests used were Rey's Auditory Verbal Learning Test (RAVLT), Rey-Osterrieth's Complex Figure Test (ROCFT), Keio version of the Wisconsin Card Sorting Test (WCST) (Igarashi et al., 2002), Verbal Fluency Test, and Raven's Colored Progressive Matrices (RCPM). RAVLT is used to evaluate the performance of verbal memory, and ROCFT is used as a measure of nonverbal visual memory. RAVLT and ROCFT were performed in the standard manner (Lezak, 1995). In RAVLT, 15 words were presented auditorily in the same sequence in five trials, ending with a free recall of the words (immediate recall). After the five trials, an interference list was presented and recalled, and then the subjects were instructed to recall the first list of words (delayed recall). In ROCFT, after the copy trial, subjects were asked to reproduce a figure from memory (immediate recall). After a 15 min pause, the subjects were asked to reproduce the figure from memory again (delayed recall). WCST is a test for executive function or cognitive flexibility involving working memory (Berman et al., 1995). It has been shown to be sensitive to dysfunction of PFC (Nelson, 1976). In WCST, categories achieved (CA), total errors (TE) and perseverative errors of Nelson (PE) were evaluated (Lezak, 1995). In the phonemic verbal fluency test, the subject was requested to retrieve in 1 min as many words as possible beginning with the Japanese syllabic characters (hiragana) "shi," "i" and "re," respectively. In the semantic verbal fluency test, the subject was requested to recall in 1 min as many words as possible belonging to a given semantic category (e.g., animals, fruit) (Lezak, 1995). RCPM was used as a general visuospatial intelligence test.

Statistical analyses. Although the selection of subjects was confined to young males in their 20's and 30's, the possible age effect on the BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457, and neuropsychological performance were examined using Pearson correlation analysis. To explore the relation between D_1 and D_2 receptors and cognitive functions, linear regression between the BP_{ND} values of each ROI and each neuropsychological performance was analyzed, and the threshold for significance was set at $p = 0.05/2 = 0.025$ to correct for two regions (PFC and HPC). Although a single dominant factor underlying the scores on all tests, i.e., general cognitive ability, might contribute to intercorrelations across the tests, what we measure with neuropsychological tests is, by nature, a dimensionality of cognitive ability. Therefore, correction of p values for multiple comparisons was done only for regions, not for multiple neuropsychological tests. To examine putative nonlinear (inverted U-shaped) relations between prefrontal dopamine receptors and frontal lobe functions, quadratic regression between the BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457 in PFC and neuropsychological performance was analyzed by SPSS package (SPSS).

To confirm the findings of the ROI analysis, parametric images of BP_{ND} (Gunn et al., 1997) were analyzed using statistical parametric mapping software (SPM2) (Wellcome Department of Imaging, Institute of Neurology, University College of London, London, UK). Normalized BP_{ND} images were smoothed with a Gaussian filter to 16 mm full-width

Table 1. Mean scores of neuropsychological tests and linear relations between and neuropsychological measures and BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457 in the prefrontal cortex and hippocampus

Neuropsychological tests	Mean scores	Prefrontal cortex r (p)		Hippocampus r (p)	
		[^{11}C]SCH23390	[^{11}C]FLB457	[^{11}C]SCH23390	[^{11}C]FLB457
RALVT immediate	57.3 ± 6.2	0.07 (0.74)	0.16 (0.47)	0.10 (0.66)	0.37 (0.09)
RALVT delayed	13.0 ± 1.5	0.14 (0.53)	0.02 (0.94)	0.08 (0.72)	0.28 (0.20)
ROCFT immediate	27.7 ± 3.9	0.11 (0.63)	0.31 (0.15)	0.21 (0.34)	0.73 (p<0.001)**
ROCFT delayed	27.3 ± 4.8	0.12 (0.58)	0.38 (0.07)	0.11 (0.60)	0.67 (p<0.001)**
WCST CA	5.4 ± 1.2	0.42 (0.049)*	0.03 (0.89)	0.21 (0.33)	0.30 (0.17)
WCST TE	11.3 ± 3.7	−0.41 (0.049)*	−0.15 (0.51)	−0.30 (0.16)	−0.51 (0.01)**
WCST PE	0.8 ± 1.4	−0.27 (0.21)	−0.18 (0.42)	−0.31 (0.15)	−0.59 (0.003)**
Phonemic verbal fluency	30.9 ± 9.3	0.21 (0.35)	0.21 (0.34)	0.20 (0.36)	0.47 (0.02)**
Semantic verbal fluency	46.1 ± 7.9	−0.07 (0.76)	0.09 (0.69)	0.06 (0.77)	0.17 (0.45)
RCPM (sec)	188.5 ± 36.0	0.10 (0.65)	−0.04 (0.87)	0.11 (0.64)	0.08 (0.70)

*$p < 0.05$. **Significant after correction for multiple statistical tests (new significance threshold: $p < 0.025$[0.05/2]).

half-maximum. Using each individual cognitive performance as covariate, regression analyses with the BP_{ND} images and the covariates were performed.

Results

The mean [^{11}C]SCH23390 BP_{ND} values of PFC and HPC were 0.41 ± 0.06 (range: 0.29–0.59) and 0.33 ± 0.09 (range: 0.20–0.53), respectively. The mean [^{11}C]FLB457 BP_{ND} values of PFC and HPC were 1.16 ± 0.21 (range: 0.82–1.58) and 1.57 ± 0.28 (range: 0.98–1.92), respectively. The mean scores of the neuropsychological data are shown in Table 1. There was no age effect on the BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457 in the two ROIs, nor on any neuropsychological performance ($p > 0.01$).

Quadratic regression analysis revealed a significant "U-shaped" relation between the BP_{ND} value of [^{11}C]SCH23390 in PFC and TE of WCST ($p < 0.001$, $r = 0.72$). (Because TE of WCST is a negative measure of frontal lobe function, the relation is not "inverted") (Fig. 1). The BP_{ND} value of [^{11}C]SCH23390 in PFC and CA of WCST also showed significant quadratic (inverted U-shaped) relation ($p < 0.001$, $r = 0.78$). However, no quadratic relation was found between the BP_{ND} value of [^{11}C]FLB 457 in PFC and any neuropsychological measures. The linear relations between neuropsychological measures and the BP_{ND} value of each ROI are shown in Table 1. As for D_1 receptors, the BP_{ND} value of [^{11}C]SCH23390 in PFC was positively correlated with CA of WCST ($p = 0.049$, $r = 0.42$), and negatively correlated with TE of WCST ($p = 0.049$, $r = -0.41$) although these relations did not survive a threshold corrected for multiple comparisons. The BP_{ND} value of [^{11}C]SCH23390 in HPC was not correlated with any neuropsychological measures. With regard to D_2 receptors, the BP_{ND} value of [^{11}C]FLB457 in HPC was positively correlated with immediate and delayed recall scores of ROCFT and phonemic verbal fluency, and negatively correlated with CA and TE of WCST. The BP_{ND} value of [^{11}C]FLB457 in PFC was not correlated with any neuropsychological measures. Figure 2 shows these relationships.

D_1 binding in PFC showed significant correlation with D_1 binding in HPC ($r = 0.74$, $p < 0.001$) and trend level correlation with D_2 binding in PFC ($r = 0.41$, $p = 0.05$), but no correlation with D_2 binding in HPC ($r = 0.27$, $p = 0.22$). D_2 binding in HPC showed significant correlation with D_2 binding in PFC ($r = 0.50$, $p = 0.02$) and trend level correlation with D_1 binding in HPC ($r = 0.36$, $p = 0.09$). D_2 binding in PFC showed no correlation with D_1 binding in HPC.

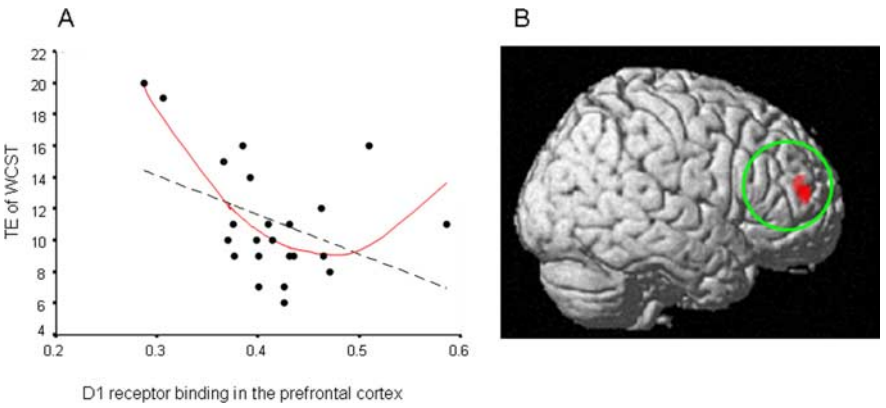

Figure 1. Quadratic (inverted U-shaped) relation between D_1 receptor binding in PFC and performance of WCST. ***A***, ROI analysis revealed a significant quadratic regression between the BP_{ND} value of [^{11}C]SCH23390 in PFC ($BP_{D1\ PFC}$) and TE of WCST. Red solid line, quadratic regression; black broken line, linear regression. Based on ROI analysis, the relation between $BP_{D1\ PFC}$ and TE can be expressed as follows: $TE = 326.92(BP_{D1\ PFC} - 0.47)^2 + 9.10$. ***B***, Using this equation, SPM analysis also revealed a significant quadratic regression between prefrontal D_1 receptor binding and TE of WCST ($p < 0.001$, uncorrected, extent threshold >30 voxels).

Using SPM2, we conduced standard voxel-based morphometry without modulation (Ashburner and Friston, 2000) to test whether the BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457 in PFC and HPC were related to the prefrontal and hippocampal gray matter concentration in the normalized images, respectively. The age and total gray matter (GM) volume were treated as confounding covariates in an analysis of covariance. The total GM volume was given by the total number of voxels within the GM compartment of each subject. The analysis revealed that there were no significant correlations between the BP values of [^{11}C]SCH23390 and [^{11}C]FLB457 in PFC and HPC and the concentration of gray matter in the prefrontal and hippocampal regions, respectively, at a threshold of $p = 0.01$, uncorrected.

Discussion

Although D_1 receptor binding in PFC showed trend-level positive linear correlations with WCST performance, quadratic regression analysis revealed significant inverted U-shaped relations between D_1 receptors in PFC and WCST performance. That is, a too high or too low level of D_1 receptor expression in PFC leads to high errors and a low number of categories achieved. However, D_2 receptor binding in PFC did not show significant relation with

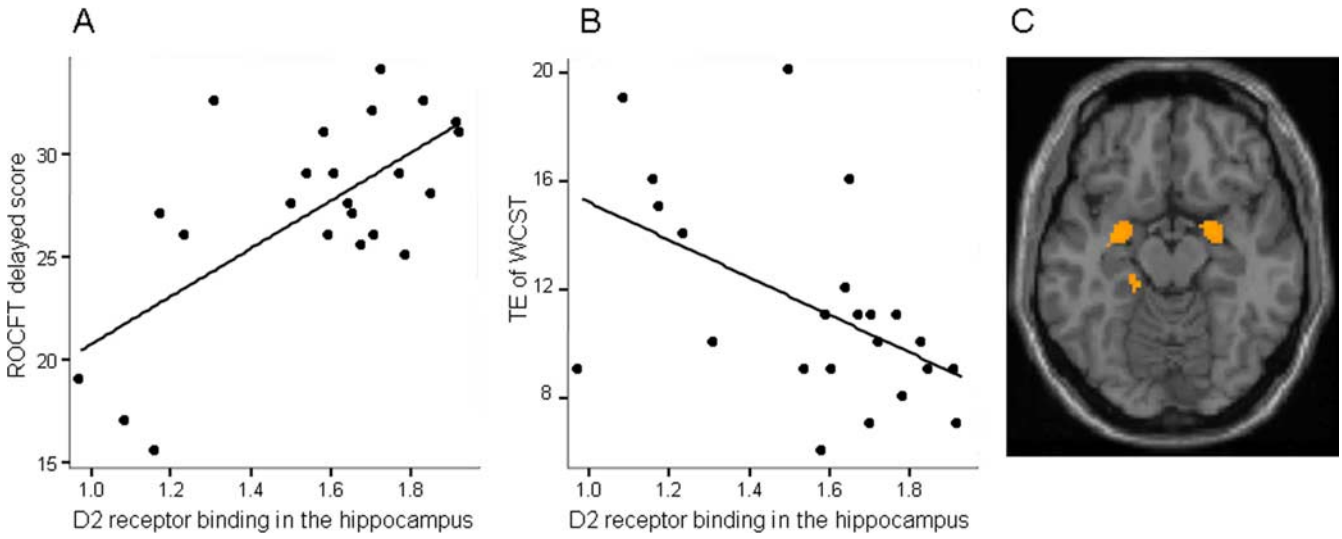

Figure 2. Correlations between D_2 receptor binding in the hippocampus and memory. ***A***, ***B***, Significant positive linear correlations between the BP_{ND} value of [^{11}C]FLB457 in the hippocampus and the delayed recall score of ROCFT and (***B***) TE of WCST revealed by ROI analysis. ***C***, The SPM result of a positive linear correlation between hippocampal D_2 receptor binding and the delayed recall score of ROCFT is shown ($p < 0.005$, uncorrected, extent threshold >30 voxels).

any neuropsychological measures. With regard to dopamine receptors in HPC, D_2 receptor binding in HPC showed positive liner correlations not only with memory function but also with frontal lobe functions, whereas D_1 receptor binding in HPC did not show significant relation with any neuropsychological measures. WCST involves a set-shifting component as well as a working memory component, although the two abilities are not mutually exclusive (Konishi et al., 1999). Working memory requires the active maintenance and manipulation of trial-unique information in a short-term memory buffer (Goldman-Rakic, 1995; Fuster, 2000). Thus, set-shifting could be regarded as updating of working memory content, and it has been demonstrated that updating of working memory content and shifting of cognitive set have a similar cognitive aspect in common (Konishi et al., 1998). Thus, in normal human subjects, the individual difference of working memory capacity could contribute to the difference in the performance of tests for cognitive flexibility.

Previous animal studies demonstrated that local injection of D_1 receptor antagonists into PFC induced impairment in working memory task in nonhuman primate (Sawaguchi and Goldman-Rakic, 1994). In a human study, systemic administration of a mixed D_1/D_2 agonist, pergolide, facilitated working memory, but the selective D_2 agonist bromocriptine had no effect, indicating that the dopaminergic modulation of working memory is mediated primarily via stimulation of D_1 receptors (Müller et al., 1998). Subsequent animal studies indicated that stimulation of D_1 receptors in PFC produces an inverted U-shaped response in working memory, with the response being optimized within a narrow range of D_1 receptor stimulation (Goldman-Rakic et al., 2000; Lidow et al., 2003; Castner and Goldman-Rakic, 2004; Seamans and Yang, 2004; Vijayraghavan et al., 2007). Recent human studies have investigated the effect of a functional polymorphism in the catechol *O*-methyltransferase gene, which has been shown to modulate the prefrontal dopamine level, on prefrontal function. The results also suggested that dopamine transmission in PFC produces an inverted U-shaped response, meaning that too little or too much dopamine signaling would impair prefrontal functions, although these studies could not identify the receptor subtype that plays a central role in this effect (Mattay et al., 2003; Williams-Gray et al., 2007).

Our PET finding is the first direct evidence in human that demonstrated an inverted U-shaped relation between D_1 receptors in PFC and executive function including working memory in normal healthy subjects. Our previous PET study revealed that, compared with normal controls, D_1 receptors in PFC were decreased in schizophrenia, which was associated with poor performance on WCST (Okubo et al., 1997). However, another PET study reported that an increase in D_1 receptors in PFC was associated with working memory deficits in schizophrenia (Abi-Dargham et al., 2002). It has been discussed that these inconsistent results might stem from several factors including differences in radioligands and patient demographics. Although the reasons for these inconsistent results need to be clarified in the future, an inverted U-shaped response can account for working memory deficits in schizophrenia whether D_1 receptors in PFC are increased or decreased in patients, because the D_1 receptor inverted U-shaped response is observed within a narrow range of the normal physiological condition (Williams and Castner, 2006; Vijayraghavan et al., 2007). An inverted U-shaped response has been suggested based on cognitive and behavioral studies, but the exact physiological mechanism of this effect has not yet been fully understood. A recent monkey electrophysiology study has demonstrated a neuron-level mechanism that constitutes the inverted U-shaped response whereby too much or too little stimulation of prefrontal D_1 receptors leads to working memory deficits. D_1 receptor stimulation had a suppressive effect on the PFC neural activities involved in a spatial working memory task. Moderate D_1 receptor stimulation spatially tunes PFC neurons that process target signals by preferentially suppressing nontarget (noisy) neural activities, whereas excessive D_1 receptor stimulation induces nonselective suppression of PFC neural activities regardless of whether the neural activities are task-related or not (Vijayraghavan et al., 2007).

Animal studies have suggested that the inverted U-shaped principle of D_1 receptor stimulation mediating working memory does not necessarily apply to other prefrontal functions (Floresco and Magyar, 2006). Therefore, it is noteworthy that prefrontal D_1 receptors were not associated with other prefrontal measures besides WCST, because fluency task by phonetic or semantic cues

and problem-solving test with visuospatial analysis are less dependent on the working memory process.

Considering that D_1 binding in PFC was not correlated significantly with D_2 binding either in PFC or HPC, D_1- and D_2-mediated working memory processes are considered to contribute differently to the completion of WCST. Although previous animal studies showed that working memory or executive function mainly depends on D_1 receptors, not on D_2 receptors in PFC (Sawaguchi and Goldman-Rakic, 1994; Seamans et al., 1998), a recent rat study demonstrated that D_2 receptors in PFC were necessary for set-shifting ability (Floresco et al., 2006). It has been suggested that when the dopamine level is high under a novel circumstance, the prefrontal network is mainly modulated by D_2 receptors. In such state, the network is likely to process multiple information (Seamans and Yang, 2004; Floresco et al., 2006). During the set-shifting stage of WCST, one needs to disengage from the previous strategy and compare alternative options under a new condition. After shifting attentional sets, one needs to learn and maintain a new strategy of WCST. In such condition, the dopamine level is considered to be moderate and D_1 receptors play a central role in stabilizing the network (Seamans and Yang, 2004; Floresco et al., 2006). We did not find any correlation between D_2 binding in PFC and WCST performances, possibly attributable to the fact that the working memory component and the set-shifting component are not entirely dissociable in WCST (Konishi et al., 1999). Instead, D_2 binding in HPC was related to WCST performances. Although the role of hippocampal D_2 receptors in set-shifting is not known, a possible interpretation is that in the initial set-shifting stage of WCST, D_2 receptors in HPC might play a role in quick learning and comparison to guide future behaviors, and once a new strategy is learned, D_1 receptors in PFC might contribute to the stability and maintenance of the novel strategy.

The association between hippocampal D_2 receptors and memory is consistent with the findings of previous PET studies (Kemppainen et al., 2003; Takahashi et al., 2007). The finding that hippocampal D_2 binding was more related to visuospatial memory than to verbal memory might stem from the fact that verbal learning is dependent on regions other than HPC, such as anterior, lateral and superior temporal lobes, which are involved in human language, although HPC plays a central role in both types of memory (Hodges and Graham, 2001). Umegaki et al. (2001) reported that injection of a D_2 receptor antagonist into HPC impaired memory performance and that the memory impairment was ameliorated by coinjection of a D_2 receptor agonist. They also found that local infusion of D_2 agonist into HPC stimulated acetylcholine release in HPC and ameliorated scopolamine-induced memory impairment (Fujishiro et al., 2005). In addition, hippocampal D_2 receptors appear to be involved in synaptic plasticity. It has been reported that D_2 antagonist inhibited long-term potentiation in HPC (Frey et al., 1990; Manahan-Vaughan and Kulla, 2003), the key mechanism underlying memory consolidation (Jay, 2003; Lynch, 2004). There is some evidence from animal studies that hippocampal D_1 receptors are also involved in memory (Hersi et al., 1995a,b; Bach et al., 1999), but supporting our PET data, Wilkerson and Levin (1999) reported that hippocampal D_1 receptors were not as responsible as D_2 receptors for memory functions.

In line with our previous study (Takahashi et al., 2007), we also found hippocampal D_2 receptors to be involved in the performance of WCST and phonemic verbal fluency, which is more dependent on PFC than semantic verbal fluency. Patients with lesions in HPC sometimes show deficits in WCST (Corkin, 2001; Igarashi et al., 2002). These observations suggest that hippocampal D_2 receptors could modulate PFC activity by the HPC–PFC pathway, which plays a significant role in the cognitive process (Laroche et al., 2000; Thierry et al., 2000). Accumulating evidence has suggested the modulatory effects of dopamine on HPC–PFC interactions (Seamans et al., 1998; Aalto et al., 2005; Tseng et al., 2007; Goto and Grace, 2008). Conceivably, dopamine influences PFC neurons directly by prefrontal D_1 receptors and indirectly by hippocampal D_2 receptors via the HPC–PFC pathway.

Müller et al. (1998) reported that the systemic administration of the mixed D_1/D_2 agonist pergolide facilitated working memory, whereas selective D_2 agonist had no effect. However, there is converging evidence from human and animal studies to suggest the involvement of D_2 receptors in cognitive functions. It was reported that the systemic administration of D_2 agonist in human improved cognitive functions including working memory and executive functions (McDowell et al., 1998), and the administration of D_2 antagonist impaired those functions (Mehta et al., 1999). In an animal study, it was reported that mice lacking D_2 receptors showed a working memory deficit (Glickstein et al., 2002). These studies, however, did not reveal the regions most responsible for these effects. Moreover, although the involvement of D_1 receptors in working memory is widely recognized, it was not clear whether D_1 receptor stimulation alone or the combination of D_1 and D_2 receptor stimulation is most effective. Our finding suggested that orchestration of prefrontal D_1 receptors and hippocampal D_2 receptors might be necessary for executive functions including working memory.

The current study has several limitations. First, although BP_{ND} is the complex value of receptor density and affinity (the inverse of Kd), previous studies indicated that the affinity does not differ according to region (Suhara et al., 1999) and that extrastriatal binding of current PET ligands is not sensitive to endogenous dopamine (Abi-Dargham et al., 1999; Okauchi et al., 2001). Still, we should keep in mind that the BP_{ND} values of [^{11}C]SCH23390 and [^{11}C]FLB457 might not necessarily be equivalents for D_1 and D_2 receptor functions, respectively. This emphasizes the need for PET investigations of the relation of BP_{ND} and presynaptic function or second messenger beyond dopamine receptors. Alternatively, multimodal imaging study combining the current method with other modalities such as functional MRI might also be advantageous in investigating the direct relation between dopamine receptor function and PFC functions. Second, we measured the level of dopamine receptor binding during a resting state rather than during cognitive tasks. It is difficult to measure endogenous dopamine release in extrastriatal regions with the current PET ligands (Abi-Dargham et al., 1999; Okauchi et al., 2001). Future study with radioligands more sensitive to endogenous dopamine release will enable us to examine its degree of receptor occupancy. Finally, attributable to limitations of the [^{11}C] radioligand, the data of [^{11}C] FLB457 binding in the striatum was not available. The striatum plays an important role in the prefrontal-hippocampus pathway. PET data in the striatum would lead to a better understanding of the interaction of these three regions. Future study with triple radioligands such as [^{11}C]SCH23390, [^{11}C] FLB457 and [^{11}C] raclopride will enable us to examine striatal and extrastriatal D_1 and D_2 receptors in the same subject.

In summary, we found that an inverted U-shaped relation existed between D_1 receptor binding in PFC and WCST performance, indicating an inverted U-shaped relation between prefrontal D_1 receptors and working memory, and that prefrontal D_2 receptor binding was not related to any frontal lobe functions.

Hippocampal D_2 receptors seem to contribute to local hippocampal functions (long-term memory) and to modulation of brain functions outside HPC (frontal lobe functions), which are mainly subserved by PFC, via the HPC–PFC pathway. Our findings suggest that prefrontal D_1 receptors and hippocampal D_2 receptors might be targets for pharmacological therapeutics for cognitive and memory impairments observed in neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease and schizophrenia.

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