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Different Roles of the Frontal and Parietal Regions in Memory-Guided Saccade: A PCA Approach on Time Course of BOLD Signal Changes

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Abstract: Although involvement of the frontoparietal regions in visually guided saccade and visuospatial attention has been established, functional difference of the frontal and parietal regions suggested in neuropsychological observations and lesion studies in animals has not been explicitly supported by functional imaging studies. Considering a possible disadvantage of cognitive subtraction in an interregional comparison, we directly compared the time course of BOLD signal changes across regions. Normal subjects performed a modified version of a memory-guided saccade task in which saccade was performed both during encoding and execution phases. In addition, the delay period was fixed and the peripheral target was presented also during the execution phase together with distracters. Therefore, visuospatial representation was likely maintained in the sensory domain during the delay phase. A principal component analysis on the time-course data separated the 20 activated areas into three groups, which largely coincided with the cerebral lobes. The frontal group included the putative human FEF and SEF, and the parietal group PEF. The frontal and occipital groups exhibited the time course of activation with two peaks corresponding to neural responses during the encoding and execution phases, and the parietal group exhibited a single-humped activation pattern corresponding to neural activity during the delay phase. The results suggest that the frontal regions are more associated with the execution of saccade, and the parietal regions with visuospatial representation, presumably in the sensory domain. *Hum Brain Mapp 23:129–139, 2004.* © 2004 Wiley-Liss, Inc.

Key words: short-term memory; attention; intention; saccadic eye movement; fMRI; principal component analysis; frontal lobe; parietal lobe

INTRODUCTION

The execution of visually guided saccade is closely associated with visuospatial attention in that both work on visuospatial representation of the target location. The involvement of frontoparietal regions in visually guided saccade and visuospatial attention has been suggested based on observation of patients with cortical lesions and lesion stud-

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ies in animals [Heide and Kömpf, 1998; Mesulam, 1981; Pierrot-Deseilligny et al., 1991]. In monkeys, three cortical eye fields that directly control the oculomotor system in the midbrain, namely, FEF [Bruce and Goldberg, 1985], SEF [Schlag and Schlag-Rey, 1987], PEF, and, more specifically, LIP [Barash et al., 1991], were identified. In functional imaging studies of normal healthy humans on visually guided saccade [Anderson et al., 1994; Beauchamp et al., 2001; Corbetta et al., 1998; Heide et al., 2001; Luna et al., 1998; Nobre et al., 2000; O'Sullivan et al., 1995; Petit and Beauchamp, 2003; Sweeney et al., 1996] and visuospatial attention [Astafiev et al., 2003; Beauchamp et al., 2001; Büchel et al., 1998; Corbetta et al., 1993, 1998; Nobre et al., 1997, 2000], several frontoparietal regions were consistently coactivated, and some of them were postulated as human homologues of the cortical eye fields. The frontal and parietal regions tended, therefore, to be regarded as components of a unitary system, and primary attention was paid to the finding that visually guided saccade and visuospatial attention share this frontoparietal system [Beauchamp et al., 2001; Büchel et al., 1998; Corbetta, 1998; Nobre et al., 1997, 2000]. In the observations of patients and the lesion studies of animals, to the contrary, the difference in the roles of the multiple eye fields has been underscored. Mesulam [1981] argued that the parietal regions are more related to the sensory map and the frontal regions to the motor programs for exploration. Evidence from the observation of patients suggests that the parietal regions are more involved in reflexive saccade and the frontal regions in intentional saccade [Heide and Kömpf, 1998; Pierrot-Deseilligny et al., 1991]. However, in functional imaging studies the difference in the roles across the multiple eye fields in visually guided saccade has not been explicitly examined to date. This may be a critical "missing link" in our system-level understanding of the neural mechanism underlying visually guided saccade, as well as that of visuospatial attention.

The major stream of current functional imaging studies, including conventional event-related fMRI studies, has been performed according to the concept of cognitive subtraction, in which a large-scale brain network that differentiates two or more cognitive states is of primary interest. The difference in the role of different regions during a specific task is secondarily inferred by comparison of differential activation between the task and control tasks across these regions. When the difference in the role between regions is of primary interest, a reduced sensitivity caused by such an indirect comparison and a risk of inappropriate control tasks may be a disadvantage of the cognitive subtraction. If the characteristics of neural activity between the different regions are directly compared, it may provide new insight into the functional organization of the brain network. In this fMRI study, we directly compared the time courses of brain activity during a single event of a memory-guided saccade task across cortical areas. The memory-guided saccade task provides an opportunity to temporally dissociate neural activity associated with visuospatial representation from that associated with ocular movement [Hikosaka and Wurtz, 1983]. We extracted all the cortical regions that participated in the task, including those processing visual input and those controlling eye movement, and these areas were segregated into a few groups on the basis of the pattern of the time course of activation. Because we had no a priori hypothesis of the exact time course of activation in these areas, the regions were segregated using a principal component analysis (PCA), which is an exploratory analysis to extract independent components of data variance. The expected results included activation in the human homologue of the cortical eye fields, including FEF in the precentral sulcus, SEF in the medial frontal cortex, and PEF in the intraparietal sulcus [Anderson et al., 1994; Beauchamp et al., 2001; Büchel et al., 1998; Corbetta et al., 1993, 1998; Heide et al., 2001; Luna et al., 1998; Nobre et al., 1997, 2000; O'Sullivan et al., 1995; Petit and Beauchamp, 2003; Sweeney et al., 1996], possibly showing different time courses of activation depending on how each region is involved in visuospatial representation and ocular movement. The dorsolateral prefrontal cortex, the involvement of which in the mnemonic component of memory-guided saccade has been suggested in the observations of patients with cortical lesions [Heide and Kömpf, 1998; Pierrot-Deseilligny et al., 1991], studies of a transcranial magnetic stimulation in humans [Brandt et al., 1998; Müri et al., 2000], and the unit-recording studies of monkeys [Funahashi et al., 1989, 1990], is also a candidate region supporting visuospatial representation. Activation of the visual cortex in response to the presentation of visual stimuli is highly likely.

SUBJECTS AND METHODS

Subjects

Nineteen normal, right-handed volunteers (11 men, 8 women; age range 18–25 years) participated in the study. None had past histories of neurological or psychiatric illness. Handedness was evaluated using the Edinburgh Handedness Inventory [Oldfield, 1971]. Written informed consent was obtained from each subject. The study was conducted according to the guidelines of the ethical committee of Tohoku Fukushi University and the Declaration of Helsinki (1991).

Tasks

Each subject performed 40 trials of a modified version of the memory-guided saccade task. Each trial began with an encoding phase, during which a peripheral target was briefly (0.5 s) presented. The peripheral target and the fixation point were subtended by a 5° visual angle, and the location of the peripheral target was pseudorandomly selected from 12 possible locations that were arranged around the central fixation point at every 30°. Each subject performed saccade to the target and memorized the location. A delay phase of 6.5 s was followed by an execution phase, during which points were briefly (0.5 s) presented at all of the 12 possible peripheral target locations, and each subject

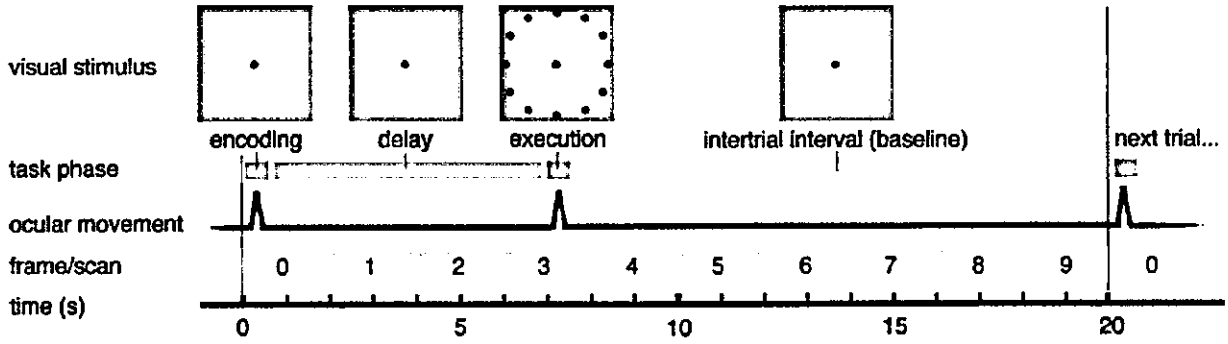

Figure 1.
Task. Examples of the presented visual stimuli, designated task phases, expected ocular movements, and fMRI scans/frames are presented along the time scale to show their temporal relationships.

had to perform saccade to the point in the same location that he/she had memorized. A subsequent intertrial interval of 12.5 s made the total duration of each trial 20 s. The central fixation point was presented throughout the task period, and each subject was required to keep his/her eyes fixed on it during the delay phase and the intertrial interval. An example of the visual stimuli and the time course of the expected ocular movement are shown in Figure 1. It should be noted that our modified version of the memory-guided task was different from the conventional one in several respects. The conventional memory-guided saccade task requires subjects to avoid performing saccade during the encoding phase, but our task required its execution because we were not interested in the effect of saccade suppression during the encoding phase. Both during the encoding and execution phases, therefore, all the processes for visually guided saccade were expected to occur. In addition, the duration of the delay phase was fixed, and the saccade was visually guided by one of the presented targets during the execution phase. During the delay phase, therefore, it was sufficient to simply maintain information of the target location to choose a target from the 12 presented possible locations, unlike the conventional memory-guided task, in which quick execution of saccade to the imaginary target is always prepared during the delay period. During the intertrial interval, due to the successive execution of trials, the subject anticipated the forthcoming visual stimulus and prepared for saccade execution for the encoding phase of the next trial. Therefore, the maintenance of the visuospatial representation of the target location was required only during the delay phase, but the requirement of cognitive processes without relevance to the target location, such as those related to anticipation and alertness, should be comparable between the delay phase and the intertrial interval. Visual stimuli were projected onto the semilucent screen attached to the head coil of the MRI scanner, and the subjects viewed the screen via a mirror. Before the fMRI measurement, each subject performed a training session of the task outside the MRI scanner until he or she could perform the task without difficulty.

MRI Data Acquisition

Whole-brain volume images composed of 16 transaxial slices each were obtained at TR of 2 s using a GE-EPI (TE60, FA90, 64 × 64 matrix, 256 mm FOV, 8 mm thickness, gapless) sequence on Siemens Vision (Siemens, Erlangen, Germany) (1.5 T). Ten scans were acquired during each trial (20 s). The temporal relationship between fMRI measurements, visual stimuli, and expected ocular movements are shown in Figure 1. Excluding the first 10 scans during a dummy trial, which was intended to familiarize the subjects to the experimental setting, 400 scans were acquired during the 800-s (20 s/trial × 40 trials) task session.

Image Processing

All image processings were performed using the Statistical Parametric Mapping (SPM 99; Wellcome Department of Cognitive Neurology, London, UK) implemented on MATLAB (Mathworks, Natick, MA). Correction for head motion, adjustment of interslice acquisition timing to the beginning of the scan, spatial normalization using the EPI-standard-brain template provided by SPM99, and smoothing with a 16-mm-wide Gaussian filter were performed as preprocessings. Ten scans obtained during each trial were designated as frames 0 to 9; frame 0 corresponded to the scan at the start of the encoding phase (Fig. 1). Preprocessed images in each frame were averaged across the 40 trials for each subject. Using the 190 generated mean images (10 frames × 19 subjects) two-way ANOVA was performed implementing the frame effect (effect of interest) and the subject effect (effect of no interest). Subtraction analysis was carried out for each of the frames from 1 to 9 against frame 0, that is, the BOLD signal during the intertrial interval immediately before the encoding phase was regarded as a baseline. The statistical threshold was set at $P < 0.05$ (corrected for multiple comparisons). The anatomical location was estimated by superimposing activation onto the T1-weighted standard brain provided by SPM99. The activated areas were identified in all the nine comparisons (frames 1–9 vs. frame 0). As a region of interest for analysis, a voxel with the activation

peak for each activated area was selected from frame in which the maximum statistical value was obtained. At each peak voxel, percent BOLD signal changes at frames 1 to 9 relative to frame 0 were regarded as a time course of the brain activity.

PCA

All the activated areas were regarded as variables, and the percent signal changes at frames 1 to 9 of 19 subjects were regarded as the measures of each activated area (9 frames × 19 subjects = 171 observations). From the measures of N activated areas, an N × N correlation matrix was calculated, and a principal component transformation was applied [Mardia et al., 1979]. Because the data comprised the variance of brain activity across frames (times) and the subjects, each principal component should represent a characteristic pattern of the time course of activation and its intersubject variability. Meaningful principal components were determined according to the cumulative contribution rate of 70%. The PCA gives a loading of each variable for each principal component. The loading indicates how much the pattern of data variance represented by each principal component explains the variance of measures in each variable. In this study, variables (activated areas) with similar loadings were likely to show the pattern of the time course of activation represented by the principal component to a similar extent. Therefore, similarity or difference in the pattern of the time course of activation between two activated areas can be assessed by a geometrical distance in a multidimensional space of the loadings for the principal components. Accordingly, all of the N activated areas were plotted in a multidimensional space of the loadings for the selected principal components, and grouped according to the distance in the space. A principal component score for each principal component for each observation (one of the 9 frames × 19 subjects) was obtained by the linear conjunction of the measures weighted by the loadings for the principal component. Therefore, a set of principal component scores can be regarded as "measures" of each principal component, which illustrate the patterns of the time course of activation and its intersubject variability represented by the principal component. All computations for the PCA were performed using MATLAB. To generate a summary time course of the signal changes of each group of cortical areas, the mean normalized signal changes were averaged across all the subjects for all the areas in a group.

RESULTS

Brain activations during the memory-guided saccade are presented in Figure 2. Activation at all frames from 1 to 9 are sequentially presented in Figure 2a to show their time courses. Figure 2b and c present the activated areas at frames 2 and 4, respectively, which show distinct spatial patterns of activation, surface-rendered onto the structural image to show their anatomical locations. Twenty activated areas were identified (Table I). Activation in the primary visual cortex, lingual gyrus, superior occipitoparietal junction, lateral occipitotemporal junction, two areas along the precentral sulcus (one at the intersection with the superior frontal sulcus and the other with the inferior frontal sulcus), and the cerebellum were observed bilaterally. The medial frontal gyrus and the anterior cingulate sulcus were also activated. These areas showed an activation pattern with two peaks around frames 2 and 6 (Fig. 2a). In contrast, activation in the posterior superior parietal lobule and the intraparietal sulcus bilaterally showed an apparently different pattern of the time course: a single-humped pattern with a plateau from frames 3 to 7 (Fig. 2a).

The results of the PCA are shown in Figure 3. The cumulative contribution rate reached 71.2% by the first three principal components (PC1, PC2, and PC3, with 41.7, 18.1, and 11.4%, respectively) and all the other principal components showed a contribution rate of less than 6%. The first three principal components were, therefore, regarded as meaningful. Loadings for the principal components were plotted for PC1–PC2 and PC1–PC3 (Fig. 3a). The 20 areas formed three groups in the three-dimensional space; the grouping coincided largely with the parcellation of the cerebral lobes. The first group was mainly composed of areas in the primary and extrastriate visual cortices, featuring markedly positive loadings for PC1 (occipital group). This group also included the areas in the bilateral cerebellar hemisphere. The second group was composed of areas in the posterior superior parietal lobule and intraparietal sulcus, having positive loadings for both PC2 and PC1 (parietal group). The third group was composed of areas in the lateral and medial frontal cortices, having positive loadings for PC1 and negative loadings for PC3 (frontal group). To illustrate the characteristics of the pattern in the time course of activation represented by each principal component, the principal component scores were plotted such that the effects of frames (time) and subject could be separately read (Fig. 3b). The plots indicate that PC1 and PC2 are characterized by the effect of frames, that is, time course of activation: two peaks, one around frames 2 and 3, and the other at frame 6 for PC1, and a single peak at frame 4 and a decrease in activation at the early frames for PC2. The effect of frames appears relatively small in PC3, although activation tended to increase at the later frames. Instead, intersubject variability in the later frames was evident in PC3. The summary time course of the signal changes for each group is shown in Figure 3c. Consistent with the impression from the visual inspection of Figure 2a, the frontal and occipital groups both showed the activation pattern with two peaks, and the parietal group a single-humped pattern with a plateau during the period during which the other two groups exhibited a dip in activation. Despite the clear distinction in PC3, the frontal and occipital groups exhibited similar double-peaked pattern, which is consistent with the fact that PC3 mainly represents intersubject variability. Compared with the occipital group, the double-peaked pattern in the frontal group was slightly obscure and similar to the single-humped pattern, consistent with the slightly larger loadings for

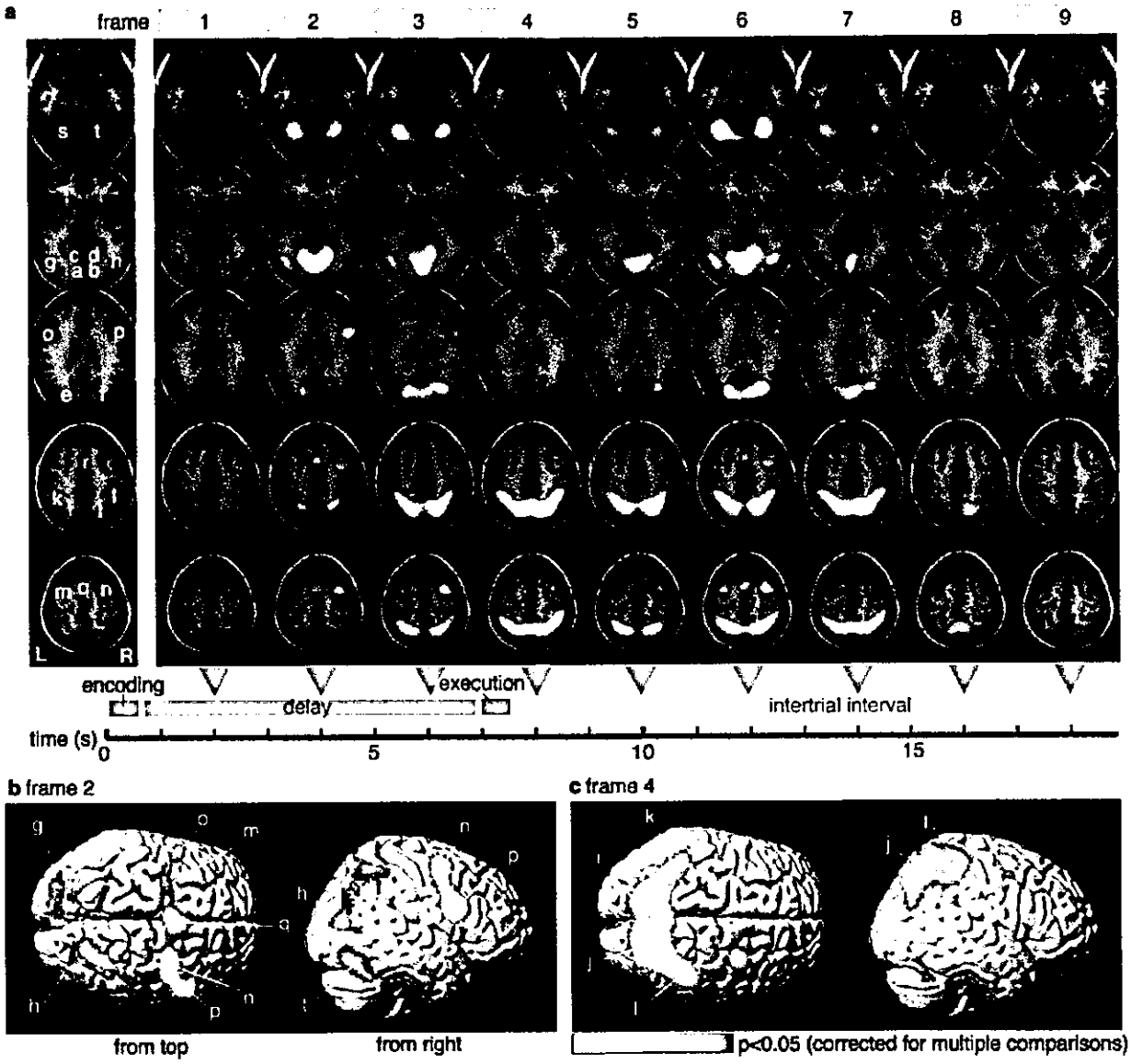

Figure 2.

Significantly activated areas. **a:** The activated areas in each of frames from 1 to 9 compared with frame 0 are superimposed on the standard anatomical T1-weighted images. Five transaxial slices, −22, 2, 26, 50, 62 mm above the AC–PC plane, are shown for each frame. Lowercase letters on the slices at the left-most column denote the location listed in Table I. The time scale together with the task phases are shown below. Scan timing of each slice was adjusted to the beginning of each volume-image acquisition (gray arrow-head). L: left, R: right. **b,c:** The activated areas in frames 2 and 4, respectively, are rendered over the surface of the standard brain.

PC2 in the frontal group than in the occipital group. Although the rise in activation in the parietal group may look slower than in the other two groups, it is likely to be an artificial impression caused by the normalization of the data. Taking the original activation data and the results of the PCA together, the rise in activation in the parietal group seems to be comparable to that in the other two groups until frame 2, but continues until frame 6, unlike the other two groups, which turn to decrease at frame 2. Although the decrease in activation after frame 6 appears to take slightly longer in the parietal group, the peak was at frame 6, as in the other two groups.

TABLE I. Activated areas

Structure	Coordinate (*x*, *y*, *z*)	Peak frame	T score	Index
Left primary visual cortex	10, −70, 4	2	6.86	a
Right primary visual cortex	−4, −76, 2	2	7.87	b
Left lingual gyrus	22, −64, −6	2	6.20	c
Right lingual gyrus	−18, −64, −4	2	7.15	d
Left superior occipitoparietal junction	30, −74, 26	6	7.82	e
Right superior occipitoparietal junction	−24, −78, 22	6	6.08	f
Left lateral occipitotemporal junction	46, −68, 6	6	5.85	g
Right lateral occipitotemporal junction	−44, −72, 2	6	6.55	h
Left posterior superior parietal lobule	20, −62, 52	6	10.63	i
Right posterior superior parietal lobule	−22, −60, 52	6	12.21	j
Left intraparietal sulcus	40, −44, 56	4	7.21	k
Right intraparietal sulcus	−32, −52, 54	4	7.17	l
Left precentral sulcus/superior frontal sulcus	34, 2, 64	6	6.51	m
Right precentral sulcus/superior frontal sulcus	−30, 0, 62	6	4.69	n
Left precentral sulcus/inferior frontal sulcus	46, 8, 26	2	5.60	o
Right precentral sulcus/inferior frontal sulcus	−50, 8, 26	2	3.61	p
Medial frontal gyrus (midline)	0, 4, 60	2	4.56	q
Anterior cingulate sulcus (midline)	4, 12, 48	2	4.79	r
Left cerebellum	34, −60, −22	6	8.59	s
Right cerebellum	−32, −62, −22	6	8.77	t

The Talairach coordinate, the frame in which the peak activation was exhibited, the T score of the activation peak, and the index (a lowercase letter, also used in Figs. 2 and 3 to indicate the location of an activated area) are shown for each major activated area.

DISCUSSION

The PCA separated the 20 activated areas into three groups according to the patterns of the time course of BOLD signal changes during the memory-guided saccade task. The grouping was largely consistent with the parcellation of the cerebral lobes. The frontal and occipital groups exhibited a double-peaked pattern, and the parietal group a single-humped pattern. Considering the empirically assumed hemodynamic response function (HRF) [Aguirre et al., 1998; Boynton et al., 1996; Dilharregui et al., 2003; Friston et al., 1994], in which vascular response to a brief neural activity exhibits a gradual increase for approximately 6 s and then a decrease close to the baseline approximately 12 s after the neural activity, the double-peaked pattern seems to be mainly composed of responses during the encoding and execution phases, and the single-humped pattern was differentiated by the neural activity during the delay phase. This suggests that the frontal and occipital groups are more associated with the execution of saccade, and/or visual processing, and the parietal group with the operation of visuosptial representation, which are roughly consistent with the proposed differences in the roles of the frontal and parietal regions in visually guided saccade [Heide and Kömpf, 1998; Mesulam, 1981; Pierrot-Deseilligny et al., 1991].

The approach adopted in this study assumed the reasonable extent of homogeneity in an HRF across the entire cortex, similar to many of the previous fMRI studies that had adopted some sort of regression analyses using estimated HRFs. The detailed characteristics of the HRF have been examined in the visual cortex [Boynton et al., 1996; Dilharregui et al., 2003; Friston et al., 1994] and the sensorimotor cortex [Aguirre et al., 1998]. There is variability in the temporal characteristics of the estimated HRFs across the studies in terms of approximately 4 s of difference in the timing of the peaks. It has been also shown that a similar extent of variability in the HRF exists across subjects [Aguirre et al., 1998]. Several fMRI studies, in which analyses did not depend on fixed HRF models, have presented rough time courses of observed BOLD signal changes similar to known HRFs in several cortical areas other than the visual or sensorimotor cortex [e.g., Calhoun et al., 2001; Clark et al., 2000; Corbetta and Shulman, 2002; Leung et al., 2000; Otten and Rugg, 2001; Petit and Beauchamp, 2002; Ranganath et al., 2001; Sawamoto et al., 2000; Yantis et al., 2002]. Although some studies presented delayed or prolonged BOLD signal responses, these responses could reasonably be attributed to the delay or prolongation of neural activity per se rather than that of the vascular response [e.g., Buckner et al., 1996; Schacter et al., 1997; Yantis et al., 2002]. These empirical findings suggest that it is reasonable to estimate the variability in the HRF across major cortical areas as less than a range of 4 s of the difference in the delay in the response peak. Furthermore, in this study, the fact that all the three groups exhibited a peak of activation at frame 6 in the summary time course (Fig. 3c) suggests that the difference in the peak of HRFs across the cortical groups is even smaller than the empirically assumed range, arguably smaller than the TR (2 s). The observed difference between the double-peaked and single-humped patterns in this study is thus not explained by this possible variability in an HRF across cortical areas or a signal from large veins [Lee et al., 1995], but it probably reflects the difference in the time course of neural

activity. The argument is also supported by the time course of the principal component score (Fig. 3b). If the difference in the time course of an HRF was solely responsible for the difference in the pattern of activation between groups, the principal component score for the PC that differentiates the groups should show a pattern that a differential component of an HRF was repeated twice. This was obviously not the case for PC2, which differentiated the parietal group from other two groups, although a small peak at frame 7 observed in a few subjects may reflect such interregional variability in an HRF. However, it may be the case for PC3, which differentiated the frontal and occipital groups.

The proportions of neurons that exhibit sustained activity during the delay phase of the memory-guided saccade task were not very different among FEF [Schall, 1991b], SEF [Schall, 1991a], and LIP [Barash et al., 1991; Paré and Wurtz, 1997] presented in previous studies on monkeys. In a human fMRI study using a delayed manual response task in which subjects had to hold a manual response during the delay period, both the premotor and posterior parietal cortices similarly exhibited a sustained activity during the delay period [Toni et al., 1999]. Compared with these previous data, the extent of the observed difference in the time course of BOLD signal changes between the frontal and parietal groups in this study may be surprising. The greater contrast in the time courses of activation in this study seems to be attributed not only to the higher sensitivity to the interregional differences inherent in our approach, but also to the nature of the task adopted. During the delay period of the conventional memory-guided saccade task, subjects always must be prepared to quickly perform saccade in response to an execution cue. Thus, the target location has to be coded as a prepared motor program. However, during the delay phase in our modified version such coding of the target location in a motor program was not necessary because the target was presented again during the execution phase. Furthermore, such coding of the target location in a motor program appears to be behaviorally inappropriate because it may conflict with the coding of the target location visually presented during the execution phase. Therefore, during the delay phase the subjects were likely to maintain the visuospatial information of the target location with little relevance to the motor program. It thus appears reasonable to assume that visuospatial information of the target location can be represented either with or without relevance to a motor program; here, we refer to the former as visuospatial representation in a motor domain and to the latter as that in a sensory domain. Accordingly, the contrast of the activation patterns in the frontoparietal network between previous studies using the conventional memory-guided saccade task and this study can be explained by the difference in the roles of the frontal and parietal regions in visuospatial representation: the frontal regions in the motor domain and the parietal regions in sensory domain. In fact, this paraphrases the argument by Mesulam [1981] on the roles of cortical areas in directed spatial attention, that is, a posterior parietal component provides an internal sensory map, and a frontal component coordinates the motor program, supporting our argument. However, the fact that the frontal group exhibited a less clear double-peaked pattern and slightly larger loadings for PC2 than the occipital group may suggest that the visuospatial representation in the motor domain also played a small role in some subjects.

There may be an alternative interpretation concerning the difference between our task and the conventional memory-guided saccade task. Unlike the conventional task, the subject performed a saccade during the encoding phase in our task. Therefore, the subject might have learned the saccade as sensorimotor association. However, we consider this explanation irrelevant because the subject performed the saccade only once during the encoding phase, and the visual stimuli presented were different between the encoding and execution phases. Furthermore, as far as we know, selective activation in the parietal cortices related to the learned sensorimotor association has not been reported to date. It may be suspected that the subjects might have verbally encoded the target location using time terms, because each target location could be assigned to a number on a clock. We consider this unlikely because the encoding and maintenance of the target location in this task was so easy that such a verbal strategy appeared to have no behavioral advantage. We explicitly instructed the subjects to memorize the target "location," and did not mention that the location could be assigned to a number on a clock. During the interview after the experiment, none of the subjects reported that he or she used such a verbal strategy during the task. Finally, the spatial pattern of activation during the delay phase, i.e., activation in the intraparietal sulcus bilaterally, is very different from the cortical network involved in the phonological loop of a verbal working memory, which features the Broca's area, premotor area, and supplementary motor area [Smith et al., 1998]. It may also be suspected that the difference in visual stimuli between the encoding and execution phases could be reflected in the difference in some preparatory processes such as preparation to inhibit saccade to the distracters during the execution phase. This is possible, but the effect was likely to be limited to the timing immediately before the execution phase due to the predictable timing of stimulus presentation. Therefore, it is highly unlikely that such a preparatory process explains the neural activity predominating during the delay phase.

Discrete activation peaks in the posterior superior parietal lobule and intraparietal sulcus observed in this study are congruent with previous functional imaging data on the memory-guided saccade [Anderson et al., 1994; O'Sullivan et al., 1995; Sweeney et al., 1996] as well as other types of visually guided saccade [Corbetta et al., 1998; Heide et al., 2001; Luna et al., 1998; Nobre et al., 2000] and visuospatial attention [Astafiev et al., 2003; Büchel et al., 1998; Corbetta et al., 1993, 1998; Nobre et al., 2000]. Although Müri et al. [1996] had assigned the human LIP homologue to the activation focus in the intraparietal sulcus, many other authors are still cautious in comparing the parietal activation foci with the findings in monkeys.

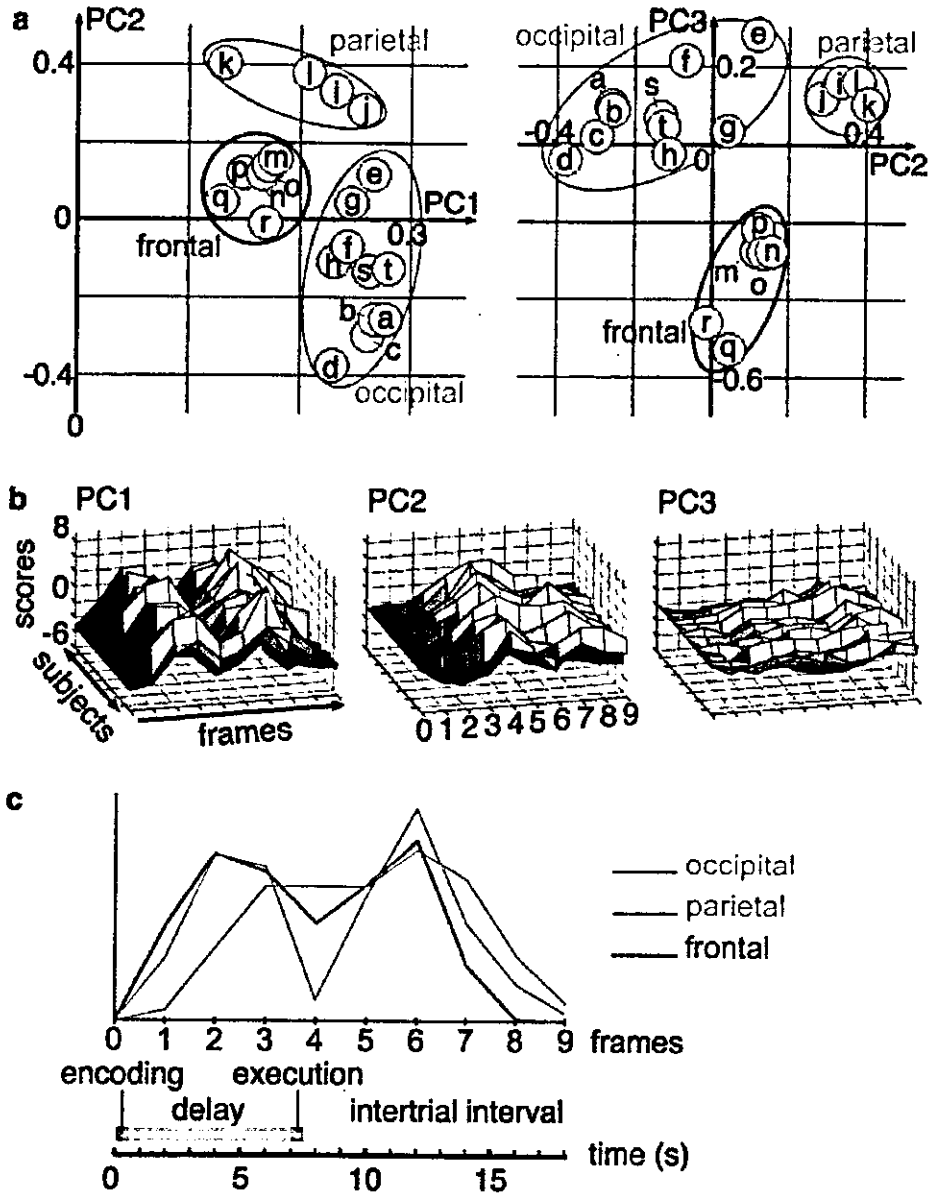

Figure 3.

Results of PCA. **a:** Plots of the loadings for three PCs. **Left:** PC2 against PC1. **Right:** PC3 against PC2. The occipital, parietal, and frontal groups are encircled in green, red, and blue, respectively. See Table I for corresponding lowercase letter indices to the location of activated areas. **b:** The principal component score (arbitrary unit) at each observation is plotted three-dimensionally (in height) against each frame (left to right) for each subject (in depth) for each of PC1 **(left)**, PC2 **(middle)**, and PC3 **(right)**. **c:** Standardized mean signal change of each group. Colors for the lines are the same as for the circles in a. The time scale together with the task phases are shown below.

Recent PET [Petit et al., 1996] and fMRI [Beauchamp et al., 2001; Corbetta et al., 1998; Heide et al., 2001; Luna et al., 1998; Nobre et al., 2000; Petit et al., 1997; Petit and Beauchamp, 2002] studies have reported more than two activation foci along the precentral sulcus. In these studies, the activation foci located superiorly have generally been considered as the classical human FEF homologue [Fox et al., 1985; Paus, 1996]. Some researchers regarded their activation foci located inferiorly as in the ventral premotor cortex [Beauchamp et al., 2001; Heide et al., 2001; Petit and

Beauchamp, 2002], comparing this region with the recently identified oculomotor area in the ventral premotor cortex of the monkey [Fujii et al., 1998]. Considering variability of the Talairach coordinates of the activation foci in the previous studies and ours, however, the number of distinct saccade-related areas along the precentral sulcus may be still a matter of debate. In fact, our superior activation foci is considerably more superiorly located ($z = 62$ and 64 mm) than the classical human FEF homologue ($44 \leq z \leq 51$ mm) [Paus, 1996]; the location of our superior activation focus is comparable to that of the third activation focus reported in a few previous studies in addition to the likely FEF and ventral-premotor activation foci [Corbetta et al., 1998; Nobre et al., 2000]. On the other hand, the recently suggested role of a region close to our inferior focus in sensory orienting [Corbetta and Shulman, 2002] may also be noteworthy.

The activation foci in the medial frontal cortex and anterior cingulate cortex are consistent with previous functional imaging data on the visually guided saccade and visuospatial attention [Beauchamp et al., 2001; Büchel et al., 1998; Heide et al., 2001; Nobre et al., 1997, 2000; Petit et al., 1996; Petit and Beauchamp, 2002]. The former focus is widely accepted as the human SEF homologue. The observed double-peaked activation pattern in this region similar to that observed in the lateral frontal regions is consistent with the results of the unit-recording studies in monkeys that suggested the similar roles of the FEF and SEF in memory-guided saccade [Schall, 1991b]. The role of the anterior cingulate cortex in the visually guided saccade is not yet clarified. A recent observation of patients with lesions in the anterior cingulate cortex has suggested an important role of this region in eye movement control [Gaymard et al., 1998].

In spite of the assumed role of the dorsolateral prefrontal cortex in the mnemonic component of the memory-guided saccade [Brandt et al., 1998; Funahashi et al., 1989, 1990; Heide and Kömpf, 1998; Müri et al., 2000; Pierrot-Deseilligny et al., 1991], no significant prefrontal activation was observed in this study. Activation in the dorsolateral prefrontal cortex reported in the previous functional imaging studies of the memory-guided saccade and visuospatial attention has not been very robust and has varied in location [Astafiev et al., 2003; Büchel et al., 1998; Corbetta, 1998; Heide et al., 2001; O'Sullivan et al., 1995; Petit et al., 1996; Sweeney et al., 1996]. Courtney et al. [1998] argued that the area for spatial working memory in the dorsolateral prefrontal cortex is located close to the FEF. In this and several previous functional imaging studies, prefrontal activation may be spatially inseparable from FEF activation due to a limited spatial resolution and interindividual anatomical variability.

Several occipital areas that have been regarded as the "dorsal visual pathway" [Ungerleider and Haxby, 1994] exhibited the double-peaked activation pattern similar to that in the frontal group. This is reasonable because the presentation of peripheral targets and execution of saccade were synchronized in our modified memory-guided task. Nevertheless, the frontal and occipital groups constituted two different groups, the difference between which was most clearly illustrated by PC3. Although we cannot exclude the possibility that PC3 reflects the different patterns of interregional variability in HRFs across the subjects, it may also be possible that PC3 reflects the intersubject difference in the relative load on the two discrete networks, that is, the frontal and occipital groups.

In summary, during the memory-guided saccade task, the frontal and occipital groups exhibited the double-peaked activation pattern that appeared to be associated with the target presentation and saccade execution, and the parietal group exhibited the single-humped pattern that appeared to reflect a neural activity during the maintenance of target location. The different time courses of activation are consistent with the different roles of the frontal and parietal regions in the visually guided saccade as suggested in the neuropsychological observations and lesion studies in animals. Because it is reasonable to consider that our modified version of the memory-guided saccade task requires subjects to maintain the location of the target predominantly in the sensory domain, the results support the postulated functional differentiation between the parietal and frontal components, that is, the former is involved in the sensory map and the latter in the motor program.

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REVIEW ARTICLE

Cocaine, reward, movement and monoamine transporters

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Recent evidence enriches our understanding of the molecular sites of action of cocaine reward and locomotor stimulation. Dopamine transporter blockade by cocaine appears a sufficient explanation for cocaine-induced locomotion. Variation in DAT appears to cause differences in locomotion without drug stimulation. However, previously-held views that DAT blockade was the sole site for cocaine reward have been replaced by a richer picture of multitransporter involvement with the rewarding and aversive actions of cocaine. These new insights, derived from studies of knockout mice with simultaneous deletions and/or blockade of multiple transporters, provide a novel model for the rewarding action of this heavily-abused substance and implicate multiple monoamine systems in cocaine's hedonic activities.
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Cocaine is a prototypical psychomotor stimulant that increases locomotor activity and elevates mood with rewarding euphoria.[1] It also produces fearful and jittery aversive effects in many who take it.[2,3] Cocaine blocks monoamine uptake by neuronal plasma membrane transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET), and can also block ligand- and voltage-gated channels with somewhat lower potencies.[4] Understanding the relationships between cocaine's molecular actions and its psychomotor stimulant and aversive properties has remained surprisingly incomplete. This review focuses on recent advances in our understanding of the complex relationships between cocaine's molecular actions and its locomotor stimulant, rewarding and aversive properties.

The 'DAT-is-it' hypothesis drove thinking about cocaine reward for at least a decade, ideas widely linked to Kuhar, Spealman and their colleagues[4–8] who gathered evidence from structure-activity relationships of transporter-blocking compounds with differential potencies at DAT, SERT and NET. They examined relationships between potencies of these compounds in tests of reward and binding affinities at each of the monoamine transporters. They each identified the best correlations for the rewarding effects of cocaine with DAT blockade. These authors also cited prior work from lesion studies[9–12] and electrical brain stimulation studies[13–17] that implicated dopamine pathways in reward mechanisms. These studies in turn built on prior pharmacologic blockade studies[18–22] that revealed altered psychostimulant self-administration with dopamine depletions or with dopamine receptor blocking drugs.

This 'DAT-is-it' hypothesis was a major motivation for work that resulted in cloning DAT cDNAs, genes and gene variants in our laboratory and others.[23–30] It drove characterization and study of DAT gene variants in humans[29,31–53] and animal models.[54,55] It led to the production and characterization of DAT-over- and under-expressing and DAT-knockout mice strains.[56–59] These studies revealed an exquisite DAT-dependence of cocaine's locomotor stimulation. By contrast, they also produced surprising results that refuted the strong 'DAT-is-it' hypothesis of cocaine reward.

Cocaine-induced locomotion: DAT is it

DAT variation in humans and mice links robustly with variation in both baseline locomotion and/or psychostimulant-induced locomotor activities. Three strains of DAT knockout or knockdown mice produced in separate laboratories each have substantially elevated locomotor activities in novel environments.[56,58,59] Supranormal levels of locomotor activity remain elevated even after several hours of habituation. When cocaine is administered, wild-type animals display substantial increases in their locomotion that are several times greater than their habituated baseline activity levels. However, DAT knockout mice display no further cocaine-induced increase in activity.[56,58,60] Neither SERT nor NET gene knockouts provide any comparable reduction in cocaine-induced locomotion.[60,61] Knockout mice have a lifetime to adapt to loss of a single gene product, providing cautions in interpretation (*see below*). Nevertheless, results from these mice clearly

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indicate that DAT is a necessary and principal site for cocaine-induced locomotor stimulation.

Genetic variation at the mouse and human DAT loci also contributes to interesting locomotor phenotypes. Mouse quantitative trait locus (QTL) studies localize a portion of the B×D recombinant inbred strain difference in baseline and psychomotor stimulant-induced locomotion near the DAT locus, while additional variance maps to a second locus that appears to regulate DAT expression.[62] Human DAT gene markers have reproducibly linked with attention deficit hyperactivity disorder (ADHD).[29,63–68] DAT polymorphisms can be linked more strongly to the hyperactivity than to the attention deficits in this disorder.[65] Variation in DAT activity is thus a significant contributor to 'baseline' locomotor activity levels, while DAT is absolutely necessary for cocaine-induced locomotor stimulation.

Cocaine, reward and aversion: rich patterns of multi-transporter involvement

This simple story for DAT and cocaine locomotion contrasts strikingly with results for rewarding properties of cocaine. We have tested DAT knockout mice in the conditioned place preference (CPP) paradigm[58] that measures preference for an environment paired with a drug. Homozygous DAT knockout mice express perfectly intact cocaine conditioned place preference. Another DAT knockout strain was also rewarded by cocaine in an operant self-administration paradigm.[69] Two strains of DAT knockouts thus provided convincing evidence that DAT was *not* necessary for cocaine reward, results fatal to any strong version of the 'DAT-is-it' hypothesis for cocaine reward.

Studies of reward in mice with deletions of the other monoamine transporters at which cocaine acts did not provide any clear alternative single transporter site for cocaine's rewarding actions. Cocaine was no less rewarding in SERT knockouts tested in the CPP paradigm[58] or in NET knockout mice.[61] The failure of any single monoamine transporter gene knockout strain to eliminate cocaine reinforcement and reward thus left open several possible roles for these transporters in cocaine reward/reinforcement in wild-type mice. These even included the possibility that non-transporter molecular sites of cocaine could be involved in cocaine reward/reinforcement.

We chose to pursue a different hypothesis: that cocaine normally works as a 'dirty drug', producing rewarding effects through simultaneous actions at more than one transporter site. Multiple molecular sites for cocaine's rewarding/reinforcing actions could provide redundancies so that no one site alone would be absolutely required for cocaine reward. Such redundancies could even be enhanced by compensatory mechanisms active in mice that develop without one or more transporters. If cocaine normally altered activities in several parallel or interactive brain systems with substantial redundancies, the systems expressing the remaining transporter(s) might thus compensate for loss of cocaine-modulated activities in knockout mouse brain systems that normally expressed the absent transporters, maintaining cocaine reward.

We tested whether DAT- and SERT-expressing systems could each provide such redundancy in the long-term absence of the other transporter. We constructed double knockout mice with deletions of both the DAT and SERT genes, examined their baseline behavioral and biochemical features, and tested their preference for cocaine.[60] Deletions of all of the DAT combined with removal of either half or all of the SERT completely eliminated cocaine reward assessed in the CPP paradigm. These results defined, for the first time, a minimal set of gene deletions necessary for elimination of cocaine reward. More recent evidence underscores the specificity of these observations. Double knockouts of both SERT and NET fail to reduce cocaine reward.[70] In fact, SERT/NET double knockouts dramatically enhance cocaine place preference.

While we have thus identified a specific group of sites whose elimination can totally block measures of cocaine reward, we have also identified potential sites for some of the aversive effects reported by cocaine users. Data from reduced or enhanced cocaine reward in the absence of specific transporters or transporter combinations can be tentatively assembled with prior data from other transporter blocking drugs as follows (see also Figure 1):

DAT appears to be the transporter most associated with rewarding properties of cocaine in conditioned place preference tests. Compounds with substantial

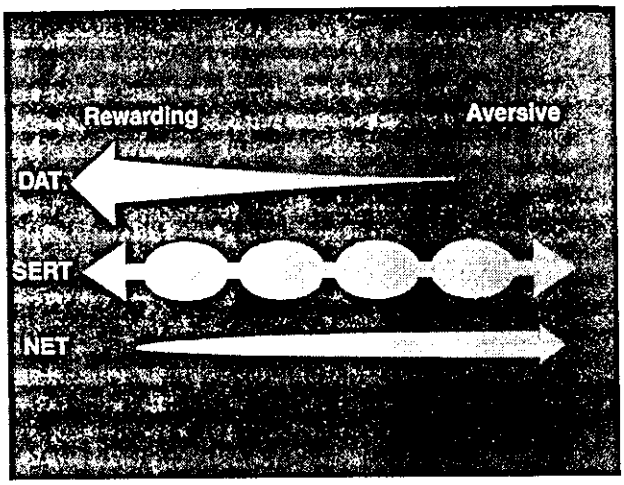

Figure 1 Model for the relative contributions of blockade of the transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET) on cocaine reward and aversive features in wild-type animals. DAT: Dopamine transporter blockade is drawn as exerting largely rewarding properties. SERT: Serotonin transporter blockade is drawn as exerting a combination of rewarding and aversive properties. Uneven distributions of these influences (lumps in the figure) are meant to suggest different properties of action at different serotonin receptor subtypes. NET: Norepinephrine transporter blockade is drawn as exerting largely aversive properties. These contributions change significantly in knockout mice, as noted in the text.

selectivity for DAT can produce place preferences and are self-administered by animals, although often less avidly than cocaine (for review see Rothman and Glowa).[71] However, as noted above, DAT is not 'it', since DAT deletion alone does not eliminate cocaine reward.[58]

Data from DAT knockouts are also tempered by results examining the role of SERT and NET in the maintenance of cocaine reward in these mice. The selective SERT blocker fluoxetine and the selective NET blocker nisoxetine both produce strong place preferences in DAT knockouts but not in wild-type mice or in heterozygote mice with one knockout DAT allele, one wild-type DAT allele and half wild-type levels of DAT expression.[70] These data clearly indicate that blockade of SERT or NET can acquire rewarding properties in DAT's lifelong absence. How much DAT removal and how long this removal needs to be carried out to achieve these striking effects remain interesting current questions. The adaptive mechanisms that make fluoxetine and nisoxetine rewarding in DAT knockouts could well contribute to the reward that cocaine confers in DAT knockout mice, since cocaine retains its ability to block both SERT and NET in these animals. Recent reports that the NET blocker roboxetine enhances nucleus accumbens dopamine in DAT knockout but not in wild-type mice[72] could contribute one biochemical substrate for the nisoxetine reward found in DAT knockouts. Such mechanisms may not be relevant for fluoxetine reward, however, since a selective SERT blocker did not increase nucleus accumbens dopamine levels in the same study.

SERT blockade could well contribute to both rewarding and aversive properties of cocaine. Such explanations seem among the most plausible current explanations for otherwise apparently contradictory data that demonstrate: (1) enhanced cocaine reward in SERT knockout mice;[58] (2) enhanced fluoxetine reward in DAT and in NET knockout mice;[70] and (3) ablated cocaine reward in DAT/SERT combined knockout mice.[60]

SERT blockade might normally produce a combination of rewarding and aversive features by augmenting serotonin actions at different serotonin receptor subtypes expressed differentially in distinct serotonergic circuits. If these rewarding and aversive features normally balanced each other to produce little net reward or aversion with SERT blockade, fluoxetine or other selective serotonin reuptake inhibitors would display minimal aversion and also little abuse liability in humans, as has been observed.[73–75] The fluoxetine reward acquired by DAT and by NET knockout mice is consistent with the idea that each of these knockouts shifts the balance between rewarding and aversive serotonergic components, although not necessarily in identical ways.

NET blockade may well contribute less to rewarding and more to aversive properties of cocaine in wildtype animals. These observations are consistent with frequent human clinical self-reports of 'jitteriness' with cocaine use.[76,77] They accord with evidence for cocaine aversive features in some animal models.[78–80] They fit with the failure of mazindol, a widely used combined DAT/NET blocker, to exhibit any striking human clinical abuse liability and with its not-infrequent discontinuation due to jitteriness and sleep disturbances.[81–83]

Caveats: brain changes in knockout mice and cocaine reward

The precise neuroadaptive changes that may contribute to the differences in the rewarding consequences of monoamine transporter blockade in single and multiple transporter knockout mice are unknown. The brains of mice of each of these knockout strains appear grossly normal, although the smaller brains of DAT KO mice are consistent with the smaller overall size of these mice.[84] Each single transporter knockout exhibits a distinctive pattern of brain neurochemical rearrangements,[58,60,61,85,86] many of which can be tentatively linked to homeostatic mechanisms triggered by increased tonic levels of extracellular neurotransmitters. Thus, the DAT knockouts that display enhanced extracellular dopamine levels[56,87] and reduced DA clearance[56,87,88] also display altered levels of expression of dopamine D1, D2 and D3 receptors levels tyrosine hydroxylase, dopamine-modulated neuropeptides, and other features.[89,90] Microarray studies of mRNA from the brains of DAT and of SERT knockouts also identify dozens of genes whose expression is consistently altered more than two-fold.[91] None of these characterizations provide evidence for large supra-additive knockout influences on any brain neurochemical, microdialysis or gene expression profile when mice with multiple knockouts have been examined to date, however. No current known neurochemical alteration follows the pattern of retained or absent cocaine reward across these multiple knockout strains.

In DAT knockouts, cocaine could also acquire novel properties by modulating dopamine acting as a 'false transmitter' in cells in which it is not synthesized. Since NET has good affinities for dopamine and SERT can even accumulate dopamine with lower affinity, some of the dopamine that is synthesized in dopaminergic neurons of DAT knockout mice could become a 'false transmitter' at adjacent norepinephrine or serotonin terminals. In a similar fashion norepinephrine could be a false transmitter at adjacent dopaminergic or serotonergic terminals in NET knockouts, and/or serotonin a false transmitter in SERT knockout dopamine or norepinephrine terminals.[92,93] While some false transmission thus seems likely in these knockouts, it seems an unlikely explanation for all of the current data derived from these mice. NET and SERT blockade by cocaine fails to provide the robust enhancements of striatal dopamine efflux in DAT knockouts that would be expected if false transmission were widespread in these mice.[85] Although cocaine and the NET blocker reboxetine do enhance dopamine efflux in the nucleus accumbens of DAT knockouts, rewarding SERT blockade by fluoxetine fails to enhance this dopamine efflux.[72] Further studies will be necessary to define how much of the cocaine, fluoxet-

ine and/or nisoxetine rewarding responses found in DAT knockouts depend on false neurotransmission. Equally plausible explanations for current data include adaptations in circuits such as those that regulate dopamine cell firing rates and dopamine release after cocaine administration.[94–103]

Conclusions

Lifelong deletions of DAT, SERT, NET or transporter combinations each distinctively alter the rewarding effects of blocking the remaining transporters. These results are consistent with the idea that cocaine normally works as a dirty drug that provides both rewarding and aversive properties by distinct actions at these three transporters. The combined observations document adaptations that occur in knockout mice. They also tentatively fit with the human abuse liability of a number of widely used drug classes whose members act differentially at these three monoamine transporters. Current data from monoamine transporter knockouts thus provide several novel pathways to thinking about cocaine therapeutics. Drugs that block cocaine's uptake-inhibiting actions at DAT and SERT but allow it to continue to block uptake by NET could provide a possible means for cocaine antagonism at 'rewarding' transporters and continued cocaine action at its 'aversive' sites. Each of the observations summarized above provides new pharmacological routes to dramatically alter the rewarding valences of cocaine.

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ORIGINAL ARTICLE

Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis

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ABSTRACT

Susceptibility to drug dependence and drug-induced psychoses is influenced not only by the pharmacological effects of the drug but also by the genetic factors of the individual. To clarify the latter, we investigated the association between methamphetamine (METH) dependence/psychosis and the *hDAT1* gene (SLC6A3) encoding the dopamine transporter, which is the primary site of METH activity in the brain. Four exonic polymorphisms of the *hDAT1* gene, 242C/T (exon 2), 1342A/G (exon 9), 2319G/A (3′UTR), and VNTR (3′UTR) were examined. Although there was no significant difference in genotypic and allelic distribution of the four polymorphisms between all METH dependence/psychosis patients ($N = 124$) and controls ($N = 160$), the patients with METH psychosis lasting for 1 month or more after discontinuance of METH consumption showed a significant excess of nine- or fewer repeat alleles of the VNTR in 3′UTR of the *hDAT1* gene ($P = 0.0054$, OR = 4.24, 95% CI = 2.46–7.31). The present study demonstrated that the presence of nine- or fewer repeat alleles of hDAT1 is a strong risk factor for a worse prognosis of METH psychosis.

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Keywords: dopamine transporter gene; amphetamine psychosis; drug dependence; Japanese; prognosis

INTRODUCTION

Genetic contributions to the etiology of substance abuse and dependence have been demonstrated by family, twin, and adoption studies.[1–4] Kendler *et al*[2] showed that twin resemblance increases from occasional use to abuse or dependence in a population-based study of twins. The estimated heritability of cocaine use was 0.39, whereas the heritabilities of abuse and dependence were 0.79 and 0.65, respectively.[5] The heritability of abuse/dependence was relatively low for sedatives and opiates, but for psychostimulants such as amphetamines, it was as high as cocaine.[6]

The primary site of biological activity of methamphetamine (METH) is the dopamine transporters in the brain. METH is taken into cytosol by dopamine transporters on the synaptic terminals of dopamine neurons, and endogenous dopamine is concurrently released through the transporters by carrier-exchange mechanisms, which results in a robust increase in the dopamine concentration in the synaptic clefts. The increase of dopamine release, especially in the accumbens, through dopamine transporters due to METH is primarily responsible for induction of its reinforcing and psychogenic effects.[7] Therefore, it is possible that variations in the dopamine transporter function produce the differences between individuals in vulnerability to METH dependence and/or

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psychosis. The *hDAT1* gene (SLC6A3), which encodes the human dopamine transporter, was reported to be associated with other dependence behaviors such as alcohol dependence[8] and tobacco smoking.[9,10] To examine the possible genetic influence of *hDAT1* gene variants on the development of METH dependence/psychosis, four exonic polymorphisms, 242C/T (exon 2), 1342A/G (exon 9), 2319G/A (3'UTR of exon 15), and VNTR (3'UTR of exon 15), of the *hDAT1* gene were analyzed in patients with METH dependence/psychosis in Japan.

The genotypic distribution and allelic frequency of three SNPs in exons 2, 9, and 15, and VNTR polymorphisms in the

Table 1 Genotypic and allelic distributions of *hDTA1* gene polymorphisms of METH dependence/psychosis

		Genotype				Allele		
242C>T	*N*	*CC (%)*	*CT (%)*	*TT (%)*	*P*	*C (%)*	*T (%)*	*P*
Control	106	98.1	1.9	0.0		99.1	0.9	
Patients	103	98.1	1.9	0.0	0.97	99.0	1.0	0.98
Duration of psychosis								
Transient	54	96.3	3.7	0.0		98.1	1.9	
Prolonged	38	100.0	0.0	0.0	0.19	100.0	0.0	0.19
Spontaneous relapse								
Positive	35	100.0	0.0	0.0		100.0	0.0	
Negative	60	96.7	3.3	0.0	0.22	98.3	1.7	0.22
Multisubstance abuse								
No	33	100.0	0.0	0.0		100.0	0.0	
Yes	69	97.1	2.9	0.0	0.26	98.6	1.4	0.27
		Genotype				Allele		
1342A>G	*N*	*AA (%)*	*AG (%)*	*GG (%)*	*P*	*A (%)*	*G (%)*	*P*
Control	159	81.1	16.4	2.5		89.3	10.7	
Patients	124	81.5	17.7	0.8	0.80	90.3	9.7	0.69
Duration of psychosis								
Transient	66	83.3	16.7	0.0		91.7	8.3	
Prolonged	47	76.6	21.3	2.1	0.50	87.2	12.8	0.28
Spontaneous relapse								
Positive	41	80.5	19.5	0.0		90.2	9.8	
Negative	71	81.7	16.9	1.4	0.75	90.1	9.9	0.97
Multisubstance abuse								
No	36	77.8	19.4	2.8		87.5	12.5	
Yes	89	80.9	19.1	0.0	0.91	90.4	9.6	0.50
		Genotype				Allele		
2319G>A	*N*	*GG (%)*	*GA (%)*	*AA (%)*	*P*	*G (%)*	*A (%)*	*P*
Control	157	58.6	36.3	5.1		76.8	23.2	
Patients	124	55.6	38.7	5.6	0.88	75.0	25.0	0.69
Duration of psychosis								
Transient	63	57.1	38.1	4.8		76.2	23.8	
Prolonged	47	51.1	42.6	6.4	0.58	72.3	27.7	0.81
Spontaneous relapse								
Positive	41	48.8	41.5	9.8		69.5	30.5	
Negative	71	57.7	39.4	2.8	0.28	77.5	22.5	0.19
Multisubstance abuse								
No	36	58.3	36.1	5.6		76.4	23.6	
Yes	88	53.4	42.0	4.5	0.76	74.4	25.6	0.70