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Ⅳ. 研究成果の刊行物・別刷

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Effects of dopaminergic and serotonergic manipulation on emotional processing: A pharmacological fMRI study

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Recent neuroimaging studies have demonstrated abnormal central emotional processing in psychiatric disorders. The dopamine (DA) systems and serotonin (5-HT) systems are the main target of psychopharmacotherapy. DA D_2 receptor antagonists and selective serotonin reuptake inhibitors (SSRIs) are widely used in psychiatric practice. Investigating the effects of these drugs on emotional processing should lead to a better understanding of the pathophysiology and pharmacotherapy of neuropsychiatric disorders. We aimed to examine effects of dopaminergic and serotonergic manipulation on neural responses to unpleasant pictures in healthy volunteers using pharmacological fMRI.

Thirteen healthy male subjects participated in a single-blind, randomized, placebo-controlled design study. Each subject participated in three fMRI sessions. In each session, participants were orally administered either 25 mg of sultopride or 50 mg of fluvoxamine or placebo prior to scanning, and neural responses to unpleasant and neutral pictures were recorded.

Despite no significant differences being found in the subjective ratings of affective pictures across three sessions, compared to placebo, acute treatments of DA D_2 receptor antagonists and SSRIs commonly attenuated the amygdala activity, although both treatments had slightly different modulatory effects on other components of the neural circuit of emotional processing. This study has shown that even acute treatment of drugs that manipulate neurotransmitter systems could affect brain activation associated with emotional processing in human brain. At the same time, our findings suggest that pharmacological fMRI could be a powerful tool for investigating the neurophysiological properties of drugs targeting neuropsychiatric disorders.

Keywords: Pharmacological fMRI; Dopamine D_2 receptor antagonists; SSRIs; Emotion

Introduction

Recent neuroimaging studies have revealed abnormal central emotional processing in patients with psychiatric disorders such as mood disorders (Drevets, 2000), schizophrenia (Paradiso et al., 2003; Takahashi et al., 2004), and anxiety disorders (Kent and Rauch, 2003). However, the majority of patients examined in neuroimaging studies, especially those with schizophrenia, were taking drugs, and their possible effects on neural responses have not been clarified.

The dopamine (DA) systems and serotonin (5-HT) systems are the main target of pharmacological treatment of these psychiatric disorders. DA D_2 receptor antagonists and selective serotonin reuptake inhibitors (SSRIs) are widely used and tolerated in clinical practice as antipsychotics and antidepressants/anxiolytics, respectively. To investigate the effects of these drugs on emotional processing will give a better understanding of the pathophysiology of psychiatric disorders and the neurophysiological properties of drugs targeting neuropsychiatric disorders.

DA systems arise from two primary midbrain areas. The mesostriatal system originates from the substantia nigra pars compacta (A9) and innervates the striatum, whereas the mesocorticolimbic system originates mainly from the ventral tegmental area (A10) and innervates the amygdala, hippocampus, and frontal cortical areas (Pralong et al., 2002). The DA systems are extensively distributed throughout the network of the limbic–cortical–striatal–pallidal–thalamic (LCSPT) circuit implicated in emotional processing (Drevets, 2000; Pralong et al., 2002). The components of the DA systems and LCSPT circuit overlap (Cardinal et al., 2002; Groenewegen and Uylings, 2000). Our PET study demonstrated that mesocorticolimbic regions (amygdala, hippocampus, thalamus, and anterior cingulate) are relatively rich in DA D_2 receptors, besides striatal regions (Okubo et al., 1999). Thus, DA D_2 receptor antagonists could be considered to

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substantially modulate emotional processing, particularly in the mesocorticolimbic systems. A recent study reported that acute administration of the DA D_2 receptor antagonist sulpiride impaired emotional memory, and the need for sensitive tests to study dopaminergic modulation of emotional processing was highlighted (Mehta et al., 2005).

Pharmacological fMRI is a non-invasive in vivo method that has the potential to investigate the effects of pharmacological manipulation on cognitive and emotional processing in the human brain, and this tool is expected to have a major impact on clinical practice and drug discovery (Honey and Bullmore, 2004). Among major neurotransmitter systems, DA systems have been widely investigated by the use of pharmacological fMRI. However, most pharmacological fMRI studies of DA systems have focused on cognitive or motor function, and pharmacological fMRI studies using emotional tasks are limited (Honey and Bullmore, 2004). An fMRI study has reported that dopaminergic drug therapy such as levodopa or DA agonists partially restored amygdala activation due to emotional task in Parkinson's disease (PD) patients who showed no significant amygdala activation during drug-off states (Tessitore et al., 2002). In addition, another fMRI study has demonstrated that amphetamine potentiated the response of the amygdala during an emotional task (Hariri et al., 2002a). Hence, using pharmacological fMRI, we aimed to investigate the effects of DA D_2 blockade on neural activity in the response to unpleasant pictures in healthy volunteers, hypothesizing that blockade of DA D_2 receptors would suppress the amygdala activity in response to emotional stimuli.

Another major neurotransmitter system, 5-HT systems, originates from 5-HT neurons in the raphe nuclei, and 5-HT fibers project to the amygdala, hippocampus, and frontal cortical area that are the key nodes of emotional processing (Buhot, 1997; Pineyro and Blier, 1999). Abnormally elevated amygdala activities in depressive patients have consistently been reported, and treatments by antidepressants such as SSRIs were reported to normalize the elevated amygdala activity (Drevets, 2000). Exaggerated amygdala responses were also reported in anxiety disorders (Rauch et al., 2003), and SSRIs are coming into use in the treatment of anxiety disorders (Kent et al., 1998).

Although it has long been thought that there is a delay of several weeks before a true antidepressant effect occurs, recent studies have led to the notion that antidepressants work within the first week (Posternak and Zimmerman, 2005), and several studies reported that even acute antidepressant treatment could facilitate positive emotional processing and work to redress negative biases in emotional processing (Harmer et al., 2003a,b). These results suggest that a single dose of an antidepressant can facilitate positive emotional processing and inhibit negative emotional processing. In fact, a recent evoked potential study has investigated the effects of acute treatment of SSRIs on emotional processing. In that study, acute administration of SSRIs attenuated cortical responses to unpleasant pictures and enhanced cortical responses to pleasant pictures (Kemp et al., 2004). However, evoked potential studies have a major limitation in that they cannot detect activity of subcortical structures. Since the amygdala receives dense serotonergic input from the raphe nuclei and has a high density of 5-HT transporters, it is considered to be a prime site for the anxiolytic action of SSRIs (Kent et al., 1998). Therefore, the need to investigate the effects of the acute administration of SSRIs on the amygdala is emphasized. We hypothesized that the acute administration of SSRIs, as well as DA D_2 antagonists, would suppress the response of the amygdala, a key node of negative emotional processing, and would modulate the response in the related brain area involved in negative emotional processing in healthy volunteers.

Methods

Participants

Thirteen healthy right-handed Japanese male subjects (mean age 29.2 ± 5.1 years, mean height 170.0 ± 4.1 cm, mean weight 65.5 ± 9.0 kg) were recruited from the surrounding community. They did not meet the criteria for any psychiatric disorder. None of the controls were taking 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 underwent an 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 Committee.

Materials

Stimulus materials were taken from the International Affective Picture System (IAPS) (Lang et al., 1997). Neutral and unpleasant pictures were selected according to the subjective ratings provided by IAPS. We employed 48 pictures from each class. Slides of the two emotional classes were matched for content (faces, human figures, animals, objects, scenery). The pictures were projected via a computer, and a telephoto lens onto a screen mounted on a head-coil. The experimental design consisted of 6 blocks for each of the 2 conditions (neutral, unpleasant) interleaved with 24-s rest periods. The order of presentation for the 2 conditions was fixed in the neutral–unpleasant sequence.

During the rest condition, subjects viewed a crosshair pattern projected to the center of the screen. In each 24-s block, 8 different pictures of the same emotional class were presented for 3 s each. During the scans, the subjects were instructed to press a selection button with the right index finger, indicating how each picture made them feel using a 3-point analog scale (1 = neutral, 2 = slightly unpleasant, 3 = extremely unpleasant).

Physical data

Participants were checked for blood pressure (BP), heart rate (HR), and respiration rate (RR) before administrations of drugs and just before scanning.

Drug administration

We used sultopride (SUL), a substituted benzamide derivative, as a selective DA D_2 receptor antagonist. SUL has higher lipophilicity than other substituted benzamide derivatives such as amisulpride or sulpiride and penetrates the blood–brain barrier more easily (Kapur et al., 2002; Mizuchi et al., 1983; Moller, 2003). For this reason, we used SUL with the purpose of minimizing the effect of DA D_2 receptor antagonist on peripheral DA receptors in blood vessels or the pituitary, which are outside the blood–brain barrier. The dosage of SUL was determined by our recent positron emission tomography (PET) study, in which the ED_{50} (concentration required to induce 50% occupancy) value of

SUL was 8.7 mg for dose and 32 ng/ml for plasma concentration, and 25 mg of SUL resulted in sufficient plasma concentration to occupy approximately 60–70% of dopamine D_2 receptors (unpublished data). It has been shown that occupancy in the approximate range of 60–70% of central dopamine D_2 receptors is needed to produce an antipsychotic effect. Higher receptor occupancy (more than 80%) is associated with extrapyramidal effects (Farde et al., 1992; Kapur et al., 2000). Since DA D_2 receptor antagonists are mainly used with the purpose of obtaining antipsychotic effects, we aimed to elucidate the effects of occupancy of about 60–70% of central DA D_2 receptors in this study. We used 50 mg of fluvoxiamine (FLU) as SSRI. A previous study reported that minimally effective dosage was 50 mg/day (Walczak et al., 1996), and our previous data demonstrated that the ED_{50} value of FLU was 7.4 mg for dose and 4.2 ng/ml for plasma concentration and that 50 mg of FLU resulted in enough plasma concentration to occupy approximately 80% of 5-HT transporters (Suhara et al., 2003).

The study was a single-blind, randomized, placebo-controlled cross-over design. Each subject participated in three fMRI sessions separated by a minimum washout period of 14 days. Because it was reported that the elimination half-life of SUL in plasma was 3.6 h (Kobari et al., 1985) and that of FLU was 15 h in human (DeVane and Gill, 1997), it was considered that a 14-day washout period was sufficient and that the order of the drugs did not affect the plasma concentration of each drug. In each session, participants were given orally either 25 mg of SUL or 50 mg of FLU or placebo (PBO) (lactose) prior to scanning in a single-blind manner so that appropriate medical treatment could be administered in the event of adverse responses. The order of drug administration was counterbalanced across the subjects. The drug administration order consisted of 6 combinations (SUL–FLU–PBO, SUL–PBO–FLU, FLU–SUL–PBO, FLU–PBO–SUL, PBO–SUL–FLU, and PBO–FLU–SUL), and we randomly assigned each of the combinations to each subject. To ensure maximum and stable plasma concentrations of SUL and FLU, SUL and PBO were given 2 h before scanning, and FUL was given 5 h prior to scanning.

Images acquisition

The images were acquired with a 1.5 T Signa system (General Electric, Milwaukee, WI). Functional images of 264 volumes were acquired with T2*-weighted gradient echo planar imaging sequences sensitive to the blood oxygenation level dependent (BOLD) contrast. Each volume consisted of 30 transaxial contiguous slices with a slice thickness of 4 mm to cover almost the whole brain (flip angle, 90°; TE, 50 ms; TR, 3 s; matrix, 64 × 64; field of view, 24 × 24 cm). High-resolution, T1-weighted anatomic images were acquired for anatomic comparison (124 contiguous axial slices, 3D Spoiled-Grass sequence (SPGR), slice thickness 1.5 mm, TE, 9 ms; TR, 22 ms; flip angle, 30°; matrix, 256 × 192; field of view, 25 × 25 cm).

Analysis of functional imaging data

Data analysis was performed with statistical parametric mapping software package (SPM02) (Wellcome Department of Cognitive Neurology, London, UK) running with MATLAB (Mathworks, Natick, MA). All volumes were realigned to the first volume of each session to correct for subject motion and were spatially normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. After normalization, all scans had a resolution of 2 × 2 × 2 mm^3. Functional images were spatially smoothed with a 3D isotropic Gaussian kernel (full width at half maximum of 8 mm). Low frequency noise was removed by applying a high-pass filter (cutoff period = 192 s) to the fMRI time series at each voxel. A temporal smoothing function was applied to the fMRI time series to enhance the temporal signal-to-noise ratio. Significant hemodynamic changes for each condition were examined using the general linear model with boxcar functions convoluted with a hemodynamic response function. Statistical parametric maps for each contrast of t statistic were calculated on a voxel-by-voxel basis. The t values were then transformed to unit normal distribution, resulting in Z scores.

To assess the specific condition effect, we used the contrasts by subtracting the BOLD signals in response to the neutral condition from those in response to the unpleasant condition (U–N contrast) in single-subject analysis. For each drug treatment condition, the U–N contrast images obtained from single-subject analysis were entered into group analysis. A random effects model, which estimates the error variance for each condition across the subjects, was implemented for group analysis. This procedure provides a better generalization for the population from which data are obtained. A one-sample t test was used to determine group activation for each drug. To compare the effect of drugs on the U–N contrast, we performed paired t tests (SUL vs. PBO, FLU vs. PBO, PBO vs. SUL, and PBO vs. FLU) to test relative differences in the pattern of neural activation by subtracting the unpleasant minus neutral U–N contrasts of PBO treatment from the U–N contrasts of drug (SUL/FLU) treatments and vice versa. Significant clusters of activation were determined using the conjoint expected probability distribution of the height and extent of Z scores with the height and extent threshold. Coordinates of activation were converted from MNI coordinates to the Talairach and Tournoux coordinates using the mni2tal algorithm (M. Brett, Cambridge, MA) (Talairach and Tournoux, 1988).

To examine individual differences in the effect of the two drugs on amygdala activation, we plotted the signal changes elicited by unpleasant condition compared to neutral condition in the amygdala. We used signal changes of the left amygdala (x, y, z = −14, −3, −22) and the right amygdala (x, y, z = 22, −3, −15), showing the largest signal reduction by SUL treatment as revealed by group analysis (paired t test). Similarly, we examined the signal changes of the left amygdala (x, y, z = −24, −12, −11) and the right amygdala (x, y, z = 26, 1, −22) for FUL treatment. We compared the mean signal changes in the left amygdala (x, y, z = −24, −12, −11) and the right amygdala (x, y, z = 26, 1, −22) elicited by unpleasant condition compared to neutral condition during PBO treatment and drug (SUL and FLU) treatments.

Results

Physical data

Two-way repeated-measures analysis of variance of BP (max), BP (min), HR, and RR showed no significant main effect of drug (P = 0.485, P = 0.744, P = 0.580, P = 0.556) nor a significant main effect of time (P = 0.514, P = 0.466, P = 0.248, P = 0.673) or

interaction ($P = 0.165$, $P = 0.123$, $P = 0.637$, $P = 0.683$), respectively.

Self-rating

Two-way repeated-measures analysis of variance of the ratings showed a significant main effect of condition ($P < 0.001$) but not a significant main effect of drug ($P = 0.66$) or interaction ($P = 0.59$). In other words, the mean ratings of unpleasant pictures were significantly greater than those of neutral pictures across the treatment group.

fMRI result

During PBO treatment, unpleasant condition relative to neutral U–N contrast revealed greater activations in the visual cortex, dorsal lateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), parietal cortex, insula, amygdala, thalamus, globus pallidus, and brainstem. During SUL treatment, U–N contrast revealed greater activations in the visual cortex, DLPFC, medial prefrontal cortex (MPFC), OFC, parietal cortex, hippocampus, thalamus, caudate body, and brainstem. During FUL treatment, U–N contrast revealed greater activations in the visual cortex,

Table 1
Brain activation in unpleasant condition relative to neutral condition during PBO, SUL, and FLU treatment

Brain region	Coordinates			BA	Z score	t value	Voxels
PBO							
R. visual cortex (LG, Cu, MOG, IOG, FG, MTG)	48	−66	−5	17, 18, 19, 37, 39	5.76	14	5404
L. visual cortex (LG, Cu, MOG, IOG, FG, MTG)	−44	−80	−3	17, 18, 19, 37, 39	5.29	11.02	
R. DLPFC (MFG, IFG)	46	15	32	9	4.53	7.65	331
L. DLPFC (IFG)	−51	7	33	9	3.82	5.5	53
R. OFC (IFG)	38	26	−18	47	3.49	4.73	59
R. OFC (IFG)	48	19	−8	47	3.27	4.27	27
L. OFC (IFG)	−36	17	−13	47	3.24	4.22	18
R. parietal cortex	30	−58	49	7	4.1	6.28	44
L. insula	−40	9	−6	13	3.36	4.47	31
R. amygdala	18	−3	−15		3.77	5.38	48
L. amygdala	−10	−1	−17		4.03	6.07	104
R. thalamus, GP	14	−2	7		3.71	5.24	66
L. thalamus, GP	−10	2	4		3.95	5.86	78
Brainstem	−4	−33	−2		4.78	8.61	356
SUL							
R. visual cortex (LG, Cu, MOG, IOG, FG)	26	−90	−4	17, 18, 19, 37	4.25	6.71	1109
L. visual cortex (LG, Cu, MOG, IOG, FG)	−28	−91	12	17, 18, 19, 37	4.97	9.45	1537
R. DLPFC (MFG, IFG)	50	26	15	8, 9, 46	4.17	6.47	673
L. DLPFC (IFG)	−48	17	23	9	4.04	6.1	377
MPFC (SFG, MFG, CG)	−2	31	41	8, 32	3.96	5.89	286
MPFC (SFG, MFG,)	−2	50	27	9	3.7	5.22	37
L. OFC (IFG)	−38	15	−11	47	3.48	4.7	45
R. OFC (MFG, IFG)	26	30	−18	11, 47	3.84	5.56	156
R. parietal cortex	32	−60	51	7	3.64	5.07	40
L. hippocampus, PHG	−28	−20	−7	27	4.28	6.82	94
R. thalamus	8	−19	8		4.26	6.76	732
L. thalamus, caudate body	−6	−17	6		4.16	6.44	
Brainstem	−2	−29	−5		4.15	6.42	
FLU							
R. visual cortex (LG, MOG, IOG, FG)	44	−71	−13	18, 19, 37	4.61	7.96	1623
L. visual cortex (LG, Cu, MOG, IOG, FG, ITG)	−38	−86	−4	17, 18, 19, 37	5.33	11.28	2154
R. DLPFC (MFG, IFG)	51	19	34	8, 9	4.44	7.34	323
R. DLPFC (IFG)	48	30	13	46	3.63	5.05	43
L. DLPFC (MFG, IFG)	−44	9	27	9, 46	4.05	6.12	273
R. parietal cortex	18	−73	48	7	3.95	5.84	43
R. parietal cortex	30	−56	49	7	3.86	5.62	123
L. parietal cortex	−30	−57	54	7	4.08	6.2	90
R. thalamus	10	−15	12		3.65	5.1	33
L. thalamus, caudate body	−10	−17	12		3.75	5.34	99
R. GP	14	2	4		3.58	4.93	48
Brainstem	−6	−28	−19		4.25	6.73	28

Significant differences were recognized at a height threshold ($P < 0.001$, uncorrected) and extent threshold (10 voxels). Coordinates and Z score refer to the peak of each brain region. BA = Brodmann area; L = left; R = right; LG = lingual gyrus; Cu = cuneus; MOG = middle occipital gyrus; IOG = inferior occipital gyrus; FG = fusiform gyrus; STG = superior temporal gyrus; MTG = middle temporal gyrus; ITG = inferior temporal gyrus; SFG = superior frontal gyrus; MFG = medial frontal gyrus; IFG = inferior frontal gyrus; CG = cingulate gyrus; AC = anterior cingulate; DLPFC = dorsal lateral prefrontal cortex; MPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; GP = globus pallidus.

DLPFC, parietal cortex, thalamus, caudate body, globus pallidus, and brainstem (Table 1 and Fig. 1). Compared to PBO, SUL treatment decreased responses of U–N contrast in the visual cortex, left temporal cortex, anterior cingulate, left amygdaloid–hippocampal region, cerebellum, and midbrain, whereas SUL treatment produced greater activation in the frontal cortex including DLPFC, MPFC, temporal cortex, parietal cortex, left insula, and left claustrum (Table 2 and Fig. 2). On the other hand, compared to PBO, FLU treatment decreased responses of U–N condition in the left OFC, right temporal cortex, right insula, right hippocampal region and left amygdaloid–hippocampal region, and right putamen, whereas FUL treatment produced greater activation in the temporal cortex and parietal cortex (Table 3 and Fig. 3).

During both SUL and FLU treatments, mean signal changes elicited by unpleasant condition compared to neutral condition were significantly less than those during PBO treatment ($t = 2.63$, $P = 0.02$ and $t = 2.93$, $P = 0.01$, respectively) in the left amygdala, but not the right amygdala ($t = 0.57$, $P = 0.57$ and $t = 1.93$, $P = 0.07$, respectively). The differences in mean signal changes between SUL and PBO were 0.36 (95% CI, 0.08–0.65) in the left amygdala and 0.07 (95% CI, −0.18–0.32) in the right amygdala. The differences in mean signal changes between FLU and PBO were 0.24 (95% CI, 0.07–0.41) in the left amygdala and 0.25 (95% CI, −0.17–0.52) in the right amygdala (Fig. 4).

Discussion

We found that acute administration of a therapeutic dose of DA D_2 antagonists and SSRIs had modulatory effects on emotional processing in the human brain, although the subjects recruited the entire neural network of the limbic–cortical–striatal–pallidal–thalamic circuit in response to unpleasant pictures while taking PBO. Our findings indicate that both acute SUL and FLU treatments manipulated the responses of the components of the circuit and commonly attenuated the activation of amygdala, a key node of the circuit.

DA D_2 receptor antagonist treatment decreased responses in the limbic areas (amygdala, hippocampus, anterior cingulate) along with the visual sensory cortex, cerebellum, and midbrain. Not

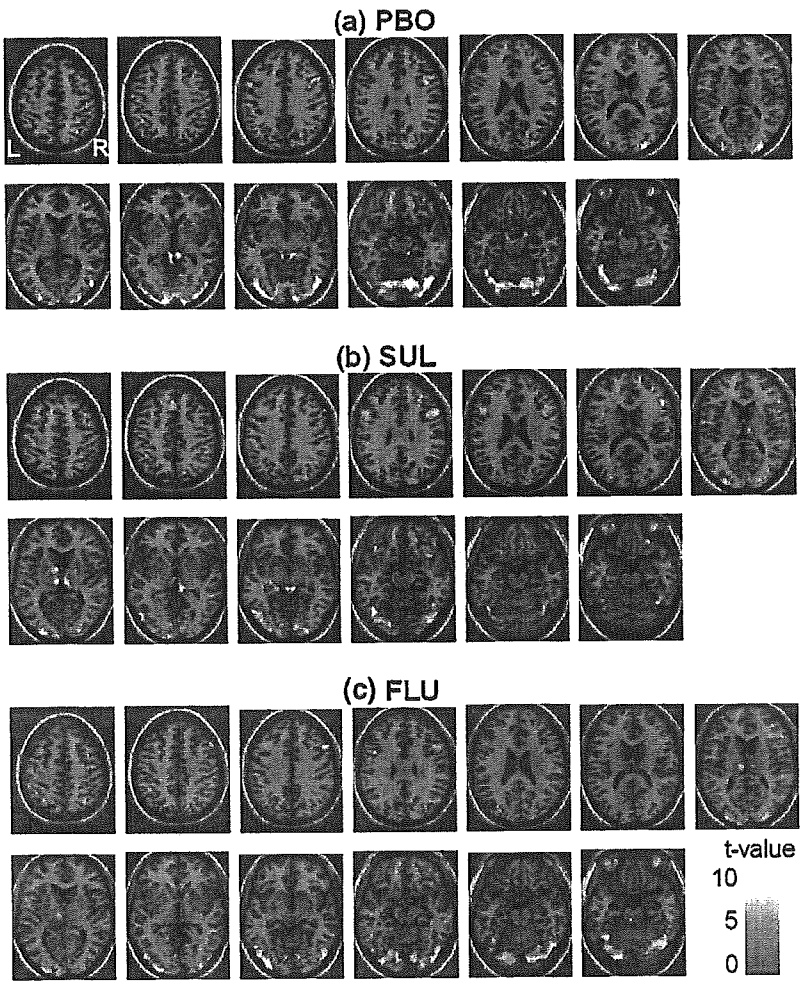

Fig. 1. Images showing dissociable brain activations in unpleasant condition relative to neutral condition during PBO treatment (a), SUL treatment (b), and FLU treatment (c). Significant differences were recognized at a height threshold ($t > 3.93$; $P < 0.001$, uncorrected) and extent threshold (10 voxels). The bar shows the range of the t value. Within the images, L indicates left and R indicates right.

Table 2
Brain regions showing significant effects of SUL treatment on neural activations in response to unpleasant condition relative to neutral condition

Brain region	Coordinates			BA	Z score	t value	Voxels
	x	y	z				
PBO vs. SUL							
L. occipital cortex (LG), cerebellum	−14	−72	−8	18	3.25	4.24	201
L. occipital cortex (Cu)	0	−62	5	30	3.43	4.6	33
L. occipital cortex (Cu)	−8	−78	33	19	3.34	4.42	13
L. occipital cortex (Cu)	−8	−70	16	31	2.96	3.69	12
L. occipital cortex (IOG)	−48	−78	−4	18	3.28	4.29	20
R. occipital cortex (Cu)	20	−92	30	19	2.96	3.7	10
L. temporal cortex (STG)	−34	16	−32	38	3.2	4.13	19
R. AC	2	34	22	32	3.18	4.1	11
L. PHG	−32	−30	−22	36	3.39	4.53	10
L. amygdala	−14	−2	−26		2.91	3.61	10
Midbrain	−10	−12	−15		3.38	4.5	19
SUL vs. PBO							
R. DLPFC (IFG)	55	30	8	46	3.19	4.12	24
L. DLPFC (MFG)	−53	17	25	9	2.88	3.55	14
L. DLPFC (IFG)	−50	5	27	9	3.01	3.77	22
MPFC (MFG)	8	29	45	8	3.17	4.08	29
R. frontal cortex	6	−32	55	4,5	2.96	3.69	46
R. frontal cortex (MFG)	26	−7	48	6	3.69	5.19	137
R. frontal cortex (MFG)	38	46	−6	10	3.17	4.08	35
R. frontal cortex (IFG)	46	22	15	45	3.79	5.44	50
L. frontal cortex (IFG)	−53	22	4	45	3.16	4.07	25
R. temporal cortex (MTG)	55	−49	−3	37	3.63	5.06	15
R. temporal cortex (MTG)	50	−33	0	21	3.16	4.06	53
L. temporal cortex (STG)	−51	−31	3	22	3.48	4.7	48
L. temporal cortex (MTG)	−55	−6	−13	21	3.25	4.24	21
R. parietal cortex	42	−47	41	40	3.62	5.03	88
R. parietal cortex	42	−30	55	40	3.15	4.04	23
R. parietal cortex	50	−13	47	3	3	3.76	19
L. parietal cortex	−38	−32	53	40	2.99	3.75	22
L. parietal cortex	−30	−42	48	40	2.97	3.71	10
L. insula	−48	−9	15	13	3.24	4.22	58
L. claustrum	−38	−25	0		3.27	4.28	93

To compare the effect of SUL on the U–N contrast, paired *t* tests (PBO vs. SUL and SUL vs. PBO) were conducted. Significant differences were recognized at a height threshold ($P < 0.005$, uncorrected) and extent threshold (10 voxels). See Table 1 legend.

surprisingly, attenuation of amygdala response by DA D_2 antagonists was in contrast to the previous pharmacological fMRI study where pharmacotherapy such as levodopa or DA agonists restored the amygdala activation in PD patients (Tessitore et al., 2002). However, the mechanisms underlying these results are not straightforward since DA could potentiate both the excitatory and inhibitory influences of afferent inputs on target neurons (Cohen et al., 2002).

Our PET study demonstrated that DA D_2 receptors are relatively dense in the mesocorticolimbic regions (amygdala, hippocampus, thalamus, and anterior cingulate), besides the striatal regions (Okubo et al., 1999). Considering the regional distributions of DA D_2 receptors, decreased activations in the amygdala, hippocampus, and anterior cingulate by DA D_2 receptor blockade indicate that the net effect of DA D_2 receptor activation is to enhance excitability of limbic regions in response to unpleasant stimuli, although we did not observe significant change of activation in the thalamus and striatal regions.

On the contrary, DA D_2 receptor blockade produced greater activations extensively in the cortical areas (frontal, temporal, and parietal). These enhanced activations in cortical areas are quite puzzling. A possible explanation is that SUL acute treatment might have increased dopaminergic transmission in the cortical area. DA D_2 antagonists are known to increase activity of A9 and A10 neurons through the feedback mechanism of presynaptic D_2-like autoreceptors (Westerink, 2002). Another substituted benzamide derivative, amisulpride, has been suggested to enhance cortical dopaminergic transmission through its preferential blockade of presynaptic D_2-like autoreceptors at optimal dose (Moller, 2003). We revealed that the registered clinical dose of SUL (300–600 mg, max 1800 mg) was about ten times higher than the estimated optimal dose by PET (unpublished data). Thus, if we used SUL at the optimal dose, it would act like amisulpride to enhance cortical dopaminergic transmission in the cortical regions.

Among the enhanced cortical areas, greater activations in the PFC are noteworthy since it is considered to be a main modulator in the neural circuit of emotional processing (Davidson et al., 2002; Drevets, 2000). There are direct and indirect connections between the amygdala and PFC (Groenewegen and Uylings, 2000; Price et al., 1996), and the PFC can attenuate amygdala activation via these connections (Hariri et al., 2000; Rosenkranz and Grace, 1999, 2001). Cognitive demands such as explicit and elaborate evaluation of stimuli that are mediated in the PFC are known to attenuate automatic amygdala activation (Hariri et al., 2000; Phan et al., 2002). In this sense, passive viewing is the ideal way to examine robust amygdala activation, but the behavioral data during the scans should be recorded. To reconcile this dilemma, we used the current paradigm, in which the participants roughly reported their subjective experience, aiming to ensure minimal cognitive demands. Since cognitive demands across the 3 sessions were identical in this design, we can rule out the effect of cognitive demands when interpreting the attenuated amygdala activation, but it remains possible that attenuated amygdala activity is partially attributable to secondary change to the principal drug effect on the PFC. However, since we observed the net effects of direct drug effect on the amygdala and possible secondary modulation by afferent input in the amygdala, we cannot differentiate between these possible mechanisms in this study.

SSRI treatment also decreased the activation in amygdaloid–hippocampal regions, as we predicted. However, unlike DA D_2 receptor antagonists, SSRI treatment reduced activation in different areas such as OFC, basal ganglia, and insula but not in the visual cortex. Moreover, SSRI treatment produced greater activation only in the temporal cortex and parietal cortex, not in the frontal cortex. Although both DA D_2 antagonist and SSRI treatment resulted in common inhibitory effects on activations of amygdaloid–hippocampal regions, the different patterns observed in other regions strongly point to different mechanisms underlying the common effects.

FLU was approved for the treatment of obsessive-compulsive disorder (OCD) but has not been officially approved for the treatment of depression in the United States. However, it is approved in many countries for the indication of depression (Hachisu and Ichimaru, 2000). It is probably no less effective than the other SSRIs in treating depression (Dalery and Honig, 2003) and no better than the other SSRIs at treating OCD (Mundo et al., 1997). FLU has greater selectivity for 5-HT vs. noradrenaline (NA) than fluoxetine and paroxetine and less selectivity than citolopram and sertraline (Wong and Bymaster, 2002). A microdialysis study

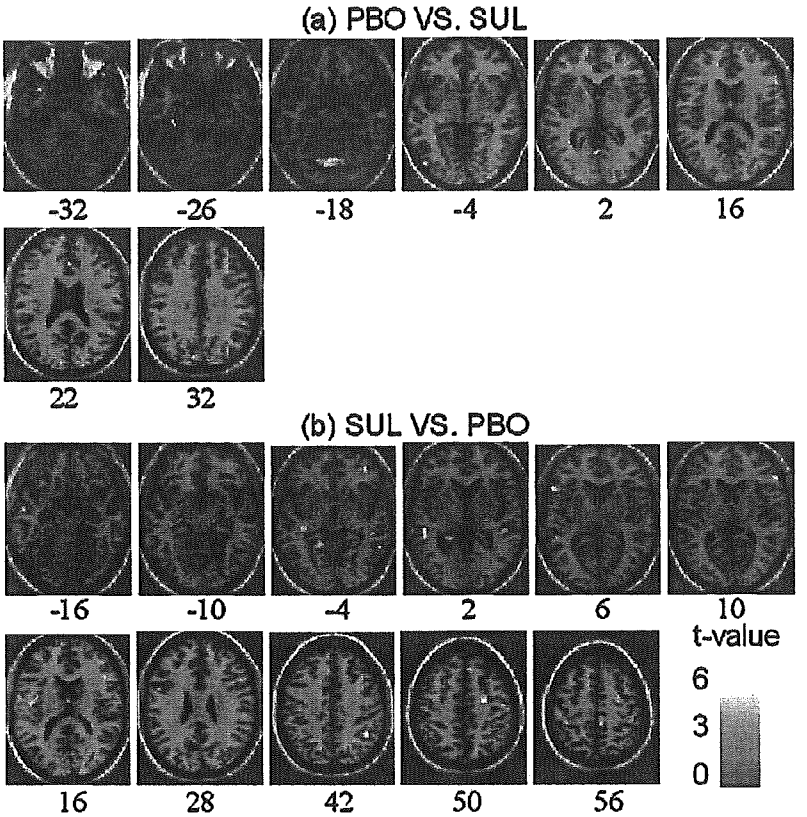

Fig. 2. Images showing manipulated brain activations in response to unpleasant condition relative to neutral condition under SUL treatment. Compared to PBO, attenuated activations were shown in the visual cortex, left temporal cortex, anterior cingulate, left amygdaloid–hippocampal region, cerebellum, and midbrain (a). Enhanced activations were found in the frontal cortex, temporal cortex, parietal cortex, left insula, and left claustrum (b). Significant differences were recognized at a height threshold ($t > 3.05$; $P < 0.005$, uncorrected) and extent threshold (10 voxels). The bar shows the range of the t value. Within the image, L indicates left and R indicates right. Numbers in the bottom row indicate the z coordinates of the Montreal Neurological Institute brain.

demonstrated that SSRIs (FLU, citalopram, sertraline, and paroxetine) did not increase NA and DA extracellular levels in the PFC, and only acute administration of fluoxetine, which has modest selectivity for 5-HT vs. NA compared with other SSRIs, increased them (Bymaster et al., 2002). Therefore, when FLU was administered acutely, the potential effect on the NA or DA system might be negligible in this study.

We understand that the effects of acute SSRI treatment on 5-HT neurotransmission are complex due to the presence of autoreceptors on the presynaptic neuron. The distribution of 5-HT transporters closely matches the regional distribution of 5-HT nerve terminal and cell bodies. They are highly expressed in the amygdala, hippocampus, thalamus, striatum, and midbrain. Intermediate density is found in the cortex and cerebellum (Parsey et al., 2000). Blockade of 5-HT transporters by acute SSRI administration preferentially increases extracellular 5-HT in the raphe nuclei. An increase in somatodendric extracellular 5-HT activates 5-HT_{1A} autoreceptor feedback system to inhibit 5-HT release in terminal projection regions (Pineyro and Blier, 1999). However, preclinical microdialysis studies have consistently reported that acute systemic administration of SSRIs increased extracellular 5-HT concentrations in the raphe nuclei, frontal cortex, hippocampus, and amygdala (Bosker et al., 1995, 2001; Dawson and Nguyen, 1998; Hatanaka et al., 2000; Invernizzi et al., 1995; Malagie et al., 1995). In the present study, acute SSRI administration might have increased 5-HT neurotransmission at postsynaptic 5-HT receptors in the amygdaloid–hippocampal regions because postsynaptic 5-HT receptors are rich in the amygdala and hippocampus (Buhot, 1997; Pineyro and Blier, 1999) and an increase of 5-HT reduced reactivity of the amygdala to sensory inputs (Stutzmann et al., 1998). There are several 5-HT receptor subtypes expressed in the amygdala, and there are both inhibitory (e.g. 5-HT_{1A}) and excitatory (e.g. $5\text{-HT}_{2A}/5\text{-HT}_{2C}$) receptors (Stein et al., 2000). In addition, because excitatory and inhibitory neurons are tightly interconnected in the local circuits, it is unlikely that a large increase in inhibition can be observed without a concomitant increase in excitation. Therefore, we cannot differentiate specific 5-HT receptor subtype effects in this study and what we observed here was the net effect of 5-HT transmission change on the amygdala.

Although the putative anxiolytic and antidepressant effect of SSRIs is generally understood to be associated with a net increase in 5-HT neurotransmission, there are conflicting results on the function of 5-HT in anxiety. Several animal studies in the literature have demonstrated that high 5-HT was associated with anxiety, indicating a major role of 5-HT in the amygdala in the generation of anxiogenic behaviors (Chaouloff, 2000; Graeff et al., 1996). On the other hand, the fact that the success of the treatment with SSRIs and acute tryptophan depletion worsens depressive symptoms in depression suggests that an increase in 5-HT transmission may be

Table 3
Brain regions showing significant effects of FLU treatment on neural activations in response to unpleasant condition relative to neutral condition

Brain region	Coordinates			BA	Z score	t value	Voxels
	x	y	z				
PBO vs. FLU							
L. OFC (IFG)	−32	26	−15	47	3.05	3.85	16
R. temporal cortex (STG)	63	−34	11	22	3.8	5.47	52
R. insula	48	1	10	13	3.45	4.64	27
R. PHG	22	−17	−21	28	3.28	4.29	29
R. hippocampus	26	−11	−16		3.5	4.75	35
L. amygdala, hippocampus	−24	−12	−11		3.6	4.99	84
R. putamen	28	−2	−3		2.91	3.61	13
FLU vs. PBO							
R. temporal cortex (MTG)	59	−47	−8	37	3.49	4.74	49
R. temporal cortex (MTG)	55	−29	−5	21	3.08	3.92	46
R. temporal cortex (STG)	48	−18	−6	22	3.14	4.02	17
L. temporal cortex (STG, MTG)	−51	−39	6	22	3.2	4.14	31
L. paricta1 cortex	−24	−72	39	7	3.84	5.57	62
L. parietal cortex	−46	−32	51	40	3.32	4.38	44

To compare the effect of FLU on the U–N contrast, paired t tests (PBO vs. FLU and FUL vs. PBO) were conducted. Significant differences were recognized at a height threshold ($P < 0.005$, uncorrected) and extent threshold (10 voxels). See Table 1 legend.

anxiolytic in humans (Kent et al., 1998). Preclinical studies reported that emotional stress increases 5-HT concentration in the amygdala and prefrontal cortex (Amat et al., 2005; Kawahara et al., 1993), and an increase of 5-HT reduces reactivity of the amygdala to excitatory sensory inputs (Stutzmann et al., 1998). This has led to the suggestion that 5-HT may act as a constraint system to inhibit primitive and impulsive reaction by reducing responsiveness of lower brain centers to emotional stress (Kent et al., 1998; Spoont, 1992). Stress from viewing unpleasant pictures might have increased endogenous 5-HT release, and SSRI might have potentiated 5-HT function to reduce reactivity of the amygdala in this study.

Interestingly, the effects of both SUL and FUL on the amygdala treatment were lateralized to the left side. It has been suggested regarding the functional laterality of the amygdala that the right amygdala may be first activated by emotional stimuli and be engaged in a rapid automatic processing of ambiguous information, while the left amygdala may be involved in a more specific sustained emotional reaction that decodes the arousal signaled by specific stimuli (Glascher and Adolphs, 2003). The reduced activity of the left amygdala might reflect reduction of the arousal. Since we used a rough self-rating score of unpleasantness, we did not have variations in terms of unpleasantness. If we had used a more detailed self-rating score of unpleasantness, the score might have detected the reduction of subjective unpleasantness. In addition, in terms of FLU treatment, it is perhaps worth remarking that, although the differences in mean signal changes elicited by unpleasant conditions compared to neutral condition in both left and right amygdala were similar, the right amygdala failed to reach a level of significance due to the greater variations in terms of the effect of SSRI treatment. Recent studies revealed that genetic variations of 5-HT transporters are associated with individual differences of right amygdala activity (Hariri et al., 2002b, 2005). Variations in the effect of SSRI treatment on the right amygdala could be attributable to genetic variations of 5-HT transporters.

Chronic successful treatments with SSRIs that normalized the elevated amygdala activity in patients with depression have been reported (Drevets, 2000). Since the therapeutic effect of SSRIs can take several weeks to appear, the mechanisms underlying the therapeutic effect of their chronic treatment are considered to be different from those of the acute pharmacological change induced by acute SSRI administration. However, our data suggest that even acute treatment of SSRIs could produce desirable preclinical

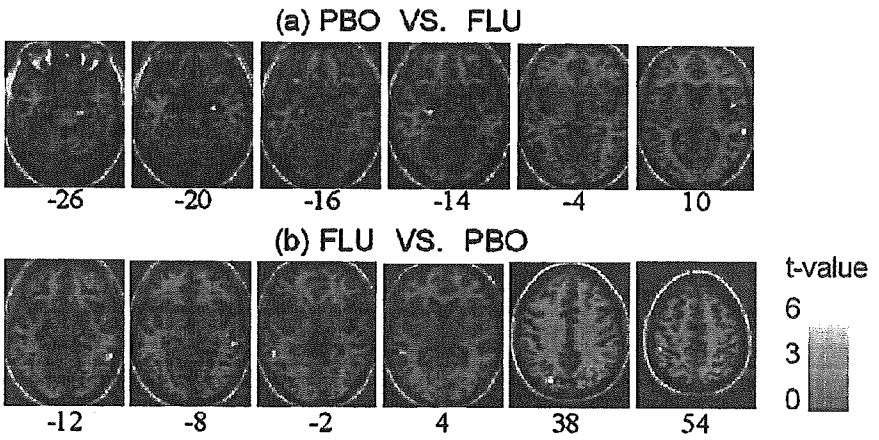

Fig. 3. Images showing manipulated brain activations in response to unpleasant condition relative to neutral condition under FLU treatment. Compared to PBO, attenuated activations were shown in the left OFC, right temporal cortex, right insula, right hippocampal region, left amygdaloid–hippocampal region, and right putamen (a). Enhanced activations were found in the temporal cortex and parietal cortex (b). Significant differences were recognized at a height threshold ($t > 3.05$; $P < 0.005$, uncorrected) and extent threshold (10 voxels). The bar shows the range of the t value. Within the image, L indicates left and R indicates right. Numbers in the bottom row indicate the z coordinates of the Montreal Neurological Institute brain.

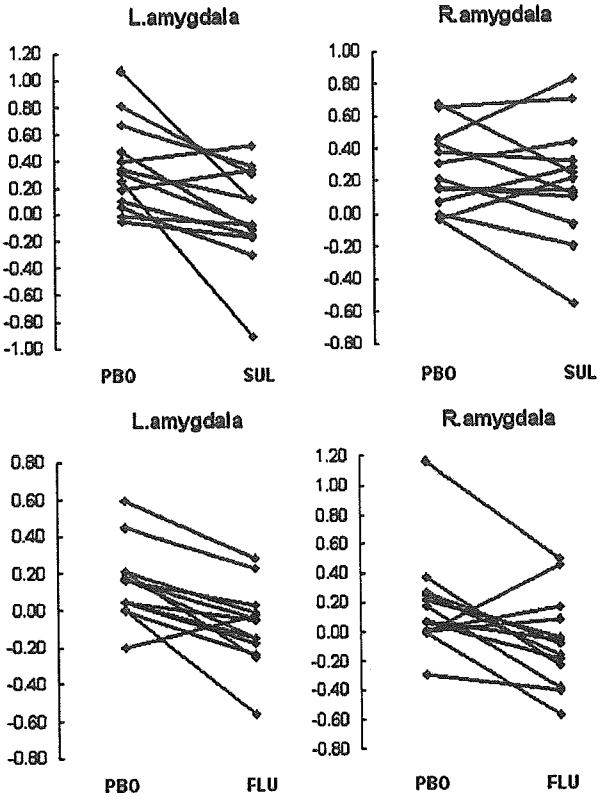

Fig. 4. Individual differences in the effect of drugs on amygdala activation elicited by unpleasant condition compared to neutral condition. The effect of sultopride on the left amygdala ($x, y, z = -14, -3, -22$) and the right amygdala ($x, y, z = 22, -3, -15$) (top). The effect of fluvoxamine on the left amygdala ($x, y, z = -24, -12, -11$) and the right amygdala ($x, y, z = 26, 1, -22$) (bottom). During both drugs treatments, mean signal changes elicited by unpleasant condition compared to neutral condition were significantly less than those during placebo treatment ($t = 2.63$, $P = 0.02$ and $t = 2.93$, $P = 0.01$, respectively) in the left amygdala, but not in the right amygdala ($t = 0.57$, $P = 0.57$ and $t = 1.93$, $P = 0.07$, respectively).

physiological changes, i.e., normalization of abnormally elevated amygdala activity, in patients with depression or anxiety disorders.

The present study has several limitations. First, it is possible that the drugs have effects not only on the specific neuronal activation but also on nonspecific vascular and respiratory systems that could, in turn, change BOLD signals. However, it could be considered that the observed regional BOLD changes mostly reflected the specific effects on neuronal responses because nonspecific effects would produce BOLD signal changes to a similar degree in any region across the brain (Honey and Bullmore, 2004). Moreover, we believed that nonspecific effects were minimized, if any, because the drugs induced minimal changes of physiological data. Second, we reported the drug effects on BOLD maps without correcting multiple comparisons. This raises the risk of type 1 errors. However, because pharmacological fMRI is a relatively new method and we do not possess sufficient information about the possible drug effects on BOLD signals across the whole brain, determining regions of interest (ROIs) a priori would be difficult. Therefore, we did not correct for multiple comparisons using ROIs.

Third, we examined the effects of DA D_2 receptor antagonists and SSRIs in healthy volunteers in this study. These drugs might not necessarily show similar actions in patients with psychiatric disorders, such as schizophrenia, mood disorders, and anxiety disorders. Studies on drug-free psychiatric disorder patients should be performed. Finally, despite significant changes of neural activation by pharmacological manipulation, behavioral results did not show significant changes. We had the subjects rate the pictures roughly using a 3-point scale. We aimed to simplify the emotional task and reduce cognitive demands during the scan since cognitive demands such as detailed evaluation or pressing several buttons could attenuate automatic emotional responses (Phan et al., 2002). Although this rough measurement might be attributable to insensitivity, conventional behavioral measurements are considered not sensitive enough to detect drugs effects (Honey and Bullmore, 2004).

In conclusion, we have shown that acute treatments of DA D_2 receptor antagonists and SSRIs commonly achieved considerable attenuation of amygdala activity, although the two treatments had different modulatory effects on other components of the neural circuit of emotional processing in healthy subjects.

The results suggest that the effects of the drug itself on BOLD signals are likely not negligible in fMRI studies aiming to investigate emotional processing in psychiatric patients taking drugs. At the same time, our findings suggest that pharmacological fMRI might be a powerful measurement tool for investigating the effects of drugs that manipulate neurotransmitter systems on emotional processing in the human brain and that this tool has potential for application in clinical practice and drug discovery.

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Pharmacological Modulations on the Human Cognitive Processes: An fMRI Study

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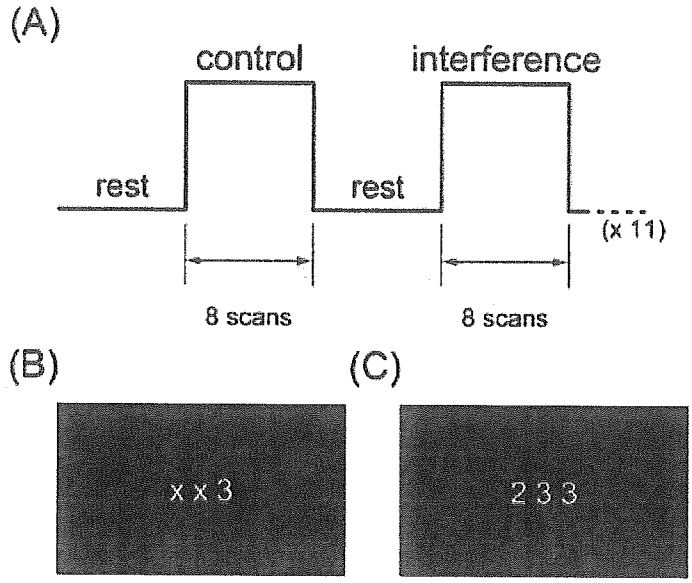

Fig. 1

Investigating modulatory effects of psychopharmacological agents in the human brain allows for not only functional characterization of particular neurotransmitter systems in the human cognition, but better understanding of pathophysiology and treatment of neuropsychiatric disorders[1]. Here we conducted a functional magnetic resonance imaging (fMRI) study to map effects of a dopamine D_2 antagonist (sultopride) on a decision-making process. In a single scanning session, ten male, right-handed, healthy subjects performed a Stroop-like cognitive interference task[2] (**Fig. 1**). In the absence of dopaminergic manipulations, comparison of blood oxygenation level dependent (BOLD) signals during the interference condition against those during the control condition revealed a widely distributed network implicated in the decision-making process with cognitive interference (**Fig. 2A**). Upon the administration of the D_2 antagonist, however, many of these regions exhibited decreased activities, and the effects were found to be most prominent in regions around the cerebellum, the thalamus, the anterior cingulate cortex, and the motor areas (**Fig. 2B**). Subsequent studies should address the role of individual components in the observed brain circuits, as well as what the decrements of activations mean in the neurophysiological context.

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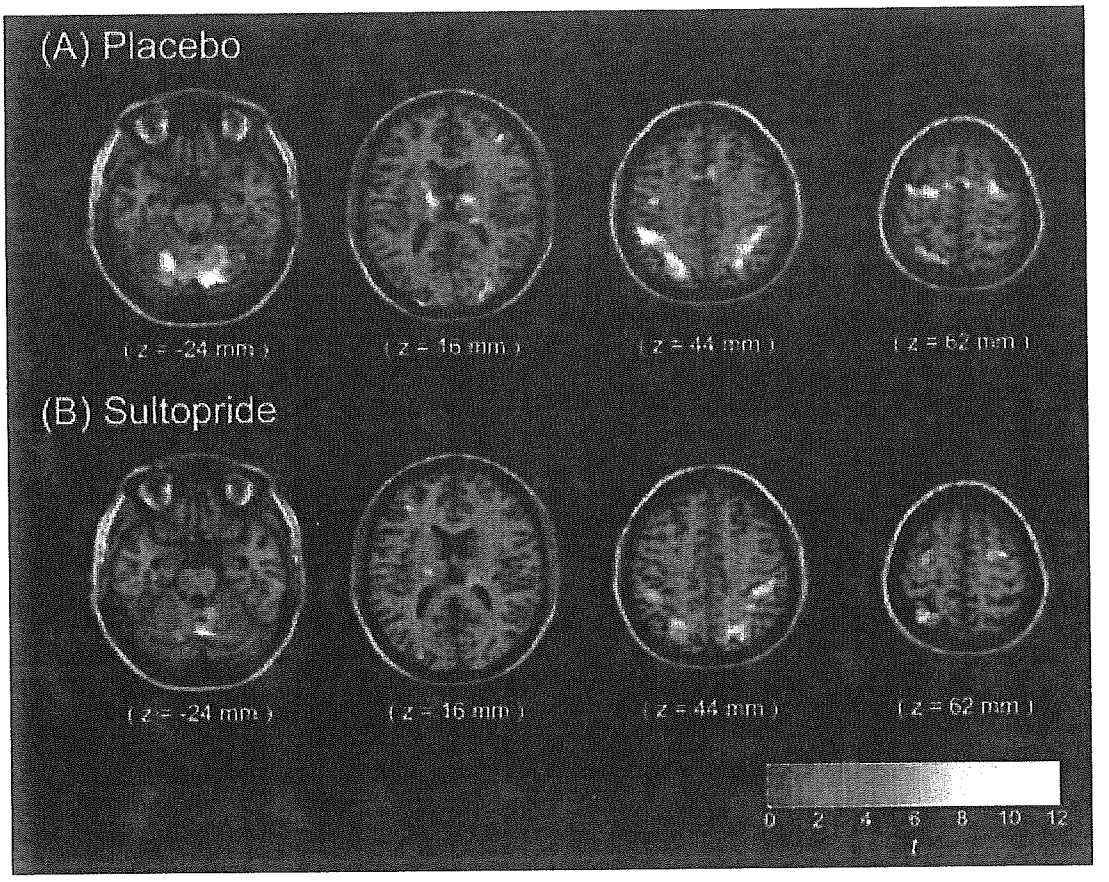

Fig. 2

Fig. 1 (A) Schematic diagram illustrating the cognitive interference task employed. A single scanning session consisted of blocks (containing eight scans) of control and interference trials interspaced by resting periods. During the trials, subjects are instructed to report by button press the identity of the number that differs from the other two. (B)-(C) Examples of the trials. During the control trials, the distractors were the letter 'x', whereas during the interference trials, the distractors were other numbers, thereby imposing higher cognitive demands.

Fig. 2 Activated regions during the interference trials in contrast to the control trials (A) with no dopaminergic manipulations and (B) under the administration of a D_2 antagonist (sultopride). The results are based on a group analysis with statistical parametric mapping (SPM) software[3] and with a statistical threshold of $P<0.001$ (uncorrected).

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Language Processing and Human Voice Perception in Schizophrenia: A Functional Magnetic Resonance Imaging Study

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Background: *Neuroimaging studies have demonstrated either reduced left-lateralized activation or reversed language dominance in schizophrenia. These findings of left hemispheric dysfunction could be attributed to language processing tasks, which activate mainly left hemispheric function. Recent functional magnetic resonance imaging studies reported right-lateralized temporal activation by human voice perception, but few studies have investigated activation by human voice in schizophrenia. We aimed to clarify the cerebral function of language processing in schizophrenia patients by considering cerebral activation of human voice perception.*

Methods: *Fourteen right-handed schizophrenia patients and 14 right-handed controls with matched handedness, sex, and education level were scanned by functional magnetic resonance imaging while listening to sentences (SEN), reverse sentences (rSEN), and identifiable non-vocal sounds (SND).*

Results: *Under the SEN-SND and SEN-rSEN contrasts including language processing, patients showed less activation of the left hemisphere than controls in the language-related fronto-tempo-parietal region, hippocampus, thalamus and cingulate gyrus. Under the rSEN-SND contrast including human voice perception, patients showed less activation than controls in the right-lateralized temporal cortices and bilateral posterior cingulate.*

Conclusions: *Our results indicate that schizophrenia patients have impairment of broader bilateral cortical-subcortical regions related to both the semantic network in the left hemisphere and the voice-specific network in the right hemisphere.*

Key Words: Language processing, human voice perception, schizophrenia, semantic network, voice-specific network, functional magnetic resonance imaging (fMRI)

Auditory hallucination and thought disturbance are the main symptoms of schizophrenia and are assumed to be associated with disturbance in language processing (Kircher et al 2002; Mitchell et al 2001; Sommer et al 2001; Woodruff et al 1997). In order to understand the neural basis of these symptoms, achieving the clarification of cerebral function when patients with schizophrenia listen to language will be a challenging target.

Functional magnetic resonance imaging (fMRI) studies have investigated the neural basis of disturbed language processing in schizophrenia (Kasai et al 2003; Kubicki et al 2003; Ragland et al 2004). Functional MRI studies have reported that normal right-handed subjects show left-lateralized activation for language processing (Gaillard et al 2002; Lehericy et al 2000; Schlosser et al 1998), whereas normal left or ambidextrous-handed subjects show bilateral activation for language processing (Hund-Georgiadis et al 2002; Pujol et al 1999; Szaflarski et al 2002). Previous fMRI studies with language listening tasks have demonstrated that schizophrenia patients show either reduced left hemispheric activation (Kiehl and Liddle 2001; Kircher et al 2001) or reversed language dominance (Menon et al 2001; Ngan et al 2003; Woodruff et al 1997), and schizophrenia patients have disturbed left hemisphere dominance for language processing (Sommer et al 2001, 2003). Similarly, fMRI studies with verbal fluency tasks have shown either reduced left frontal activation (Artiges et al 2000; Curtis et al 1998; Yurgelun-Todd et al 1996) or reversed activation in the frontal cortices (Crow 2000; Sommer et al 2001, 2003). These findings of hypo or reversed activation of schizophrenia could depend on the nature of language processing tasks, which mainly activate the left hemisphere in normal subjects.

In schizophrenia, investigating cerebral response to human voice is significant since human voice perception is known to closely associate with the generative mechanism of functional auditory hallucination like languages or specific identifiable sounds (Hayward 2003; Hunter and Woodruff 2004). Recent fMRI studies have shown that the human-voice-specific area is located in the superior temporal sulcus (STS) with right hemisphere dominance in normal subjects (Belin and Zatorre 2003; Belin et al 2002; Belin et al 2000). When auditory hallucination appears, patients with schizophrenia have demonstrated increasing cerebral activation in the temporal cortex (Bentaleb et al 2002; Dierks et al 1999; Woodruff et al 1997). If cerebral activation in the right hemisphere is increased by human voice perception, language dominance in the temporal cortices could be reversed when schizophrenia patients are listening to language. Recent studies have indicated the importance of investigating right-hemisphere language function as a social perception in schizophrenia (Abdi and Sharma 2004; Mitchell and Crow 2005; Onitsuka et al 2005; Williams et al 2004). However, to our knowledge, no studies concerning language processing of schizophrenia have taken cerebral activation by human voice perception into account. Therefore, we investigated how cerebral activation for language processing and human voice perception in schizophrenia patients are different in comparison to normal subjects.

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The aim of our research was to clarify cerebral function by language processing in patients with schizophrenia by considering activation by human voice perception.

Methods and Materials

Subjects

Fourteen schizophrenia patients (12 males and 2 females, mean age 31.6 years, SD = 7.0) meeting the DSM-IV criteria for schizophrenia were studied. Diagnoses were made by HTak, YO, and the attending psychiatrists on the basis of a review of their charts and a conventionally semi-structured interview. Exclusion criteria were current or past substance abuse and a history of alcohol-related problems, mood disorder, or organic brain disease. Eleven patients were recruited from the outpatient unit of Asai Hospital, and 3 were recruited from the inpatient unit. Ten of the 14 patients were the same as in our previous fMRI studies investigating emotional neural responses (Takahashi et al 2004). As for the subtypes of schizophrenia, 13 patients were paranoid and one had undifferentiated schizophrenia. Thirteen of the 14 patients were receiving neuroleptics (mean risperidone equivalent daily dosage = 3.6 mg, SD = 3.5; 8 patients, risperidone; 1 patient, perospirone; 1 patient, olanzapine; 1 patient, zotepine; 2 patients, sulpiride), and one was not receiving any neuroleptics. Mean illness duration was 9.6 (SD = 9.7) years. Clinical symptoms were assessed by the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962). The ratings were reviewed by HTak and YO after the patient interview, and disagreements were resolved by consensus; consensus ratings were used in this study. Further, sum scores for positive and negative symptoms were calculated, with the positive symptom subscale including the following eight items: conceptual disorganization, mannerisms and posturing, hostility, grandiosity, suspiciousness, hallucinatory behavior, unusual thought content, and excitement. The negative symptom subscale included these three items: emotional withdrawal, motor retardation, and blunted affect. The mean score of BPRS was 13.9 (SD = 7.4). The mean positive symptom score was 1.9 (SD = 2.4), and the mean negative symptom score was 3.6 (SD = 2.9). The control group consisted of 14 normal subjects (10 males and 4 females, mean age 29.1 years, SD = 7.8), who were recruited from the surrounding community. The candidates were carefully screened and standardized interviews were conducted by trained psychiatrists (HTak and YO). They did not meet the criteria for any psychiatric disorders. None of the control subjects was taking alcohol or medication at the time, nor did they have a history of psychiatric disorder, significant physical illness, head injury, neurological disorder, or alcohol or drug dependence. Patients tended to have a lower educational status but there was no significant difference in the mean period of education between the controls and patients (mean ± SD; patients 13.2 ± 2.0 years, control subjects 14.4 ± 1.8 years; $p = .15$, t-test). All of the patients and control subjects were right-handed, as investigated by the Edinburgh Handedness Inventory (EHI) (mean ± SD; control subjects 92.3 ± 10.3; patients 91.5 ± 9.9, $p > .05$, t-test) (Oldfield 1971). Previous fMRI studies defined a right-handed subject as a subject with an EHI score of more than 50 or 52 (Springer et al 1999; Szaflarski et al 2002). Therefore, we defined a right-handed subject as "a subject with an EHI score of more than 52." In our study, 16 patients were initially included, but two ambidextrous patients (EHI: less than 52) were then excluded from the analysis. There was no significant difference in the mean of the EHI score between the two groups. They all underwent MRI to rule out cerebral anatomic abnormalities. After the procedures had been fully explained to the subjects, written informed consent was obtained in accordance with the guidelines of the Asai Hospital Ethics Committee.

Experimental Design

In a single session, three types of stimuli were presented: forward-played sentences (SEN); reverse sentences (rSEN), the same sentences, but played in reverse; and identifiable nonvocal sounds (SND). The duration of each stimulus was 20 sec, and rSEN, SND, and SEN were played in sequence to each subject. Before each sound, the subjects listened to silence from the headphones for 20 sec (rest condition). Each set was 120 sec, and consisted of these three sound conditions and the rest condition. One session consisted of 8 sets, with a total scanning time of 960 sec (Figure 1). As identifiable non-vocal sounds for the SND condition, sounds of a shower, washing machine, bell, and computer printer were used. The sentences were in Japanese, spoken by two speakers, one male and one female, and represented a single topic per set, with one session consisting of four topics, each repeated twice randomly. Concerning the contents of the sentences, each topic was expressed by one or two sentences, consisting of 6–7 phrases including compound sentences. These sentences used conjunctional phrases or long adjuncts. Therefore, each subject was required to comprehend complex situations and understand the connection of the phrases or sentences (Appendix 1). In our experiment, two voices, one male and one female, were used alternately for the sound contents of sentences and reverse sentences. The material of the sentences included the linguistic section of the contents of Wechsler's Memory Scale – Revised, translated into Japanese (Sugishita 2001; Wechsler 1987). In our research, we used Japanese sentences for Japanese native subjects because research of semantic processing of language has shown left-

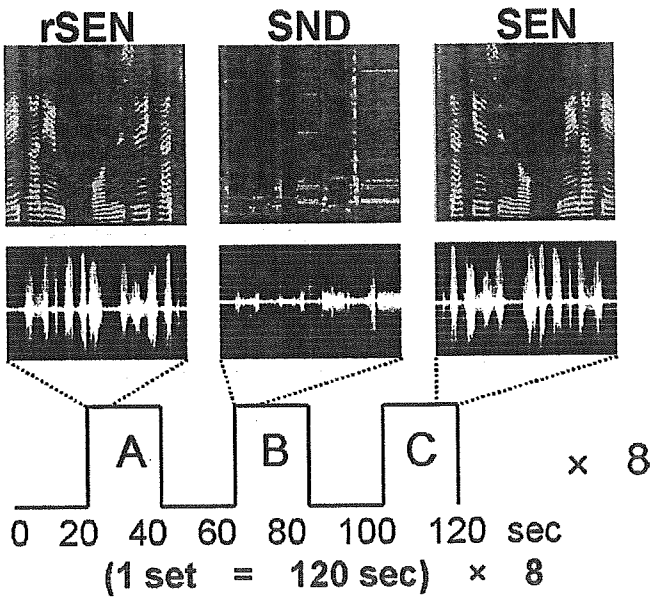

Figure 1. **(A)** Reverse sentences (rSEN), **(B)** Identifiable non-vocal sounds (SND), **(C)** Sentences (SEN). The top row shows sound spectrograms under the three sound conditions. The horizontal axis shows the time domain. The vertical axis shows the frequency (about 15–20000 Hz) of the tone domain. The middle row shows the time-domain waveforms. The horizontal axis shows the tone domain. The vertical axis shows the power of the sound.

lateralized activation in the fronto-tempo-parietal region by native languages (Gaillard et al 2004; Homae et al 2002; Kansaku et al 2000; Springer et al 1999).

We used reverse sentences for the human voice condition. Several phonetics studies have indicated that human voice generated by air passing through the vocal tract or vocal cords has a specific frequency or spectrum of the voice (Joos 1948; Kohler 1984; Simos et al 1997). Reverse sentences have the same spectrum domain as forward sentences (Figure 1) and maintain the character of the human voice. A neuropsychological study has demonstrated that reverse speech is a sound that has very little lexical or semantic information (Binder et al 2000). Additionally, several previous fMRI studies have used reversed speech as non-semantic vocal sound (Burton et al 2001; Howard et al 1992; Price et al 1996). Furthermore, if subjects listen to reverse words or reverse phrases, they may guess the meaning of the term or contents (Burton et al 2001). Therefore, instead of using reverse words or reverse phrases, as non-semantic vocal sound we used reverse sentences of sufficient length to preclude guessing their meaning. We performed a preliminary study in which we asked 30 normal subjects, different from those of this fMRI study, what the reverse sentences sounded like without introducing them in advance (Appendix 2). All subjects answered that the sound was a human voice although they could not understand the contents.

Instruments Used for Presentation of Stimuli

Stimuli were presented by digital-audio file from a computer. The sounds were presented at 8 bits and the sampling rate was 8 kHz. Subjects listened to the sound stimuli through headphones attached to an air conductance sound delivery system (Commancer X6, MRI Audio System, Resonance Technology Inc., Los Angeles, California).

Functional MRI Acquisition

The images were acquired with a 1.5 Tesla Signa system (General Electric, Milwaukee, Wisconsin). Functional images of 240 volumes were acquired with T2*-weighted gradient echo planar imaging sequences sensitive to blood oxygenation level dependent (BOLD) contrast. Each volume consisted of 40 transaxial contiguous slices with a slice thickness of 3 mm to cover almost the whole brain (flip angle, 90°; time to echo [TE], 50 msec; repetition time [TR], 4 sec; matrix, 64 × 64; field of view, 24 × 24).

Behavioral Data

In order to ensure that the subjects actively participated in the fMRI study, each subject was asked a series of questions regarding the contents of each condition (SEN, rSEN, SND) immediately after fMRI scanning. For the SEN condition, the questionnaire consisted of four questions regarding the situation relevant to the sentences, and four questions regarding the proper nouns used in the sentences. For the rSEN condition, the subjects were asked whether they could recognize the sound as human voice, and whether they could discriminate between the voice of a male and a female. For the SND condition, we asked each subject to identify the names of the sound stimuli.

Image Processing

Data analysis was performed with statistical parametric mapping software SPM2 (Wellcome Department of Cognitive Neurology, London, United Kingdom) running with MATLAB (Mathworks, Natick, Massachusetts). All volumes were realigned to the first volume of each session to correct for subject motion, and they were spatially normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. After normalization, all scans had a final resolution of 3 × 3 × 3 mm^3. Functional images were spatially smoothed with a 3-D isotropic Gaussian kernel (full width at half maximum of 8 mm). Low-frequency noise was removed by applying a high-pass filter (cutoff period of 80 sec) to the fMRI time series at each voxel. A temporal smoothing function was applied to the fMRI time series to enhance the temporal signal-to-noise ratio. The significance of hemodynamic changes in each condition was examined using the general linear model with boxcar functions convoluted with a hemodynamic response function. Statistical parametric maps for each contrast of the *t*-statistics were calculated on a voxel-by-voxel basis. The *t*-values were then transformed to unit normal distribution, resulting in *z*-scores.

Statistical Analysis

Group analysis was performed on the data for 14 control subjects and 14 schizophrenia patients using a random effect model on a voxel-by-voxel basis. Three trials (rSEN, SND, and SEN conditions) were presented by each explanatory variable, and they were each convoluted with a standard hemodynamic response function taken from SPM2 to account for the hemodynamic response lag. At first, we analyzed the one-sample *t*-test for cerebral activation of the control and patient groups, respectively. Second, we investigated the difference of the mean of cerebral activation between control and patient groups using the two-sample *t*-test. The *t*-statistics were calculated for contrast among the three trials.

Cognitive Systems

To assess the specific condition effect, we used the contrasts of reverse sentences minus identifiable non-vocal sounds (rSEN–SND) and sentences minus identifiable non-vocal sounds (SEN–SND), and sentences minus reverse sentences (SEN-rSEN). In the rSEN-SND and SEN-SND contrasts, we used cerebral activation when the subjects listened to identifiable non-vocal sound (SND) as a baseline in order to investigate whether cerebral activation by language processing or human voice perception was greater than that of listening to non-vocal sound. The contrast of rSEN–SND included human voice perception. The contrast of SEN–SND was assumed to represent the activation due to not only human voice perception but also language processing including lexical-semantic processing. The content of SEN-rSEN was assumed to represent the activation due to lexical-semantic processing (Koeda et al, in press).

A random effect model that estimates the error variance for each condition across the subjects was implemented for group analysis. Contrast images obtained from single-subject analysis were entered into group analysis. A one-sample *t*-test was applied to determine group activation for each effect. Significant clusters of activation were determined using the conjoint expected probability distribution of the height and extent of *z*-scores with the height and extent threshold. Coordinates of activation were converted from MNI coordinates to the Talairach and Tournoux coordinates (Talairach and Tournoux 1988) using the mni2tal algorithm (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml).

Region of Interest (ROI) Analysis

We investigated the activated voxel numbers in the regions of interest (ROIs). The peak coordinates of activation in the control group were used as the common coordinate ROIs for both

controls and patients. Under the SEN-SND and the SEN-rSEN contrasts, ROIs were set at the bilateral triangular portion of IFG (tri-IFG; SEN-SND: [±51, 21, 24], SEN-rSEN: [±48, 18, 24]), the posterior superior temporal gyrus (pSTG; SEN-SND: [±63, −24, 0], SEN-rSEN: [±51, −36, −12]), and the inferior parietal lobe (IPL; SEN-SND: [±42, −72, 36], SEN-rSEN: [±30, −75, 42]). Under the rSEN-SND contrast, ROIs were set at the anterior middle temporal gyrus (aMTG; [±57, 9, −6]). These ROIs were set as spheres of 30-mm radius to clarify the broader cortical activation in the fronto-tempo-parietal region, and statistical threshold was set at random effect model, $p = .001$, uncorrected. Furthermore, we calculated the Laterality Index (LI), which was used in previous fMRI studies (Springer et al 1999; Szaflarski et al 2002) (LI = (L−R) / (L+R) × 100; L: activated voxel numbers in the left hemisphere, R: activated voxel numbers in the right hemisphere) in these regions (threshold: random effect model, $p = .001$, uncorrected). A p value (SEN-SND and SEN-rSEN contrasts: .05/6, rSEN-SND contrast: .05/2) was used in order to prevent type I errors in the multiplicity of statistical analysis.

Correlation Analysis

We investigated the correlation between cerebral activation of the local area and symptoms of schizophrenia in each patient by using ROI analysis. These ROIs were set as spheres of 20-mm radius to clarify the activation of the specific subcortical regions as well as the cortical regions. We selected the location of each ROI at the peak of cerebral activation of the control group. These ROIs were in the following bilateral sites: frontal lobe (middle frontal gyrus, operculum and triangular gyrus of inferior frontal gyrus), temporal lobe (anterior and posterior areas), parietal lobe, hippocampus, thalamus, and posterior cingulate.

Results

Performance

The mean percentages (± SD) of the performance ratio to the questionnaire of the control subjects for SEN, rSEN, and SND were 86.6 ± 12.5%, 89.3 ± 12.8%, and 85.7 ± 12.8%, and those of the schizophrenia patients were 88.3 ± 13.3%, 82.1 ± 11.7%, and 85.7 ± 12.8%, respectively. A mixed analysis of variance (mixed ANOVA) in the percentages of correct answers to the questionnaire did not show a significant difference with one repeated, within-subject factor (type of stimulus): F three contrasts (2,52) = .552, $p = .574 > .05$, one between-subjects factor (group): F groups (1,26) = .349, $p = .560 > .05$, and interaction F contrasts × groups (2,52) = 1.525, $p = .227 > .05$. After the experiment, all control subjects and patients were asked about the difference of gender by listening to reverse sentences made by a male voice and a female voice (Appendix 2). All could discriminate the gender difference.

Functional MRI Data

SEN-SND and SEN-rSEN Contrasts. In the one-sample t-test, the control group showed cerebral activation under the SEN-SND contrast in the left middle frontal gyrus (MFG), left IFG, right anterior STS (aSTS), aMTG, left inferior parietal lobe (IPL), left hippocampus, left thalamus, and bilateral posterior cingulate (Figure 2, left column of the SEN-SND contrast, and Table 1 Control subjects: group analysis, random effect model, $p = .001$ uncorrected, extent threshold 50 voxels, $z = 3.31$). On the other hand, cerebral activation of the patient group was observed in the MFG, IFG, and hippocampus in the left hemisphere and bilateral temporal cortices, (Figure 2, middle line of the SEN-SND

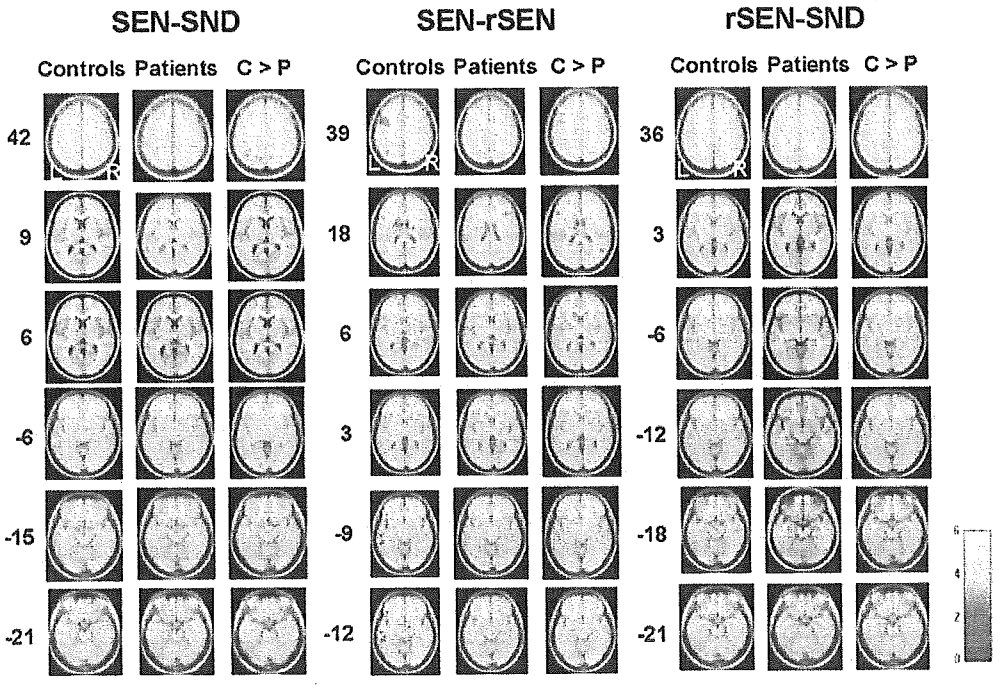

Figure 2. Cerebral activation in language processing under SEN-SND and SEN-rSEN contrasts, and cerebral activation in human voice perception under rSEN-SND contrast. In each contrast, the left column shows the results of one-sample t-test in control subjects, the middle column shows the results of one-sample t-test in schizophrenia patients, and the right column shows the difference of cerebral activation of control subjects over schizophrenia patients by two-sample t-test. Numbers along the left side of the columns of the three contrasts represent the z coordinates of Talairach and Tournoux coordinates. rSEN, reverse sentences; SEN, sentences; SND, identifiable non-vocal sounds; C, controls; P, schizophrenia patients.