fMRI data

Within-group comparisons

Both groups showed similar activation patterns in the neutral vs. rest contrast and in the pleasant vs. rest contrast (Fig. 1, top and bottom). Moreover, activation patterns in the neutral vs. rest contrast and the pleasant vs. rest contrast were also similar. In fact, significant activation in response to pleasant pictures relative to neutral pictures was seen only in the visual cortex (lingual gyrus) across groups (height threshold: $z > 4.75$ and extent threshold > 30 voxels). However, the two groups showed different activation patterns during the unpleasant condition compared to the rest condition (Fig. 1, middle). The controls demonstrated significant activation in response to unpleasant pictures relative to neutral pictures in the bilateral primary and secondary visual cortex, bilateral amygdala, bilateral hippocampal regions, medial prefrontal cortex (MPFC), right orbitofrontal cortex (OFC), bilateral thalamus, left caudate nucleus, cerebellum, and midbrain. Patients demonstrated significant activation in response to unpleasant pictures relative to neutral pictures in the bilateral primary and secondary visual cortex and left amygdala (Table 1 and Fig. 2).

Between-group comparisons

The group comparison of the U − N contrasts showed that schizophrenic patients demonstrated less activation in the right amygdala, bilateral hippocampal regions, MPFC, left visual cortex, left putamen, left caudate nucleus, left posterior thalamus, cerebellum, and midbrain (Table 2 and Fig. 3). No significantly greater activation was identified in schizophrenic patients in the between-group comparison of the U − N contrasts. We did not use between-group analysis for the pleasant minus neutral (P − N) contrast due to its meager activation across groups, possibly resulting from insufficient elicitation of pleasantness.

Correlations with BOLD signal change

There were no correlations between dosage of neuroleptics and signal change in the brain regions where patients showed decreased activation. In addition, no correlations were found between the BPRS score and signal change in these regions (Pearson's correlation analysis, $P > 0.05$).

Table 2
Brain regions with relatively less activation (unpleasant minus neutral) in 15 schizophrenic patients compared with 15 normal controls

Brain region	Brodmann's area	Coordinates[a]			z score[b]
		x	y	z	
Left lingual gyrus	18	−8	−88	−11	3.18
Left hippocampal region	30, 35	−14	−32	−12	3.83
Right hippocampal region	28	26	−24	−9	3.14
Right amygdala		24	−3	−13	3.39
Left thalamus		−22	−25	1	3.08
Left putamen		−20	12	9	2.88
Left caudate nucleus		−16	14	14	2.87
Medial prefrontal cortex	9	−2	54	25	3.21
Cerebellum		−14	−46	−23	3.06
Midbrain		−6	−26	−10	3.15

[a] Talairach and Tournoux coordinates in the local point maximal activation included in the cluster.

[b] Activation differences were considered significant at height threshold ($z > 2.57$; $P < 0.005$, uncorrected) and extent threshold (30 voxels).

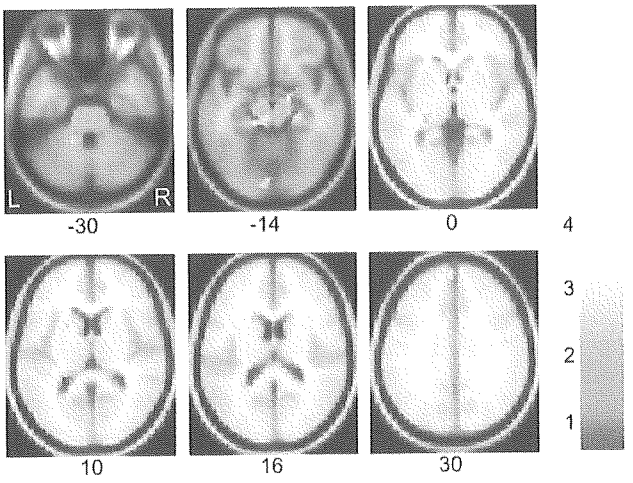

Fig. 3. Images showing brain area of relatively less activation (unpleasant minus neutral) in 15 schizophrenic patients compared with 15 normal controls. The bar shows the range of the z score. Within the image, L indicates left and R indicates right. Activation differences were considered significant at height threshold ($z > 2.57$; $P < 0.005$, uncorrected) and extent threshold (30 voxels). Numbers in the bottom row indicate the z coordinates of the Montreal Neurological Institute brain.

Discussion

In control subjects, we identified the neural circuit of the automatic emotional response to unpleasant pictures in the amygdaloid–hippocampal region, thalamus, OFC, MPFC, basal ganglia, cerebellum, midbrain, and visual cortex. We used evocative pictures with minimal cognitive demands so as to examine the automatic emotional response that requires no elaborate rating or categorization of stimuli for the subjects. Facial expressions do not necessarily elicit strong emotions, and cognitive demands such as discriminating analogous facial expressions might affect brain activations (Critchley et al., 2000; Hariri et al., 2000; Keightley et al., 2003; Lange et al., 2003; Taylor et al., 2003). Passive emotional tasks with minimal cognitive demands might activate the amygdala and other subcortical regions more often than emotional tasks with greater cognitive demands (Phan et al., 2002). Thus, we successfully observed robust activation in widespread cortical and subcortical regions as reported in previous studies (Lane et al., 1997a,b).

The cortical–basal ganglia–thalamic circuit has been implicated in cognitive or emotional processing (Alexander et al., 1986). The circuit involving the components of the cortical–basal ganglia–thalamic circuit along with the amygdala appears to be involved in the control of emotional behavior (Groenewegen and Uylings, 2000; Price et al., 1996), and dysfunction of this circuit is considered to cause mood disorder (Drevets, 2001).

Despite similar categorizations of pictures for the controls, patients demonstrated less activation in the amygdaloid–hippocampal region, MPFC, thalamus, basal ganglia, cerebellum, midbrain, and visual cortex. This finding represented evidence of functional abnormalities in the neural circuit involving the cortical–basal ganglia–thalamic circuit and the amygdala in schizophrenia. Our patients showed decreased cerebellar activation as well. Considering the fact that neuroimaging studies of schizophrenia using a variety of cognitive tasks have demonstrated a disruption in the cortical–cerebellar–thalamic–cortical circuit

(CCTCC) leading to cognitive deficits (Andreasen et al., 1999), the functional abnormalities in the CCTCC along with the limbic area might lead to the emotional dysfunction in schizophrenia. Our results could also be interpreted in this manner, supporting the notion that schizophrenic patients have disruption in the distributed neural circuit, although cortical regions vary depending on the task (Andreasen et al., 1999).

The amygdala and PFC are considered to be key nodes of the neural circuit of emotional processing, the former a main signal generator and the latter a modulator (Davidson, 2002; Drevets, 2000). Previous fMRI studies using facial expressions showed decreased activation in the bilateral amygdala (Schneider et al., 1998) or left amygdala (Gur et al., 2002) in schizophrenia. However, our patients showed significantly less activation in the right amygdala. These inconsistent findings might be due to differences in the emotional tasks. The left amygdala activation has been consistently reported in the processing of emotional facial expressions (Calder et al., 2001), and the right amygdala has been suggested to have a higher affinity with picture processing (Keightley et al., 2003; Markowitsch, 1998). It has also been suggested that cognitive or attention-demanding aspects of the emotional task could attenuate amygdala activation (Critchley et al., 2000; Hariri et al., 2000; Keightley et al., 2003; Taylor et al., 2003). Using affective pictures and minimizing cognitive demands, we demonstrated robust activation in the right amygdala as well as in the left amygdala in controls. This result concerning right amygdala activation could be attributed to group differences. Another interpretation of patients showing less activation in the right amygdala might be possible. Several lines of evidence have suggested the functional laterality of the amygdala, that is, the right amygdala may engage in a rapid automatic processing of ambiguous information, while the left amygdala may participate in conscious evaluation of significant stimuli (Critchley et al., 2000; Markowitsch, 1998; Morris et al., 1998, 1999; Phelps et al., 2001; Wright et al., 2001). Taking this into account, schizophrenic patients might have relatively intact function of conscious processing of significant information, leading to a categorization of pictures similar to that of controls, but impairment of the rapid, automatic processing of salient stimuli. In other words, patients could assign significance to stimuli through conscious processing, but they might have diminished automatic emotional response to external stimuli.

A PET study using IAPS reported that schizophrenic patients showed decreased activation in the right amygdala in response to non-aversive pictures (Taylor et al., 2002). In that study, non-aversive pictures elicited robust activation in the bilateral amygdala and aversive pictures failed to elicit greater activation in the amygdala relative to non-aversive pictures. In other words, their non-aversive pictures were not "neutral" and they might have contained emotionally salient features. In this regard, our result is consistent with this previous finding. By contrast, another recent PET study using IAPS reported decreased activation in the left amygdala in schizophrenia (Paradiso et al., 2003). Unfortunately, the study did not set up a neutral condition. Without such a condition, it remains unclear whether the decreased activation in response to unpleasant pictures stems from impairment in emotional processing of unpleasant pictures or in a more basic cognitive function such as visual perception or object recognition. We ruled out the latter possibility by comparing the activation in response to neutral stimuli across groups. Obviously, more research is needed on the abnormal amygdala function in schizophrenia.

Within the PFC, another key node of the neural circuit of emotional processing, we found decreased activation in the MPFC in patients. The MPFC was commonly activated in studies about emotional response in healthy subjects, and its activation was not specific to particular emotion or induction methods with or without cognitive components (Phan et al., 2002). The MPFC is assumed to play general roles in emotional processing such as attention to emotion, identification or regulation of emotion (Reiman et al., 1997; Teasdale et al., 1999), and guiding motivational behavior by modulating or appraising autonomic emotional responses (Drevets, 2001; Epstein et al., 1999; Phillips et al., 2003). The decreased activation in the MPFC in our patients appears to be an important finding with respect to abnormal motivational behavior in schizophrenia. In contrast, the PET study using IAPS showed hyperactivation in the MPFC in schizophrenia, contrary to the author's expectation (Taylor et al., 2002). These contradictory results therefore emphasize the need for further studies of the activation of the MPFC in schizophrenia.

The present study has several limitations. First, most of the patients were taking neuroleptic medications, possibly affecting neural activation. They were, however, taking atypical neuroleptics, and at relatively low doses. To our knowledge, there has been no previous study on the effect of neuroleptics on the BOLD response of emotional processing. Compared to typical neuroleptics, atypical neuroleptics have shown less influence on BOLD contrast in the motor area or thalamus during a finger-tapping task (Braus et al., 1999; Muller and Klein, 2000). Future studies with neuroleptic-naive patients, where the effect of neuroleptics can be controlled, will clarify this possible limitation. Second, we could not demonstrate any correlation between signal changes and BPRS scores in patients, possibly due to a lack of dispersion in the psychopathology of the patients, most of them being non-deficit outpatients with mild psychiatric symptoms. Third, the unpleasant pictures contained emotional features ranging from fear to disgust, and we could not differentiate the processing of particular emotions. In the processing of fear, the amygdala plays a central role. In contrast, the basal ganglia rather than the amygdala is considered to be essential in the processing of disgust (Calder et al., 2001; Phan et al., 2002). Thus, activation in the components of the neural circuit, the amygdala and basal ganglia, might reflect both emotional processing. Finally, we have difficulties in measuring emotional behaviors. This point has implications for the interpretation of the discrepancy between normal behavioral result and the abnormal neural activations observed in schizophrenic patients, as was also reported in previous studies (Gur et al., 2002; Paradiso et al., 2003; Schneider et al., 1998; Taylor et al., 2002). Our task could be regarded as an emotion-induction task. However, the finding needs to be interpreted cautiously because, strictly speaking, our task was testing the access to autonoetic perception of elicited emotions. It might be possible that the ability of schizophrenic patients to access their emotions (categorization of feeling) was different from that of normal controls. Our behavioral results might not necessarily reflect gut-level elicited emotion that drives emotional behavior. Autonomic data such as skin conductance responses would help to measure gut-level emotional response.

In conclusion, we investigated the automatic emotional response in healthy controls and schizophrenic patients. By using a task with minimal cognitive demands, we identified robust activation across the neural circuit of emotional processing including the amygdaloid–hippocampal region, prefrontal cortex, thalamus, and basal ganglia in response to unpleasant stimuli in the controls.

Schizophrenic patients demonstrated less activation in the components of the circuit. In particular, decreased activation in the right amygdala and MPFC, the key structures in the circuit, could be related, respectively, to diminished automatic emotional response to external stimuli and impairment in regulating emotional responses to guide emotional behavior in schizophrenia.

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Magnetic brain activity elicited by visually presented symbols and Japanese characters

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A standard model of word reading postulates that the posterior inferior temporal cortex is involved in the processing of written words. This processing probably occurs within 200 ms after stimulus presentation. In order to characterize this process more precisely, we conducted a MEG study during a reading task in nine right-handed normal Japanese subjects. The subjects were required to respond to a word pertaining to the human body so that all stimuli would be subject to the same semantic processing. The trials for non-target conditions, such as kanji words, meaningful kana words, kana pseudowords and symbols were analysed to avoid possible P300 effect. The magnetic response peak of around 200 ms for symbols was smaller than any of the other three letter conditions. This result may suggest that M200 reflects the word-specific process such as visual word form recognition. *NeuroReport* 15:771–775 © 2004 Lippincott Williams & Wilkins.

Key words: Kanji; Kana; Language; M200; MEG; Semantic processing; Symbol; Visual word form recognition

INTRODUCTION

The ability to read words is one of our most important skills. Some lesion studies have revealed the importance of the posterior inferior temporal cortex (PITC) for processing visually presented words and letters [1]. The crucial role of PITC in word recognition has been further supported by studies using PET [2] and fMRI [3]. Further evidence for word-specific processing in PITC has been obtained from electric stimulation studies [4]. These imaging modalities have recently become widely favored, and they are also being used for detailed localization of the parts of the human brain activated during such a process. However, neither PET nor fMRI technique has the temporal resolution necessary to uncover the time course of events within the neuronal networks, since they measure the changes triggered in blood flow.

In order to answer questions about the dynamics of brain activity related to reading, the use of electrophysiological methods such as EEG and MEG, which provide high temporal resolution in the range of milliseconds, is highly appropriate. Nobre [5] performed intracranial recordings in the inferior temporal sulcus/fusiform gyrus and observed that letter string-specific activation peaked 150–200 ms after stimulus onset, followed 200 ms later by semantically sensitive activation in the medial temporal areas. Reading printed words may target the posterior fusiform and lingual gyri for visual processing in a proposed word recognition model [6]. In recent studies using MEG, the magnetic response peak at about 200 ms (M200 component) to the visual presentation of words has been found in PITC [7,8]. The M200 (M180 in [8]) component of the evoked magnetic field was larger for the processing of words and false font stimuli compared with nonverbal stimuli. In a PET study [2], although the areas in the left medial extrastriate visual cortex were activated by visually presented pseudowords that obey English spelling rules as well as by actual words, these areas were not activated by nonpronounceable strings of letters or letter-like forms. Considering first the differences between pseudowords and nonsense letter strings, a string of letters that follows the spelling rules of English could be seen as a legitimate visual word form. Secondly, a difference could also be due to the pronounceability of particular letter strings. Pseudowords and real words are pronounceable presentations, whereas false fonts and illegitimate strings of letters are not. A third possible explanation for the reduced activity of the PIT areas to nonpronounceable stimuli is that there will be no subsequent semantic processing. If the third hypothesis is true, M200 will depend on semantic processing. In other words, semantic processing will be included in the component of M200. The absence of activation in PITC for nonpronounceable strings of letters or letter-like forms [2] might be explained in terms of the lack of semantic processing. It is still not known whether lexical-semantic processing already begins in the latency range of the M200 component. To explore this issue further, we designed a MEG experiment to compare the PIT activities after the visual presentations of symbols, which have no visual word form or

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pronounceability but have semantics, kanji words and kana words that are both pronounceable and have meanings, and kana pseudowords that can be pronounced but have no meaning. It was of great interest to us whether there would be any difference in M200 between symbols and words, and between pseudowords and real words. Modern Japanese sentences are written in kanji (morphograms) and kana (syllabograms) combinations without spaces between words. Kanji were brought from ancient China and each kanji has semantic value as well as phonetic, whereas kana were constructed later as simplifications of kanji but represent only Japanese short syllables (mora). Kanji are used for writing most nouns, stem of verbs, adverbs and adjectives. In contrast, kana are usually used to write inflectional endings, conjunctions, particles, foreign words and onomatopoeic expressions. There are about 2000 kanji characters and 71 kana characters in daily use. This mixed usage of kanji and kana in the Japanese writing system has brought a unique pathological condition in brain lesion patients showing severe kana alexia with relatively well-preserved kanji reading [9]. However, a MEG study reported that there was no difference between kanji and kana processing [7]. The main goal of the present study was to investigate whether symbols would elicit the M200 component in a similar way as words. The second aim was to clarify the difference in processing between real words and pseudowords and between kanji and kana. If the M200 component reflects a part of the semantic processing, it would emerge for symbols like for actual word and pseudoword stimuli. However, if the M200 component reflects some processing stage specific to language between the morphological and semantic processing such as the visual word form recognition, the M200 component to symbols would be less than that to character stimuli.

MATERIALS AND METHODS

Subjects: Nine healthy native Japanese-speaking subjects (three females and six males), aged between 20 and 52 (mean 29.2±8.4 years), participated in the current experiment. They were all right-handed as confirmed by a modified version of the Edinburgh Inventory [10], and had normal, or corrected-to-normal, vision. The protocol had been approved by the Ethical Committee of the Graduate School of Tokyo Medical and Dental University. Informed consent was obtained from all participants after the nature and possible risks of the experiment were explained.

Stimuli: Four non-target and two target conditions were used (Table 1). Non-target conditions consisted of kanji words, meaningful kana words, kana pseudowords and symbols (136 stimuli or 23% expectations for each kind); target conditions, which pertained to the human body, comprised kanji words and meaningful kana words (24 times or 4% for each kind). A white semilucent screen was placed at a distance about 30 cm from the eyes and each stimulus was presented in the center of the screen with a visual angle delimited to about 2° vertically and either 2 or 4° horizontally under the control of a computer (Valustar, NEC, Japan). The stimuli were black on a white background. The kanji and meaningful kana word lists consisted of the same words, although they appeared in different character types, and the numbers of letters also differed due to the nature of the different character types. The symbols were recruited from the symbol and wingdings font of Microsoft Word. The experiment consisted of six sessions, with each session comprising five blocks of trials. For each block, six types of stimuli

Table 1. Examples of kanji words (one character), kana words (two characters), kana pseudowords (two characters) and symbols.

Stimuli		Examples				592 (100%)
Kanji words	Nontarget	皿 (dish)	土 (soil)	服 (clothes)	北 (north)	136 (23%)
Kana words		さら (dish)	つち (soil)	ふく (clothes)	きた (north)	136 (23%)
Kana pseudowords		れは (meaningless)	せあ (meaningless)	のゆ (meaningless)	つあ (meaningless)	136 (23%)
Symbols		(floppy disk)	(air plane)	(bomb)	(sandglass)	136 (23%)
Human body kanji words	Target	足 (foot)	首 (neck)	肩 (shoulder)	胸 (chest)	24 (4%)
Human body kana words		あし (foot)	くび (neck)	かた (shoulder)	むね (chest)	24 (4%)

Target stimuli are shown in the lower portion. The trials of these stimuli were excluded from the analysis.

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were arranged in a pseudo-randomized order for 1.2 s per word or symbol, but no more than 3 stimuli in the same condition appeared consecutively. The intertrial interval varied randomly from 0.3 to 0.5 s. Ten-second intervals were inserted after each block of 20 stimuli, and blinking and swallowing, prohibited during the block of stimuli to minimize artifacts, were permitted during these intervals.

Procedure: The subjects were required to lie on a bed in a dimly lit, sound-attenuated, magnetically shielded room. They were asked in advance to click the castanets whenever a word pertaining to the human body was presented. By this task, all stimuli would be processed semantically while the vigilance of the subjects was monitored.

Recordings of event-related magnetic fields (ERFs) were carried out in the using a Magnes 2500, 148-channel, whole-head system manufactured by Biomagnetic Technologies (San Diego, CA, USA) with a band pass of 0.1–400 Hz and digitized at a rate of 1024 Hz for 1000 ms including a 100 ms pre-stimulus baseline before stimulus presentation. Epochs containing a magnetic field in which the difference between maximum and minimum potentials > 4000 fT were deemed to have artifacts and were excluded from averaging. The averaged waveforms were digitally filtered using a lowpass filter of 30 Hz. The ERF waveforms elicited by the target stimuli are likely to be superimposed by large P300. Target stimuli would also be affected by motion preparation components. To avoid this, we excluded the target stimuli from the following analysis. The number of responses included in the averaging was ≥ 74 for each type of presentation and for each subject. The root mean square (RMS), i.e., the sum of the square root of all 148 sensor amplitudes mean averaged over the following time window for the components, were used to evaluate the magnitude of the magnetic field obtained. The average RMS for a 150–250 ms period was adopted as the magnitude of M200. The point during a 150–400 ms period showing the maximum RMS was adopted as the M200 peak, and the time from the stimulus onset to that point as the M200 latency. If the maximum was reached at 150 or 400 ms, the point with the highest amplitude nearest the 200 ms point was adopted as the M200 peak. For each condition, a single signal source was estimated from the 38-channel data for the posterior half of each hemisphere. Source analyses based on a single equivalent current dipole modeling (ECD) were estimated using 38 sensors in the temporo-parieto-occipital regions on each hemisphere. Only data meeting the following five criteria were accepted: (1) a correlation between the theoretical fields generated by the model and the observed fields > 0.90; (2) a goodness-of-fit (a parameter used to determine how well the observed measurements and the resulting dipole fit agree with the model) > 90%; (3) a 95% confidence volume for the location of the dipole < 2.14 cm^3 (corresponding to the volume of an 8 mm radius globe); (4) ECDs located on the cortex in MR images; (5) temporal stability of ECDs for > 10 ms associated with the preceding four criteria. Criterion (4) was checked by visual inspection. Statistical analyses were carried out using repeated measure ANOVA. The Greenhouse-Geisser correction procedure was used where appropriate.

RESULTS

Behavioral data: Response accuracy (mean ± s.d.) in the kanji and kana conditions during recordings was 95.0 ± 4.6 and 95.8 ± 5.5%, respectively. Accuracy of all the subjects was > 83% in each of the conditions, allowing all of them to enter the succeeding analysis.

Event-related fields: Figure 1 shows grand-averaged MEG waveforms for the four non-target conditions. Under each of the four conditions, visual inspection revealed three components: M150 peaking at 150 ms, M200 at 200 ms and M400 at 400 ms after stimulus onset. For the amplitude of M200, the symbol condition showed a smaller amplitude than the kanji word, kana word and kana pseudoword conditions. Figure 2 presents the grand-averaged RMS of 9 subjects for the magnetically evoked fields of all 148 channels. No difference among the four conditions was found for the amplitude or latency of M150. However, RMS waveforms began to differ between experimental conditions about 170 ms after the stimulus onset. The waveforms for the symbol condition appeared to begin later and persist longer. ANOVAs revealed significant main effects of stimulus condition for the M200 amplitude ($F(3,24)=4.42$, $\epsilon=0.596$, $p < 0.05$) and latency ($F(3,24)=2.73$, $\epsilon=0.728$, $p < 0.01$) in the left hemisphere, indicating that M200 was reduced and delayed for symbols compared to any of the other conditions. M200 did not differ between kanji words and kana words on both hemispheres. Localization of M200 showed inter-individual variability, due mainly to differences in cortical anatomy, and therefore different distributions of neural activity in MEG sensors. The sources of M200, which showed satisfactory dipole solutions on the left hemisphere, were localized in the vicinity of the fusiform gyrus (6 of 9 subjects for kanji words, 5 for kana words, 5 for kana pseudowords and 4 for symbols), inferior temporal gyrus (1 for kanji words, 2 for kana words, 1 for kana pseudowords and 1 for symbols), angular gyrus (1 for kana words and 2 for kana pseudowords) and lingual gyrus (1 for kanji words and 1 for symbols). Figure 3 shows an example of the determination of the source of the M200 electrical currents, located in the vicinity of the fusiform gyrus. The location did not differ significantly between any two of the four conditions.

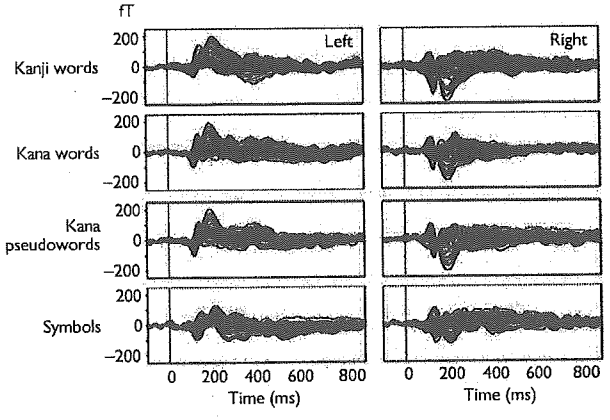

Fig. 1. Grand-averaged (n=9) event-related field (ERF) waveforms elicited by four non-target conditions (kanji words, kana words, kana pseudowords and symbols) during the semantic task recorded from 38 sensors in the temporo-parieto-occipital regions on each hemisphere. Three magnetic components can be detected (M150, M200 and M400).

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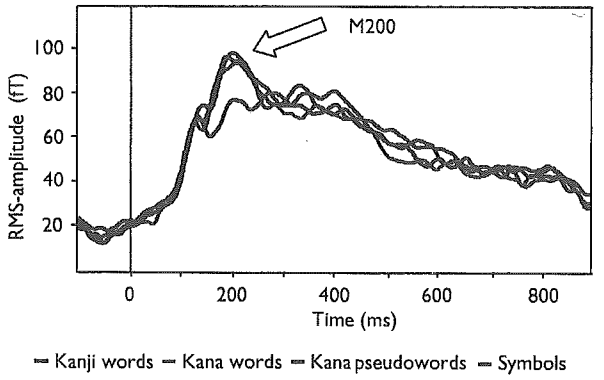

Fig. 2. Grand-averaged root mean square (RMS) waveforms recorded from the whole head are shown separately for the kanji word condition (red), kana word condition (yellow), kana pseudoword condition (green) and symbol (blue) condition. The M200 component in the symbol condition is smaller and later than in the other three conditions.

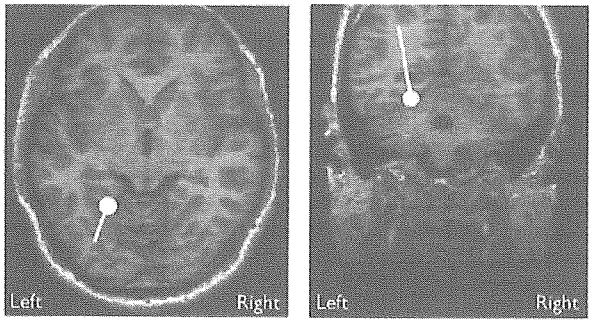

Fig. 3. White circle with a bar indicates one representative equivalent current dipole (ECD) source estimated during the M200 time window in the kanji word condition from one subject superimposed onto horizontal and coronal MRI scans. There was no difference among the four conditions.

DISCUSSION

The present experiment assessed the response of PITC during semantic judgment of kanji words, kana words, kana pseudowords and symbols, and compared the magnitude of activations between words and symbols. The MEG data revealed that M200 for symbols was smaller than for any other condition. One possible explanation is that the processing of words may activate the neural substrates that subserve visual word form recognition. Consistent with this perspective, the present study revealed greater activation in PIT during the processing of real words and pseudowords relative to symbols. An alternative explanation would be that at least a part of M200 is involved in phonological processing and that, in the case of symbols, little phonological processing occurs. However, recent studies using fMRI [11,12] have revealed that the left inferior prefrontal cortex plays a critical role in phonological processing, inconsistent with lesion deficit studies with neurological patients [13]. Therefore the plausibility of the second interpretation seems very slight. The third explanation that no semantic processing following early visual processing can result in the absence of M200 must be abandoned, because symbols have meaning and are subject to semantic processing in spite of the fact that they cannot be pronounced.

In the current study there was no effect of lexicality on M200 localized in PITC. This result is in line with studies using fMRI [14] and PET [2] that failed to find reliable activation differences between actual words and pronounceable nonwords in these areas. However, a PET study of word-naming [15] demonstrated less activation for real words than pseudowords. In contrast, a recent study using event-related fMRI of lexical decision [16] reported the reverse result, namely, stronger activation for real words than pseudowords was obtained in bilateral occipito-temporal brain areas. PET and fMRI studies have produced conflicting findings probably as a result of design and task differences. Brain activations in a block design may have been influenced by strategic effects on task performance like a stereotypic response, whereas they were elicited by individual events in an event-related design. When subjects were required to articulate the stimuli, different (although likely overlapping) and more extensive populations of neurons would be engaged compared to a lexical decision task. In contrast to PET and fMRI studies, the fact that there is no difference in the component peaking around 200 ms between actual words and pseudoword was consistently shown in a cortical surface ERP study [5] and MEG studies (1 M in [17]). Based on the present result, it seems that pseudowords may be processed in a similar way to real words in the vicinity of PITC when participants are not required to give any overt response and when an event-related design is used.

The results that the M200 responses for kanji and kana were similar in shape and consequently the locations of ECDs to kanji and kana did not differ are in accord with the previous findings [7], suggesting that kanji and kana may be processed similarly. In our previous MEG study [18] the source of M200 was localized in the vicinity of the fusiform gyrus for both kanji and kana nouns, although the amplitude of the component for kanji was larger than that for kana nouns. Coupled with the lesion study [19] indicating that there was no neuroanatomical relationship between impairments of certain high cortical functions, such as the reading of morphograms and syllabogarms, and lesion sites, our results provide converging evidence that kanji words and kana words may be processed in the same anatomical regions. Koyama [7] interpreted the kanji-kana dissociation in reading as reflecting the greater graphic complexity of some kanji. A limitation of this study that should be noted was the use of the ECD modeling. The fundamental principle of localizing the putative source depends on the basic assumption that it is reasonable to consider a single discrete source for the phenomena in question that can be appropriately mathematically modeled [20]. Many early (latency up to ~100 ms poststimulus) fields such as sensory evoked fields have a high goodness of fit to a single ECD model. When such sources are mapped onto the corresponding MRI, the locations are found to fall within the appropriate sensory cortex. This model provides validity to the source localization of such phenomena. This is less likely to be true for later (longer latency) evoked fields components that likely involve widely distributed cognitive processing that cannot be reasonably modeled with a single or simple set of sources. M200 response most probably represents the summed activity from multiple intracranial generators. Although most of the localizations during M200 period were estimated in the vicinity of the

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PITC, a few subjects showed activations in the angular gyrus. We previously reported that the ECDs for the particles (Joshi in Japanese), which are always written in kana, were mainly located in the supramarginal and angular gyri, while those for nouns (both in kanji and kana) tended to be located in the posterior-inferior-temporal areas [18]. It remains unclear whether spatially distinct sources may reflect a different aspect of the encoding process that leads to word recognition.

CONCLUSION

The present study demonstrated that M200 for symbols was smaller than any other letter condition, but there were no differences between actual words and pseudowords or between kanji and kana. These results provide evidence that M200 may reflect the prelexical process such as visual word form recognition.

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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.
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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

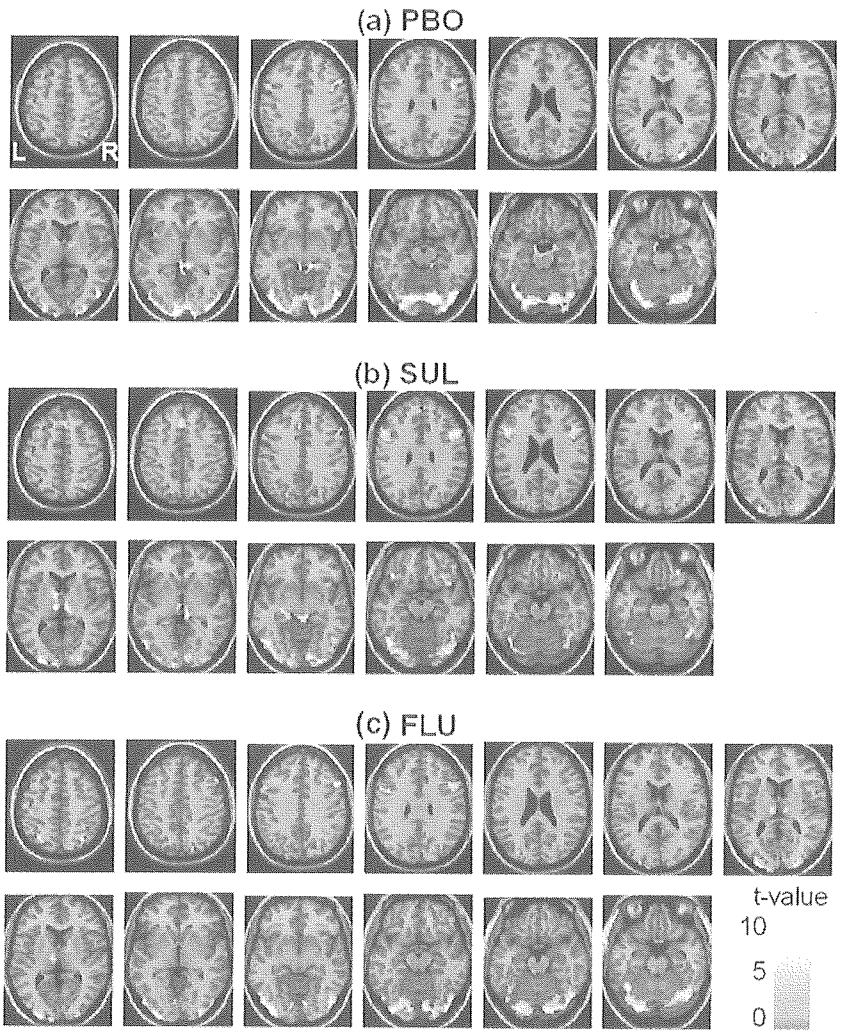

Fig. 1. Images showing dissociable brain activations in unpleasant condition relative to neutral condition during PBO treatment (a), SUL treatment (b), and FLU treatment (c). Significant differences were recognized at a height threshold ($t > 3.93$; $P < 0.001$, uncorrected) and extent threshold (10 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

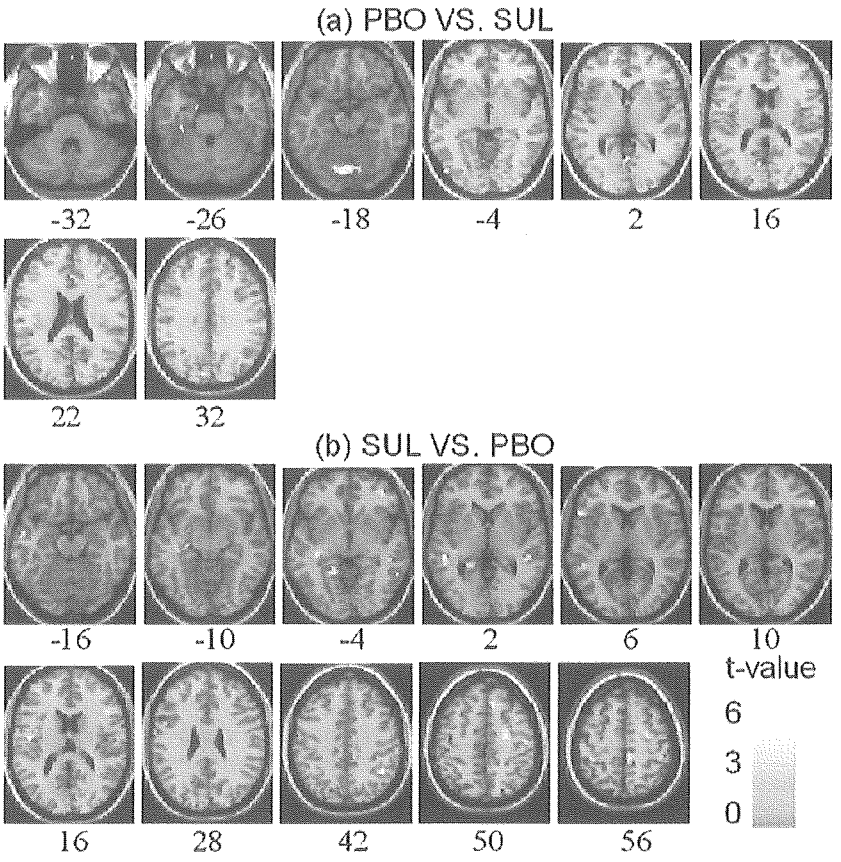

Fig. 2. Images showing manipulated brain activations in response to unpleasant condition relative to neutral condition under SUL treatment. Compared to PBO, attenuated activations were shown in the visual cortex, left temporal cortex, anterior cingulate, left amygdaloid–hippocampal region, cerebellum, 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. parietal 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

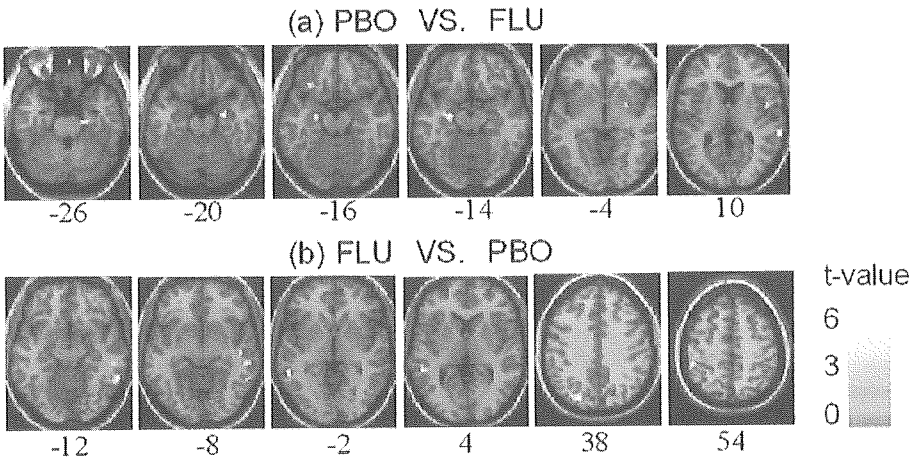

Fig. 3. Images showing manipulated brain activations in response to unpleasant condition relative to neutral condition under FLU treatment. Compared to PBO, attenuated activations were shown in the left OFC, right temporal cortex, right insula, right hippocampal region, left amygdaloid–hippocampal region, 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.

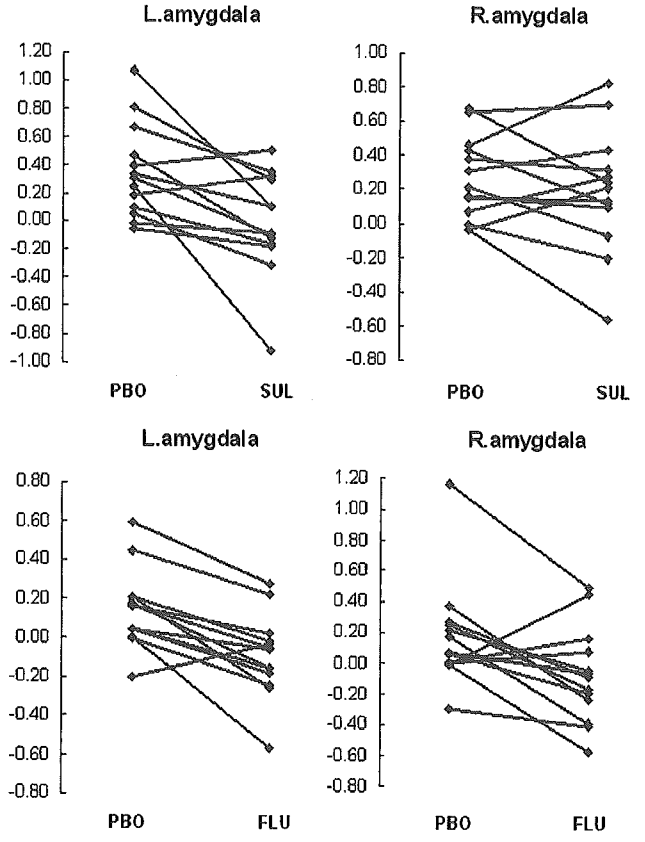

Fig. 4. Individual differences in the effect of drugs on amygdala activation elicited by unpleasant condition compared to neutral condition. The effect of sultopride on the left amygdala ($x, y, z = -14, -3, -22$) and the right amygdala ($x, y, z = 22, -3, -15$) (top). The effect of fluvoxamine on the 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.

Acknowledgments

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