method because the specific binding (target region minus caudate) has a peak at this time during PET measurement (Ito et al. 1998). AUC of the caudate adjusted by the injected dose of (S,S)-[$^{18}$F]FMeNER-$D_2$ did not significantly differ between pre- and post-administration of nortriptyline (paired $t$ test; $p$=0.27). For these analyses, the software package PMOD (PMOD Technologies, Zurich, Switzerland) was used.

NET occupancy was calculated by the following equation:

$$\text{Occupancy}(\%) = \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{baseline}}} \times 100,$$

where $\text{BP}_{\text{baseline}}$ is $\text{BP}_{\text{ND}}$ in the drug-free state, and $\text{BP}_{\text{drug}}$ is $\text{BP}_{\text{ND}}$ after administration of nortriptyline. The relationship between the dose or plasma concentration of nortriptyline and NET occupancy is described by the following equation:

$$\text{Occupancy}(\%) = \frac{C}{C + \text{ED}_{50}} \times 100,$$

where $C$ is the dose or plasma concentration of nortriptyline, and $\text{ED}_{50}$ is the dose or plasma concentration required to induce 50% occupancy (Suhara et al. 2003; Takano et al. 2006).

## Results

Typical summated PET images (120–180 min) before and after administration of 75 mg of nortriptyline are shown in Fig. 1. Typical time-activity curves of the thalamus and caudate before and after administration of 75 mg of nortriptyline are shown in Fig. 2. In the AUC ratio method, the mean NET occupancies in the thalamus by nortriptyline doses were 16.4% at 10 mg, 33.2% at 25 mg, and 41.1% at 75 mg. NET occupancies in the LC ranged from 41.6% to 90.3%, but they were not dose-dependent. Those in the hippocampus and ACC by the AUC ratio method and in all regions by SRTM and MRTM could not be estimated because occupancies or $\text{BP}_{\text{ND}}$ revealed very high or negative values in many cases. The mean plasma concentration of nortriptyline was less than the lower limit (<20.0 ng/mL) at 10 mg, 23.7 ng/mL at 25 mg, and 50.5 ng/mL at 75 mg. NET occupancy in the thalamus by the AUC ratio method and plasma concentration of nortriptyline before and after the

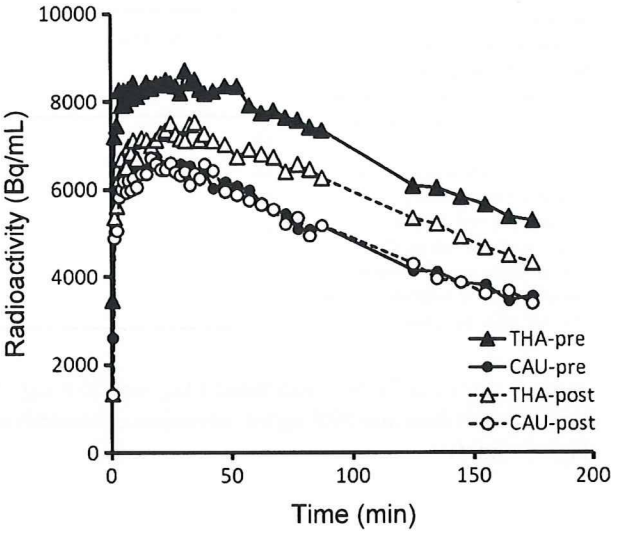


**Fig. 2** Typical time-activity curves of thalamus and caudate before and after administration of 75 mg of nortriptyline. *THA-pre* thalamus before administration, *CAU-pre* caudate before administration, *THA-post* thalamus after administration, *CAU-post* caudate after administration

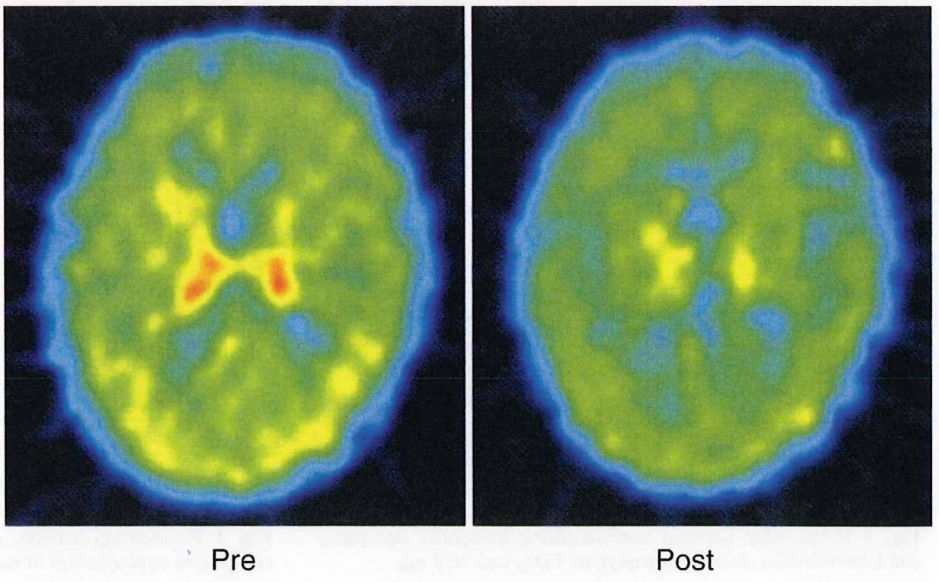


**Fig. 1** Typical summated positron emission tomography images (120–180 min) before and after administration of 75 mg of nortriptyline

**Table 1** Norepinephrine transporter occupancy in the thalamus by area under the curve ratio method and plasma concentration of nortriptyline

Plasma concentration *pre* is plasma concentration of nortriptyline just before injection of the tracer. Plasma concentration *post* is plasma concentration of nortriptyline at 180 min after injection

| Nortriptyline dose (mg) | NET occupancy (%) | Plasma concentration (ng/mL) | |
|---|---|---|---|
| | | Pre | Post |
| 10 | 22.8 | – | – |
| 10 | 10.0 | – | – |
| 25 | 36.9 | 25.5 | 21.6 |
| 25 | 29.4 | 26.3 | 21.2 |
| 75 | 33.8 | 30.4 | 45.6 |
| 75 | 48.3 | 69.9 | 56 |

scans are shown in Table 1. Estimated $ED_{50}$ was 76.8 mg of administration dose and 59.8 ng/mL of plasma concentration (Figs. 3 and 4).

## Discussion

NET is an important site of action of antidepressants such as tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors. Several studies have been reported regarding 5-HTT occupancy by antidepressants (Meyer et al. 2001, 2004; Suhara et al. 2003; Takano et al. 2006). However, NET occupancy by antidepressants in human brain has not been reported because of a lack of suitable radioligands for NET. One human study using [$^{11}$C](S,S)-MRB reported no significant dose dependency of NET occupancy at different doses of atomoxetine (Logan et al. 2007). (S,S)-[$^{18}$F]FMeNER-$D_2$ has recently been developed as a radioligand for the measurement of NET binding with PET (Schou et al. 2004). Quantification methods of NET bindings in the human brain using this ligand have been established (Arakawa et al. 2008; Takano et al. 2008a, b). Previous animal studies using (S,S)-[$^{18}$F]FMeNER-$D_2$ showed dose-dependent change in NET occupancy up to almost 100% after steady-state infusion of different doses of atomoxetine (0.003–0.12 mg/kg per hour) (Seneca et al. 2006; Takano et al. 2009). Consistent with animal experiments, the present human studies showed that NET occupancy in the thalamus by nortriptyline occurred in a dose-dependent manner. On the other hand, dose-dependent NET occupancy was not observed in the LC. This might in part reflect the difference in NET inhibitory effect by antidepressants between the LC and thalamus.

SSRIs are widely considered as the first choice of treatment for depression. However, it is known that about one third of the patients with major depression do not respond to SSRIs (De Wilde et al. 1983; Dick and Ferrero 1983; Guelfi et al. 1987; Martin et al. 1987; Stokes 1993). Recent studies suggest that the treatment of depression with newer antidepressants that simultaneously enhance both serotonergic and norepinephrinergic neurotransmissions can be expected to result in higher response and remission rates compared to SSRIs (Kampf-Sherf et al. 2004; Papakostas et al. 2007; Thase et al. 2007).

5-HTT occupancy was reported to be over 80% at clinical doses of SSRIs during the treatment of depression (Meyer et al. 2001, 2004; Suhara et al. 2003). However, the percentage of NET occupancy during clinical optimal dosing has not yet been reported. A previous study reported that a plasma concentration range between 50 and 139 ng/mL of nortrip-

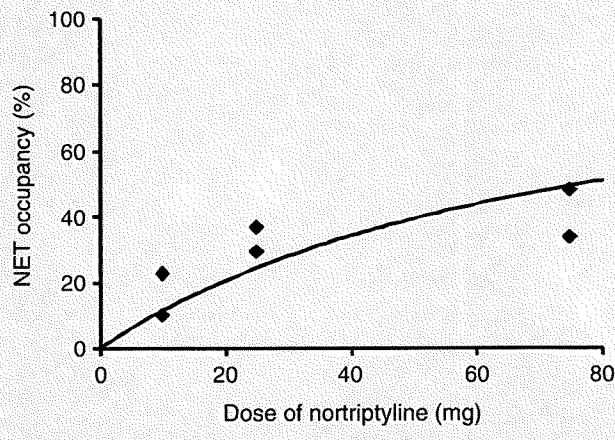


**Fig. 3** Relationship between norepinephrine transporter occupancy and administration dose of nortriptyline. $ED_{50}$ was 76.8 mg

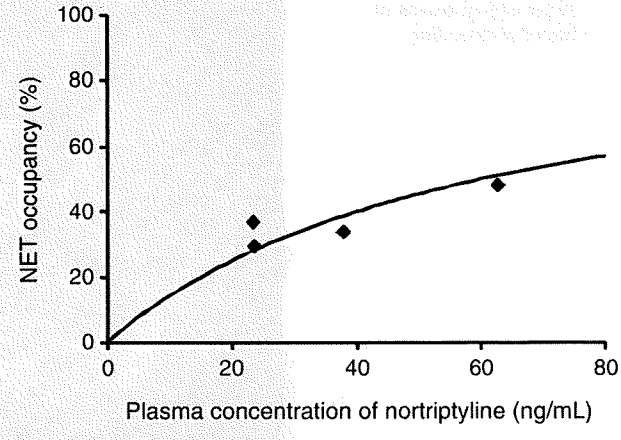


**Fig. 4** Relationship between norepinephrine transporter occupancy and plasma concentration of nortriptyline. $ED_{50}$ was 59.8 ng/mL

tyline was effective after 2 weeks of treatment for patients with depression (Asberg et al. 1971). Another double-blind, randomized study suggested that less than 150 ng/mL of nortriptyline was effective compared with greater than 180 ng/mL after 6 weeks of treatment (Kragh-Sorensen et al. 1976). Thus, it was generally appreciated that the effective plasma concentration of nortriptyline was between 50 and 150 ng/mL (Sadock and Sadock 2007). Extrapolating from these plasma concentrations and $ED_{50}$ value of the present study, the expected NET occupancy of nortriptyline can be assumed to be in a range of 50–70%. Further, NET occupancy studies in humans will be needed to evaluate the relation with the clinical effects of antidepressants.

There are several limitations in this study. First, we measured healthy subjects with a single oral dose of nortriptyline, and the dosing schedule was not randomized. Moreover, the test–retest reproducibility of (S,S)-[$^{18}$F]FMeNER-$D_2$ has not been reported. The present study was a pilot and preliminary study for further studies on patients with chronic treatment to allow discussion of the clinical conditions. Second, we could only estimate NET occupancy in the thalamus and LC by the AUC ratio method. The reason for not estimating other occupancies would be the PET procedures in this study. The present PET scan time was short, and the injected dose of (S,S)-[$^{18}$F]FMeNER-$D_2$ was small compared with previous studies. Another reason would be the characteristics of (S,S)-[$^{18}$F]FMeNER-$D_2$, which showed relatively low specific bindings. Additionally, arterial blood samplings, which would allow conducting the occupancy plot (Cunningham et al. 2010), could not be performed. Further study with arterial input function will be needed to estimate the accuracy of NET occupancy obtained by the AUC ratio method. Third, although we have reported that a linear correlation was observed in $BP_{ND}$ values between the ratio method and kinetic analysis using arterial blood data (Arakawa et al. 2008), the ratio method can induce systemic underestimation of NET occupancy. This underestimation may have affected the outcome of this study. Fourth, previous study reported that E-10-hydroxynortriptyline, an active metabolite of nortriptyline, had shown antidepressant properties (Nordin et al. 1991). In this study, we did not measure the plasma concentrations of E-10-hydroxynortriptyline, and the effect of this metabolite on NET occupancy was unknown.

## Conclusion

NET occupancy by nortriptyline corresponding to the administration dose and plasma concentration of nortriptyline was observed from 16.4% at 10 mg, 33.2% at 25 mg, and 41.1% at 75 mg in human brain using PET with (S,S)-[$^{18}$F]FMeNER-$D_2$. Estimated $ED_{50}$ was 76.8 mg of administration dose and 59.8 ng/mL of plasma concentration.

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# Electroconvulsive Therapy Decreases Dopamine $D_2$ Receptor Binding in the Anterior Cingulate in Patients With Depression: A Controlled Study Using Positron Emission Tomography With Radioligand [$^{11}$C]FLB 457

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***Objective:*** Electroconvulsive therapy (ECT) has been confirmed as one of the most effective treatments in drug-resistant major depression. However, the mechanism of ECT is still poorly understood. Although several lines of studies have focused on its effect on dopamine neurotransmission, the effects of ECT on dopamine $D_2$ receptors in a living human brain have not been investigated. Using positron emission tomography (PET) scans with the radioligand [$^{11}$C]FLB 457, we aimed to evaluate the effect of ECT on extrastriatal $D_2$ receptor binding in medicated patients with major depressive disorder (MDD).

***Method:*** Seven patients with a *DSM-IV* diagnosis of MDD underwent PET scans before and after a series of 6–7 treatments with bilateral ECT. Eleven healthy controls were scanned for comparison. All participants were scanned at the National Institute of Radiological Sciences, Chiba, Japan, between November 2000 and September 2005. The parametric images of [$^{11}$C]FLB 457 binding were generated on the basis of a simplified reference tissue model. Voxel-based methods were used to assess the effect of ECT on $D_2$ receptor binding.

***Results:*** There were no significant differences in $D_2$ receptor binding between patients with MDD and controls. All 7 patients showed clinical improvements in response to ECT treatment ($P<.001$). Significant changes in $D_2$ receptor binding, a mean of 25.2% reduction, were found in the right rostral anterior cingulate (AC) following ECT ($P<.001$).

***Conclusions:*** Electroconvulsive therapy decreased $D_2$ receptor binding in the rostral AC in MDD patients responding to ECT. Our finding suggests that one of the biologic mechanisms of ECT could be related to dopaminergic alteration in the rostral AC.

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Electroconvulsive therapy (ECT) has been confirmed as one of the most effective treatments in drug-resistant major depression.[1] Among several neurotransmitter systems, such as the serotonergic, noradrenergic, and GABAergic neurotransmitter systems, on which ECT has been reported to have an effect,[2,3] it has also been suggested that the dramatic and early improvements of severe psychomotor retardation and appetite loss after ECT were related to the dopaminergic effect.[4] In addition, ECT has been widely used, with documented efficacy, in the treatment of different mental disorders involving dopaminergic dysregulation, such as mania, schizophrenia, and catatonia, and it has also led to prolonged improvement in some patients with Parkinson's disease and effectively treated neuroleptic malignant syndrome.[1] Such clinical effects suggest that dopamine neurotransmission plays a crucial role in the mechanism of the action of ECT.

Several lines of studies have focused on the effect of ECT on dopamine neurotransmission. Both animal and human studies[5,6] have suggested that repeated ECT enhances dopamine-mediated behaviors. Some animal studies[7,8] have indicated that both acute and chronic electroconvulsive shock (ECS) at the head increases dopamine release in the frontal cortex and the striatum. A human study[9] also demonstrated increases in the concentrations of homovanillic acid in cerebrospinal fluid after ECT.

Animal behavioral studies[10,11] have demonstrated that ECS stimulates dopamine $D_2$ receptor functions. However, only a few animal studies have investigated $D_2$ receptor binding after ECS, and the results have not been consistent.[12,13] In addition, the effects of ECT on $D_2$ receptors in humans have not been investigated.

Recently, a positron emission tomography (PET) study[14] using [$^{18}$F]setoperone revealed that chronic ECS decreases serotonin 5-$HT_2$ receptors in nonhuman primates. In a human PET study,[15] decreased regional cerebral glucose metabolism was reported after ECT, but the effect on specific neurotransmitter systems has not been studied.

Common symptoms of depression, such as anhedonia and amotivation, can be viewed as impairment in the

Table 1. Clinical Characteristics of Patients

| Patient | Age, y[a] | Sex | Drug | Dose, mg/d | Length of Treatment, wk | No. of Episodes | Duration of Illness, mo | Length of Current Episode, mo | Current Clinical Features | GAF Score at Pre-ECT | HDRS Score | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Pre-ECT[b] | Post-ECT[c] |
| 1 | 32 | M | Paroxetine hydrochloride | 40 | 8 | 3 | 8 | 2 | Melancholia | 51 | 16 | 4 |
| 2 | 53 | M | Fluvoxamine | 125 | 8 | 3 | 56 | 2 | Melancholia | 40 | 17 | 3 |
| 3 | 36 | F | Fluvoxamine | 100 | 20 | 3 | 31 | 5 | Melancholia | 35 | 28 | 13 |
| 4 | 35 | F | Fluvoxamine | 150 | 16 | 2 | 11 | 4 | Melancholia | 55 | 12 | 5 |
| 5 | 52 | M | Paroxetine hydrochloride | 40 | 70 | 3 | 21 | 1 | Melancholia | 31 | 26 | 11 |
| 6 | 58 | M | Paroxetine hydrochloride | 40 | 31 | 1 | 9 | 9 | Melancholia | 35 | 21 | 14 |
| 7 | 33 | M | Paroxetine hydrochloride | 40 | 47 | 2 | 20 | 2 | Melancholia | 45 | 16 | 2 |

[a]Mean (SD) age was 42.7 (11.1) years.
[b]Mean (SD) pre-ECT HDRS score was 19.43 (5.83).
[c]Mean (SD) post-ECT HDRS score was 7.43 (5.06).
Abbreviations: ECT = electroconvulsive therapy; F = female; GAF = Global Assessment of Functioning; HDRS = 21-item Hamilton Depression Rating Scale, Japanese version; M = male.

control of dopaminergic reward and motivational systems.[16] Those systems are reportedly closely connected with the dopamine mesolimbic-cortical pathway functionally and anatomically.[17]

The radioligand [$^{11}$C]FLB 457 is a well-known dopamine $D_2/D_3$ antagonist that has picomolar in vitro affinity to both $D_2$ and $D_3$ receptors and has proven useful for the quantification of extrastriatal $D_2$ receptors in the human brain.[18] We have previously demonstrated that the measurements of cortical $D_2$ receptor binding with [$^{11}$C]FLB 457 are reproducible and reliable.[19] In the present study, using [$^{11}$C] FLB 457, we measured $D_2$ receptors in patients with major depressive disorder (MDD) before and after ECT to clarify the effect of ECT on the dopaminergic role in MDD.

## METHOD

### Subjects

Seven inpatients (mean age = 42.7 years, SD = 11.1 years; 5 men and 2 women; all right-handed) with MDD who had consented to ECT for clinical reasons, such as poor medication response and patient's own preference, at Nippon Medical School, Tokyo, or at Asai Hospital, Togane, Japan, and 11 age-matched healthy volunteers (mean age = 40.5 years, SD = 11.3 years; all men; all right-handed) participated in this study (Table 1).

The 7 patients met *DSM-IV* criteria for MDD. Based on conventional unstructured interviews and medical histories, we excluded patients with psychiatric disorders other than MDD, such as schizophrenia, substance abuse, anxiety disorder, and personality disorder. All patients were being treated with selective serotonin reuptake inhibitors (SSRIs), and their daily doses remained unchanged throughout the study (Table 1). Their median duration of treatment before ECT was 20 weeks (range, 8–70 weeks). Their medications for anxiety and sleep disturbances included lorazepam (1.5 mg/d) and flunitrazepam (1–2 mg/d).

The severity of symptoms was assessed using the Japanese version of the 21-item Hamilton Depression Rating Scale (HDRS)[20] on the same day as each PET scan. The HDRS was evaluated by 3 trained psychiatrists. The ratings were reviewed after the interviews; disagreements were resolved by consensus, and the consensus ratings were then used in this study. Each patient's Global Assessment of Functioning[21] score was assessed by the treating physicians on the day before the patient's first ECT session.

The healthy volunteers were recruited from the surrounding community. Based on unstructured psychiatric screening interviews, they were free of current and past psychiatric or major medical disease and had no relatives with neuropsychiatric disorders.

This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan; the Ethics Committee of Nippon Medical School, Tokyo, Japan; and the Ethics Committee of Asai Hospital, Togane, Japan. After we provided complete explanation of the study, written informed consent was obtained from all patients and healthy volunteers.

### Electroconvulsive Therapy Procedure

The patients received a series of 6–7 bilateral ECT sessions, 2–3 per week. Electroconvulsive therapy was administered with a square-wave, brief-pulse, constant-current device (Thymatron DGX; Somatics Inc, Lake Bluff, Illinois). Before the stimulus, subjects received atropine (0.4 mg), propofol (2.0 mg/kg), and succinylcholine (0.75 mg/kg), with dose adjustment as needed. Standard bifrontotemporal placement of electrodes was used for the treatment. Stimulus intensity was determined as a function of age, initially, and was increased until an adequate electroencephalographic seizure activity of 25 seconds or longer was obtained. Treatment was terminated when patients were judged by the treating physicians to have shown adequate symptom response.

### Positron Emission Tomography Procedure

Patients underwent baseline PET scans with [$^{11}$C]FLB 457 on the day before their first ECT session. Follow-up PET studies were performed on the day after the last ECT session. Healthy volunteers were scanned once for comparison. All PET scans were conducted at the National Institute of Radiological Sciences, Chiba, Japan, between November 2000 and September 2005. Patients and healthy volunteers were placed in a supine position with eyes closed, and head-fixation devices (Fixter Instruments, Stockholm, Sweden) with thermoplastic attachments made to fit the individuals were used.

Radioligand [$^{11}$C]FLB 457 was synthesized by O-methylation of the corresponding precursors with [$^{11}$C] methyliodide with high specific radioactivity, which was obtained by a reduction of [$^{11}$C]$CO_2$ with $LiAlH_4$ in an inert atmosphere with specially designed equipment.[22] The radiochemical purities were higher than 95%.

After a transmission scan with a $^{68}$Ge-$^{68}$Ga source, a bolus of 81.4–248.0 MBq of [$^{11}$C]FLB 457 was injected into the antecubital vein with a 20-mL saline flush. Specific radioactivity of [$^{11}$C]FLB 457 was 44.5–393.6 GBq/μmol at the time of injection. Dynamic PET data were acquired for 90 minutes using CTI-Siemens ECAT EXACT HR+ (CTI-Siemens, Knoxville, Tennessee) in 3D mode without arterial sampling.

T1-weighted magnetic resonance imaging (MRI), by Philips Intera, 1.5 Tesla (Philips Medical Systems, Best, The Netherlands), was acquired from all patients and healthy volunteers. The scan parameters were 1-mm-thick 3D T1 images with a transverse plane (TR/TE 21/9.2 ms, flip angle 30°, matrix 256 × 256 mm).

### Data Analysis

All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4 (full width at half maximum [FWHM] = 7.5 mm).

The parametric images of [$^{11}$C]FLB 457 binding were generated on the basis of a 3-parameter simplified reference tissue model.[23,24] This model gives parametric images of $R_1$ (relative delivery of radioligand normalized to the cerebellum) and binding potential ($BP_{ND} = f_{ND}B_{avail}/K_D$), in which $f_{ND}$ is the free fraction in the nondisplaceable compartment, $B_{avail}$ is the density of receptors available to bind radioligand in vivo, and $K_D$ is the dissociation constant for the radioligand.[25] The cerebellum was used as the reference region because of its negligible $D_2$ receptor density for calculation.[26] The cerebellar tissue concentration of radioactivity was obtained from regions of interest for the cerebellum, which were manually delineated on the individual MRIs coregistered to the PET summated images for 90 minutes using SPM2 (Wellcome Department of Cognitive Neurology, London, United Kingdom).

Analysis of parametric images of $BP_{ND}$ and $R_1$ was undertaken using SPM2 to investigate differences in the brain.[27] A ligand-specific template image[28] was used to define the stereotactic transformation parameters for the $BP_{ND}$ and $R_1$ images of [$^{11}$C]FLB 457. Normalized $BP_{ND}$ and $R_1$ images were smoothed with a Gaussian filter to 8-mm full-width half-maximum.

### Group Comparisons and Correlation

Statistical analyses of the $BP_{ND}$ and $R_1$ data were performed with SPM2 using voxel-based paired or unpaired *t* tests. The $BP_{ND}$ and $R_1$ differences were estimated for the following 3 contrasts: (1) patients before and after ECT, (2) healthy volunteers versus patients before ECT, and (3) healthy volunteers versus patients after ECT. For those analyses, the significant voxel level threshold was set at $P < .001$, uncorrected, with a minimal cluster size of > 100 voxels. The resulting *t* values were converted to *z* scores, with brain locations reported as x, y, and z coordinates in Montreal Neurological Institute space with approximate Brodmann areas identified by mathematical transformation of SPM2 coordinates into Talairach space.[29,30]

For significant regions, relation to the clinical effect was investigated. The percent changes in $BP_{ND}$ values in significant clusters after ECT and the correlation between changes in $BP_{ND}$ values and those of HDRS scores were evaluated. The following procedures were performed in SPM2: (1) the detected significant cluster was extracted as a volume of interest (VOI) and (2) individual mean $BP_{ND}$ values in the VOI were acquired by applying the detected significant cluster extracted as a VOI to each of the normalized $BP_{ND}$ images. The Spearman correlation method was used to examine the relationship between changes in $BP_{ND}$ variables and changes in HDRS scores. We also investigated individual mean $BP_{ND}$ values for controls and $R_1$ values for patients before and after ECT in the above VOI by applying the detected significant cluster extracted as a VOI to each of the normalized $BP_{ND}$ images of controls and the normalized $R_1$ images of patients, respectively.

### Statistical Analysis of Clinical Data

The paired *t* test was used to statistically analyze the difference between HDRS scores 1 day before the first session and 1 day after the last session of ECT. Values of $P < .05$ were considered significant.

## RESULTS

All patients showed clinical improvement in response to ECT (Table 1). The baseline HDRS score was reduced from a mean ± SD of 19.43 ± 5.83 to 7.43 ± 5.06 (paired *t* test: $t_6 = 8.92$, $P < .001$).

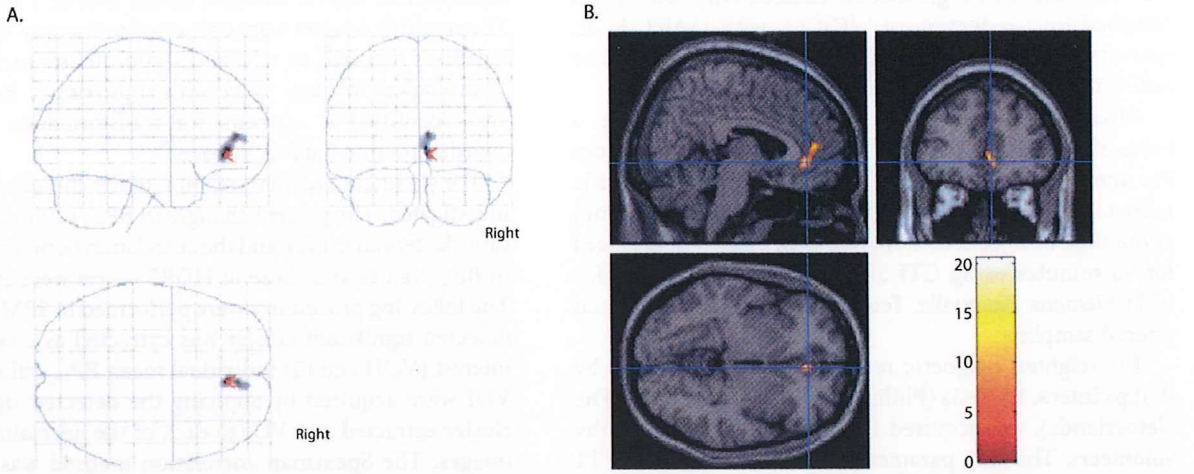
A voxel-based paired *t* test showed significant reduction of $BP_{ND}$ in patients after ECT compared with patients before ECT in the right anterior cingulate (AC) (area 24), which extended rostrally to mesial regions of area 32 and to some voxels in area 10 (Table 2 and Figure 1). In contrast,

**Table 2. Effect of ECT on [$^{11}$C]FLB 457**

| | | Cluster Level | | | Voxel Level | | | | | MNI[a] | | | Talairach[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | Brodmann Area | *P* (corrected) | $K_E$ | *P* (uncorrected) | *P* (FWE-corrected) | *P* (FDR-corrected) | *t* | *z* | *P* (uncorrected) | x | y | z | x | y | z |
| rAC | 10/24/32 | <.001 | 109 | <.001 | .042 | .067 | 20.5 | 4.92 | <.001 | 6 | 40 | −4 | 5 | 35 | −3 |
| | | | | | .133 | .077 | 16.85 | 4.69 | <.001 | 6 | 48 | 10 | 5 | 44 | 8 |
| | | | | | .308 | .078 | 14.62 | 4.51 | <.001 | 4 | 42 | 0 | 3 | 38 | 0 |
| | | | | | 1 | .085 | 9.8 | 3.99 | <.001 | 8 | 54 | 8 | 7 | 49 | 6 |

[a]The MNI column contains spatial coordinates in MNI canonical magnetic resonance imaging.
[b]The Talairach column contains spatial coordinates in the Talairach atlas.[30]
Abbreviations: FDR = false detective rate, FWE = family-wise error, $K_E$ = number of voxels in the cluster, MNI = Montreal Neurological Institute, rAC = right anterior cingulate.

**Figure 1. Map of *t* Values Showing the Voxels With Significant Decrease in [$^{11}$C]FLB 457 Binding Potential After Electroconvulsive Therapy (ECT)[a,b]**




[a]*P* < .001, uncorrected.
[b]Detected areas exceed an uncorrected *P* value of .001 with 100 or more contiguous voxels. In the left panel, all clusters throughout the whole brain are demonstrated in the "glass brain" in SPM2. The red arrow indicates a significant cluster of 109 voxels in the right anterior cingulate. The right panel shows the *t* statistic for the effect of ECT superimposed on the SPM2 canonical single-subject T1 image. The color scale shows the *t* values.

no changes in $R_1$ were seen in the patients between pre-ECT and post-ECT scans. Significant increases of $BP_{ND}$ in patients after ECT compared with patients before ECT were not found.

Voxel-based unpaired *t* tests demonstrated no significant differences in $BP_{ND}$ either for healthy volunteers versus patients before ECT or for healthy volunteers versus patients after ECT.

In the post-ECT patients, there was a mean ± SD reduction in $D_2$ receptor binding of 25.2% ± 7.5% in the VOI compared with the pre-ECT patients (Figure 2). No significant correlations were observed between changes in $BP_{ND}$ values and changes in HDRS scores. The mean ± SD individual $BP_{ND}$ values for controls and for patients before and after ECT were 0.81 ± 0.26, 0.92 ± 0.28, and 0.70 ± 0.27, respectively.

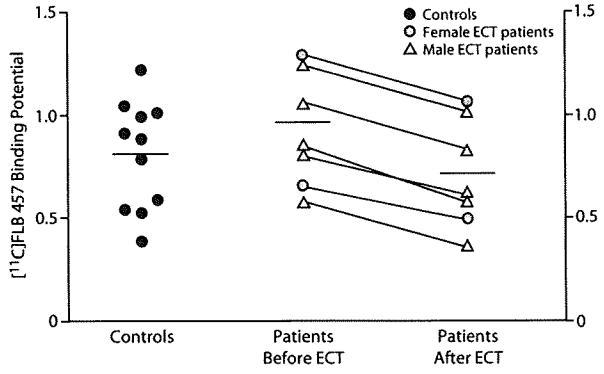
The mean ± SD individual $R_1$ values for patients before and after ECT were 0.81 ± 0.06 and 0.79 ± 0.04, respectively. The paired *t* test revealed that there were no changes in $R_1$ values in the right AC cluster in patients between the pre-ECT and post-ECT scans ($t_6 = 1.13$, $P = .302$).

## DISCUSSION

The major finding of this study was that ECT induced a mean ± SD significant decrease of 25.2% ± 7.5% in $D_2$ receptor binding in the right rostral (AC).

Our previous study[31] indicated that extrastriatal [$^{11}$C]FLB 457 was not sensitive to endogenous dopamine. In healthy volunteers, interindividual variance in $BP_{ND}$ has been suggested to be due mainly to variability in $B_{max}$ rather than $K_D$.[32] In clinical PET studies, $BP_{ND}$ is thus commonly used as an index of receptor density.[33] It is therefore reasonable to assume that the decreased [$^{11}$C]FLB 457 binding observed in this study reflects a reduction of $D_2$ receptor density in the anterior cingulate of patients with MDD after ECT. Providing that there was no neuronal cell loss after ECT,[34] the reduction of receptor density levels after

Figure 2. [$^{11}$C]FLB 457 Binding Potential of Right Anterior Cingulate in 11 Controls and 7 Depressed Patients Before and After 1 Course of Electroconvulsive Therapy (ECT)[a]




[a]Horizontal lines indicate means.

ECT could be attributable to mechanisms adaptive to increased dopamine levels in the synaptic cleft.[7,9] The detected area, the rostral region of the AC, receives dense dopamine innervation principally from the ventral tegmental area.[35] Moreover, in the medial prefrontal cortex, which would include part of the detected area, dopamine was reported to show slow clearance from the synaptic cleft.[36] Since the density of $D_2$ receptors has been reported to be sensitive to synaptic concentrations of dopamine, $D_2$ receptors could be down-regulated in response to higher dopamine availability in the rostral AC.[37] Chronic stimulation with $D_2$ receptor agonists can lead to down-regulation of $D_2$ receptors,[38] and repeated ECS is reported to sensitize $D_2$ receptors.[10,11] Taken together, our finding of decreased $D_2$ receptor binding in the rostral AC could be interpreted as a down-regulation of $D_2$ receptors after the ECT-induced dopamine release and $D_2$ receptor stimulation in the same area.

There has been only 1 animal study on $D_2$ receptors in the AC after ECS.[12] However, it was an autoradiographic study using [$^3$H]spiroperidol, demonstrating that ECS had no effect on $D_2$ receptor binding in the AC. The differences in the method of ECT administration, the species differences, and the ligand selectivity could explain the difference in results.

The rostral AC, connecting reciprocally to both the dorsal AC and the subgenual AC,[39] is considered to act as a bridge between attention and emotion[40] and to consist of the limbic-cortical circuits that maintain homeostatic mood control against stress. The region has been postulated as being one of the distinct targets that different modes of treatment modulate, resulting in a variety of complementary chemical and molecular adaptations and homeostatic effects that establish a normal mood state.[41] In addition, anhedonia and amotivation, which are frequent symptoms of depression, have been hypothesized to be associated with a dysfunction of the subgenual prefrontal cortex (part of which is the rostral AC) to maintain tonic dopaminergic-dependent, reward-related activity.[42] According to these lines of thought, one of the most relevant factors of the mechanism of antidepressant action would be the amelioration of the function of this region to regulate mesolimbic-cortical dopamine neuron activity.

We found no significant correlation between the changes of $BP_{ND}$ in the rostral AC and the changes in HDRS score. The small sample size and the lack of diversity of patients made it difficult to investigate the correlation between ECT effects and clinical improvements. In a related study, Marano et al[43] reported that ECT increased brain-derived neurotrophic factor during a course of ECT and that the change was accompanied by a significant decrease in HDRS score. This finding indicates that $D_2$ receptor binding change might have been most significant not after, but during, a course of ECT; a correlation might have been detected between HDRS score change and $BP_{ND}$ change during a course of ECT. Furthermore, it might have been valuable to assess the motivation/anhedonia or psychomotor retardation scores, in addition to HDRS scores, as those factors are reported to have close relations to the dopaminergic effect. Further studies are needed to clarify this issue.

Our finding—decreased $D_2$ receptor binding in the AC after ECT—might point to the mechanism of ECT, not the mechanism of antidepressant action. Electroconvulsive therapy has a distinct efficacy in the treatment of different mental disorders, such as mania and schizophrenia, and has also produced improvement in some patients with Parkinson's disease.[1] To clarify this question of whether our finding might point to the mechanism of ECT or the mechanism of antidepressant action, investigations of the effect of ECT on $D_2$ receptors in patients with different mental disorders will be required.

Significant differences in [$^{11}$C]FLB 457 binding between patients with depression and control subjects were not detected in this study. Results of neuroimaging studies of striatal $D_2$ receptor binding in MDD have been inconsistent. For instance, increased dopamine $D_2$ receptor binding or sensitivity[16,44–46] has been found in depressive and/or suicidal patients. Meanwhile, no difference in $D_2$ receptor binding was found in 2 recent studies.[47,48] There had been, however, only 1 in vivo study measuring extrastriatal $D_2$ receptor binding of patients with MDD, and it demonstrated no difference in [$^{11}$C]FLB 457 binding between 7 people with depression and 7 healthy controls.[49] Our result was consistent with this study. Nevertheless, both studies may have been affected by methodological limitations, such as small sample size, differences in sex distribution of each group, and differences in medications. Further study is needed to clarify this issue.

In our study, the additional analysis of $BP_{ND}$ in the right AC of controls revealed relatively large interindividual variability of $BP_{ND}$ values (Figure 2). This result is supported by

a former study[50] that demonstrated a mean ± SD $BP_{ND}$ value in the AC of healthy volunteers of 0.79 ± 0.2 (N = 10; 7 men and 3 women; age, 22–38 y). In this respect, our finding that $D_2$ binding did not differ between patients with MDD and controls despite the fact that all $BP_{ND}$ values in the right AC of patients with MDD had decreased after ECT might be explained.

Several confounding factors need to be considered for the interpretation of the present results. Since our sample size was small, our data might not assume normality of distribution. Therefore, we conducted additional analysis using a nonparametric voxel-based approach (SnPM; Wellcome Department of Cognitive Neurology, London, United Kingdom) to confirm our result. Nonparametric voxel-based analysis also revealed significant reduction of $BP_{ND}$ in the right AC in patients after ECT compared with patients before ECT (Montreal Neurological Institute coordinates: x = 4, y = 44, z = 4; pseudo $t = 4.85$; $P = .008$).

Although ECT might alter blood flow,[51] we could not detect a change in $R_1$ values between patients before and after ECT, suggesting that altered blood flow was unlikely to be responsible for the decreases in $BP_{ND}$. Our method for quantification required the use of the cerebellum as a reference region for estimation of nonspecific binding and free ligand concentration. Functional or structural abnormality of the cerebellum in mood disorders might hamper its use as a reference.[52,53] There is also a possibility that ECT could induce differences in tracer kinetics for the cerebellum. In addition, a more recent study[54] with [$^{11}$C]FLB 457 in rodents has shown that it cannot be excluded that a small percentage of the activity in the cerebellum may represent specific binding to $D_2$ receptors. However, cerebellar time-activity curves normalized by injected radioactivity did not differ significantly between patients before and after ECT.

We found no main effect of ECT or ECT-by-time interaction using repeated-measures analysis of variance with Greenhouse-Geisser correction (ECT: $F_{1,12} = 0.03$, $P = .865$; ECT-by-time interaction: $F_{2.28,22.4} = 0.281$, $P = .784$). It should be noted that there was no significant difference between the patients before and after ECT with respect to the injected specific radioactivity and injected mass in [$^{11}$C]FLB 457 (radioactivity injected: $t_6 = -0.81$, $P = .449$; mass of [$^{11}$C]FLB 457 injected: $t_6 = 0.90$, $P = .402$). The mean ± SD values for injected mass for patients before and after ECT were 0.43 ± 0.17 μg and 0.49 ± 0.17 μg, respectively. Increased receptor occupancy of unlabeled radioligand could lead to lower $BP_{ND}$ values, constituting a possible source of error. In simulation studies, for $D_2$ receptor density of the AC, an injected mass of 0.5 μg has been shown to result in a radioligand occupancy of 5%, while an injected mass of 1.0 μg corresponded to 8% occupancy.[50] On the basis of this analysis, the reported difference of 0.43–0.49 should account for 1% or 2% difference in $BP_{ND}$.

Regarding the possible contribution of atropine, propofol, and succinylcholine to our findings, only the effects of atropine and propofol against $D_2$ receptors have so far been investigated. Atropine has been reported not to occupy striatal or nigral dopamine $D_2$ receptors.[55] Propofol has been reported not to interact strongly with $D_2$ receptors ($K_{50}$ values, affinity constant, for propofol binding to $D_2$ receptors > 150 μM).[56] Taken together, it is unlikely that these medications used with ECT contributed to decreased $D_2$ receptor binding in the AC.

In this study, all patients were established on SSRI (fluvoxamine or paroxetine) treatment and were defined as treatment nonresponders on the basis of the clinical judgment of the attending physicians. Among depressive patients receiving paroxetine (modal daily dose, 50 mg; range, 30–50 mg), an increase in $D_2$ receptor binding has been reported in the anterior cingulate gyrus in treatment responders but not in nonresponders.[57] Paroxetine has also been reported to improve social functioning in A1+ allelic patients with posttraumatic stress disorder to a greater extent than in A1− allelic patients with posttraumatic stress disorder.[58] In that study, A1+ allelic patients with lower $D_2$ receptor density of basal ganglia, relative to A1− allelic patients, were speculated to respond to paroxetine via up-regulation of $D_2$ receptors. Chronic treatment of depressed patients with fluvoxamine (daily dose, 300 mg) has been shown not to alter $D_2$ receptor binding in the basal ganglia.[59] For patients with obsessive-compulsive disorder, repeated administration of fluvoxamine (mean ± SD daily dose, 233 ± 50 mg) has been reported to increase $D_2$ receptor binding in the basal ganglia.[60] The relationship between the effect of fluvoxamine and changes in $D_2$ receptor binding in the AC has not been clarified. Taken together, although the effects of SSRIs cannot be ruled out completely, it is unlikely that SSRIs contributed to the reduction in $D_2$ receptor binding in the AC of treatment nonresponders in the present study.

In conclusion, using PET with [$^{11}$C]FLB 457, we measured $D_2$ receptors in patients with MDD before and after ECT and found that ECT decreased $D_2$ receptor binding significantly in the rostral region of the right AC. The present findings suggest that the down-regulation of brain $D_2$ receptors might be induced after ECT as a compensatory change secondary to the modulation of the dopamine system in the region. We provide evidence that one of the biologic mechanisms of ECT could be related to dopaminergic alteration in the rostral AC.

***Drug names:*** atropine (Atropen and others), fluvoxamine (Luvox and others), lorazepam (Ativan and others), paroxetine (Paxil, Pexeva, and others), propofol (Diprivan and others), succinylcholine (Quelicin and Anectine).

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