The aim of the study was to test: (i) if D2/D3 binding in three functional subsections of striatum is different in patients with severe major depressive episodes than in controls; and (ii) if this difference is normalized after electroconvulsiv therapy (ECT).

Methods: Nine inpatients were examined with positron emission tomography (PET) and the radioligand [11C]raclopride before and after an average of 8.4 ECT sessions. Treatment response was assessed using the Montgomery–Åsberg Depression Rating Scale. Nine age- and sex-matched controls were examined twice with PET and [11C]raclopride.

Results: [11C]raclopride binding was significantly lower in all three subsections of striatum in patients compared to controls (Cohen’s d2/3, 1.14–1.68; P = 0.003–0.027). Montgomery–Åsberg Depression Ratings decreased significantly after ECT (P < 0.001; Cohen’s d2/3, 2.9). ECT had no statistically significant effect on [11C]raclopride binding, although post-ECT binding estimates were more similar to those obtained in controls in all subsections of striatum.

Conclusion: Using PET and [11C]raclopride, we found support for the notion that severe major depressive episodes are associated with significantly lower dopamine D2/D3 binding in all three subsections of striatum compared to controls. We noted no significant effect on D2/D3 binding in the patient group after response to ECT.

Keywords: depression, dopamine, electroconvulsive therapy, positron-emission tomography, raclopride.

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Major depressive episodes (MDE) within unipolar and bipolar disorder are the leading causes of years lived with disability worldwide, exceeding even that of cardiovascular diseases. Development of novel treatments are hampered by a limited understanding of the pathophysiology underlying MDE. Using positron emission tomography (PET), our group and others have shown in the living human brain of both control subjects and patients with depressive episodes that for most antidepressant drug treatments the mechanism of action involves inhibition of the serotonin (5-HT) transporter (5-HTT) and in some cases also the norepinephrine transporter. In established antidepressant treatment only two-thirds of patients with major depression achieve remission.

The dopamine (DA) system has been suggested to be involved in the pathophysiology of MDE. Post-mortem data are somewhat contradictory, although three studies suggest higher D1 and D2/D3 receptor density in the caudate, putamen, nucleus accumbens, and amygdala. In vivo studies of the dopamine system have focused on the striatum, with a few exceptions. [11C]raclopride is a PET radioligand suitable for quantification of D2/D3 receptor binding, particularly in the basal ganglia in the living human brain. Four previous publications have reported comparative [11C]raclopride D2/D3 receptor binding data for MDE patients and control subjects. Here patients have had either decreased binding, increased binding or no significant difference in binding compared to controls.

However, these studies may have been limited by including outpatients only, a subgroup typically less ill than patients hospitalized due to MDE. In severe depression, psychomotor retardation and Parkinsonism may be observed, and psychomotor disturbances have been shown to correlate with [11C]raclopride binding in major depression.

Electroconvulsive therapy (ECT) is a well-established and effective treatment of MDE within unipolar and bipolar disorder. It is typically given to severely depressed patients. Despite being in clinical use for almost 70 years, there is only limited knowledge of its molecular mechanism of action in humans. The dopamine system is one of several systems suggested to be modulated by ECT. This claim is supported: (i) by microdialysis experiments in small animals and PET experiments in large animals receiving ECT; (ii) by documented efficacy in the treatment of disorders associated with decreased dopamine transmission, such as Parkinson’s disease; (iii) by analysis of DA metabolites in cerebrospinal fluid of ECT patients; and (iv) by one PET study using [11C]FLB-457 for quantification of D2/D3 receptor binding in extrastriatal regions.
The aim of the present study was to determine D2/D3 receptor density in three functional subsections of the striatum, as measured with PET and the radioligand \(^{[11C]}\)raclopride in a group of severely depressed inpatients: (i) cross-sectionally, compared to healthy controls; and (ii) longitudinally, before and after a series of ECT treatments. Considering previous reports suggesting involvement of dopaminergic neurotransmission in the pathophysiology of MDE, the main hypothesis in this study was that a difference in D2/D3 receptor binding at PET2 would be detected using PET. A secondary hypothesis was that ECT would lead to a change in D2/D3 receptor binding towards that of the control group.

### Methods

The study was performed in accordance with the Declaration of Helsinki, fifth revision. It was approved by the regional ethical committee in Stockholm, Sweden, and by the radiation safety committee at Karolinska Sjukhuset, Stockholm, Sweden. The design was a non-blinded longitudinal, PET study comparing \(^{[11C]}\)raclopride binding in the striatum of patients with depressive episodes before and after a series of electroconvulsive treatments with that of control subjects matched for age (±3 years) and sex examined at two occasions, at the same time intervals as the patients (±1 week).

Recruitment of patients was performed at psychiatric wards at the Psychiatric Clinic Southwest and Northwest, SLSO, Stockholm, Sweden. Patients planned for ECT treatment of MDE were approached and informed of the study. Recruitment of control subjects was performed via advertisement in a daily newspaper and through a home page for scientific volunteers. No study-related procedure was performed before oral and written consent were obtained.

The inclusion criteria consisted of the following: (i) signed informed consent; (ii) healthy according to a clinical interview (apart from MDE in patient group); (iii) healthy according to physical examination; (iv) age ≥ 18 years; and (v) c use of alcohol disorder or drug addiction; (vi) history or presence of epilepsy, any brain disorder, or injury or loss of consciousness for more than 5 min; and (vii) treatment with D2/D3-receptor binding drugs or other drugs with known effects on the dopamine system.

No change in pharmacological treatment was allowed between the two PET experiments. ECT was administered with a square-wave, brief-pulse, constant current device (the Mecta 5000Q, MectaCorp, Lake Oswego, OR, USA and the Thymatron DGX, Somatics LLC, Lake Bluff, IL, USA). Patients were anesthetized with sodium thiopental (induction dose 4–6 mg/kg, dose adjustments as needed) and muscle relaxation was given with succinylcholine (0.75 mg/kg). The initial electrical stimulus dose was decided by age. During treatment, patients were monitored with electroencephalography (EEG) and seizure length was manually determined based on the EEG. Blood pressure and pulse were monitored during treatments. The following treatments were adjusted depending on patient responses to previous treatments and other relevant clinical factors. The number of ECT and electrode positioning were based on the clinical effect as determined by the physician responsible for the treatment.

PET2 was done when the patient was in remission or after the ECT treatment series, whichever took place first. Patients were diagnosed according to the criteria of the ICD-10.28 Symptoms were quantified at time of PET using the Montgomery–Åsberg Depression Rating Scale (MADRS).29 In addition, the following rating scales were applied: Clinical Global Impression – Severity Rating Scale (CGI-S)30 and the CORE scale,31 for quantification of overall clinical impression and of melancholic features.

A plaster helmet was made for each subject and used with a head fixation device to minimize head motion during PET.22 The helmet is constructed to orient the axial slices of the image volume parallel to the plane defined by the masticus acusticus externus and the lateral angle of the orbita. One subject (Patient 4) was imaged using the Sigma, 1.5-T MRI system (GE Medical Systems, Milwaukee, Wisconsin, USA) whereas all other subjects were imaged with the 3 Tesla GE Discovery model MR750.

\(^{[11C]}\)raclopride was prepared as described previously.33 The injected radioactivity for patients ranged between 392–504 MBq (439 ± 52 [mean ± SD]) for PET1 and 235–541 (439 ± 108) for PET2 (P = 1.00). The specific radioactivity was 556 ± 346 GBq/μmol for PET1 and 424 ± 357 GBq/μmol for PET2 (P = 0.47) corresponding to an injected mass ranging between 0.14–0.88 μg (0.42 ± 0.30) for PET1 and 0.14–1.53 μg (0.58 ± 0.42) for PET2 (P = 0.40). The injected radioactivity for controls ranged between 296–534 MBq (397 ± 98) for PET1 and 156–561 (411 ± 135) for PET2 (P = 0.81 within controls; 0.27 between patients and controls). The specific radioactivity was 148 ± 49 GBq/μmol for PET1 and 206 ± 75 for PET2 (P = 0.07 within controls; 0.01 between patients and controls) corresponding to an injected mass ranging between 0.72–2.76 μg (1.08 ± 0.64) for PET1 and 0.3–2.11 μg (0.82 ± 0.52) for PET2 (P = 0.37 within controls; 0.02 between patients and controls).

In each PET experiment a saline solution containing \(^{[11C]}\)raclopride was injected into an antecubital vein as a bolus (<10 s). The cannula was then immediately flushed with 10 mL saline. All subjects were examined using a high-resolution research tomograph (HRRT; Siemens Molecular Imaging, Knoxville, TN, USA) with a maximum spatial resolution of ~2 mm full-width-half-maximum.34 Transmission scans were performed prior to each PET measurement in order to correct for signal attenuation. Brain radioactivity was measured continuously for 5 min. The radioactivity was reconstructed in consecutive time frames: four 15-s, four 30-s, six 1-min, six 3-min, and four 6-min frames.

Regions of interests (ROIs) were defined using an atlas of functional, connectivity-based, subdivision of striatum defined in the MNI space.5,3,6 The atlas was projected to the dynamic PET data in two steps, first via transformation to each subject’s MR image using the FLIRT and FNIRT functions in FSL5 (FMRIB Software Library v5.0), and then via co-registration to a summation PET image, using the MR-to-PET transformation matrix. Time activity curves were extracted and non-displaceable binding potential (BP\(_{\text{ND}}\)) was calculated using simplified reference tissue modeling (SRTM)37 with cerebellar gray matter as reference region.

Statistical analyses and data visualization were performed using R (Version 3.3.3). If not stated otherwise all descriptive statistics are reported as means ± SD. For hypothesis testing, BP\(_{\text{ND}}\) was the parameter of interest.

The effects of diagnostic group, time, and time-by-group interaction on the three ROI and D2/D3 receptor binding were investigated by means of repeated measures, between-group analysis of variance according to the following regression model:

\[
BP_{\text{ND}} = B_0 + B_1 \text{Group} + B_2 \text{Time} + B_3 \text{Group} \times \text{Time} + B_0 + \varepsilon,
\]

where \(B_0\) represents subject-specific intercept.

Due to the fact that the second PET examination had to be excluded for two patients (reason given below) the group analysis in the regression model does not include all relevant cross-sectional data. Alpha was set to 0.05.

Correlations between disease severity scores (i.e., CORE and MADRS) and PET outcomes (i.e., BP\(_{\text{ND}}\)) were investigated using Pearson’s correlation coefficient. Primary hypothesis results were corrected for multiple comparisons across ROI using the Bonferroni method. Effect sizes were quantified using Cohen’s \(d\), a paired version of the classical Cohen’s \(d\) where the standard deviation of the difference scores is used as denominator.38 The primary advantage of this approach is that the estimate can be directly used in a power calculation (e.g., using G*Power) for a paired design. Difference in injected radioactivity, specific radioactivity, and injected mass was tested using unpaired t-test.
Results

Nine patients with a depressive episode within unipolar or bipolar disorder and nine control subjects (six women, three men) were recruited. Depressive symptoms indicated moderate to severe depressive episodes (MADRS, 35.0 ± 7.3, range 21–44). Nine age- and sex-matched control subjects were recruited. Both patients and controls were healthy according to interview and physical examination. They were all examined with PET at two occasions (Tables 1,2).

The cross-sectional comparison was performed in nine MDE patients and nine controls for PET1 and seven patients and seven controls for PET2. The binding potential in all three subdivisions of the striatum was significantly lower in the MDE group than in the control group.

Table 1. Clinical characteristics

| Patient | Age (years) | Sex | Polarity | Drug | Number of ECT | Electrode position | Time from last ECT to PET2 (days) | MADRS | CGI-S | CORE |
|---------|-------------|-----|----------|------|---------------|-------------------|---------------------------------|-------|-------|-------|
| 1       | 50          | M   | Unipolar | lorazepam 1 mg 2 × 3, propiomazine 25 mg 1 × 1, zopiclone 7.5 mg 1 × 1 | 9               | RUL              | 44                              |       |       | 23    |
| 2       | 52          | F   | Unipolar | propiomazine 25 mg 1 × 1, oxazepam 10 mg 1 × 1, nitrazepam 5 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1, duloxetine 30 mg 1 × 1 | 7               | RUL              | 28                              | 5     | 2     | 18    |
| 3†      | 58          | M   | Bipolar | nitrazepam 2.5 mg 2 × 1, oxazepam 10 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1, duloxetine 30 mg 1 × 1 | 11              | RUL              | 36                              | 12    | 6     | 2     |
| 4       | 38          | F   | Bipolar | oxazepam 10 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1 | 7               | RUL              | 41                              | 9     | 5     | 3     |
| 5†      | 63          | F   | Bipolar | oxazepam 10 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1, acamprosate 333 mg 3 + 1 + 1,itraconazole 100 mg 1 × 1 | 12              | RUL/BIL          | 41                              | 13    | 6     | 3     |
| 6       | 39          | F   | Unipolar | oxazepam 10 mg 1 × 1, nitrazepam 5 mg 2 × 1, venlafaxine 225 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1, duloxetine 30 mg 1 × 1, sertraline 100 mg 1 × 1 | 10              | RUL              | 35                              | 14    | 5     | 3     |
| 7       | 43          | M   | Unipolar | oxazepam 10 mg 1 × 1, nitrazepam 30 mg 1 × 1, lamotrigine 30 mg 1 × 1, duloxetine 60 mg 1 × 1, lithium–sulfate 42 mg 2 × 2 | 6               | RUL              | 38                              | 9     | 6     | 3     |
| 8       | 44          | F   | Bipolar | oxazepam 5 mg 1 × 1, zopiclone 7.5 mg 1 × 1, lamotrigine 300 mg 1 × 1, duloxetine 30 mg 1 × 1, sertraline 100 mg 1 × 1 | 8               | RUL              | 31                              | 17    | 5     | 3     |
| 9       | 71          | F   | Unipolar | venlafaxine 300 mg 1 × 1 | 6               | RUL              | 21                              | 5     | 4     | 2     |

Mean ± SD: 48.1 ± 11.3; 51.3 ± 11.7

†Discontinued 4 days prior to PET1. Plasma concentration at time of PET1 = 115 nM.

‡Excluded from longitudinal analysis.

BIL, bilateral; CGI-S, Clinical Global Impression-Severity; ECT, electroconvulsive therapy; MADRS, Montgomery–Åsberg Depression Rating Scale; post-ECT, at time of PET2; pre-ECT, at time of PET1; RUL, right unilateral.

Table 2. [11C]raclopride binding data

| Group        | Subject | PET1 (BPND) Striatal subsection | PET2 (BPND) Striatal subsection |
|--------------|---------|---------------------------------|---------------------------------|
|              |         | Sensorimotor | Limbic | Executive | Sensorimotor | Limbic | Executive |
| Patients     | 1       | 2.62          | 2.61   | 2.58      | 2.61          | 3.09   | 2.98      |
|              | 2       | 1.67          | 1.45   | 1.51      | 3.62          | 2.94   | 3.27      |
|              | 3       | 1.80          | 2.02   | 1.94      | MD            | MD     | MD        |
|              | 4       | 4.24          | 3.13   | 3.88      | 3.88          | 2.97   | 3.64      |
|              | 5       | 4.05          | 2.67   | 3.45      | MD            | MD     | MD        |
|              | 6       | 2.34          | 2.20   | 2.41      | 2.91          | 2.31   | 2.66      |
|              | 7       | 3.07          | 2.56   | 2.98      | 3.38          | 2.00   | 2.71      |
|              | 8       | 2.52          | 1.88   | 2.33      | 3.19          | 2.13   | 2.83      |
|              | 9       | 2.76          | 2.61   | 2.76      | 2.95          | 2.68   | 2.97      |
| Mean ± SD   |         | 2.75 ± 0.79   | 2.34 ± 0.55 | 2.64 ± 0.72 | 3.22 ± 0.44 | 2.58 ± 0.44 | 3.01 ± 0.35 |

Controls

| Group        | Subject | PET1 (BPND) Striatal subsection | PET2 (BPND) Striatal subsection |
|--------------|---------|---------------------------------|---------------------------------|
|              |         | Sensorimotor | Limbic | Executive | Sensorimotor | Limbic | Executive |
| Mean ± SD   |         | 3.64 ± 0.52   | 3.00 ± 0.20 | 3.22 ± 0.28 | 3.65 ± 0.31 | 3.02 ± 0.31 | 3.27 ± 0.24 |

†Adjusted for multiple comparisons.

BPND, [11C]raclopride binding potential; MD, missing data.
group in PET1 ($BP_{ND}$): 2.75 ± 0.79, 2.34 ± 0.55, and 2.64 ± 0.72 versus 3.65 ± 0.52, 3.00 ± 0.20, and 3.22 ± 0.29 in the sensorimotor, limbic, and executive striatum of patients and controls, respectively. The effect size (Cohen’s $d$) varied between 1.14 and 1.69 (Figs 1,2; Table 2).

The patients were examined before and after a series of ECT, the number of sessions of which was determined by the treating physician. None of the ongoing drug treatments were altered during the course of the study. No ECT was interrupted because of side-effects. All patients received right unilateral ECT according to d’Elia.39 For one subject, the electrode position was switched to bilateral due to slow response (total number of ECT = 12). All treatments resulted in at least 25 s of generalized seizure, as determined from an EEG recording. The average number of ECT was 8.4 ± 2.3. For two patients the post-treatment PET-examination had to be excluded from analysis: for one of the patients the equipment malfunctioned, making quantification impossible. For the other patient, excessive head movement was noted (4 mm displacement as seen in the SPM realignment plot). This caused an artifact in the registered radioactivity in the reference region creating evidently inflated $BP_{ND}$ in cerebral regions (e.g., in centrum semiovale, a region containing only white matter, a $BP_{ND}$ of 1 was observed). Correction for motion did not yield different results. The longitudinal analysis was thus performed in seven subjects.

The binding potential in all three subdivisions of the striatum were an average of 10–17% higher after the ECT series compared to baseline. Negligible differences were noted in the control group between PET1 and PET2. However, the group-over-time interaction in the analysis of variance was not significant in any of the three regions ($P = 0.15, 0.23$, and 0.3 in the sensorimotor, executive, and limbic subsections of striatum, respectively). See Appendix S1 for regression output. The effect size (Cohen’s $d$) varied between 0.37 and 0.65 (Figs 1,2; Table 2).

As all but one patient was treated with only unilateral ECT, we explored potential side differences in $[^{11}C]$raclopride binding. No significant differences between the left and right hemisphere were detected (data not shown).
There was no significant correlation between $BP_{ND}$ and MADRS, CORE, or $\Delta$MADRS ($r = -0.58–0.41$, $P = 0.17–0.79$). When comparing with clinical severity score, a potential association between $\Delta BP_{ND}$ and $\Delta$CORE was observed, but the effect was too weak to yield a conclusive result ($r = 0.68–0.78$, $P = 0.06–0.09$, Figure S1 in Appendix S1). There were no significant correlations between the values of individual MADRS items and $BP_{ND}$, or the corresponding deltas (data not shown).

Neither was there any correlation between the number of ECT treatments and change in $BP_{ND}$.

**Discussion**

$[^{11}C]$raclopride binding was analyzed in three established functional subsections of the striatum, as defined by Tziortzi et al.36,40 In theory, this disentangles changes related to drive and motivation (limbic striatum), cognition (executive striatum), and movement (sensorimotor striatum). Although no specific assessments of cognitive or motor function were done in the present study, it is of some interest that MDE patients typically show lower performance in tests related to all these subsections41–45 where $[^{11}C]$raclopride binding was significantly lower in the MDE patients.

The observed effect sizes were large in all three connectivity-based subsections of the striatum compared to healthy controls, before ECT (Cohen’s $d_p$, 1.14–1.69). Previously published data have shown lower, higher, and similar $[^{11}C]$raclopride binding in MDE and control subjects. However, these data were based on patients with less severe depressive symptoms than in the current study.17-item Hamilton Rating Scale for Depression, 17.2 $\pm$ 2.6, 46 18.6 $\pm$ 3.0, 47 20.4 $\pm$ 3.2, 17 and 7.3 $\pm$ 3.7,11 all indicating mild to moderate depressive episodes. The patients in our sample were all planned for ECT, a treatment mainly used in severe MDE.22 That the episodes were severe is supported by the symptom ratings collected at the time of the first PET examination (MADRS, 35.0 $\pm$ 7.3). One possible explanation for the discrepancy may thus be that only severe MDE are associated with lower $[^{11}C]$raclopride binding than in controls.

The dopamine system is one of several monoamine systems suggested to be modulated by ECT.44 This suggestion is supported: (i) by studies in small and large animals; and (ii) by analysis of monoamine metabolites in cerebrospinal fluid of ECT patients and by PET experiments in large animals, non-human primates, and psychiatric patients. Two reports describe conflicting results regarding changes in [carbonyl-(11)C]WAY100635 binding to 5-HT1A receptors (non-significant versus global decreases) in MDD patients after ECT treatment.45,46 Also, decreases in $[^{13}F]$spiperone binding to 5-HT2A receptors have been described after ECT treatment of MDD patients and in non-human primates.47,48 But not in minipigs where ECT has been associated with increased binding of $[^{11}C]$MDL100,907.49,50 There is one previous publication on extrastriatal DA D2/D3-receptor binding in relation to clinical ECT using the PET radioligand $[^{11}C]$FLB-457,51 but no study to date has examined the effect of ECT on D2/D3-receptor binding in the striatum with $[^{11}C]$raclopride. In the present study, the analysis of an effect of group over time, which was done in a subgroup only as explained above, showed no significant effect, although a numerical increase was found in the patient (10–17%) but not the control group. In theory, such an increase could be related to increased D2/D3-receptor density and/or decreased DA concentration. Increased responsiveness to the dopamine receptor agonist apomorphine has been demonstrated after ECT for MDD treatment.50 Furthermore, electroconvulsive shock enhanced apomorphine induced motor activity in reserpine-pretreated mice.52 Finally, ECT has been shown to be effective for the treatment of Parkinson’s disease.52 Thus, an increase in raclopride $BP_{ND}$ would more likely rather be due to increased D2/D3-receptor density than reduced dopamine concentration in the striatum. It should be noted that the largest increase was detected in Patient 2, who also had the longest delay from last ECT to PET2 (5 days; Tables 1,2). However, the two subjects with the second longest delay (3 days) did not show a large increase, despite achieving remission post-treatment.

Does the low raclopride binding in the depressed patients represent reduced D2/D3 receptor density or increased synaptic dopamine concentrations displacing radioligand binding to D2/D3 receptors? $[^{11}C]$raclopride binding has been shown to be sensitive to physiological changes in both D2/D3-receptor densities and in DA levels.43,44,53 Given the key role of dopamine in motivation and reward, and the cardinal symptom of anhedonia in major depression, a hyperdopaminergic tone in the striatum would seem unlikely.54,55 Low D2/D3 receptor density and/or reduced dopaminergic transmission would, however, be in line with the profoundly increased prevalence of MDE in patients suffering from Parkinson’s disease, a known hypodopaminergic state.52 Thus, the low raclopride binding demonstrated in the patients with MDE likely reflects low D2/D3 receptor binding, although high striatal dopamine concentrations cannot be ruled out. Neither can an underestimation of the difference in D2/D3 receptor binding between MDE patients and controls due to a putative low endogenous dopamine concentration during severe MDE.

No significant correlation between MADRS and $[^{11}C]$raclopride binding was detected, whereas a potential association between $\Delta$CORE and $\Delta BP_{ND}$ in two of the three regions was observed, although this correlation did not reach strict statistical significance. The study consists of a limited sample of patients with severe depression, all of whom responded to ECT. The effect sizes in the longitudinal analysis varied between Cohen’s $d_p$, 0.37–0.65. Given that there exists a true population effect of this magnitude, to detect it a sample size of 20–60 subjects is necessary to achieve 80% power (calculated using G*Power56 with paired two-sided $t$-test with an alpha of 0.05). We therefore do not rule out the possibility that the observed correlations reflect biological associations between dopaminergic neurotransmission and cognition, although a larger sample would be required to investigate it.

This study has some limitations. The complex protocol and logistics involving transportation of severely depressed patients through Stockholm made recruitment cumbersome. Also, the symptomatology of severe depression includes negativity and indecisiveness making voluntary enrollment in research less attractive for the approached patients than might have been the case if they had been in remission. This restricted the sample size of the cross-sectional data to nine pairs and may have introduced a bias in that patients not volunteering to participate may represent a subgroup with other clinical characteristics. Due to technical difficulties the longitudinal data were even smaller.

Four of the patients suffered from MDE within bipolar disorder, and five within unipolar disorder (Table 1). It has been suggested that MDE within bipolar and unipolar disorder may have phenomenological differences,58 and hence different biological underpinning. Our data do not support the latter, as there was no significant difference in $[^{11}C]$raclopride binding between the groups, although the binding was numerically lower in the unipolar group. We suggest that this issue is best addressed in a well-powered cross-sectional comparison of patients with bipolar and unipolar depression.

Six of the patients were on antidepressant treatment (Table 1). Preclinical data on nucleus accumbens indicate that chronic antidepressant treatment may be associated with increased D2/D3-receptor binding of several ligands.59 In non-human primates, acute citrulopram administration (1.0 mg/kg) has been shown to increase striatal $[^{11}C]$raclopride binding with 33%.55 At least one report suggests that $[^{11}C]$raclopride binding is unaffected by chronic treatment with norepinephrine and 5-HT reuptake inhibitors.50 None of the subjects in the current study were exposed to drugs with direct effect on DA availability or with a clinically relevant affinity for D2-receptors (Table 1). As for SSRI and SNRI exposure, there was no significant difference in $[^{11}C]$raclopride binding between the patients exposed to SSRI or SNRI and the non-exposed patients. Two patients were treated with lamotrigine. Acute but not chronic lamotrigine exposure has been shown to alter DA concentrations in microdialysis from hippocampus of freely moving rats.58 One patient was treated with lithium. Chronic lithium treatment has been shown
to decrease DA release concentration in nucleus accumbens and prefrontal cortex in rodents. In conclusion, if anything, the exposure to antidepressants and mood stabilizers in our patient sample during the study period may have increased [11C]raclopride binding in the treated patients, which would then lead to an underestimation of the true effect size of MDE on [11C]raclopride binding. It should be noted that there are no records available on the drug treatment prior to 1 week before study enrollment. Thus, it cannot be concluded to what extent previous antidepressant treatment may have influenced the results.

The PET experiments in the control group were done with a significantly larger radioligand mass than in the patient group. In theory, too large a mass could lead to an underestimation of $B_{PD}$. However, it is unlikely that the relatively low injected mass would result in a detectable mass-effect for [11C]raclopride.14

In conclusion, using [11C]raclopride and PET, we quantified D$_2$/D$_3$ binding in three functional subsections of the striatum in patients with severe MDE before and after ECT, and in a group of age- and sex-matched control subjects. We found support for the notion that severe MDE is associated with significantly lower Diamine D$_2$/D$_3$ binding in all three subsections of striatum, compared to controls.

No significant increase in D$_2$/D$_3$ binding could be detected in the patient group after response to ECT. All effect sizes were moderate to large, suggesting that the longitudinal sample was too small to be used to reject the null hypothesis.

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Disclosure statement
The authors declare no conflict of interest.

Author contributions
Concept and design of the study: C.H., J.L., L.F., T.S. Acquisition and analysis of data: B.L., J.L., J.S., M.S., M.T. Drafting the manuscript and/or figures: B.L., C.H., J.L., J.S., L.F., M.S., M.T., T.S.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Appendix S1. Supporting Information.