Dose-escalation in the picture: pharmacological and imaging studies in depression
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SEROTONIN TRANSPORTER BINDING WITH $[^{123}I]\beta$-CIT SPECT IN MAJOR DEPRESSIVE DISORDER VERSUS CONTROLS: EFFECT OF SEASON AND GENDER.

Submitted

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Abstract

Background
The serotonin system is undoubtedly involved in the pathogenesis of major depressive disorder (MDD). More specifically the serotonin transporter (SERT) serves as a major target for antidepressant drugs. There are conflicting results about SERT availability in depressed patients versus healthy controls (HC).

Aim
To measure SERT availability and study effects of age, gender and season of scanning in MDD patients versus HC.

Methods
We included 49 depressed outpatients (42.3 ± 8.3 [SD] years) with a Hamilton Depression Rating Scale above 18, who were drug-naive or drug-free for ≥4 weeks, and 49 age- (±2 years) and sex-matched HC. Subjects were scanned with single photon emission computed tomography (SPECT) using $^{[123]}\beta$-CIT. SERT availability was expressed as specific to non-specific binding ratios (BPND) in midbrain (MID) and diencephalon (DIENC) with cerebellar binding as a reference.

Results
In crude comparisons between patients and HC, we found no significant differences in MID or DIENC SERT availability. In subgroup analyses, depressed males had numerically lower MID SERT availability than HC, whereas in women SERT availability was not different (significant diagnosis*gender interaction; p= 0.048). In DIENC we found a comparable diagnosis*gender (p= 0.002) and an additional smoking*gender (p= 0.036) interaction. In MID the season of scanning showed a significant main-effect (p= 0.018) with higher SERT availability in winter.

Conclusions
Differences in MID and DIENC SERT availability in MDD patients compared with HC are modified by gender. The season of scanning is a covariate in MID. The diagnosis*gender and gender*smoking interactions on SERT availability should be considered in future studies of the pathogenesis of MDD.
Introduction

Major depressive disorder (MDD) is a highly prevalent and disabling disease, often treated by selective serotonin reuptake inhibitors (SSRIs). SSRIs block the serotonin transporter (SERT), which lowers the reuptake of serotonin (5-HT) from the synaptic cleft and increases neurotransmission. Despite the fact that the working-mechanism of antidepressants supports the monoamine deficiency theory, the pathogenesis of MDD remains unclear. Therefore, differences in SERT availability in patients and healthy controls (HC) have been studied previously.

Postmortem studies have shown reduced or unchanged concentrations of SERTs in MDD-patients compared with HC, but these studies may be biased by retrospective data collection, previous antidepressant use, suicidal behavior apart from MDD or non-selective ligands (reviewed by Stockmeier). Cerebral SERTs in humans can be quantified in-vivo with single photon emission computed tomography (SPECT) and positron emission tomography (PET). The first SERT radioligand, iodine-123-labeled 3-2-carbomethoxy-3β-(4-iodophenyl)tropane ([123I]β-CIT) binds both to SERTs and dopamine transporters (DATs). Diencephalon (DIENC) and brainstem (MID) [123I]β-CIT binding predominantly reflect SERT, while striatal [123I]β-CIT uptake reflects DAT.

Studies comparing depressed patients with HC have reported either decreased, unchanged, or increased SERT availability in MDD patients (Table S7.1). A negative correlation between SERT availability and severity of depression (measured by Hamilton Depression Rating Scale (HDRS) scores) was reported in patients with primary MDD or Wilson’s disease. Discrepant results among studies may be explained by differences in scanning techniques, analytic methods, and subject sampling, although the effects of additional variables and their interaction might also explain conflicting results. Staley et al. reported lower SERT availability in the DIENC of MDD female patients versus HC, and suggested that this interaction accounted for the contradictory results between studies. Furthermore, in HC significant effects on SERT availability have been reported for gender, smoking behavior, aging and season of scanning.

Our objectives were to quantify SERT availability in MDD patients versus HC while accounting for these potential covariates and possible interactions, and to correlate SERT availability with depression severity. Therefore, we compared [123I]β-CIT SPECT scans of drug-free MDD patients with age- and sex-matched HC.

Methods

Subjects

After approval by the institutional ethical committee and written informed consent, we recruited depressed patients from primary care, and our outpatient department (October 2003-August 2006). Patients were eligible if they were 25-55 years old, had a diagnosis of MDD (diagnosed by structured clinical interview for DSM-IV (SCID Patient Version)), had a HDRS score >18, and were drug-free or used no more than one antidepressant (stopped for >4 weeks and ≥ 5 half-lives of this antidepressant before scanning) for the present MDD-episode. Exclusion criteria were pregnancy (or desire to become pregnant), bipolar disorder, psychotic features, primary anxiety and/or substance abuse disorders and acute, severe suicidal ideation. We allowed secondary co-morbid anxiety and/or substance abuse.

We individually matched each patient by gender and age (±2 years). HC were in good physical health and had never used psychotropic medication. Exclusion criteria were current or lifetime psychiatric disorder(s) according to the SCID (including abuse or addiction disorders), a Beck Depression Inventory (BDI) score >9, alcohol use >4 units per day (last month) or a 1st-degree relative with psychiatric disorder(s). We allowed HC to have incidentally used illicit drugs unless
criteria for a DSM-IV disorder were met, but prohibited illicit drug use the month prior to scanning. Patients and HC received €50 and €40, respectively. No restrictions were made with respect to smoking behavior.

**Procedure & SPECT-imaging**

We performed all scans 230 ±18 (SD) minutes after intravenous injection of approximately 100 MBq $[^{123}I]\beta$-CIT, when the radioligand was at equilibrium for SERT binding in brain areas expressing high densities of SERTs. Radiosynthesis of $[^{123}I]\beta$-CIT and image acquisition were described earlier. We performed SPECT imaging using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, Strichmann Medical Equipment; Cleveland, OH) with a full-width at half-maximum resolution of 6.5 mm, throughout the 20 cm field-of-view (http://www.neurophysics.com).

**Image Analysis**

After attenuation correction and reconstruction in 3D mode (http://www.neurophysics.com), we selected regions of interest (ROIs) for midbrain (MID), DIENC and cerebellum (CER) by using validated templates (see Figure 2.3). One examiner (HGR), blinded for diagnosis positioned all ROIs in two series. Intra-class correlation coefficients were ≥0.98 for all ROIs. If the two series differed by >5%, scans were re-evaluated by a second investigator (JB). In the analyses we averaged the counts for the two series.

We assumed activity in CER to represent non-displaceable activity (non-specific binding and free radioactivity). We calculated the binding potential (BP) as the rate of specific to non-displaceable (ND) binding ($BP_{ND} = \frac{\text{dilution}_{ND} - \text{dilution}_{ND}}{\text{dilution}_{ND}}$) for MID and DIENC. $BP_{ND}$ is proportional to transporter number under equilibrium conditions.

**Statistics**

General linear models were used to analyze differences in $BP_{ND}$ in MID and DIENC between depressed patients and HC using the following modeling strategy.

We first compared mean $BP_{ND}$ in MDD patients versus HC in univariable (‘crude’) models, only containing the main effect of diagnosis (categorical: MDD/HC). We then fitted multivariable models by adding variables to the model, which in the literature have been reported to influence $BP_{ND}$. These variables included: gender (categorical: male/female), age (continuous), smoking (categorical: yes/no), season of scanning (categorical: “winter” October-March/ “summer” April-September). In addition, a number of specific two-way interactions were examined, again because they have been reported as significant in previous studies, which included: diagnosis*gender, diagnosis*smoking, gender*smoking (‘full multivariable models’). Three-way interactions were not examined because of the relatively small sample-size (from a statistical perspective). The Akaike’s Information Criterion (AIC) was used to judge whether the two-way interactions improved the model. If a two-way interaction did not improve the fit of the model, it was removed from the model in order to facilitate the interpretation of the model (‘reduced multivariable models’). Main effects always remained in the model, irrespective of their significance, in order to report their (lack of) impact. Diencephalon and midbrain data were analyzed separately, but the same set of variables and interaction terms were examined using the same modeling approach. If significant interactions were present, we performed post-hoc analyses in order to report the absolute differences in SERT availability in the involved subgroups. Differences between subgroups were analyzed within the framework of the multivariable model and tested for significance using the residual variance estimate of the model. For explorative analyses see the supplementary appendix.

We examined the association of $BP_{ND}$ with HDRS scores using linear regression models in patients, correcting for covariates used in the multivariable models. We used SPSS (version 15.0.1) for statistical procedures (www.spss.com). We expressed all means ±SD, except in Figures 7.1 and S7.1, where ±SEM was used for legibility.
Results

We studied 17 male and 32 female patients with MDD, versus 17 male and 32 female HC (Table 7.1). Patients smoked significantly more (n = 27; 55.1%) than HC (n = 11; 22.4%; χ² = 11.2; df = 2; p = 0.004). Among HC we included more Caucasians (n = 44; 89.8%) than among patients (n = 31; 63.3%; χ² = 11.9; df = 3; p = 0.008). We scanned 67% of patients and 55% of HC in winter (χ² = 1.55, df = 1, p = 0.213). In one female patient insufficient CER was scanned as a reference, and in three patients and one control MID slices were insufficient; these were omitted in the analyses. Of 15 patients who used antidepressants during their lives, 3 used antidepressants for the current episode. One patient used mirtazapine until 4 weeks before scanning, all others stopped antidepressants 6-132 months before scanning. Illicit drug abuse mainly involved cannabis. Lifetime MDMA-use occurred in none of the patients and in one female control (<10 tablets; last use 8 months before scanning).

Table 7.1. Baseline characteristics of MDD patients and healthy controls (stratified by gender).

<table>
<thead>
<tr>
<th></th>
<th>MDD patients</th>
<th>Healthy controls (HC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n= 17)</td>
<td>Female (n= 32)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.2 ±7.8</td>
<td>41.8 ±8.6</td>
</tr>
<tr>
<td>Current cigarette smokers; n (%)</td>
<td>12 (70.6)</td>
<td>15 (46.9)</td>
</tr>
<tr>
<td>Alcohol use &gt;8 Units/ week; n (%)</td>
<td>3 (17.6)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Race: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>12 (70.6)</td>
<td>19 (59.4)</td>
</tr>
<tr>
<td>Creole</td>
<td>2 (11.8)</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (11.8)</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>MDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity ‡</td>
<td>23.2 ±4.9</td>
<td>25.4 ±4.7</td>
</tr>
<tr>
<td>First episode; n (%)</td>
<td>9 (52.9)</td>
<td>18 (56.3)</td>
</tr>
<tr>
<td>Drug-naïve; n (%)</td>
<td>12 (70.6)</td>
<td>22 (68.8)</td>
</tr>
<tr>
<td>Melancholic; n (%)</td>
<td>12 (70.6)</td>
<td>24 (75.0)</td>
</tr>
<tr>
<td>Atypical; n (%)</td>
<td>0</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Suicidal thoughts, plan or attempt; n (%)</td>
<td>7 (41.2)</td>
<td>10 (31.3)</td>
</tr>
<tr>
<td>Duration of episode: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 months</td>
<td>5 (29.4)</td>
<td>8 (25.0)</td>
</tr>
<tr>
<td>5 months – 2 years</td>
<td>8 (47.1)</td>
<td>21 (65.6)</td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>4 (23.5)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Age of first episode (years)</td>
<td>35.9 ±10.3</td>
<td>35.8 ±10.4</td>
</tr>
<tr>
<td>Co-morbidity: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>4 (23.5)</td>
<td>7 (21.9)</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>1 (5.9)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Alcohol dependence</td>
<td>1 (5.9)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Drug (alcohol, cannabis, benzodiazepines) abuse</td>
<td>4 (23.5)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>SPECT scan in winter; n (%)</td>
<td>9 (52.9)</td>
<td>24 (75.0)</td>
</tr>
</tbody>
</table>

Numbers represent means ± SD

‡ Significantly more smoking in MDD patients than in HC (χ² = 11.0; 1 df; p < 0.001)
† Trend of lower alcohol use in MDD patients than in HC (χ² = 3.75; 1 df; p = 0.053)
‡ Severity measured by Hamilton Depression Rating Scale (17-items) in MDD patients and by Beck Depression Inventory in HC

SERT availability in MDD patients versus healthy controls (‘crude models’)

Mean MID BPND in patients (0.62 ±0.22 [SD]) was not significantly different from HC (0.63 ±0.19; F1,94 = 0.118; p = 0.733). BPND in DIENC was 1.15 (±0.24) in patients and 1.09 (±0.26) in HC (F1,97= 1.209; p = 0.274).

Multivariable models of SERT availability in MDD patients versus healthy controls

Midbrain

For MID, the full multivariable model (including diagnosis, gender, age, smoking, season of scanning, diagnosis*gender, diagnosis*smoking, and gender*smoking) was subsequently reduced by removing the non-significant interaction terms diagnosis*smoking, and
gender*smoking (AIC-decrease = 2.478; Figure 7.1A). This reduced multivariable model had a significant diagnosis*gender interaction \( (F_{1,87} = 4.039; p = 0.048) \). In post-hoc comparisons, MDD males showed a trend of lower \( \text{BP}_{\text{ND}} \) compared with male HC (difference = -0.124; \( t_{87} = -1.718; p = 0.089 \)), while female patients and HC did not differ (difference = 0.047; \( t_{87} = 0.898; p = 0.372 \)). Furthermore, the main effect of season of scanning was significant \( (F_{1,87} = 5.814; p = 0.018) \). Scans performed in winter showed on average 18\% higher \( \text{BP}_{\text{ND}} \) than scans made in summer \( (F_{2,87} = 3.248; p = 0.044) \). The main effects of age and smoking were also included in this reduced model, but were not significant \( (p = 0.227 \) and \( p = 0.582 \), respectively; Figure 7.1A).

**Figure 7.1.** \( \text{BP}_{\text{ND}} \) values for midbrain and diencephalon.

Multivariable models for \( \text{BP}_{\text{ND}} \) in MDD patients (MDD) versus HC stratified for gender. Values represent estimated means ±SEM.

A. Midbrain \( (n = 94) \), corrected for main effects of diagnosis \( (p = 0.414) \), gender \( (p = 0.128) \), season of scanning \( (F_{1,87} = 5.814; p = 0.018) \), smoking \( (p = 0.582) \), age \( (p = 0.227) \) and diagnosis*gender interaction \( (F_{1,87} = 4.039; p = 0.048) \).

* Post-hoc differences \( t_{87} = -1.718; p = 0.089 \) between: MDD males versus HC males.

B. Diencephalon \( (n = 97) \), corrected for main effects of diagnosis \( (p = 0.476) \), gender \( (p = 0.277) \), season of scanning \( (p = 0.679) \), smoking \( (p = 0.223) \), age \( (p = 0.247) \) and diagnosis*gender interaction \( (F_{1,87} = 10.127; p = 0.002) \), gender*smoking interaction \( (F_{1,88} = 4.541; p = 0.036) \) and diagnosis*smoking interaction \( (p = 0.127) \).

* Post-hoc difference \( t_{88} = -2.384; p = 0.019 \) between: smoking HC males versus non-smoking HC males. ** Post-hoc differences \( t_{88} > 2.643; p < 0.01 \) between: smoking MDD males versus smoking HC males, non-smoking MDD females versus non-smoking HC females.

**Diencephalon**

For DIENC, the full multivariable model (including the same variables as MID) could not be reduced as all three interactions terms (diagnosis*smoking, diagnosis*gender and smoking*gender) improved the fit of the model as evaluated by the AIC (Figure 2B). This full multivariable model showed significant diagnosis*gender \( (F_{1,88} = 10.227; p = 0.002) \) and smoking*gender \( (F_{1,88} = 4.541; p = 0.036) \) interactions. The diagnosis*smoking interaction was not significant \( (p = 0.127) \). In post-hoc comparisons, smoking MDD males had significant lower \( \text{BP}_{\text{ND}} \) compared with smoking male HC (difference = -0.304; \( t_{88} = -2.643; p = 0.016 \)). In non-smoking MDD males \( \text{BP}_{\text{ND}} \) was numerically lower than in non-smoking male HC (difference = -0.140; \( t_{88} = -1.340; p = 0.184 \)). Contrary, non-smoking female patients had higher \( \text{BP}_{\text{ND}} \) than non-smoking female HC (difference = 0.211; \( t_{88} = 3.064; p = 0.003 \)) with almost no difference in \( \text{BP}_{\text{ND}} \) between MDD and HC in smoking females (difference = 0.057; \( t_{88} = 0.616; p = 0.539 \)). Furthermore, male smoking HC had significantly higher \( \text{BP}_{\text{ND}} \) than non-smoking male HC (difference = 0.279; \( t_{88} = -2.384; p = 0.019 \)), while in female HC \( \text{BP}_{\text{ND}} \) was not affected by smoking (difference = 0.032; \( t_{88} = 0.371; p = 0.712 \)). The main effects of season of scanning and age were also included in this model, but were not significant \( (p = 0.679 \) and \( p = 0.247 \), respectively; Figure 7.1B).

**Relation of SERT availability and severity of MDD**

With linear regression models, we found no significant relation between HDRS-scores and \( \text{BP}_{\text{ND}} \), neither in MID, nor in DIENC when accounting for gender, age, smoking and season of scanning.
Discussion

In the present – until now largest – study of MDD patients versus HC, we aimed to quantify SERT availability in MDD patients versus HC while accounting for covariates and interactions, and to correlate SERT availability with depression severity. We did not find significant differences in SERT availability in MID or DIENC in crude comparisons. However, a significant diagnosis*gender interaction existed in MID and DIENC, combined with a significant gender*smoking interaction in DIENC only. Depressed males, but not females, had lower MID SERT availability compared with HC. In DIENC depressed smoking males had significantly lower MID SERT availability compared with smoking male HC, while non-smoking female patients had higher SERT availability than female HC. Furthermore, the season of scanning influenced SERT availability in MID, with higher SERT availability in winter. We found no clinically relevant correlation of HDRS-scores with SERT availability.

Comparison with previous studies

Our results replicate earlier reports of similar SERT availability in MID and DIENC in MDD patients and HC.14-18 Other studies reported increased19 or decreased7-13 SERT availability in MDD patients versus HC. However, none of these studies – except two11;13 – investigated the effect of gender and no study corrected for season. Furthermore, we replicated a significant contribution of season of scanning on MID BPND.24

The diagnosis*gender interaction in MID and DIENC is our most important finding. Contrary to Staley and colleagues,11;21 we found a different direction of this interaction in DIENC: significantly lower BPND in MDD males in MID (-17%) and DIENC (-18%), and higher BPND in MDD females in MID (+9%, non-significant) and DIENC (+13%, significant) than in HC. Staley et al. found 1% lower DIENC SERT availability in MDD males, and 22% lower DIENC SERT availability in MDD females. We found a gender*smoking interaction in DIENC (with highest BPND in smoking healthy males), while Staley found higher SERT availability in the brainstem (attributable to males). Our findings suggest that a failure to stratify for a diagnosis*gender interaction may obscure differences between patients and HC.

Methodological explanations for inconsistent findings

Despite technical differences between studies (scanning protocols, radioligands, image analyses), variation in the selection of HC (e.g. having relatives with psychiatric diagnoses) or patients (from different source populations) is the most probable explanation for inconsistent findings. Previous studies recruited patients in general psychiatric outpatient and university clinics.7;8;10;12;18;19;28 We recruited 65% of our patients from primary care settings. We adequately diagnosed patients by SCID, and required an HDRS >18 for inclusion. Thus, we recruited severe and often melancholic patients that were drug-free, with 69% of the patients drug-naïve. Three studies7;13;29 included larger proportions of drug-naïve patients. Like Parsey et al.,10 we observed (non-significant -15%) lower MID SERT availability in drug-naïve patients (results available on request). Additionally, some studies suggested that anxiety disorders influence SERT availability,30 and MDD with co-morbid anxiety may differ from ‘pure MDD’. However, this was not observed in our sample (results available on request).

Role of SERT in the pathogenesis of MDD

SERTs evacuate extracellular 5-HT from the synapse. Observed differences in SERTs between patients and HC may represent differences in the number of SERT containing neurons, in the number of SERTs per neuron or a combination of both.

Two major mechanisms for the role of SERT in MDD are hypothesized.30 First, increased SERT availability reduces 5-HT from the synapse more easily, which might lower 5-HT transmission, possibly leading to MDD. Second, as the brain might apply compensation mechanisms to retain homeostasis, a decreased 5-HT transmission by MDD may result in downregulation (decrease) of
SERT in order to increase 5-HT transmission. A sequential occurrence of these two mechanisms could also be hypothesized: an initially increased SERT availability destabilizes (with or without an additional factor) and leads to MDD, which is followed by a decrease in SERT to compensate for decreased 5-HT transmission.

Differential effects of MDD on SERT availability between sexes may be explained via sex-hormones. Estrogen replacement after ovariectomy increased SERT mRNA and SERT availability in female rats and in hypothalamic regions of female macaques. Depressed women may have significantly higher 24h mean levels of diurnal estradiol rhythms, and may have higher testosterone levels compared with HC. Testosterone may increase SERT availability by conversion to estrogen by aromatase, which is especially available in DIENC. This could explain our finding of increased SERT availability in DIENC in females. In depressed men, the sex steroid testosterone is decreased, with 34-61% biochemical hypogonadism in depressed males compared with 6-14% in HC. This lack of testosterone in MDD may reduce SERT availability by reduced conversion to estrogen. Replacement of testosterone in castrated male rats increased SERT mRNA and SERT availability. Since we did not measure sex hormones, and Best et al. found no relation between menstrual cycle or sex hormones and SERT availability in HC, these explanations remain speculative and should be examined further.

We replicated a main effect of season demonstrated previously in DIENC in 12 healthy women and mesencephalon in 29 HC. Neumeister et al. observed decreased SERT availability in winter. In contrast, Buchert et al. found increased SERT availability in winter, which was also found in our study. Serotonin modulates the effects of photic input in the suprachiasmatic nucleus (SCN). The SCN imposes a circadian rhythm by affecting hormonal and autonomic output (reviewed by Buijs). Serotonin release in the SCN is highest during waking and activity. Because raphe neurons show regular high firing rates during waking and decreased firing during sleep, it could be hypothesized that during winter, with decreased daylight, more serotonergic activity is needed, which may be mediated via the raphe input into the SCN. Increased serotonergic activity (increased free synaptic serotonin) may result in a compensatory increase in SERTs. Nevertheless, the small size of the SCN (~0.27 mm³) by itself cannot explain higher SERT availability found by SPECT or PET.

Limitations of the present study

The cerebellum (especially the vermis) contains small amounts of SERT, which could result in an underestimation of BPND in patients and HC, expected to be 7% at most. Although a systematic underestimation of SERT due to DAT- or NET-rich areas in MID (substantia nigra and locus coeruleus, respectively) cannot be ruled out, we think this does not differentially affect patients or HC.

Second, lower levels of endogenous 5-HT (e.g. in MDD) could result in less competition with radioligands, increasing the specific binding measured. This was demonstrated in rhesus monkeys with [123I]β-CIT SPECT, but not in humans. After tryptophan depletion (artificially reducing endogenous 5-HT) no differences in SERT availability were observed but the radioligand ([11C]DASB) in that study does not bind to the 5-HT recognition/translocation site, and may not be suitable to image such changes in extracellular 5-HT.

Third, we used previously validated ROIs in instead of Magnetic Resonance Imaging for coregistration. Because these templates cover larger brain areas, BPND in small regions (raphe nuclei) cannot be determined. This potential measurement error (non-differential for patients and HC), might have increased variance in our measurements, despite very good intra-rater correlation coefficients. Additionally, we did not correct for non-uniform photon attenuation and partial volume effects in our gender-analyses. Greater skull thickness might underestimate BPND in males, and smaller MID and DIENC in females might suppress BPND compared with males. However, these factors are unlikely to explain the observed interactions.
Fourth, the 4-week washout of antidepressants (binding to SERT) may be too short.48 Because all but one patient stopped antidepressants ≥ 6 months before scanning, we think no substantial bias of the BPND assessment was introduced by competitive binding by traces of previous antidepressants.

Fifth, we allowed previous incidental use of illicit drugs (marijuana/cannabis n = 10, or MDMA n = 1) in our HC. Because heavy use of MDMA (>50 MDMA tablets) can damage serotonin neurons,49 we performed an additional analysis in which we excluded data from the MDMA-user in the HC group. However, this exclusion did not affect our results (results available on request). Sixth, we did not check personal or family history of psychiatric illnesses in HC, nor did we test for alcohol or drug abuse.

Conclusion
We showed lower SERT availability in MID and DIENC in depressed males and higher SERT availability in DIENC in depressed (non-smoking) females compared with HC. We replicated a seasonal influence on MID SERT availability, and found a gender*smoking interaction on DIENC SERT availability. This study points to complex effects of gender, smoking and season on the serotonergic system in the pathogenesis of MDD.

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Conflicts of interest
None

References


Supplementary appendix: explorative analyses

METHODS
In addition to the analyses described in the main text, in exploratory analyses we examined whether potentially relevant other factors (alcohol use\(^{10}\) lifetime antidepressant use\(^{10}\) and duration of depression episode) had an impact on BP\(_{\text{ND}}\) by adding them as main effects to the final multivariable model. Because our exploratory models used small subgroups for assessment of further confounding in multivariable models, we only present these analyses as hypothesis generating, exploratory models.

RESULTS
Midbrain
For MID differentiation of patients who never took antidepressants (drug-naïve) versus those who did improved the reduced model (AIC-decrease= 1.399). Drug-naïve male and female patients had non-significantly lower BP\(_{\text{ND}}\) than patients with a history of antidepressant use (data not shown).

Differentiation of the duration of the current episode, stratified as ≤2 years or >2 years (‘chronic’ depression) improved the reduced model (AIC-decrease= 7.289). Men depressed for more than 2 years had significantly higher BP\(_{\text{ND}}\) than men depressed ≤2 years (difference 0.333; t\(_{85}\) = -3.102; p = 0.003), men depressed ≤2 years had significantly lower BP\(_{\text{ND}}\) than patients with a history of antidepressant use (data not shown).

Diencaphalon
Differentiation between patients with or without a secondary diagnosis of alcohol abuse/dependence improved the model for DIENC (AIC-decrease= 0.602). Smoking, alcohol-abusing male patients had numerically higher BP\(_{\text{ND}}\) than smoking male patients not abusing alcohol (difference 0.717; t\(_{85}\) = -1.175; p = 0.064). Non-smoking, female, alcohol-abusing patients had significantly lower BP\(_{\text{ND}}\) than non-smoking, female patients not abusing alcohol (difference -0.388; t\(_{85}\) = 2.182; p = 0.032; Figure S7.1B).

Figure S7.1. BP\(_{\text{ND}}\) values for midbrain and diencephalon (exploratory models)

exploratory models for BP\(_{\text{ND}}\) in MDD patients (MDD) versus HC stratified for gender. Values represent estimated means ±SE. AIC = Alcohol abuse/dependence.

A. Midbrain (n= 94), corrected for main effects of duration of episode (F\(_{2,85}\)= 4.777; p= 0.011), gender (p= 0.294), age (p= 0.380), smoking (p= 0.636), season of scanning (F\(_{2,85}\)= 6.194; p= 0.015), and duration of episode*gender interaction (F\(_{2,85}\)= 3.953; p= 0.023).

** Post-hoc differences for MDD males between: episodes ≤2 years versus >2 years (t\(_{85}\)= 3.102; p= 0.003), episodes ≤2 years versus HC (t\(_{85}\)= 2.795; p= 0.006).

B. Diencaphalon (n= 97), corrected for main effects of alcohol abuse/dependence (p= 0.521), gender (p= 0.489), season of scanning (p= 0.511), age (p= 0.486), smoking (p= 0.067) and alcohol abuse/dependence*gender interaction (F\(_{2,85}\)= 7.236; p= 0.001), gender*smoking interaction (F\(_{2,85}\)= 7.112; p= 0.009) and alcohol abuse/dependence*smoking interaction (p= 0.099).

* Post-hoc differences t\(_{85}\)= 2.182; p= 0.032 between non-smoking MDD females without, versus with alcohol abuse/dependence.
Table S7.1. Previous studies measuring SERT availability in MDD patients versus healthy controls.

<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Population (mean age)</th>
<th>N</th>
<th>SERT Imaging and ROI*</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahonen 200414</td>
<td>Drug free MDD pts (34 ±?) Controls (35 ±?)</td>
<td>10</td>
<td>[123I]-ADAM SPECT, 10min, 5h, 7h MidBr, Thal, Caud, Putam, Pons, Cer</td>
<td>Nonsign. Differences of V3'' in MidBr (7% higher in patients); large interindividual variation in V3'' in MidBr. Other ROIs no sign. differences.</td>
<td>Abstract of preliminary results only; little information provided. Gender not reported. Apparently no matching of controls and patients. Recruitment of patients unspecified.</td>
</tr>
<tr>
<td>Catafau 200615</td>
<td>Drug-free MDD pts (36 ±11) Controls (36 ±11)</td>
<td>10</td>
<td>[123I]-ADAM SPECT, 4h MidBr, Thal, Striatum, Cer</td>
<td>Slightly lower but nonsign. differences in SERT availability in MidBr (-4%), Thal (-11%) in MDD patients vs controls.</td>
<td>Patients were drug-free for &gt;6m. Age matched historic control group, no matching for sex. Unequal male-female distribution in MDD vs. controls. No information on family history of controls. Recruitment of patients unspecified.</td>
</tr>
<tr>
<td>Dahlström 200018</td>
<td>Drug-naive MDD pts (13.5 ±2.5)</td>
<td>31</td>
<td>[123I]β-CIT SPECT, 1, 4, 24h Striatum (24h), PFC, Thal (4h), MidBr (4h), Occ</td>
<td>MidBr V3'' in patients sign. higher (8%) at 1hr compared to controls (difference not sign. at 4h, again sign. at 24h). Prefrontal and Thal differences not sign. different</td>
<td>Control-group is due to ethical considerations not depressed but a psychiatrically affected group of adolescents. MDD patients and controls are not matched for age and sex. No information on family history of controls. No effect of age, gender or co-morbidity found. Recruitment of outpatients in university clinic.</td>
</tr>
<tr>
<td>Herold 200616</td>
<td>Drug-free MDD pts (42 ±12) Controls (36 ±13)</td>
<td>21</td>
<td>[123I]-ADAM SPECT, 4h MidBr, Cer</td>
<td>MidBr V3'' in patients sign. higher (8%) at 1hr compared to controls (difference not sign. at 4h, again sign. at 24h). Prefrontal and Thal differences not sign. different</td>
<td>All patients were drug-free for &gt;2m. No matching for age or sex. Unequal male-female distribution in MDD vs. controls. Study also reports on occupancy after 1 week of treatment with CIT 10 mg (n=13). Recruitment of patients unclear.</td>
</tr>
<tr>
<td>Ichimiya 200219</td>
<td>Drug-free MDD pts (44.1 ±13.5) or BD (41.7 ±8.8) Controls (42.3 ±14.5)</td>
<td>13</td>
<td><a href="+">11C</a>McN5652 PET MidBr, Thal, Cer</td>
<td>Sign. higher (22%) in Thal BP in complete patient group vs controls (in MDD only (23%), no change in MidBr</td>
<td>6 patients had BD; 10 patients had been treated with antidepressants &gt;6w before scanning (1 patient with BD 2w). Age matched (groupwise??) controls, not for sex. Recruitment of outpatients in university and general psychiatric clinics.</td>
</tr>
<tr>
<td>Joensuu 200719</td>
<td>Drug-naive MDD pts (38.8 ±8.6) Controls (30.6 ±8.9)</td>
<td>29</td>
<td>[123I]nor β-CIT SPECT, 6, 24h MidBr, Cer</td>
<td>Sign. lower V3'' in patients (10%) in MidBr vs controls. No correlation with SERT and MDD severity rating.</td>
<td>Although mentioned as such, incomplete matching on gender and age. 14/29 pts had HDRS ≤18. Recruitment of outpatients for psychodynamic psychotherapy in university clinic. No sign. effects of gender (included patients mostly F) or season.</td>
</tr>
<tr>
<td>Lehto 200617</td>
<td>Drug-naive MDD pts (28.3 ±7) Controls (30.6 ±9.2)</td>
<td>29</td>
<td>[123I]nor-β-CIT SPECT, 5 min, 6h, 24h MidBr, Striatum, Cer</td>
<td>Sign. lower SERT (-10%) in MDD patients vs controls. No differences between melancholic, atypical, nondifferentiated MDD subtypes. Linear inverse correlation between SERT and atypical score. No correlation between SERT and HDRS.</td>
<td>Patients and controls were (groupwise??) matched for age and sex. No substantial rationale given for relation between SERT availability and atypical dimension of MDD. Recruitment of outpatients in university clinic.</td>
</tr>
</tbody>
</table>
### Table S7.1. Previous studies measuring SERT availability in MDD patients versus healthy controls. (Continued)

<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Population (mean age)</th>
<th>Sex</th>
<th>N</th>
<th>SERT Imaging and ROI*</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malison 19985</td>
<td>Drug-free</td>
<td>MDD pts (44 ±10)</td>
<td>M + F</td>
<td>15</td>
<td>[123I]-β-CIT SPECT, 24h Brainstem, Striatum, Occ</td>
<td>Sign. reduction (-19%) in brainstem V3'' of MDD vs. controls, but not (-11%) in striatum. No correlation with SERT and MDD severity ratings</td>
</tr>
<tr>
<td>Kugaya 20045</td>
<td>Controls (45 ±11)</td>
<td>M + F</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer 20015</td>
<td>Drug-free</td>
<td>MDD pts (37 ±8)</td>
<td>M + F</td>
<td>13</td>
<td>[11C]DASP PET striatum, Cer</td>
<td>No sign difference in striatum V3'' of MDD vs controls. Sign. effect of age.</td>
</tr>
<tr>
<td>Meyer 2004a5</td>
<td>Controls (?)</td>
<td>M + F</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer 2004b5</td>
<td>Drug-free</td>
<td>MDD pts (35 ±11)</td>
<td>M + F</td>
<td>20</td>
<td>[11C]DASP PET Bilateral anteromedial PFC, dorsolateral PFC, Ant. Cing, Caudate, Putam, Thal, MidBr, Cer</td>
<td>No difference in SERT availability between MDD and controls for any region. Within patients (but not in controls) sign. correlations between increased Dysfunctional Attitude Scale (DAS) scores and increased SERT avail. Significant increase in SERT in patients vs. controls in all regions for 8 patients with high DAS scores (&gt;190) vs controls.</td>
</tr>
<tr>
<td>Meyer 2004b5</td>
<td>Controls (35 ±11)</td>
<td>M + F</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newberg 20059</td>
<td>Drug-free</td>
<td>MDD pts (38 ±?)</td>
<td>M + F</td>
<td>7</td>
<td>[123I]-ADAM SPECT, 4h MidBr, Cer</td>
<td>MidBr SERT sign. lower (7%) in MDD patients vs. controls. SERT MidBr correlated significantly with scores on HDRS.</td>
</tr>
<tr>
<td>Parsey 200610</td>
<td>Controls (38 ±15.4)</td>
<td>M + F</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parsey 200610</td>
<td>Drug-free</td>
<td>MDD pts (38.0 ±13.4)</td>
<td>M + F</td>
<td>25</td>
<td>[11C]McN5652 PET MidBr, Putam, Amyg, Thal, Hippoc, Ant. Cing, Cer</td>
<td>Sign. lower (-20%) BP'' in all regions in MDD patients vs controls. Post-hoc assigned to amygdala and midbrain regions. Drug-naive patients had sign. lower BP'' in amygdala and midbrain vs non drug-naive patients. No correlation with BP and depression severity ratings</td>
</tr>
</tbody>
</table>
Table S7.1. Previous studies measuring SERT availability in MDD patients versus healthy controls. (Continued)

<table>
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<tr>
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<th>N</th>
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<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reivich 2004(^{18})</td>
<td>Drug free MDD pts (22-56) Controls (23-59) M + F</td>
<td>4 4</td>
<td>(^{11}C)(+)McN5652 PET Bilateral Frontal, Cing, Thal, Pons, Cer</td>
<td>Sign. larger BP in left frontal (+17%) and right cingulate (+24%) in patients vs controls. Thalamus and pons no sign. difference</td>
<td>MDD patients were drug free for ≥ 5 half-lives of the drug, ≥2w for MAO-I and 23 w for fluoxetine. Patients were not matched for age or sex; no information on family history of controls. Wide age-range without possibility to correct for age-effects on SERT availability. Large SDs for thalamus or pons ROIs. Recruitment of outpatients.</td>
<td></td>
</tr>
<tr>
<td>Staley 2006(^{11})</td>
<td>Drug-free MDD pts (38.8 ±9.7) Controls (38.9 ±10) M + F</td>
<td>32 32</td>
<td>(^{123}I)β-CIT SPECT, 24h Brainstem, Diencephalon, Striatum, Cer</td>
<td>Sign. reduction (-12%) in diencephalons V3'' of MDD vs. controls, but not in brainstem. Interaction MDD by sex on diencephalon V3'': women -22% in MDD, men -1%.</td>
<td>Subjects were (group??) matched for sex, age and smoking status. No information on family history of controls. Recruitment of patients unspecified.</td>
<td></td>
</tr>
<tr>
<td>Willeit 2000(^{12})</td>
<td>Drug-free patients with SAD (30.5 ±8) Controls (29.0 ±5.5) M + F</td>
<td>11 11</td>
<td>(^{123}I)β-CIT SPECT, 4 h, 24h Thal, HypoThal, MidBr, Pons, Cer</td>
<td>Sign. reduction (-15%) in Thalamus and Hypothalamus V3'' of MDD vs. controls, but not (-8%) in MidBr-Pons. No correlation with SERT and depression severity ratings</td>
<td>5 subjects had been treated with antidepressants ≥6m before scanning. Groupwise matching for age and sex. All differences in SPECT acquisitions 24h p.i., at 4h p.i. no significant differences found. Recruitment of outpatients of university clinic.</td>
<td></td>
</tr>
</tbody>
</table>

* Naming of identical regions as provided in the studies. Reference region in italics.

Abbreviations: Amyg = Amygdala, Ant. Cing = Anterior Cingulate, BD = Bipolar Disorder, BP = Binding Potential, Caud = Caudatus, Cer = cerebellum, CIT = citalopram, h = hour, Hippoc = Hippocampus, HypoThal = Hypothalamus, min = minute, m = month, MDD = Major Depressive Disorder, MidBr = Midbrain, Occ = Occipital lobe, PAR = paroxetine, PFC = Prefrontal Cortex, Putam = Putamen, SAD = Seasonal Affective Disorder, Thal = Thalamus, V3'' = BP, w = week