Dose-escalation in the picture: pharmacological and imaging studies in depression
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Evidence why paroxetine dose-escalation is not effective in major depressive disorder: A randomized-controlled trial with assessment of serotonin transporter occupancy

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Abstract

Background
Dose-escalation is often used in depressed patients who fail to respond to standard doses of SSRIs, but clinical efficacy is equivocal.

Aim
To reassess the efficacy of paroxetine dose-escalation and quantify whether paroxetine dose-escalation increases occupancy of the serotonin transporter (SERT) more than placebo dose-escalation, in a randomized controlled trial.

Methods
We recruited 107 non-psychotic, unipolar depressed outpatients (18-70 yrs; Hamilton Depression Rating Scale (HDRS_{17}) >18) from primary care and psychiatric outpatient departments. After 6 weeks open-label paroxetine 20 mg/day (T0), non-responding patients (HDRS_{17} decrease <50%; n=60) were randomized to double-blind paroxetine (30-50 mg/day as tolerable) or placebo dose-escalation (paroxetine 20 mg/day + placebo). Patients were followed until 6 weeks after randomization (T1). Forty-nine patients, drug free at study-entry, underwent SPECT-scanning before treatment and were scanned repeatedly at T0 and T1. Paroxetine serum concentrations and SERT occupancy were determined at T0 and T1 (n=32).

Results
We terminated the dose-escalation trial after an interim analysis. Thirty non-responding patients were randomized to paroxetine (46.7 ±5.5 mg/day), 27 to placebo dose-escalation. Response-rates were 10/30 (33.3%) and 10/27 (37.0%), respectively. Repeated measurement analyses showed no significant effect for treatment (p=0.88, exceeding a priori stopping rules for futility [p>0.5]). Overall dropout was higher for placebo (26.7%) than paroxetine (3.3%; p=0.03). Paroxetine dose-escalation increased paroxetine serum concentrations (p<0.001). SPECT measurements (12 patients randomized to paroxetine (46.9 ±4.8 mg) and 14 to placebo dose-escalation) showed no significant increase of midbrain SERT occupancy (2.5 ±26.4%, paroxetine; 3.1 ±25.8% placebo; p=0.687) nor in diencephalon (p=0.529).

Conclusions
Paroxetine dose-escalation in depressed patients has no clinical benefit over placebo dose-escalation. This is explained by the absence of significant increases of SERT occupancy by paroxetine dose-escalation, despite increased paroxetine serum concentrations. (ISRCTN register nr. ISRCTN44111488)
Introduction

Major depressive disorder (MDD) is often treated with antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs). Unfortunately, response and remission rates are modest (30-50%), which require additional strategies to gain remission.\textsuperscript{3}\textsuperscript{3}\textsuperscript{1}\textsuperscript{2} Switching\textsuperscript{3} and augmentation\textsuperscript{4} have recently been evaluated. A third, and frequently applied option is dose-escalation, recommended in treatment guidelines\textsuperscript{1}\textsuperscript{2} and frequently used preceding other strategies. Only the recent NICE guideline is more reluctant in recommending dose-escalation.\textsuperscript{5} Although an individual patient may improve after dose-escalation, this could also represent a delayed drug response or reflect the natural course of the disease. Prolonged (up to 10 weeks), unaltered treatment with fluoxetine 20 mg/day improved the response rates of initial week 6 non-responders.\textsuperscript{6} Theoretically, the concept of dose-escalation assumes linear dose-response relationships which have not been proven for SSRIs.\textsuperscript{7}\textsuperscript{8}\textsuperscript{9} Therefore, the efficacy of dose-escalation of SSRIs has been questioned.\textsuperscript{5}\textsuperscript{8}\textsuperscript{10}

Previous studies did not show improved clinical effectiveness of dose-escalation, but had serious methodological weaknesses.\textsuperscript{6}\textsuperscript{11}\textsuperscript{17} All previous studies increased dosages probably too early (mostly after 3-4 weeks) and too abruptly, which may have obscured true dose-escalation effects by delayed effect of the standard doses and selective early drop-out of patients receiving true dose-escalation.\textsuperscript{6}\textsuperscript{10}\textsuperscript{11} Moreover, no study provided a rationale why dose-escalation was ineffective.

The primary molecular target of SSRIs is the serotonin transporter (SERT). Imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) allow in-vivo labeling of SERT in the brain, which can be used to study their occupancy. To date, several imaging studies measured SERT occupancy after short or prolonged treatment with SSRIs (Table S9.1).\textsuperscript{18}\textsuperscript{31} Particularly, Meyer et al.\textsuperscript{20} showed 60-80% SERT occupancy after standard clinical doses of SSRIs, and demonstrated curvilinear dose-response relationships for SERT occupancy by SSRIs. However, high doses of SSRIs were rarely studied,\textsuperscript{30} and dose-escalation was never studied.

Taking into account previous methodological criticisms, and considering the molecular target of SSRIs, we have tested whether paroxetine dose-escalation increases SERT occupancy, and improves depressive symptoms more than placebo dose-escalation. We performed a 6 week, multicenter, randomized study in depressed patients not responding to 6 weeks of paroxetine at 20 mg/day. As a novel extension to previous clinical trials, and in order to elucidate the neurobiological basis for an expected lack of benefit of dose-escalation, we included a SPECT imaging approach. Herewith, we quantified whether paroxetine dose-escalation increased SERT occupancy more than placebo dose-escalation. This enabled us to relate clinical findings to the neurobiological correlate of SERT occupancy.

Methods

Participants

Following approval by the institutional ethical committee and written informed consent, we recruited outpatients (18-70 years) from primary care, our outpatient department, and public psychiatric settings between October 2003 and February 2007. Inclusion criteria were: MDD determined by the structured clinical interview for DSM-IV (SCID),\textsuperscript{32} and a Hamilton Depression Rating Scale (17 items; HDRS\textsubscript{17})\textsuperscript{33} score above 18. All participants were drug-free or had undergone no more than one antidepressant treatment (other than paroxetine) at an effective dose for $\geq 6$ weeks for the present MDD-episode. By the latter criterion, we avoided treatment resistance as potential bias for inefficacy of dose-escalation. Exclusion criteria, apart from pregnancy (or wish), were bipolar disorder, psychotic features, neurological cognitive impairments (i.e. dementia),...
primary anxiety and/or substance abuse disorders and acute, severe suicidal ideation. Contrary, we allowed secondary co-morbid anxiety and/or substance abuse to increase applicability of our findings.

Interventions

Patients were treated by their referring physician or were referred to our outpatient department. After assessment at study-entry, all patients were treated open-label with paroxetine 20 mg/day for 6 weeks (see Figure 2.1). When severe adverse effects occurred, dosages were reduced to 10 mg/day and again increased to 20 mg/day after one week. We randomized all patients who did not achieve ≥5% decrease in HDRS17 score after 6 weeks, relative to study entry. They received a true paroxetine or a placebo dose-escalation added to paroxetine 20 mg/day. Dose-escalation was provided in blue capsules containing 10 mg paroxetine or placebo. Randomization was stratified for treatment setting (SPECT-group, outpatient department AMC, primary care, public psychiatry), gender and age. Within strata, we applied a minimization method to achieve a balanced distribution. We concealed allocation by using an independently operated computer program.

Dose-escalation consisted of incremental steps of one capsule every 5 days towards a maximum of 50 mg/day (20 mg + 3 capsules). Patients were allowed to increase at a slower pace (e.g. by 7 days) or stop further escalation (e.g. 20 mg + 2 capsules) according to adverse effects.34 No dosage adjustments were allowed during the last 3 weeks of the study. We checked adherence by pill-counts and anamnesis.35

Outcomes and measurements

Primary clinical outcomes were HDRS17-scores, and the proportion of patients achieving response (≥50% decrease in HDRS17) or remission (HDRS17 ≤7). Secondary outcomes were total and specific (adverse effects / inefficacy) dropout rates, the Maier and Bech 6 item subscales of the HDRS17, the Inventory for Depressive Symptomatology self-rated (IDS-SR 30)38 scores, the occurrence of adverse effects and health-related quality of life (MOS-SF36; physical and mental component scales standardized to a general Dutch population).39

We administered questionnaires at study-entry, randomization (T0), and 6 weeks after randomization (T1). Depressive symptoms were also monitored at week 1, 2 and 4 using the Maier and Bech subscales and IDS-SR30 (see Figure 2.1). Three trained investigators administered clinician-rated questionnaires. Agreement between raters was good (intraclass correlation coefficient = 0.98). Raters and patients were blinded for treatment.

Subgroup for SPECT imaging

From all patients who entered the trial, we recruited patients who were drug-free (>4 weeks and ≥5 half-lives of a previous antidepressant) as potential candidates for SPECT imaging. These patients were asked to participate in the SPECT sub-study if their age was between 25-55 years to reduce variability in SERT measurements by age.40 Forty-nine patients could thus be recruited for a first SPECT scan. None of these patients reported past or present use of 3,4-methylenedioxymethamphetamine. We made a second scan in those patients who completed 6 weeks of paroxetine treatment (n= 44; including 12 responders), while only randomized non-responders (n= 32) were invited for a third scan at the end of the study. We treated SPECT patients at the AMC outpatient department. Medication was supplied in pillboxes.

SPECT imaging and analysis

We performed SPECT imaging at study-entry (baseline-scan), T0 and T1 (see Figure 2.1) between 2 to 10 pm according to previously described procedures.41 We made all scans 230 ±18 (SD) minutes after intravenous injection of approximately 100 MBq iodine-123 labeled 2β-carbomethoxy-3β-(4-iodophenyl)-tropane ([123I]β-CIT), when the radioligand is at equilibrium for SERT binding in brain areas expressing high densities of SERTs.42 To prevent thyroid uptake of [123I], all subjects received
oral potassium-iodide solution. 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). Blood for paroxetine serum concentrations (PSC) was collected at T0 and T1 immediately before scanning. Serum was stored at -20° C until analysis. PSC were determined in May 2007 using a validated HPLC-MS/MS method (therapeutic range 10-75 µg/L; see appendix). The lower limit of quantification was 5 µg/L, the lower limit of detection was 0.3 µg/L.

After attenuation correction and reconstruction in 3D mode (http://www.neurophysics.com), we defined regions of interest (RoIs) for midbrain, diencephalon and cerebellum by using validated templates (see Figure 2.3).41 One examiner, blinded for scan session (baseline-scan/T0/ T1), positioned all RoIs in two series. Intraclass correlation coefficients were >0.97 for all RoIs. If the two series differed by >5%, scans were re-evaluated by a second investigator. In the analyses we averaged the counts for the two series.

Using activity in cerebellum as indicator of non-displaceable activity (non-specific binding and free radioactivity),43 we calculated specific to non-specific binding ratios per scan as

$$BP_{ND} = \frac{\text{Activity}_{CTR}}{\text{Activity}_{ND}}.$$  

$$BP_{ND}$$ is proportional to transporter number under equilibrium conditions.44 In a different study, we found high reproducibility of SERT imaging with $[^{123}]$β-CIT SPECT after repeated scanning of subjects, using the same camera and scanning-protocol (de Win et al., submitted). As primary outcomes, we calculated SERT occupancies at T0 or T1 relative to untreated baseline-scan SERT availability: OCC$_{T0}$ or T1 = .

Power and interim analysis
We performed a-priori power-calculations for two co-primary endpoints: (a) To detect a difference of ≥5-points in HDRS$_{17}$ scores between paroxetine and placebo dose-escalation, while assuming a common standard deviation of 7 and using a one-tailed $\alpha = 0.025$ and $\beta = 0.05$, sample sizes of 60 per group were required; (b) For response rates (assumed to be 50% and 30% for paroxetine vs. placebo dose-escalation) a two-tailed $\alpha = 0.05$ and $\beta = 0.20$ required 110 participants per group. Because previous dose-escalation studies indicated no benefits relative to placebo dose-escalation, we planned an interim analysis after SPECT data for had been collected on at least 30 randomized patients in the SPECT subgroup. Stopping criteria, using the most informative continuous scores in a mixed model, were predetermined using the O'Brien and Fleming$^{45}$ approach, were undisclosed while performing the interim analysis, and were $p<0.0026$ in case of superiority and $p>0.50$ for futility.

Data analyses
Analyses were performed while blinded for treatment allocation. We based endpoint analyses on intention to treat (ITT), with last-observation carried forward (LOCF). To examine the effectiveness of paroxetine vs. placebo dose-escalation, we compared the proportion of patients with response, remission and drop-outs at the end of study using $\chi^2$ or Fisher’s exact test. We examined differences in mean continuous endpoints by ANCOVA with treatment as factor and value at randomization (T0) as covariate.

We used linear mixed models to assess differences in trends over time between groups in Maier, Bech and IDS-SR$_{30}$ scores. Mean scores for these questionnaires were modeled as a function of the randomized group (paroxetine vs. placebo dose-escalation), score at randomization, and time since randomization (categorical, four levels). The interaction between time*group was added to the model to test whether trends over time were different between the two treatment groups. We used the Akaike Information Criteria to choose the best fitting variance/covariance structure (unstructured, compound symmetry or first-order auto-regressive) for each outcome parameter.

To examine changes in SERT occupancy between T0 and T1, we used ANCOVAs with treatment as factor, and SERT occupancy at T0 and age as covariates. In order to obtain maximum information of dose-escalation in these analyses, we excluded patients that were likely non-adherent to paroxetine at T0 or T1 (PSC < 5 µg/l). Thereafter, we plotted SERT occupancy against
dose and PSC. We modeled dose-response in an $E_{\text{max}}$ model as $\text{OCC}= a \frac{\text{PSC}}{(b + \text{PSC})^2}$, in which $a$ represents maximal SERT occupancy and $b$ the PSC with 50% SERT occupancy. We calculated $a$ and $b$ by fitting a nonlinear regression model that minimizes the sum of squares of the residuals. For quantification of differences in SERT occupancy between final responders and non-responders, we used ANCOVA models corrected for differences at To, age and baseline-scan SERT availability in diencephalon. We performed all analyses in SPSS v15.0.1.1 (www.spss.com).

Results

Patient disposition

One-hundred and seven patients (mean age 43.8 ±9.8) started open-label paroxetine (Figure 9.1). The response-rate in the open phase was 27/107 (25.2%), and 60 non-responding patients were randomized for the double-blind phase. Randomization over the 2 treatment-arms resulted in comparable groups (Table 9.1). Fifty-one patients completed the 6 week randomization phase including 31 from the SPECT study. We obtained at least 1 post-randomization HDRS$_{17}$ score for 57 patients.

HDRS$_{17}$ scores at study-entry (-6 weeks) were comparable in To responders vs. non-responders (ANOVA, $F_{1,85}=1.972$, $p=0.164$). At T0, HDRS$_{17}$ scores ($\pm SD$) were 7.8 ±3.62 (66.6 ±14.3% decrease) in responders vs. 20.5 ±6.25 (17.7 ±20.3% decrease) in non-responders.

Figure 9.1. Recruitment and flow of participants.

* Three patients who refused dose-escalation after randomization, never ingested study drugs and refused further questionnaires, were excluded for endpoint analysis.

1 One SPECT patient dropped out early due to inefficacy, but for all SPECT-patients clinical data could be obtained. For SPECT analyses, 6 patients were excluded: 1 patient missed the T1 scan (placebo dose-escalation), 3 patients were likely non-adherent at T0 (paroxetine serum concentration <5µg/l; all paroxetine dose-escalation), and 2 were likely non-adherent at T1 (1 paroxetine dose-escalation, 1 placebo dose-escalation).
### Table 9.1. Characteristics of non-responding MDD patients after 6 weeks of open treatment with paroxetine 20 mg/day (trial population).

<table>
<thead>
<tr>
<th></th>
<th>All patients (n= 60)</th>
<th>Paroxetine DE (n= 30)</th>
<th>Placebo DE (n= 30)</th>
<th>SPECT subgroup (n= 32)</th>
<th>Paroxetine DE (n= 16)</th>
<th>Placebo DE (n= 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at baseline (years)</strong></td>
<td>41.9 ±9.1</td>
<td>42.9 ±10.3</td>
<td>42.5 ±7.7</td>
<td>40.4 ±7.7</td>
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<tr>
<td><strong>Female sex - n (%)</strong></td>
<td>21 (70.0)</td>
<td>19 (63.3)</td>
<td>11 (68.8)</td>
<td>10 (62.5)</td>
<td></td>
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<tr>
<td><strong>Marital status - n (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>Single (never married)</td>
<td>15 (51.7)</td>
<td>12 (40.0)</td>
<td>6 (37.5)</td>
<td>5 (31.3)</td>
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<tr>
<td>Married</td>
<td>8 (27.6)</td>
<td>6 (20.0)</td>
<td>7 (43.8)</td>
<td>4 (25.0)</td>
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<tr>
<td>Divorced</td>
<td>4 (13.8)</td>
<td>11 (36.7)</td>
<td>2 (12.5)</td>
<td>7 (43.8)</td>
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<tr>
<td>Widowed</td>
<td>2 (6.9)</td>
<td>1 (3.3)</td>
<td>1 (6.3)</td>
<td>0</td>
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<tr>
<td><strong>Educational level - n (%)</strong></td>
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<td></td>
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<tr>
<td>Low</td>
<td>6 (20.0)</td>
<td>11 (36.7)</td>
<td>2 (12.5)</td>
<td>3 (18.8)</td>
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<tr>
<td>Intermediate</td>
<td>19 (63.3)</td>
<td>15 (50.0)</td>
<td>10 (62.5)</td>
<td>10 (62.5)</td>
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<td></td>
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<tr>
<td>High</td>
<td>5 (16.7)</td>
<td>4 (13.3)</td>
<td>4 (25.0)</td>
<td>3 (18.8)</td>
<td></td>
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</tr>
<tr>
<td><strong>Income €/month (median; 25 &amp; 75 quartiles)</strong></td>
<td>1485 (875-1769)</td>
<td>1197 (715-1820)</td>
<td>1177 (715-1530)</td>
<td>1185 (610-2277)</td>
<td></td>
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</tr>
<tr>
<td><strong>Current smoking - n (%)</strong></td>
<td>13 (43.3)</td>
<td>16 (53.3)</td>
<td>6 (37.5)</td>
<td>11 (68.8)</td>
<td></td>
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<tr>
<td><strong>Alcohol use: n (%)</strong></td>
<td></td>
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<tr>
<td>≤ 2 Units/week</td>
<td>20 (66.7)</td>
<td>21 (70.0)</td>
<td>10 (62.5)</td>
<td>11 (68.8)</td>
<td></td>
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<tr>
<td>3-7 Units/ week</td>
<td>6 (20.0)</td>
<td>7 (23.3)</td>
<td>4 (25.0)</td>
<td>5 (31.3)</td>
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<td>8-21 Units/ week</td>
<td>2 (6.7)</td>
<td>1 (3.3)</td>
<td>1 (6.3)</td>
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<tr>
<td>&gt;22 Units/ week</td>
<td>2 (6.7)</td>
<td>1 (3.3)</td>
<td>1 (6.3)</td>
<td>0</td>
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<tr>
<td><strong>Race - n (%)</strong></td>
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<tr>
<td>Caucasian</td>
<td>17 (56.7)</td>
<td>19 (63.3)</td>
<td>9 (56.2)</td>
<td>13 (81.2)</td>
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<td>Creole</td>
<td>4 (13.3)</td>
<td>7 (23.3)</td>
<td>2 (12.5)</td>
<td>3 (18.8)</td>
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<td></td>
</tr>
<tr>
<td>African</td>
<td>6 (20.0)</td>
<td>1 (3.3)</td>
<td>3 (18.8)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (10.0)</td>
<td>3 (10.0)</td>
<td>2 (12.5)</td>
<td>0</td>
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<tr>
<td><strong>MDD</strong></td>
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<tr>
<td>HDRS&lt;sub&gt;17&lt;/sub&gt; at study-entry (-6 weeks)</td>
<td>24.5 ±4.7</td>
<td>25.5 ±5.0</td>
<td>25.6 ±5.0</td>
<td>24.4 ±4.6</td>
<td></td>
<td></td>
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<tr>
<td>HDRS&lt;sub&gt;17&lt;/sub&gt; (T0)*</td>
<td>20.1 ±2.6</td>
<td>1.0 ±5.9</td>
<td>21.3 ±7.4</td>
<td>19.3 ±5.1</td>
<td></td>
<td></td>
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<tr>
<td>Maier at (T0)*</td>
<td>10.0 ±3.0</td>
<td>10.5 ±3.1</td>
<td>10.4 ±3.6</td>
<td>10.1 ±2.9</td>
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<tr>
<td>Bech (T0)*</td>
<td>10.5 ±2.9</td>
<td>11.3 ±3.0</td>
<td>11.0 ±3.4</td>
<td>10.8 ±3.1</td>
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<tr>
<td>IDS-SR&lt;sub&gt;17&lt;/sub&gt; (T0)*</td>
<td>38.1 ±21.3</td>
<td>40.8 ±12.2</td>
<td>40.3 ±11.6</td>
<td>39.9 ±11.8</td>
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<tr>
<td>First episode - n (%)</td>
<td>17 (56.7)</td>
<td>21 (70.0)</td>
<td>9 (56.3)</td>
<td>9 (56.3)</td>
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<td></td>
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<tr>
<td>No of episodes</td>
<td>1.6 ±0.8</td>
<td>1.7 ±1.8</td>
<td>1.6 ±0.8</td>
<td>2.3 ±2.4</td>
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<tr>
<td>Drug-naïve - n (%)</td>
<td>24 (80.0)</td>
<td>19 (63.3)</td>
<td>14 (87.5)†</td>
<td>8 (50.0)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used AD in current episode - n (%)</td>
<td>3 (10.0)</td>
<td>5 (16.7)</td>
<td>1 (6.3)</td>
<td>2 (12.5)</td>
<td></td>
<td></td>
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<tr>
<td>Melancholic - n (%)</td>
<td>23 (76.7)</td>
<td>19 (63.3)</td>
<td>12 (75.0)</td>
<td>12 (75.0)</td>
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<tr>
<td>Duration of episode: n (%)</td>
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<tr>
<td>&lt;5 months</td>
<td>9 (30.0)</td>
<td>5 (16.7)</td>
<td>7 (43.8)</td>
<td>3 (18.8)</td>
<td></td>
<td></td>
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<tr>
<td>duration 5 months – 2 years</td>
<td>19 (63.3)</td>
<td>22 (73.3)</td>
<td>7 (43.8)</td>
<td>11 (68.8)</td>
<td></td>
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<tr>
<td>duration &gt; 2 years</td>
<td>2 (6.7)</td>
<td>3 (10.0)</td>
<td>2 (12.5)</td>
<td>2 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of first episode (years)</td>
<td>37.1 ±9.1</td>
<td>38.1 ±11.8</td>
<td>38.4 ±8.8</td>
<td>34.4 ±10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Co-morbidity – n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>5 (16.7)</td>
<td>7 (23.3)</td>
<td>0</td>
<td>4 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysthymia</td>
<td>2 (6.7)</td>
<td>0</td>
<td>1 (6.3)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug (alcohol, cannabis, benzodiazepines) abuse / dependence</td>
<td>2 (6.7)</td>
<td>1 (3.3)</td>
<td>1 (6.3)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MOS-SF36</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical*</td>
<td>41.2 ±9.0</td>
<td>40.3 ±10.7</td>
<td>41.2 ±8.6</td>
<td>43.9 ±9.9</td>
<td></td>
<td></td>
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<tr>
<td>Mental*</td>
<td>26.1 ±8.4</td>
<td>25.7 ±10.2</td>
<td>25.3 ±5.9</td>
<td>22.3 ±6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SERT availability baseline-scan (BPND)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>N/A</td>
<td>N/A</td>
<td>0.553 ±0.119</td>
<td>0.657 ±0.217</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diencephalon</td>
<td>N/A</td>
<td>N/A</td>
<td>1.157 ±0.226</td>
<td>1.134 ±0.247</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SERT occupancy (% of BPND in Bsl-scan)</strong>†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>N/A</td>
<td>N/A</td>
<td>73.2±17.1</td>
<td>82.2±18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diencephalon</td>
<td>N/A</td>
<td>N/A</td>
<td>63.8 ±15.4</td>
<td>70.3 ±12.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers represent means (±standard deviation) unless specified otherwise. * at randomization (T0). † sign. difference between SPECT patients randomized to conditions (Fisher’s exact p = 0.026).

† n = 29; excluding three patients who were likely nonadherent at T0 (PSC <5μg/l). BPND = Binding Potential (non-displaceable), DE = dose-escalation, MDD = Major Depressive Disorder, PSC = Paroxetine serum concentration.
Clinical effectiveness of paroxetine vs. placebo dose-escalation

During dose-escalation (T0-T1), 1, 8 and 21 patients reached final doses of 30, 40 and 50 mg/day respectively. The placebo group escalated to a comparable number of capsules (χ² = 0.895, df = 2, p = 0.639). Adherence based upon pill-counts was comparable between both groups (Fisher’s exact, p = 0.492).

Paroxetine dose-escalation did not yield better outcomes in depression severity and health-related quality of life compared to placebo dose-escalation (ITT; Table 9.2). The robustness of this finding was confirmed in the longitudinal analysis (mixed model). Changes over time in the Maier subscale and IDS-SR30-scores (Figure 9.2), and Bech subscale-scores (available on request), were comparable between the two groups. Overall dropout was higher with placebo (26.7%) than with paroxetine dose-escalation (3.3%; p = 0.03). Paroxetine dose-escalation had significantly more adverse effects than placebo dose-escalation, but this did not result in higher discontinuation rates due to adverse effects (Table S9.2). Instead, adverse effects by paroxetine dose-escalation moderately decreased over time, suggestive of habituation.

Table 9.2. Depression and health-related quality of life scores after 6 weeks paroxetine vs. placebo dose-escalation (T1); all patients and SPECT subgroup.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 57)</th>
<th>SPECT subgroup (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paroxetine DE (n = 30)</td>
<td>Placebo DE (n = 27)</td>
</tr>
<tr>
<td>Mean dosage mg/day</td>
<td>46.7 ±1.00</td>
<td>NA</td>
</tr>
<tr>
<td>HDRS₁₇</td>
<td>16.1 ±1.22</td>
<td>15.3 ±1.28</td>
</tr>
<tr>
<td>Maier subscale</td>
<td>7.5 ±0.61</td>
<td>7.5 ±0.64</td>
</tr>
<tr>
<td>Bech subscale</td>
<td>8.1 ±0.63</td>
<td>8.1 ±0.66</td>
</tr>
<tr>
<td>Response* - n (%)</td>
<td>10 (33.3)</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td>Remission* - n (%)</td>
<td>4 (13.3)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>IDS-SR₃₀</td>
<td>34.8 ±1.83</td>
<td>32.5 ±2.05</td>
</tr>
<tr>
<td>MOS-SF36</td>
<td>Physical 41.8 ±0.93</td>
<td>42.4 ±1.06</td>
</tr>
<tr>
<td></td>
<td>Mental 29.6 ±1.37</td>
<td>27.3 ±1.57</td>
</tr>
</tbody>
</table>

Scores at endpoint of the study are based on intention to treat, with last observation carried forward for early drop-outs. Values are means ±standard error, corrected for (mean) scores at randomization (To) (ANCOVA). * ≥50% decrease in HDRS₁₇ with baseline score (-6 weeks) as reference; Fisher’s exact test.¹ HDRS₁₇ ≤7; Fisher’s exact test.² Due to missing values: n = 29 paroxetine DE and n = 33 placebo DE for all patients.³ Due to missing values: n = 29 paroxetine DE and n = 22 placebo DE for all patients. DE = dose-escalation.

Figure 9.2. Changes over time in Maier and IDS-SR scores after randomization.

Points represent mean Maier (A) and IDS-SR (B) scores (±SEM) adjusted for scores at randomization (To) for paroxetine (n= 30) and placebo (n = 27) dose-escalation. Mixed model analysis (Maier: n= 57, IDS-SR: n= 53): overall difference between paroxetine vs. placebo dose-escalation for Maier scores t₄₉,10₄,6₀₀ = 2.095, p = 0.880, and for IDS-SR F₄₉,₅₁₉ = 1.516; p = 0.213.¹ Mean IDS-SR score at week 4 differed significantly in favor of placebo dose-escalation (t₃₉,₁₆₁ = 2.11; p = 0.040). DE = dose-escalation.
SERT occupancy and clinical response

Of 32 randomized patients in the SPECT subgroup, only 3 (9%) previously used mirtazapine or fluoxetine in the current episode (1 patient stopped mirtazapine 4 weeks prior to scanning, others stopped <2 months before scanning). One patient missed the T1 scan. Based upon PSC at randomization or T1, five patients in the SPECT-subgroup (4 with paroxetine and 1 with placebo dose-escalation; Fisher’s Exact: p = 0.172; see Figure 9.1) with PSC <5 µg/l were considered non-adherent, despite adherence according to pill-counts. We excluded these five patients for analyses of changes in SERT occupancy after dose-escalation, leaving 26 T1 scans analyzable for diencephalon occupancies after true or placebo dose-escalation. Paroxetine dose-escalation increased mean PSC from 36.2 to 154.3 µg/l (paired t-test; p < 0.001), while mean PSC in placebo dose-escalation remained unchanged (Table S9.3). At randomization (T0), mean SERT occupancies (±SEM) for the paroxetine dose-escalation group were 76.2 ±4.70% in midbrain and 64.3 ±4.60% in diencephalon. For the placebo dose-escalation group these were 84.6 ±4.95% and 72.2 ±3.08%, respectively. Neither paroxetine nor placebo dose-escalation significantly increased SERT occupancy further (Figure 9.3A). Plotting PSC vs. SERT occupancy showed that PSCs > 50 µg/l were not associated with further increases of SERT occupancy in midbrain or diencephalon (Figure 9.3BC). Furthermore, individual changes in PSC (To to T1) were not significantly associated with changes in occupancy in midbrain (F1,24 = 0.101; p = 0.754) and diencephalon (F1,17 = 1.332; p = 0.259; Figure 9.1).

Figure 9.3. SERT occupancy during randomized dose-escalation of paroxetine. Changes over time and relation with paroxetine serum concentration.

A. Mean SERT occupancy (±SEM) for paroxetine dose-escalation and placebo dose-escalation at randomization (T0) and after 6 weeks of dose-escalation (T1). SERT occupancy was calculated as percentage of initial available SERTs (expressed as BPNO) at baseline (-6 weeks) scans (see text). Changes in SERT occupancy between To and T1 for paroxetine dose-escalation and placebo dose-escalation were non-significant in ANCOVA models correcting for age and differences in To SERT occupancy (MIDBRAIN: F1,22 = 0.167; p = 0.687; DIENCEPHALON: F1,22 = 0.409; p = 0.529). For 1 patient insufficient midbrain was scanned at study entry to compute subsequent SERT occupancies.

B & C. Data for randomized patients used from both To (open circles) and T1 (diamonds). Dose - occupancy relationships are modeled as OCC = x - 100. For Midbrain (B): a = 86.0 ±4.03 (SE), b = 2.65 ±1.39 (n = 30 at To and n = 27 at T1). For Diencephalon (C): a = 73.3 ±2.28, b = 3.93 ±1.07 (n = 32 at To and n = 29 at T1). Dashed lines represent 95% confidence interval of fitting.

DE = dose-escalation, OCC = occupancy, PAR = paroxetine, PLAC = placebo. PSC = paroxetine serum concentration.
We explored whether SERT occupancy was related to clinical response at T1 irrespective of paroxetine or placebo dose-escalation. Responders at T1 (n= 12) had numerically higher SERT occupancy (±SEM) in midbrain (91.2 ±5.8%) and diencephalon (69.2 ±2.8%) than non-responders (n= 14; 77.8 ±5.1% and 63.8 ±2.6%, respectively; ANCOVA: p= 0.107 and p= 0.178). These models used baseline-scan diencephalon SERT availability as covariate as this accounted for a major part of variance (F1,21= 4.831, p= 0.039 and F1,22= 10.407, p= 0.004 respectively). Contrary, T1 SERT occupancy in midbrain or diencephalon did not significantly predict the percentage decrease in HDRS17 in linear regression, or response status in logistic regression (neither when corrected for baseline-scan SERT availability in diencephalon or age).

**Discussion**

In this randomized trial we examined clinical effectiveness of dose-escalation in MDD patients, who were non-responders to 6 weeks of 20 mg/day paroxetine, and explored potential underlying mechanisms. Despite markedly increased drug exposure, paroxetine dose-escalation to 30-50 mg/day did not improve depressive symptoms more than placebo dose-escalation, but was associated with more adverse effects. Concomitantly, increased paroxetine serum concentrations were not associated with substantially greater SERT occupancy, indicating that standard paroxetine doses (20 mg/day) already resulted in maximum SERT occupancy.

**Clinical outcomes of dose-escalation in non-responders**

The dose-response relationship for paroxetine was previously examined in fixed dose, parallel group designs. Twenty mg/day and higher paroxetine doses yielded similar clinical improvements. Similar findings were reported from parallel-group, fixed dose studies of other SSRIs. Accordingly, the usefulness of dose-escalation in non-responders to paroxetine and other SSRIs has been questioned. However, the underlying studies had methodological shortcomings, and dose-escalation remains a recommended standard approach for non-responders.

If dose-escalation is applied too early (before week 6), randomization of ‘late responders’ will likely dilute the difference between true and placebo dose-escalation, resulting in potentially false negative findings. While one study reported randomized dose-escalation of sertraline after 6 weeks of treatment, this was compromised by a non-randomized dose-increase 2 weeks prior to randomization.

The benefits of dose-escalation might be under-estimated if actively-treated patients drop-out early due to adverse effects or become more non-adherent. Our schedule for dose-escalation did not increase drop-out. Hypothetically, patients receiving a paroxetine dose-escalation might have interpreted the increased level of adverse effects as subjective clue of greater drug effects, encouraging them to persevere in the trial. At first sight a misbalance in adherence is suggested with 4 patients with paroxetine vs. 1 patient with placebo dose-escalation having low PSCs. However, this was not due to dose-escalation. As mentioned in Figure 9.1, in three patients, low PSCs at T0 already classified them as likely non-adherent, with only 1 paroxetine vs 1 placebo dose-escalation patient becoming likely non-adherent during dose-escalation (T0-T1). Therefore, we think that neither adverse effects nor non-adherence account for the observed inefficacy of dose-escalation.

Thus, the present study overcomes methodological limitations of previous SSRI dose-escalation studies by using a randomized, placebo-controlled, double-blind dose-escalation in non-responders to 6 weeks treatment with a standard dose of paroxetine. We also avoided treatment resistance as a factor for inefficacy of dose-escalation by inclusion of patients who received no more than one effective antidepressant trial for the current episode. Under these conditions paroxetine was not superior to placebo in dose-escalation. Moreover, our study was
more inclusive than most previous ones and thus may be more applicable to ‘real-world’ first-line antidepressant treatment. This may also explain why we observed lower response and remission rates than previous studies.

**Neurobiological effects of dose-escalation**

Our pharmacokinetic and imaging measurements were designed to explore why paroxetine dose-escalation would or would not improve treatment outcomes. Our imaging of SERT occupancy bypasses potential bias by inclusion of patients with ultrarapid drug-metabolism, which is often causally linked to non-response. Hypothetically the clinical selection of non-responders eligible for dose-escalation, might represent a selection of patients not reaching high levels of SERT occupancy.

Comparing SERT occupancies of different SSRI doses faces several methodological challenges. Firstly, the assessment of occupancy requires knowledge on the available number of SERTs. Due to inter-individual differences in available SERTs, only assessments with individual drug-free baseline-scans yield reliable data. Secondly, a given drug dose may yield a range of serum concentrations due to inter-patient pharmacokinetic differences. Hence, associations based upon serum concentrations are more reliable than those based upon administered dose. Finally, intra-individual comparisons of occupancy changes following dose-escalation are more powerful than those with historic data.

Against this background, Voineskos et al. recently reported high SERT occupancies in striatum (~85%), thalamus (~79%), and midbrain (~98%) in 12 depressed patients exposed to >4 weeks of venlafaxine 225-450 mg/day, sertraline 150-200 mg/day or citalopram 60-80 mg/day in a ["C]DASB PET study. They concluded that high doses significantly increased occupancy compared to an average of 80% SERT occupancy determined in previous studies with standard SSRI doses, which would favor the concept of dose-escalation. However, they did not determine occupancy relative to baseline scans of the same patients without medication, nor at standard doses. We performed drug-free study-entry scans, in addition, >90% of patients did not use antidepressants for the current episode of MDD. Furthermore, low therapeutic dosages of several SSRIs also yielded high SERT occupancy in most studies. The present study resolves this controversy by showing that 4-fold increases of PSC upon paroxetine dose-escalation did not significantly increase SERT occupancy. This offers an explanation for our findings: SERT occupancy is limited by a ceiling effect. A PSC achieved with a 20 mg/day paroxetine dose is sufficient to yield maximum SERT occupancy (Figures 9.3B,C). If low doses already yield maximum SERT occupancy, dose-escalation cannot be expected to increase treatment efficacy, which is in line with our clinical findings. Our results do not necessarily challenge the relationships between dose, SERT occupancy and clinical response but rather suggest that these relationships exist mainly at low and sub-therapeutic doses. Furthermore, the relationship between SERT occupancy and response might be confounded by other factors such as SERT gene polymorphisms.

In a recent study Owens et al. showed increased SERT occupancy with increasing paroxetine CR doses (12.5-75 mg/day) in an ex-vivo model using human transporter transfected cells, which might be at odds with our findings. However, validation of this ex-vivo method (in cultured cells) with concomitant in-vivo SPECT or PET SERT occupancy (the gold standard) is not yet available. Additionally, Zitterl et al. found a significant relation between SERT occupancy and clinical response in obsessive compulsive disorder treated with clomipramine (150 mg/day), but did not study the effects of dose-escalation in their study. Therefore, our study optimally quantifies the neurobiological effects of dose-escalation of antidepressants in patients.

**Critique of methods**

For logistic reasons, we used ["I"]β-CIT for SPECT imaging, which is a non-selective radioligand, and also binds to dopamine transporters (DAT; e.g. substantia nigra) and norepinephrine transporter (NET; e.g. locus coeruleus). Furthermore, imaging studies indicated increased striatal DAT binding after treatment with paroxetine, especially when the occipital cortex was used as a reference. Nevertheless, uptake in midbrain and diencephalon is considered to reflect
predominantly SERT, as these structures are rich of SERT relative to DAT and NET. Therefore, although this non-selectivity might have concealed changes in SERT occupancies due to additional DAT or NET binding, we think our findings in diencephalon and midbrain mainly reflect SERT occupancy. Nevertheless, it would be challenging to replicate our study using a selective ligand for SERT like \([^{11}C]DASB\) for PET or \([^{123}I]ADAM\) for SPECT imaging.

Our study did not investigate secondary effects of paroxetine. Many adaptive pre- and postsynaptic effects of chronic administration of SSRIs have been documented, including neuroadaptive alterations in serotonin receptors and intracellular signalling pathways, as well as time-dependent effects on neurogenesis. These hypothetical additional effects of dose-escalation remain to be investigated. Nevertheless, neither the results of our trial, nor the findings in previous randomized controlled trials indicate that dose-escalation is an efficacious strategy for SSRI non-responders in MDD.

The fourfold increase of PSC after dose-escalation from 20 to 50 mg/day may question adherence of patients in the open phase of the study. However, paroxetine inhibits the cytochrome P450 enzyme 2D6, also responsible for the metabolism of paroxetine. Therefore nonlinear increases of PSC reflect normal paroxetine pharmacokinetics.

Our study was discontinued after an interim analysis with relatively small patient numbers. However, the criteria for premature trial termination regarding futility had been pre-specified, making it highly unlikely that we overlooked clinically relevant differences. Moreover, the neurobiological parts of our study provide a rationale why even with much larger patient numbers no substantially different outcome can be expected. On the other hand, premature stopping reduced the power to examine whether subgroups of patients were more responsive to dose-scalation.

The present study was not designed to test the efficacy of paroxetine per se, as this is well established in patients with severe MDD (HDRS 17>18). Therefore, we did not include a pure placebo arm. Rather we investigated dose-escalation, and accordingly only included placebo during dose-escalation. This approach is similar to e.g. the STAR*D project, which interestingly reported similar response rates for open treatment with citalopram.

**Conclusion**

Previous studies had failed to demonstrate a clinical benefit of dose-escalation by SSRIs, but had methodological limitations. Addressing those limitations, our trial replicates that dose-escalation of paroxetine above the 20 mg/day standard dose has no additional clinical benefit. As a novel extension, we revealed the underlying neurobiological mechanism for this inefficacy: maximum SERT occupancy was already reached with the standard dose. Similarly high SERT occupancies reported with low doses of other SSRIs suggest that our conclusion may be applicable to the entire drug class. However, this does not exclude that dose-escalation has clinical benefits for antidepressants with additional molecular targets, e.g. the norepinephrine transporter, such as venlafaxine. This drug has shown dose-dependency of the clinical response in fixed-dose studies.

If dose-escalation is not promising for paroxetine and presumably other SSRIs, two clinical options remain for the treatment of non-responders to standard doses. These are either continuation of treatment until 10 weeks while waiting for a potential delayed response, or a change to a different and potentially more effective treatment strategy. Both strategies will further improve response-rates, but studies directly comparing these strategies have not yet been performed.
Acknowledgements

The authors wish to thank the patients in this study for their participation, and especially thank the patients that were willing to participate in the SPECT-study. We thank all participating general practitioners in the area of Amsterdam Oost and Zuidoost, Hoofddorp, Nieuw Vennep, and Abcoude for their inclusions and referrals for the study. Mrs. E. Miedema M.D. and Mrs. M.C. ten Doesschate M.D. were indispensable for their help in rating questionnaires. Mrs M. Haages managed randomization and maintained blinding. This study was financed by a grant from the Netherlands Organisation for Health Research and Development (ZonMw), program Mental Health, education of investigators in mental health to H.G. Ruhé (OOG; #100-002-002). We especially thank Professor M.E. Thase M.D. for his constructive comments on a previous version of the manuscript.

Conflicts of interest

All authors reported no biomedical financial interests or potential conflicts of interest.

References


Supplementary appendix: methods of paroxetine serum concentrations and results.

Paroxetine serum concentrations

PSC were determined in May 2007 using a previously validated HPLC-MS/MS method. A Thermo Finnigan (San Jose, CA, USA) Surveyor HPLC system was used, equipped with a Surveyor autosampler. Separation was carried out on an Agilent Eclipse XDB-CN column, 100 x 2.1 mm, particle size 3.5 µm (Agilent, Amstelveen, The Netherlands). A mixture of 60% (v/v) 2 mM ammonium acetate (pH 3.2) and 40% (v/v) acetonitrile was used as mobile phase. Paroxetine was extracted from serum by liquid-liquid extraction. Briefly, 500 µL of serum samples were mixed with sodium carbonate solution (0.5M). An internal standard solution (disopyramide 20 µg/l) was added and the mixture was vortexed for 5 seconds. Thereafter, 1-chlorobutane/ethylacetate mixture (60/40; v/v) was added and vortexed for 10 minutes. After centrifugation for 10 minutes (3000g), the upper layer was transferred to a second vial, and evaporated under a nitrogen atmosphere at 40 °C. After addition of methanol the solution was vortexed and transferred onto the HPLC-MS/MS system. Selected reaction monitoring (SRM) was used for drug quantification (SRM paroxetine 330.1/192.0 and SRM disopyramide 340.2/239.0). Calibration curves were constructed by adding known amounts of paroxetine to blank serum (5-75 µg/L range). The lower limit of quantification was 5 µg/L, which was 50% below the lower end of the therapeutic range of paroxetine in serum (10-75 µg/L range). The lower limit of detection was 0.3 µg/L.
<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Population (mean age)</th>
<th>N Follow-up Design</th>
<th>Intervention / control</th>
<th>SERT Imaging and ROI</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catafau 2006&lt;sup&gt;28&lt;/sup&gt;</td>
<td>Drug-free MDD pts (36 ±11) M + F</td>
<td>10 Bsl, 4-6 w</td>
<td>Paroxetine 20 mg</td>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-ADAM SPECT, 4h MID, Thal, Striatum, Cer</td>
<td>In 9 patients a 2&lt;sup&gt;nd&lt;/sup&gt; scan was made. Occupancies were: MID 66.4 ± 9.5%, Thal 63.0 ± 9.6%, Striat 61.3 ± 10.8%</td>
<td>Considerable variation in occupancy observed. Authors suggest that SERT occupancy is probably not related to SSRI response.</td>
</tr>
<tr>
<td>Cavanagh 2006&lt;sup&gt;31&lt;/sup&gt;</td>
<td>MDD pts treated N/A mean AD use 26.3 ± 12.8m</td>
<td>24 Variety of drugs used: monotherapy (n=17); venlafaxine (75-300 mg), SSRIs (20-60 mg), tricyclic (150 mg), Mirtazapine (30 mg); combination (n=7)</td>
<td>No occupancy percentages available. No significant difference in SERT residual activity between responders and non-responders. Wide range of SERT availability</td>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-β-CIT SPECT, 3.3h BrSt/Dienc, Occ</td>
<td>Patients were not scanned before treatment (bsl), instead a comparison of binding potentials was made. Highly heterogeneous group considering drug treatment.</td>
<td></td>
</tr>
<tr>
<td>Erlandsson 2005&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Healthy volunteers (32; 25-52) M</td>
<td>16 Bsl, 2-7d</td>
<td>Citalopram at different dosages (10-60 mg) for different durations (2-7 days). Volunteers were randomized to one of 7 different dosing regimens</td>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-ADAM SPECT, 4h MID, Thal, Striatum, Cer</td>
<td>No mean occupancy for separate dosing regimens given. Maximum MID occupancy 84%</td>
<td>Study aims at finding the best time frame (single scan protocol) for scanning with [&lt;sup&gt;123&lt;/sup&gt;I]-ADAM. Considerable variation in relation occupancy and blood-levels of citalopram observed.</td>
</tr>
<tr>
<td>Herold 2006&lt;sup&gt;29&lt;/sup&gt;</td>
<td>Drug-free MDD pts (42 ±12) M + F</td>
<td>21 Bsl, 7d</td>
<td>Citalopram 10 mg, scans were made 6-7 hours after last application of oral dose</td>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]-ADAM SPECT, 4h MID, Cer</td>
<td>In 13 patients a 2&lt;sup&gt;nd&lt;/sup&gt; scan was made. Mean MID occupancy was 61% (range 37-88%) Considerable variation in occupancy observed. No correlation of occupancy and decrease in depression severity.</td>
<td></td>
</tr>
<tr>
<td>Hiltunen 1998&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Healthy volunteers (30; 25-35) M + F</td>
<td>5 Bsl</td>
<td>1 patient received citalopram 30 mg 3hr prior to injection; 1 patient received citalopram 20 mg 1 hr after injection.</td>
<td>[&lt;sup&gt;123&lt;/sup&gt;I]nor-β-CIT SPECT, 0-24 h a.o. aCG, MID, Thal, BasGang, Cer</td>
<td>After citalopram 30 mg 3hr prior to injection specific binding in the midbrain was 52% less than in untreated subjects. For venlafaxine and citalopram 20 mg no data given. Study aims at establishing tracer-properties of [&lt;sup&gt;123&lt;/sup&gt;I]nor-β-CIT. Specific binding of [&lt;sup&gt;123&lt;/sup&gt;I]nor-β-CIT is 33% higher compared to [&lt;sup&gt;123&lt;/sup&gt;I]-β-CIT (in other subjects).</td>
<td></td>
</tr>
<tr>
<td>Kent 2002&lt;sup&gt;48&lt;/sup&gt;</td>
<td>Patients with Social Fobia (35 ±11) M + F</td>
<td>5 Bsl, 3-6m Paroxetine 20-40 mg (titrated by clinical response) (3-6m)</td>
<td>Oclo by paroxetine: MID 98±3%, Thal 81±6%, Striat 75±7%, Hip 92±12%, Amyg 94±7%, CingA 81±22%</td>
<td>Higher occupancy of SERT than after acute challenge with higher doses (see&lt;sup&gt;67&lt;/sup&gt;). Unexpected decrease in V&lt;sub&gt;T2&lt;/sub&gt; in Cerebellum and white matter, indicative of decrease in non-specific distribution volume of ligand after chronic treatment with paroxetine.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table S9.1. Studies of SERT occupancy after treatment with serotonergic antidepressants
### Table S9.1. Studies of SERT occupancy after treatment with serotonergic antidepressants (Continued)

<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Population (mean age) Sex</th>
<th>N</th>
<th>Follow-up</th>
<th>Design Intervention / control</th>
<th>SERT Imaging and ROI</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klein 200626</td>
<td>Healthy volunteers (26.8 ±4.3) M</td>
<td>25</td>
<td>Bsl, 6h</td>
<td>Citalopram (10 mg or 20 mg), escitalopram (5 mg, 10 mg, 20 mg). Volunteers were randomized to one of 5 different drug/dosing regimens</td>
<td><strong>[123I]</strong>-ADAM SPECT, 4h MID/Thal, Cer</td>
<td>Occupancy of 60 ±6%, 64 ±6% and 75 ±5% for 5, 10 and 20 mg escitalopram. For 10 and 20 mg citalopram occupancy 65 ±10% and 70 ±6%</td>
<td>Study aims to determine acute occupancy of SERT after single dose drug administration of citalopram or escitalopram</td>
</tr>
<tr>
<td>Klein 200725</td>
<td>Healthy volunteers (28 ±7) M</td>
<td>15</td>
<td>Bsl, 10d</td>
<td>Citalopram 20 mg, escitalopram 10 mg. Volunteers were randomized to one of the dosing groups</td>
<td><strong>[123I]</strong>-ADAM SPECT, 4h, 52h MID/Thal, Cer</td>
<td>Occupancy escitalopram 81.5 ±5.4%, citalopram 64.0 ±12.7%</td>
<td>Increased occupancy observed for escitalopram, also represented in ( E_{\text{max}} ) curves. ( E_{\text{max}} ) curves constructed with data for 4h and 52h post injection scans in 1 model</td>
</tr>
<tr>
<td>Kugaya 2003, 200446;48</td>
<td>Volunteers (37.4 ±14.3) M + F</td>
<td>9</td>
<td>Bsl, 8d, 16d Bsl, 3w, 6w</td>
<td>Citalopram 40 mg (8d), Citalopram 40 mg + Buproprion 100 mg (8-16d) Paroxetine 20 mg (6w)</td>
<td><strong>[123I]</strong>-β-CIT SPECT, 24h BrSt, Dienc, Cer</td>
<td>Occ by citalopram at 8d: BrSt 51.4%, Dienc 39.4%; no sign. change thereafter Occ by paroxetine at 1-3w: BrSt 36.5%, Dienc 29.6% &amp; at 6w BrSt 32.6%, Dienc 23.4%</td>
<td>Increased DAT-binding in striatum during prolonged SSRI treatment. Buproprion (100-200 mg) only has no influence on DAT-binding in striatum in study 2 higher Bsl Dienc SERT availability predicted better treatment response at week 6.</td>
</tr>
<tr>
<td>Laasonen-Balk 200470</td>
<td>Patients with MDD (42.7 ±7) M + F</td>
<td>18</td>
<td>Bsl, 6m (±1m drug-free)</td>
<td>Open treatment with various antidepressants (n= 3) and/or benzodiazepines or supportive counselling</td>
<td>**[11C]**DASB PET, 0-1½ h Striat, Thal, CingA, PFC, Cer</td>
<td>Binding in MID was sign. increased in responders to treatment (corrected for age and sex)</td>
<td>Low depression severity at Bsl (HDRS 13.9 ±6.7); only three patients received antidepressants, and six benzodiazepines only. Increased SERT binding in responders suggests decreased midbrain density of SERT during MDD</td>
</tr>
<tr>
<td>Meyer 200119</td>
<td>Patients with MDD, HDRS ≥16 Rx-free (32 ±8) M + F</td>
<td>12</td>
<td>Bsl, 4w Bsl, 4w</td>
<td>Open treatment with paroxetine 20 mg (n= 7), 10 mg (n=1) or citalopram 20 mg (n= 4). No treatment</td>
<td>**[11C]**DASB PET, 0-1½ h Striat, Thal, CingA, PFC, Cer</td>
<td>1. Occ in Striat after paroxetine 20 mg: 83 ± 5%, and after citalopram 20 mg: 77 ±10. Occ in Thal after paroxetine 20 mg: 75-78% ±8, and after citalopram 20 mg: 65-70% ±7.5. Occ in CingA after paroxetine 20 mg: 76-77% ±15, and after citalopram 20 mg: 77-79% ±29.</td>
<td>No relationship was found between HDRS-score and occupancy level in any ROI. Striatal Occ increased with higher serum-levels of paroxetine, with app. 85% occ at serum levels of 28 μg/l. References to other studies that describe a hyperbolic relationship of serum level and occupancy. Test-retest in healthy subjects showed a mean difference of -3.7% ±3.7 (range -8 – 2%) over 4 weeks. Mean absolute difference was 10.9% ±2.9.</td>
</tr>
</tbody>
</table>
Table S5.1. Studies of SERT occupancy after treatment with serotonergic antidepressants (Continued)

<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Results</th>
<th>Population (mean age)</th>
<th>Sex</th>
<th>N</th>
<th>Follow-up</th>
<th>Design Intervention / control</th>
<th>SERT Imaging and ROI</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meyer 200420</td>
<td>1. Patients with MDD 2. Patients with MDD and co-morbid anxiety disorder 3. Healthy subjects (20-50) M + F</td>
<td>29 Bsl, 4w</td>
<td></td>
<td></td>
<td>MDD: citalopram 20-40 mg, fluoxetine 20 mg, sertraline 50-100 mg, paroxetine 20 mg, venlafaxine 75 mg MDD+anxiety: citalopram 40-60 mg, fluoxetine 40-60 mg, sertraline 150-200 mg, paroxetine 40-60 mg, venlafaxine 150-255 mg Healthy subjects: citalopram 1-10 mg, fluoxetine 1-10 mg, sertraline 5-25 mg, paroxetine 5-10 mg, venlafaxine 2.4-35.7 mg</td>
<td>[11C]DASB PET, 0-1½ h Striat, Thal, CingA, PFC, Cuneus, MID, Cer</td>
<td>Mean striatal occ was 81.4 ±7.2% for citalopram (20-40 mg), 76.2 ±7.5 for fluoxetine (20 mg), 85.0 ±7.0% for sertraline (50-100), 84.5 ±6.0% for paroxetine and 81.7 ±2.4 for venlafaxine (75 mg). Occ for Thal: 72.3 ±7.6%, 69.1 ±9.4%, 76.8 ±4.3%, 74.7 ±15.7% and 71.3 ±10.2% resp. Occ for MID: 87.5 ±7.7%, 82.3 ±9.3%, 91.8 ±14.0%, 93.4 ±7.7% and 91.0 ±7.7% resp.</td>
<td>12 patients also described in Meyer et al. 200119 2 MDD-patients received subtherapeutical doses in 'Healthy' group; in 5 patients of MDD group dosages were secondarily increased and also analysed (twice) in MDD + anxiety group. No relation between striatal occupancy and clinical remission or percentage change in Hamilton depression scores.</td>
<td></td>
</tr>
<tr>
<td>Parsey 200069</td>
<td>Healthy volunteers (38 ±12) M</td>
<td>2 Bsl</td>
<td></td>
<td></td>
<td>2 of 6 volunteers received paroxetine 60m or 80 mg prior to scanning (blocking study); blocking compared to PET before using paroxetine</td>
<td>[11C]McN56 SPECT, 0-2½ h MID, Thal, Hip, Amyg, CingA, mTL</td>
<td>Occ by 60 mg paroxetine ‘pretreatment’ (n=1): Amyg 64.8%, Hip 46.0%, Thal 38.4%, Midbr 83.9%, CingA 26.4%</td>
<td>Mainstuyd (n=6) aims to quantify characteristics and protocol for [11C]McN5652.</td>
<td></td>
</tr>
<tr>
<td>Parsey 200621</td>
<td>Healthy volunteers</td>
<td>17 Bsl, 4-6d</td>
<td></td>
<td></td>
<td>Sertraline 25 mg, 50 mg and 100 mg (4 days at designated dosage)</td>
<td>[11C]DASB PET, 0-1½ h Striat, Thal, Cing, DLO/M PFC, Amyg, Hip, Insula, MID, Occ, Cer</td>
<td>Average across 15 RoIs: max occupancy 106.8 ±8.3% range 126.9 ±35.9 in OPFC to 79.3 ±10.4% in Thal</td>
<td>Wide range of RoI’s taken. Study also examines SERT availability in cerebellum and examines its role as reference. Specific SERT binding was found higher in the cerebellar vermis compared to the cerebellar grey. In conclusion no ideal reference region for in vivo SERT studies can be recommended.</td>
<td></td>
</tr>
<tr>
<td>Pirker 199571</td>
<td>Patients with MDD (44-26-71) M + F 2. ‘Healthy’ controls (42.3-24-70) M + F</td>
<td>13 1 scan some where in treatment</td>
<td></td>
<td></td>
<td>Citalopram 20 mg (n=5), 40 mg (n=6) and 60 mg (n=1) for at least one week; one untreated patient.</td>
<td>[123I]β-CIT SPECT, 2, 4, 16, 20, 24h Thal, hypothal, MID, Pons, Cer</td>
<td>No difference in binding in Striat between patients and controls. Citalopram patients showed sign. decrease in Thal, hypothal, MID compared to controls. No difference in binding between patients with citalopram 20 mg or 40 mg.</td>
<td>Study included patients with bipolar disorder (n=1) and conversion disorder (n=1). Control group included 4 patients with peripheral neurological disease. Citalopram-dosing was not blinded and higher dosages may be the result of an unclear selection process. Specific bound in Thal, hypothal and Midbr region peaked at 4h; in Striat this was reached after 16-24h.</td>
<td></td>
</tr>
<tr>
<td>Suhara 200322</td>
<td>Healthy volunteers (22.0 ±2.3) M 2. Patients with MDD (35.7 ±12.1)</td>
<td>27 1 scan</td>
<td></td>
<td></td>
<td>1. Clomipramine 5-50 mg, fluvoxamine 12.5-50 mg as single administration 2. Clomipramine 20-250 mg, fluvoxamine 25-200 mg as long-term administration</td>
<td>[11C]McN56 SPECT, 0-1½ h Thal</td>
<td>1. Clomipramine occ ≥83.9-100% at dosages 5-50 mg; fluvoxamine occ ≥27.7-87.7% at dosages 12.5-50 mg. 2. Clomipramine occ ≥261.3-100% at dosages 20-250 mg; fluvoxamine occ ≥76.6-93.6% at dosages 25-200 mg.</td>
<td>Hyperbolic relationship between oral dose, plasma concentration and occ. for clomipramine and fluvoxamine. Patient data suggest that the duration of treatment with fluvoxamine may be correlated with occ. This does not hold for clomipramine.</td>
<td></td>
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</tbody>
</table>
### Table S9.1. Studies of SERT occupancy after treatment with serotonergic antidepressants (Continued)

<table>
<thead>
<tr>
<th>Author &amp; Date (reference)</th>
<th>Population (mean age)</th>
<th>Sex</th>
<th>N</th>
<th>Follow-up</th>
<th>Design</th>
<th>Intervention / control</th>
<th>SERT Imaging and ROI</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shang 200772</td>
<td>Healthy controls (23.6 ± 6.3) M + F?</td>
<td>8 Bsl, 9d</td>
<td>Venlafaxine 150 mg (4 days stable dose)</td>
<td>[123I]β-CIT SPECT, 23h</td>
<td>Dienc, MID, Striat, Cere</td>
<td>Occ in Dienc: 52.5 ± 4.7%, in MID: 55.7 ± 4.5%</td>
<td>Study also investigates influence of venlafaxine on striatal DAT availability, which increases 10.7 ± 3.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takano 2006a73</td>
<td>Healthy controls (24.3 ±4.8) M</td>
<td>6 Bsl, 5h, 26h, 53h</td>
<td>Fluvoxamine 50 mg once</td>
<td>[11C]DASB PET, 0-1½ h</td>
<td>PFC, Thal, Striat, Amyg, Hip, Cer</td>
<td>Mean occ of the 5 regions of 6 subjects were 72.9 ± 4.9% at 5 hours, 50.3 ± 11.0% at 26 hours, and 24.7 ± 15.3% at 53 hours. 5h occ: Thal 71.8 ± 5.8%, Amyg 71.6 ± 12.8%; 53h occ: Thal 24.2 ± 12.3%, Amyg 27.4 ± 18.2%</td>
<td>Study investigates time-course of occupancy of SERT after one (low) dose of fluvoxamine. In Emax model dependent measurements at 3 timepoints are used as independent data-points</td>
<td></td>
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<tr>
<td>Takano 2006b72</td>
<td>Healthy controls (24.1 ±2.4) M</td>
<td>15 Bsl, 1.6h, 25h, 53h</td>
<td>1. single duloxetine dose (5, 20, 40, 60 mg) (n= 3) 2. duloxetine 60 mg for 7 days then stopped (n= 3)</td>
<td>[11C]DASB PET, 0-1½ h</td>
<td>Thal, Cer</td>
<td>Thal occ: 35.3-86.5% at increasing dosages: at 6hrs occ: 43.6 ±8.8% (5 mg), 71.3 ± 3.5% (20 mg), 80.6 ± 4.8% (40 mg), 81.8 ± 4.3% (60 mg) 2. Thal occ: 84.3 ± 2.8% (7d), 71.9 ± 2.6% (9d), 47.1 ± 3.7% (11d)</td>
<td>Study concludes that more than 40 mg is required to attain 80% occupancy. Instead of plasma concentrations that decrease in a one-exponential way after stopping, occupancy appears to decrease linearly and slower than plasma concentrations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tauscher 199974</td>
<td>Patient with BN and MDD (18) F</td>
<td>1 scan</td>
<td>Fluoxetine 60 mg. Until 1 week before scan addition with doxepine 75 mg.</td>
<td>[123I]β-CIT SPECT, 4, 24h</td>
<td>Thal, hypoThal, Striat</td>
<td>Compared to controls an occ in Thal and hypoThal of app. 41% was expected by fluoxetine. Higher Striat DAT than in controls; in accordance with other MDD-patients</td>
<td>Methodologically poor. Results compared to a historic control group (n= 13, M + F; age 46 ±20; age range 24-71).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viinamäki 199875</td>
<td>1. Patients with PersDis (25 &amp; 34) M 2. Healthy controls (25±3 &amp; 34±1) M</td>
<td>2 Bsl, 1-1.5y</td>
<td>Psychodynamic psychotherapy or no treatment</td>
<td>[123I]β-CIT SPECT, 4, 24h</td>
<td>Thal, hypoThal, Striat</td>
<td>(Successfully) treated patient 1: increase in binding in MID: 34%, mPFC 43%, Thal 31%; with no changes in untreated patient 2; levels of binding post-treatment approach levels of controls</td>
<td>Patients were both abusing alcohol; patient 1 abstained during treatment; patient 2 did not. Patient 1 had low, and patient 2 had high HDRS-scores at baseline and follow-up. Controls used alcohol occasionally, no data on HDRS are given. Methodologically poor, study is described poorly.</td>
<td></td>
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</tr>
<tr>
<td>Voineskos 200730</td>
<td>Patients with MDD (36 ±9) Healthy controls (32 ±9) M+F</td>
<td>&gt;4w Bsl</td>
<td>Venlafaxine 225 mg, 45.0 mg, sertraline 150 mg, 200 mg, citalopram 60 mg, 80 mg</td>
<td>[11C]DASB PET, 0-1½ h</td>
<td>Striat, MID, Thal, PFC,ACg, Cer</td>
<td>Occ Striat: 85.8 ± 3.4% (venlafaxine), 85.8 ± 2.3% (sertraline) and 85.4 ± 2.0% (citalopram) Occ MID: 99.5 ± 4.1% (venlafaxine), 98.2 ± 3.2% (sertraline) and 95.7 ± 1.4% (citalopram) Occ Thal: 77.6 ± 4.7% (venlafaxine), 76.3 ± 7.9% (sertraline) and 82.2 ± 3.6% (citalopram)</td>
<td>No baseline scans were performed in patients. Instead as reference baseline scans of controls were used to determine occupancy.</td>
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</tbody>
</table>

Abbreviations: Amyg = Amygdala, aCG = anterior Cingulate Gyrus, BasGang = Basal Ganglia, BN = Bulimia Nervosa, BrSt = BrainStem, Bsl = Baseline, CingA = Cingulate Anterior, d = days, DAT = Dopamine Transporter, Dienc = Diencephalon, h = hours, Hip = Hippocampus, HDRS = Hamilton Depression Rating Scale, hypOThal = Hypothalamus, m = months, MDD = Major depressive disorder, mTL = medial temporal lobe, MID = Midbrain, mPFC = medial PreFrontal Cortex, OC = Occipital Cortex, Occ = Occupancy, PersDis = Personality Disorder, PFC = PreFrontal Cortex, RoI = Regions of Interest, Striat = Striatum, Thal = Thalamus, w = weeks, y = years. Most studies used antidepressants: SERT occ. = SERT occupancy relative to non-specific binding (cerebellum).
**Table S9.2.** Drop-outs and emerging adverse effects reported more than once between T0 and T1 (ITT).

<table>
<thead>
<tr>
<th></th>
<th>Paroxetine DE (n = 30)</th>
<th>Placebo DE (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drop-outs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>1/30 (3.3%)</td>
<td>8/30 (26.7%)</td>
<td>0.026</td>
</tr>
<tr>
<td>Inefficacy</td>
<td>0/30 (0.0%)</td>
<td>3/30 (10.0%)</td>
<td>0.237</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>0/30 (0.0%)</td>
<td>4/30 (13.3%)</td>
<td>0.112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Adverse effect rates</strong></th>
<th>Paroxetine DE (n = 30)</th>
<th>Placebo DE (n = 27)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% all*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To</td>
<td>≥2x RP</td>
<td>End RP</td>
<td>Rec. AE†</td>
</tr>
<tr>
<td>Headache</td>
<td>69.0</td>
<td>46.7</td>
<td>70.0</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>67.2</td>
<td>63.3</td>
<td>70.0</td>
</tr>
<tr>
<td>Sweating</td>
<td>63.8</td>
<td>56.7</td>
<td>80.0</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>63.8</td>
<td>66.7</td>
<td>63.4</td>
</tr>
<tr>
<td><strong>Orthostatic hypotension</strong></td>
<td>46.6</td>
<td>50.0</td>
<td>53.3</td>
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<tr>
<td><strong>Constitution</strong></td>
<td>44.8</td>
<td>36.7</td>
<td>50.0</td>
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<tr>
<td><strong>Libido</strong></td>
<td>41.4</td>
<td>36.7</td>
<td>56.7</td>
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<tr>
<td><strong>Agitation</strong></td>
<td>41.4</td>
<td>33.3</td>
<td>50.0</td>
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<tr>
<td><strong>Appetite</strong></td>
<td>39.7</td>
<td>46.7</td>
<td>46.7</td>
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<tr>
<td><strong>Blurry vision</strong></td>
<td>37.9</td>
<td>40.0</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>Tremor</strong></td>
<td>36.2</td>
<td>36.7</td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Dizziness</strong></td>
<td>34.5</td>
<td>36.7</td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>34.5</td>
<td>26.7</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>Parasthesia</strong></td>
<td>32.8</td>
<td>23.3</td>
<td>36.7</td>
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<tr>
<td><strong>Sleep disturbances</strong></td>
<td>31.0</td>
<td>23.3</td>
<td>43.3</td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td>29.3</td>
<td>33.3</td>
<td>33.3</td>
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<tr>
<td><strong>Weakness</strong></td>
<td>29.3</td>
<td>16.7</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Diarrhea</strong></td>
<td>24.1</td>
<td>16.7</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>Urinary retention</strong></td>
<td>24.1</td>
<td>13.3</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Other sexual dysfunction</strong></td>
<td>22.8</td>
<td>16.7</td>
<td>26.7</td>
</tr>
<tr>
<td><strong>Palpitations</strong></td>
<td>22.4</td>
<td>16.7</td>
<td>26.7</td>
</tr>
<tr>
<td><strong>Rash</strong></td>
<td>10.3</td>
<td>6.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

* Adverse effects are listed in order of overall frequency (mentioned more than once in Randomization Phase).
† Recurrence of adverse effect was determined if an adverse effect was initially present during the open phase, disappeared at the end of the open phase, and reemerged during the randomization phase. Columns represent reemerging cases (numerator) of all subjects with the adverse effect initially present, but in whom it disappeared at end of the open phase (denominator). Values in brackets represent this rate as percentages.
‡ Sign. difference paroxetine DE vs. placebo DE (p<0.05; Fisher’s exact test).
§ Sign. difference paroxetine DE vs. placebo DE (p<0.01; Fisher’s exact test).
DE = dose-escalation, T0 = time of randomization at end of open phase, ≥2x RP = mentioned more than once in randomization phase, Rec. AE = Recurrence of adverse effect.

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**Table S9.3.** Changes in paroxetine serum concentrations (μg/l) in the SPECT subgroup due to paroxetine or placebo dose-escalation.

<table>
<thead>
<tr>
<th></th>
<th>Paroxetine DE (n= 11)</th>
<th>Placebo DE (n= 15)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T0</strong></td>
<td>36.2 (22.7-52.8)</td>
<td>60.3 (41.0-83.3)</td>
<td></td>
</tr>
<tr>
<td><strong>T1</strong></td>
<td>154.3 (112.4-202.7)</td>
<td>52.2 (32.9-75.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values represent means with 95% confidence intervals, computed on square-root transformed paroxetine serum concentrations. Probable adherent patients only; in 1 patient (paroxetine dose-escalation) the measurement of paroxetine concentration was missing. The change in paroxetine serum concentrations after paroxetine dose-escalation vs. placebo dose-escalation was significant (ANCOVA correcting for paroxetine serum concentration at T0: F1,23= 59.938; p <0.001). 
* significance of difference between T0 and T1; paired T-test. DE = dose-escalation.
Figure S9.1. Changes in paroxetine serum concentration versus changes in occupancy (randomization to endpoint).

Regression lines show linear relationship (with 95% CI) between increase in paroxetine serum concentration (PSC) and change in occupancy of midbrain (circles; beta= 0.03 ±0.08; F_{1,24}= 0.101; p= 0.754) and diencephalon (diamonds; beta= 0.07 ±0.06; F_{1,27}= 1.332; p= 0.259)