Psychiatry Research: Neuroimaging 221 (2014) 155–161

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

# Occupancy of serotonin transporters in the amygdala by paroxetine in association with attenuation of left amygdala activation by negative faces in major depressive disorder

Henricus G. Ruhé [a,b,*], Michiel Koster [a], Jan Booij [c], Marcel van Herk [d], Dick J. Veltman [e], Aart H. Schene [a,f,g]

[a] Program for Mood Disorders, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[b] University of Groningen, University Medical Center Groningen, Mood and Anxiety Disorders, Department of Psychiatry, Groningen, The Netherlands
[c] Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[d] Department of Radiation Oncology, Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
[e] Department of Psychiatry VUMC, Vrije Universiteit, Amsterdam, The Netherlands
[f] Department of Psychiatry, Radboud University Medical Center, Nijmegen, The Netherlands
[g] Donders Institute for Brain, Cognition and Behavior, Radboud University, Nijmegen, The Netherlands

ARTICLE INFO

*Article history:*
Received 23 August 2013
Received in revised form
25 November 2013
Accepted 12 December 2013
Available online 19 December 2013

*Keywords:*
Major depressive disorder
Amygdala
Paroxetine
Serotonin transporter
Single photon emission computed tomography
Functional magnetic resonance imaging

ABSTRACT

Amygdala hyperactivation in major depressive disorder (MDD) might be attenuated by selective serotonin reuptake inhibitors (SSRIs), but the working mechanism remains unclear. We hypothesized that higher amygdala serotonin transporter (SERT) occupancy by paroxetine results in greater attenuation of amygdala activation by negative facial expressions in MDD patients. We treated fifteen MDD patients (22–55 years) with paroxetine 20–50 mg/day. After 6 and 12 weeks, we quantified (1) clinical response ($\geq$50% decrease in Hamilton Depression Rating Scale (HDRS), (2) SERT occupancy in both amygdala measured by repeated [$^{123}$I]β-CIT single photon emission computed tomography (SPECT), and (3) amygdala activation when viewing fearful and angry (negative) faces with repeated functional MRI scans. Response rates were 4/15 and 9/15 at 6 and 12 weeks, respectively. Attenuation of left amygdala activation was associated with amygdala SERT occupancy ($P$=0.006) and response ($P$=0.015). This association may provide a rationale for decreased limbic activity seen during treatment of MDD. It might also explain the rapid decrease in negative attentional bias and amygdala activation caused by SSRIs.


## 1. Introduction

In major depressive disorder (MDD), both amygdala respond hyperactively to negative valence stimuli, e.g. facial expressions on functional MRI (fMRI) (Groenewold et al., 2013). This hyperactivation can be attenuated by selective serotonin reuptake inhibitors (SSRIs) (Fu et al., 2004; Harmer et al., 2006; Ruhe et al., 2012; Sheline et al., 2001; Victor et al., 2010).

Given the target of SSRIs, the observed attenuation of amygdala activation after SSRIs may be caused by serotonin transporter (SERT) blockade in the amygdala. Previous reports have addressed SERT measurements in the amygdala (Rhodes et al., 2007; Smith and Porrino, 2008). In the amygdala, the SERT is the monoaminergic transporter that is available most abundantly compared to dopamine or norepinephrine transporters (Smith and Porrino, 2008). One of the first multimodal neuroimaging studies combining positron emission tomography (PET) and fMRI reported that lower availability of SERT in the left amygdala was associated with increased left amygdala activation by an emotional task in healthy men (Rhodes et al., 2007). Increased activation of the amygdala was also observed in carriers of the 's' allel of the promotor region of the SERT gene (5-HTTLPR; associated with lower SERT-availability) (Hariri et al., 2005) and transgenic mice lacking the SERT showed increased anxiety-like behavior (Holmes et al., 2003). Nevertheless, it remains unclear whether similar associations exist in depressed patients.

Lower SERT availability is expected to result in more serotonin in the synaptic cleft. This is also expected when prolonged treatment with SSRIs is provided (Blier and De Montigny, 1994). Therefore, the association of higher left amygdala activation with lower SERT (and higher synaptic serotonin concentrations) availability might be in contrast with the fact that prolonged SSRI treatment attenuates amygdala activation. This discrepancy was acknowledged by Rhodes

* Corresponding author at: Program for Mood and Anxiety Disorders, University Center for Psychiatry, University Medical Center Groningen, Suite 5.16 (CC44), Hanzeplein 1, 9700 RB, Groningen, The Netherlands. Tel.: +31 50 361 2367; fax.: +31 50 361 1699.
*E-mail address:* H.G.Ruhe@UMCG.NL (H.G. Ruhé).

0925-4927/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pscychresns.2013.12.003

et al., who proposed that (i) SSRIs might either induce stronger restraint of the amygdala by other brain structures (e.g. anterior cingulate and prefrontal cortex, as proposed in the corticolimbic model of MDD Phillips et al., 2003, 2008, or (ii) an intra-individual change in serotonin levels (by SSRIs) may have different effects than comparisons between subjects with different (untreated) serotonergic tone, e.g. due to polymorphisms of the 5-HTTLPR.

Importantly, no study yet reported on amygdala SERT occupancy after pharmacological treatment in relation to attenuated amygdala responses to facial expressions. This may elucidate whether SSRIs have direct effects on amygdala function. In previous work, part of a larger randomized controlled trial investigating dose-escalation of paroxetine in depressed patients, we assessed SERT occupancy by paroxetine in the midbrain in 32 patients (Ruhe et al., 2009b), while in a subgroup of 18 patients we also measured paroxetine-related changes in amygdala activation during viewing of negative facial expressions (Ruhe et al., 2012). In the present multimodal neuroimaging study, we combined Single Photon Emission Computed Tomography (SPECT) SERT occupancy findings and our findings of magnetic resonance imaging (MRI) to investigate whether amygdala SERT occupancy during treatment with paroxetine is associated with the observed attenuation of amygdala activation during viewing of negative faces in MDD patients.

## 2. Methods

### 2.1. Participants

Following approval by the institutional ethical committee and written informed consent, we recruited outpatients (25–55 years, both sexes) with MDD (determined by structured clinical interview for DSM-IV, First et al., 1999) and a 17-item Hamilton Depression Rating Scale ($HDRS_{17}$, Hamilton, 1960) score above 18. All participants were drug-free and had received no more than one pharmacological antidepressant treatment (other than paroxetine) at an effective dose for $\geq 6$ weeks for the present MDD-episode (Ruhe et al., 2009b). Exclusion criteria were bipolar disorder, psychotic features, neurological/cognitive disorders (i.e. dementia), primary anxiety and/or substance abuse disorders, pregnancy and acute, severe suicidal ideation.

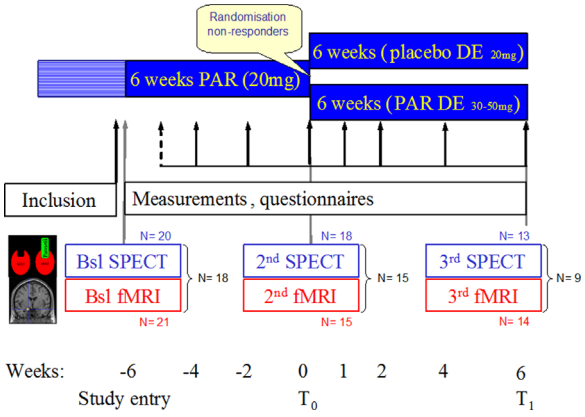


**Fig. 1.** Design of the study. Patients entered open treatment with paroxetine 20 mg/day after acquisition of a study-entry SPECT scan and a functional Magnetic resonance Imaging (fMRI) scan. After 6 weeks (T0) these scans were repeated in all patients who completed 6 weeks of treatment. Patients who did not respond ($<50\%$ improvement in 17-item Hamilton depression rating scale) to paroxetine 20 mg/day were then randomized to receive a double-blind paroxetine dose-escalation (aiming to reach 50 mg/day) or a placebo dose-escalation *added to* paroxetine 20 mg/day and were followed for another 6 weeks (T1). By design and to reduce the burden of radiation, repeated SPECT scans were obtained in randomized patients only. Numbers indicate actual number of successful scans obtained for each scan type (blue and red) and the combination of SPECT-fMRI scans (black). DE, dose-escalation. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

### 2.2. Treatment

After assessment at study-entry, all patients were treated open-label with paroxetine 20 mg/day for 6 weeks at our outpatient department. After 6 weeks (T0), responders remained on 20 mg/day while non-responding patients ($<50\%$ decrease in $HDRS_{17}$-score relative to study entry) were randomized (stratified for age; Fig. 1) (Ruhe et al., 2009b). Randomized patients received a double-blind paroxetine dose-escalation (aiming to reach 50 mg/day) or a placebo dose-escalation *added to* paroxetine 20 mg/day. No dosage adjustments were allowed during weeks 9–12 of the study. Medication was supplied in pillboxes. We checked adherence by pill-counts, paroxetine serum concentrations (PSC; weeks 6 and 12) and patient self-report.

### 2.3. Measurements and outcomes

The MRI-protocol was added to the study-protocol after the original study was planned, in order to further investigate underlying mechanisms of paroxetine treatment in MDD. Primary neuroimaging outcomes were SERT occupancy and changes in blood oxygen level dependent (BOLD) signals representing amygdala activation when viewing negative faces (relative to scrambled faces) at 6 weeks (T0) and 12 weeks (T1) (Fig. 1). All patients participated in three fMRI-sessions as described earlier (Ruhe et al., 2012). Because the purpose of the original study was to study SERT occupancy after a randomized dose-escalation and to reduce the burden of radiation, a third SPECT scan was only made when patients had been randomized. Clinical outcomes were changes in $HDRS_{17}$-scores, obtained at three time-points: study-entry, randomization (T0), and 6 weeks after randomization (T1). Agreement between (blinded) raters was high (intraclass correlation coefficient $=0.98$).

### 2.4. SPECT imaging

SPECT scans were acquired, corrected for attenuation and reconstructed as described earlier (Ruhe et al., 2009a;b,c). Briefly, to assess in vivo binding to SERTs (De Win et al., 2005), iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)-tropane ($[^{123}I]\beta$-CIT) SPECT scans were acquired approximately 230 min after intravenous injection (approximately 110 MBq), on a brain-dedicated scanner (Neurofocus 810; see Supplemental Methods for more details).

### 2.5. fMRI imaging and paradigm

We performed fMRI imaging using a 3 T Intera MRI scanner (Philips, Eindhoven, NL), details of the scanning procedure were described earlier (also see Supplemental Methods for more details) (Ruhe et al., 2012). Briefly, subjects performed an event-related emotional faces task (Wolfensberger et al., 2008). Subjects were instructed to make sex judgments to control for attention lapses. We presented four human face stimuli: angry, fearful, happy and neutral (Ekman and Friesen, 1976), with scrambled faces as baseline condition. Because negative faces (angry and fearful) showed highest amygdala activations (relative to scrambled faces) (Ruhe et al., 2012), we report on the negative faces contrast.

### 2.6. Analyses of neuroimaging data

After standard preprocessing of scans (detailed description in Supplemental Methods), we modeled the BOLD-responses for the negative facial expressions. This provided individual first-level contrast images at study-entry, T0 and T1, which were entered into a second level (random effects) one-way ANOVA with three timepoints as factor. We used SPM5 (Statistical Parametric Mapping; Wellcome Trust Center for Neuroimaging, London, UK; http://www.fil.icon.ucl.ac.uk/spm/), operated under Matlab version 7.3.0.267 (2006b; the Mathworks, Natic, Massachusetts, USA).

For the present study, we extracted the individual's contrast-estimates for each timepoint from the second-level one-way ANOVA. We used a standard volume of interest approach in SPM with a 10 mm sphere (5 mm radius) around the voxel with the maximum activation by negative faces ($P<0.05$, uncorrected, extent threshold 3 voxels; left MNI $-18$, $-8$, $-15$ ($z=3.51$), right 22, $-4$, $-18$ ($z=1.78$)). We calculated $\Delta$T0 and $\Delta$T1 contrast-estimates, by subtracting the individual's amygdala study-entry estimate from those at T0 and T1 (for left and right amygdala separately), representing the change in amygdala activation per subject at T0 and T1. We describe an alternative, secondary approach to extract contrast estimates based on the manually drawn regions of interest (ROIs) for both amygdala (see below) in the supplemental material.

ROIs for left and right amygdala, midbrain and cerebellum (excluding the vermis) were manually drawn by one author (M.K.) for all subjects using the 3D high-resolution structural MRI (sMRI), with a random 50% of scans left-right reversed. We defined boundaries for the amygdala mainly according to Sheline et al. (2001) and for midbrain according to Ghaemi et al. (2002) (full criteria provided in Supplemental Table S1). The drawing of ROIs was trained and supervised by two neuroanatomists. Intraclass correlation coefficient was excellent (left amygdala $=0.97$ (95% CI 0.92–0.99); right amygdala $=0.95$ (0.88–0.98)) tested