Patients with obsessive–compulsive disorder have increased 5-HT$_{2A}$ receptor binding in the caudate nuclei

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Abstract

The pharmacological efficacy of serotonergic-acting drugs suggest that patients with obsessive–compulsive disorder (OCD) may have alterations in their cerebral serotonergic (5-HT) receptor system, and previous neuroimaging studies of OCD patients have shown abnormalities in several fronto-subcortical regions. In this study we investigated cerebral 5-HT$_{2A}$ receptor binding in 15 untreated OCD patients and in 15 age- and gender-matched healthy volunteers by magnetic resonance imaging and $^{[18]}$Faltanserin positron emission tomography (PET). Eleven of the patients were rescanned with PET after receiving treatment with a selective serotonin reuptake inhibitor (SSRI). The distribution volumes of specific tracer binding (DV$_3k$) were calculated for 12 brain regions, and comparisons were made between:

(1) healthy volunteers vs. untreated OCD patients, (2) healthy volunteers vs. treated OCD patients, and (3) OCD patients before and during treatment. When comparing the distribution volume for specific fronto-subcortical brain regions, significantly higher values were recorded in the caudate nuclei in OCD patients (DV$_3k$: 0.24 ± 0.14) compared to the healthy control group (DV$_3k$: 0.15 ± 0.13) (p < 0.05, Wilcoxon matched-pairs test). This difference between groups was not present after treatment with SSRIs. There was no correlation between the severity of OCD symptoms and 5-HT$_{2A}$ receptor binding. An increase in 5-HT$_{2A}$ receptor binding is found in the caudate nuclei of untreated patients with OCD. The up-regulation in 5-HT$_{2A}$ receptors might be compensatory for a lack of serotonin in the feedback loop between the thalamus and orbito-frontal cortex, the caudate nuclei, and the globus pallidus.

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Key words: OCD, positron emission tomography (PET), serotonin 5-HT$_{2A}$ receptors.

Introduction

Obsessive–compulsive disorder (OCD) is a psychiatric disorder, which affects 2–3% of the population worldwide (Karno et al., 1988; Weissman et al., 1994). It has been estimated that up to 10% of patients from outpatient psychiatric clinics suffer from OCD, making it one of the most common diagnoses in psychiatry, after phobias, substance abuse and, major depression. The disease attacks men and women equally and it has a mean age of onset of approx. 20 yr of age.

OCD has been classified as an anxiety disorder by both the American Psychiatric Association (DSM-IV; APA, 1994), and the World Health Organization (ICD-10; WHO, 1992–1994). OCD symptoms include recurrent, unwanted thoughts (obsessions) and conscious, ritualized acts (compulsions) usually attempting to deal with the apprehension generated by the obsessions. OCD is frequently a chronic and debilitating condition as the obsessions and compulsions engage the patient for numerous hours every day.

To maximize treatment outcome in OCD, various treatment approaches need to be combined. There is extensive evidence that indicates that selective serotonin re-uptake inhibitors (SSRIs) and behavioural
therapy applying the principles of exposure and response prevention (Cottraux et al., 2001; Lindsay et al., 1997), are highly effective in reducing the symptoms of OCD. Multiple randomized, double-blind, placebo-controlled studies have demonstrated the efficacy of SSRIs alone (Jenike et al., 1990a,b; Ravindran et al., 1999; Zohar and Judge, 1996).

The cause of OCD remains unknown. However, family-genetic studies suggest that there may be multiple aetiological subtypes (Pauls et al., 1986; Pauls and Leckman, 1986). A review of the OCD functional brain-imaging literature reveals a remarkable amount of data suggesting abnormal functioning along specific, fronto-subcortical brain circuits comprising the orbito-frontal cortex, cingulate cortex, caudate nuclei, and the thalamus. Previous positron emission tomography (PET) studies have examined cerebral glucose consumption in OCD patients in comparison to healthy controls and in OCD patients before and after treatment. In untreated OCD, there is evidence of an elevated glucose metabolism in the fronto-subcortical brain circuit (Baxter et al., 1987, 1988; Perani et al., 1995; Saxena et al., 2001; Swedo et al., 1989), and a normalization of glucose consumption in response to pharmacological treatment (Baxter et al., 1992; Hansen et al., 2002; Perani et al., 1995; Saxena et al., 1999, 2002; Swedo et al., 1992). Further, during provoked OCD symptoms, brain activation with elevated cerebral blood flow in the fronto-subcortical brain circuit has been found (McGuire et al., 1994; Rauch et al., 1994).

In addition to the abnormal functioning along specific fronto-subcortical brain circuits in OCD, it has been suggested that OCD is caused by a dysfunction in the serotonergic system, since SSRIs have a clinical effect in OCD. Furthermore, recent data suggest that the activation of 5-HT$_{1A}$ and/or 5-HT$_{2C}$ receptors may be important for the improvement of OCD symptoms. Thus, some hallucinogens with 5-HT$_{2}$ agonist properties have been reported to have a beneficial effect, e.g. LSD and psilocybin (Delgado and Moreno, 1998; Hanes, 1996; Moreno and Delgado, 1997). In addition, administration of the serotonergic agent m-chlorophenylpiperazine (m-CPP), which binds to 5-HT$_{1A}$/5-HT$_{2}$ receptors, have been shown to exacerbate symptoms in some OCD patients (Hollander et al., 1992). This neuroendocrine and behavioural effect of m-CPP in patients with OCD are, however, antagonized by the non-selective 5-HT antagonist metergoline, suggesting that the response of m-CPP is mediated by 5-HT$_{1}$ and/or 5-HT$_{2}$ receptors (Pigott et al., 1993).

So far, there has been a surprising lack of data on the serotonergic receptor system with no contribution from post-mortem brain studies or imaging studies. In this study we investigated whether OCD patients show alterations in their 5-HT$_{1A}$ receptor system compared to normal controls, and whether such abnormalities could be reversed by successful medical treatment. We hypothesized that OCD patients have alterations in their density of serotonin 5-HT$_{1A}$ receptors in regions of the fronto-subcortical brain circuit, namely the gyrus rectus (part of the orbito-frontal cortex), anterior cingulate cortex, thalamus, and the caudate nuclei. It was also hypothesized that these changes would normalize following successful SSRI treatment, since in PET studies of major depression a reduction in 5-HT$_{1A}$ binding is found following medical treatment (Attar-Levy et al., 1999; Meyer et al., 2001).

### Method

#### Patients and controls

Fifteen outpatients, eight women and seven men, with a mean age of 38 yr (range 18–73 yr) were recruited from the Anxiety Disorder Unit in the University Department of Psychiatry, Copenhagen. Diagnosis and clinical evaluation were made on the basis of a semi-structured interview designed for the study by two trained psychiatrists (E.S.H. and T.G.B.). All patients fulfilled the DSM-IV and the ICD-10 criteria for OCD, and the severity of OCD symptoms were assessed by the Yale–Brown Obsessive–Compulsive Scale (YBOCS; Goodman et al., 1989). For assessment of comorbidity with other psychiatric conditions at the time of study, patients were interviewed according to the Brief Psychiatric Rating Scale (BPRS) and Hamilton Depression Scale (HAMD). During the interviews we diagnosed the following Axis II disorders: Two had dependent personality disorder, two had obsessive–compulsive personality disorder and one patient had histrionic personality disorder.

A general somatic and neurological examination was performed in all subjects. There was no suspicion of substance abuse in any of the subjects and laboratory screening for drug or alcohol were thus not performed. Subjects were rated with YBOCS and HAMD at the time of the PET scan both before and after treatment. Responders to treatment were defined as those that rated either ‘much improved’ or ‘very much improved’ on item 18 of the YBOCS at the time of the second PET scan.

Four patients did not complete the post-treatment PET-study; one patient was excluded from the study because of a concurrent depression, whilst the other three did not wish to undergo a second scan.
All patients were declared physically healthy and all but one patient, who had suffered from migraine, had no previous history of neurological illness, including Tourette’s Syndrome or other tic disorders, nor had they experienced any major head trauma. The duration of OCD symptomatology ranged from 1 to 39 yr with a mean of 16 yr (S.D. = 9.7 yr). The age of onset in one patient was 35 yr and in another 48 yr.

All patients had obsessions as well as compulsions of varying severity. Five patients had checking behaviour as their predominant symptom, five washing rituals, one patient a mixed symptomatology with repetition rituals, counting and need for symmetry and exactness, and two patients suffered from ritualistic eating. None of the two latter patients suffered from anorexia nervosa; their OCD symptoms were related to way of chewing, arranging elements of food, dishes, etc. in a ritualistic order.

Eight of the patients had a history of comorbidity: Five patients had suffered from depression, one patient had had a single phobia, one had suffered from anorexia nervosa and one patient had had generalized anxiety symptoms. However, none of these comorbid conditions had been manifest for at least 6 months prior to the study. Six patients had a family history of OCD and two patients had a family history of depression among first-degree relatives.

In 11 patients, previous attempts to treat OCD symptoms had been made, either by contact with a psychiatrist, a general practitioner or a psychologist. The 10 patients who had received psychotherapy had stopped several months before the onset of the study, and the seven patients who had been pharmacologically treated had been completely drug free at least 4 wk prior to entering the study. Common for all patients, however, was that previous attempts to treat within the last 12 months prior to the study had been unsuccessful. Clinical data are presented in Table 1.

Control subjects were recruited through newspaper advertisements. Fifteen gender- and age-matched healthy volunteers, eight women and seven men, with a mean age of 39 yr (range 21–75 yr) were included in the study. Before inclusion all controls were interviewed by a psychiatrist using the same scales as those used for the patients. One control subject was excluded due to a YBOCS score of 4. None of the subjects had past or present psychiatric or neurological disorders. Furthermore, none of the volunteers were receiving medical treatment acting on the central nervous system.
Written informed consent was obtained from patients and controls according to the Declaration of Helsinki II, and the study was approved by the Ethics Committee of Copenhagen and Frederiksberg [(KF) 02–058/99].

Treatment

The patients received SSRI treatment daily, either paroxetine (60–80 mg), sertraline (50–150 mg), fluoxetine (60–80 mg), or citalopram (60–80 mg). Plasma drug levels were not monitored, but all patients showed good compliance and relatively few had side-effects. One patient refused pharmacological treatment and received counselling only. The patients were not allowed to and did not receive psychotherapy from outside the study. At inclusion, the patients received weekly consultations and later, after initializing drug treatment, they had consultations every 4 wk. The re-scans were performed at the earliest 12 wk after starting the pharmacological therapy (median 26.5 wk, range 12–38 wk).

MRI studies

All subjects had a structural MRI scanning performed with a 1.5 T Vision scanner (Siemens, Erlangen, Germany) using a 3D MPRAGE sequence (TI/TE/TR = 100/4.4/11.4 ms, flip angle 8°). The images were acquired as sagittal plane scans with a spatial resolution of 1.1 × 1.2 × 1.2 mm³. The number of planes was 158 and the in-plane matrix dimensions were 256 × 192.

[¹⁸F]altanserin PET studies

PET imaging of the regional distribution of 5-HT₂A receptors was done with [¹⁸F]altanserin, which is a 5-HT₂A antagonist. [¹⁸F]altanserin has high affinity for the 5-HT₂A receptor with a Kᵢ = 0.13 nM, which is at least a 20-fold greater than Kᵢ for other 5-HT subtypes (Tan et al., 1999). The radiosynthesis of [¹⁸F]altanserin was prepared according to a previously described method by Lemaire et al. (1991). Quality control was performed using analytical TLC and HPLC. For each PET study 0.4–3.5 GBq of [¹⁸F]altanserin was produced with a radiochemical purity greater than 98% and a mean specific activity of 169 GBq/µmol (range 71–300 GBq/µmol).

Catheters were inserted in both cubital veins for tracer injection and blood sampling respectively. [¹⁸F]altanserin was administrated as a combination of a bolus injection and a continuous infusion to obtain tracer equilibrium both in plasma and brain tissue. Subjects received a maximum dose of 3.7 MBq/kg bodyweight [¹⁸F]altanserin, given as a bolus-infusion ratio of 1.75 h, according to Pinborg et al. (2003).

PET scans were performed with an 18-ring GE Advance scanner (GE, Milwaukee, WI, USA) operating in 3D acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution of 5 mm. The technical specifications have been described by DeGrado et al. (1994) and Lewellen et al. (1996).

Ninety minutes after the bolus injection of [¹⁸F]altanserin the subjects were placed in the scanner. Subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbitomeatal line, and positioned to include the cerebellum in the field of view using a short 2-min transmission scan. An individual head holder was made to ensure relative immobility. All subjects were scanned in a resting state.

Dynamic 3D emission scans (five frames of 8 min) started 120 min after administration of [¹⁸F]altanserin. Prior to this scan a 10-min transmission scan was obtained for correction of tissue attenuation, using retractable ⁶⁷Ga/⁶⁸Ga pin sources. The transmission scans were corrected for tracer activity by a 5-min emission scan performed in 2D mode.

Data were reconstructed into a sequence of 128 × 128 × 35 voxel matrices, each voxel measuring 2.0 × 2.0 × 4.25 mm, with software provided by the manufacturer (GE, Milwaukee, WI, USA). A 3D re-projection algorithm with a transaxial Hann filter (6 mm) and an axial ramp filter (8.5 mm) was applied. Corrections for dead-time, attenuation, and scatter were performed.

Five venous blood samples were drawn at mid-scan times 4, 12, 20, 28 and 36 min after starting the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 ml of plasma was counted in a well counter for determination of radioactivity. Three of the five blood samples drawn at 4, 20 and 36 min were also analysed for percentage of parent compound ([¹⁸F]altanserin) using reverse-phase HPLC following the procedure described by Pinborg et al. (2003).

Data analysis

Reconstructed and decay-corrected PET images and MRI images were transferred to Hewlett Packard UX workstations. Images were co-registered using a Matlab-based program, IPS (Interactive Point Selection), developed in-house, based on selection of at least six common anatomical locations in the two images. The transformation between the two sets of
points was estimated in a least-squares sense, and the MRI image was re-sliced into PET space.

Regions-of-interest (ROI) analysis was performed using a Matlab-based program, Editroi, also developed in house. ROIs were drawn on the co-registered MRI images and transferred to the PET images. Twelve regions were selected: cerebellum, gyrus rectus (part of the orbito-frontal cortex), ventral lateral frontal cortex, hippocampus, anterior cingulate, insula, caudate nuclei, lentiform nuclei, thalamus, temporal cortex, parietal cortex and dorsal lateral prefrontal cortex. (see Figure 1). The ROIs were determined by inspection of the MRI images with reference to the brain atlas of Talairach and Tournoux (1988), and two investigators (K.H.A. and S.G.H.) blinded to the clinical diagnosis, agreed on the ROI positions. A mean count density was obtained for each anatomical structure by averaging values across all slices in which the structure appeared. Count densities were expressed in MBq/cc.

Decay-corrected plasma curves were corrected for the presence of radiolabelled metabolites of [18F]altanserin, as determined by the HPLC analysis. Brain and plasma curves were visually inspected to ensure the presence of steady-state in both brain and plasma activity.

The steady-state method requires that equilibrium is present both in blood and brain tissue (Lassen, 1992). When steady-state is present, the distribution volume (DV) can be calculated as the ratio of tissue radiotracer concentration to plasma radiotracer concentration. In receptor studies the DV of a ROI (DVROI) represents the sum of the specifically bound DV (DV3) and the non-specifically bound (DV2). DVROI is related to binding parameters as follows:

\[ DV_{ROI} = DV_2 + DV_3 = f_1 \frac{B_{\text{max}}}{K_d} \] (ml/ml),

where \( f_1 \) is the free tracer fraction (not bound to plasma proteins) assuming that the concentration of free ligand in tissue water equals the concentration in plasma water, \( B_{\text{max}} \) is the concentration of receptors available for binding, and \( K_d \) is the affinity constant of [18F]altanserin. From eqn (1) it can be seen that DVROI is a linear function of \( B_{\text{max}} \) provided that \( K_d \) is the same. Due to overall physicochemical constancy of the brain tissue, DV2 is often assumed to equal the DV of a ROI devoid of receptors (DVref). Inserting \( DV_{ref} = DV_2 \) and rearranging eqn (1) yields the outcome measure used in this study:

\[ DV_3 = f_1 \frac{B_{\text{max}}}{K_d} = DV_{ROI} - DV_{ref} \] (ml/ml),

which also can be given as eqn (3) using cerebellum as a reference:

\[ DV_3 = \frac{C_{ROI} - C_{cerebellum}}{C_{plasma}} \] (ml/ml).
Statistical analysis

Statistical analysis of the regional DV_i data for gyrus rectus, anterior cingulate cortex, thalamus and the caudate nuclei were performed using Wilcoxon matched-pairs tests, with a significance level at p < 0.05. Three separate comparisons were analysed: (1) healthy volunteers vs. untreated OCD patients (n = 15), (2) healthy volunteers vs. treated patients (n = 11) and (3) OCD patients before and during treatment (n = 11). A paired test was used, as the controls and patients were carefully matched to counteract the effect of ageing (Meltzer et al., 1998; Rosier et al., 1996; Sheline et al., 2002) and gender (Biver et al., 1996) and for the evaluation of treatment, patients acted as their own controls. Similarly, Wilcoxon matched-pairs tests, corrected for multiple comparisons with a Bonferroni correction (p < 0.007) were performed for the seven ROIs not included in the fronto-subcortical circuit when looking for global changes in the brain.

The difference between DV_i for all ROIs in patients/controls or patients before and after treatment were analysed by linear mixed models. It was first tested whether the mean difference was the same in all ROIs and then whether this common difference was zero. The difference was allowed to be correlated within patients or patients/controls respectively.

Correlation analysis (ANCOVA) with age as a covariate was conducted to evaluate possible treatment effects. Correlations were evaluated between the YBOCS scores and per cent change in DV_{ISOCD} for gyrus rectus, anterior cingulate, caudate nuclei and thalamus, as determined before and after treatment.

In order to identify clusters with particularly increased/decreased binding, e.g. responders vs. non-responders, Mann-Whitney tests were performed with a significance level of p < 0.05 and p < 0.007, as explained above.

As an additional explorative analysis to identify possible ROIs involved, a statistical parametric mapping (SPM 99; Wellcome Department of Cognitive Neurology, London, UK) analysis was performed. DV_i images were generated [eqn (3)] and spatially normalized into Montreal Neurological Institute stereotactic space using the corresponding MRI image and SPM 99 software. Normalized DV_i images were smoothed with a gaussian filter to 12 mm full-width half maximum (SPM 99). Hypothesis testing was performed on a voxel × voxel basis, comparing the same three contrasts as mentioned above using an ANCOVA test with age as a nuisance in SPM 99, at p < 0.05 (corrected). Also paired t tests at p < 0.05 (corrected) were performed in SPM 99.

Table 2. Distribution volume (DV_i ± s.d.) for all regions of interest in healthy controls and patients with OCD (pre-treatment)

<table>
<thead>
<tr>
<th>Region</th>
<th>Controls (n = 15)</th>
<th>OCD patients pre-treatment (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyrus rectus</td>
<td>1.37 ± 0.42</td>
<td>1.36 ± 0.51</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>1.29 ± 0.47</td>
<td>1.31 ± 0.51</td>
</tr>
<tr>
<td>Caudate nuclei*</td>
<td>0.15 ± 0.14</td>
<td>0.23 ± 0.15</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.29 ± 0.14</td>
<td>0.33 ± 0.15</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>1.41 ± 0.49</td>
<td>1.44 ± 0.50</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>1.39 ± 0.50</td>
<td>1.40 ± 0.51</td>
</tr>
<tr>
<td>Ventral lateral frontal cortex</td>
<td>1.15 ± 0.43</td>
<td>1.19 ± 0.49</td>
</tr>
<tr>
<td>Dorsal lateral prefrontal cortex</td>
<td>1.26 ± 0.48</td>
<td>1.29 ± 0.49</td>
</tr>
<tr>
<td>Insula</td>
<td>1.31 ± 0.47</td>
<td>1.32 ± 0.48</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.45 ± 0.27</td>
<td>0.50 ± 0.23</td>
</tr>
<tr>
<td>Lentiform nuclei</td>
<td>0.47 ± 0.17</td>
<td>0.48 ± 0.18</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.60 ± 0.46</td>
<td>1.52 ± 0.45</td>
</tr>
</tbody>
</table>

The ROIs above the line belong to the fronto-subcortical brain circuit.

* Indicates that DV_i for the caudate nuclei are significantly higher for OCD patients (pre-treatment) than for the controls (p < 0.05, Wilcoxon matched pairs test). The non-specific binding defined as: C_{cerebellum}/C_{plasma} is shown at the bottom of the table.

Results

Clinical data

The 15 patients participating in the study had the following clinical scores (median ± s.d.): BPRS 1.0 ± 1.9 and HAMD 6.0 ± 2.9, which correspond to values found in the healthy subjects. The YBOCS score in the unmedicated patients were 30 ± 6.8. Following treatment, the YBOCS score dropped by 40% (t = 5.402, f = 14, p = < 0.001) (see Table 1). Four patients were non-responders with a post-treatment YBOCS score > 24, while the remainder responded to treatment with YBOCS scores < 22. All patients that completed the second PET scan (n = 11) were responders. None of the controls scored on any of the psychiatric scales.

5-HT_2A receptor binding

Tables 2–4 show the distribution volumes (DV_i) for [^{3}F]altanserin binding in all ROIs. Untreated OCD patients have a higher binding of [^{3}F]altanserin in both the left and the right caudate nucleus when compared to a matched control group (f = 14, p < 0.05) (see Figure 2). No significant differences were found for DV_i in any of the other regions for the conditions tested.
Table 3. Distribution volume (DV$_i$ ± s.o.) for all regions of interest in healthy controls and patients with OCD (post-treatment)

<table>
<thead>
<tr>
<th>Region</th>
<th>Controls (n = 11)</th>
<th>OCD patients post-treatment (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyrus rectus</td>
<td>1.42 ± 0.39</td>
<td>1.29 ± 0.50</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>1.33 ± 0.45</td>
<td>1.33 ± 0.53</td>
</tr>
<tr>
<td>Caudate nuclei</td>
<td>0.18 ± 0.15</td>
<td>0.28 ± 0.20</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.31 ± 0.14</td>
<td>0.33 ± 0.13</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>1.46 ± 0.46</td>
<td>1.44 ± 0.54</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>1.46 ± 0.51</td>
<td>1.41 ± 0.54</td>
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<td>Ventral lateral frontal cortex</td>
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</tr>
<tr>
<td>Dorsal lateral prefrontal cortex</td>
<td>1.34 ± 0.45</td>
<td>1.36 ± 0.54</td>
</tr>
<tr>
<td>Insula</td>
<td>1.38 ± 0.46</td>
<td>1.26 ± 0.52</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.49 ± 0.29</td>
<td>0.51 ± 0.26</td>
</tr>
<tr>
<td>Lentiform nuclei</td>
<td>0.48 ± 0.15</td>
<td>0.48 ± 0.18</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.53 ± 0.31</td>
<td>1.38 ± 0.43</td>
</tr>
</tbody>
</table>

The ROIs above the line belong to the fronto-subcortical brain circuit. The non-specific binding defined as: $C_{\text{cerebellum}}/C_{\text{plasma}}$ is shown at the bottom of the table.

Statistical testing using linear mixed models of differences in DV$_i$ in controls and OCD patients showed that all regions could be assumed to have the same expected difference between patients and controls. Furthermore, this shared expected difference was found not to be significantly different from zero. The significance of these fixed effects was tested in a model that took the large correlation between differences in DV$_i$ within the same matched pair into account.

The distribution volumes are generally higher (on average 9%) in all ROIs, in unmedicated OCD patients compared to the matched control group (n = 15) and slightly higher (4%) for the treated OCD patients compared to the matched controls (n = 11). For the OCD patients an averaged decline in all ROIs of DV$_i$ of 3% occurred after treatment (n = 11). None of these changes were, however, statistically significant. No differences were found in the non-specific binding, as defined from the cerebellar DV$_i$ (Wilcoxon matched-pairs test).

There was no difference in 5-HT$_{2A}$ binding between patients with a family history of depression and/or OCD, and patients without such a family history. In addition, there was no difference between SSRI drug-naive patients and those who on previous occasions had received SSRI therapy. Further, there was no difference between responders and non-responders to SSRI treatment, and no correlation between duration of SSRI treatment and differences in 5-HT$_{2A}$ binding of OCD patients before and after treatment. Exclusion of the patient who received clinical counselling only (n = 10) did not alter the results.

There was no statistically significant correlation between YBOCS score and DV$_i$ for the gyrus rectus, anterior cingulate, caudate nuclei, and thalamus; nor were there any statistically significant correlations between the relative (before and after treatment) changes in these variables (data not shown). The results obtained using the SPM analysis of DV$_i$ images were similar to the results obtained by the ROI analysis, in that the SPM analysis did not reveal any statistically different clusters/ROI between any of the groups tested. Due to the restrictive statistics involved in testing without any a-priori hypothesis, SPM analysis failed to identify that untreated OCD patients have higher [$^{18}$F]alanserin binding in the caudate nuclei compared to controls.

**Discussion**

We find that in unmedicated patients with OCD, DV$_i$ for [$^{18}$F]alanserin binding to 5-HT$_{2A}$ receptors in the caudate nuclei is increased compared to an age- and...
gender-matched control group. It should be pointed out that the relatively low density of 5-HT$_{2A}$ receptors in the caudate nuclei complicates the detection of alterations present, because of the relative uncertainty in the determinations. Test–retest data for $[^{18}F]$altanserin after bolus injection is approx. 10% (Smith et al., 1998) which is in the same order of magnitude as for the bolus-infusion approach (Pinborg, Adams, Knudsen, unpublished data). It is unknown to what extent there is a biological day-to-day variation, and therefore, it is not possible to assess the variability that stems from methodological instability alone. This possibly explains why the small and statistically insignificant decrease in DV$_3'$ of the caudate nuclei following SSRI treatment caused a loss of statistically significant difference between groups. We cannot exclude that the latter finding is an effect of the limited sample size in the within-subject study of SSRI treatment ($n = 11$) and that the study may, therefore, lack power to detect effects of SSRI treatment on the number of 5-HT$_{2A}$ receptors. The statistical difference between unmedicated OCD patients and controls was observed with the ROI analysis only, whereas both the ROI and SPM analyses confirmed that there were no global alterations in the 5-HT$_{2A}$ receptor density in OCD patients as compared to gender- and age-matched controls. SPM, however, takes a data-driven approach which might be useful for the generation of hypotheses but is less suitable for detection of subtle differences in predefined regions. Further, SPM involves filtering with a 12-mm gaussian filter and is, thus, less likely to pick up smaller signal changes in regions located close to the ventricles, because of partial volume effects.

The location of changes to the caudate nuclei is in agreement with findings from other groups where OCD patients have been found to have a significantly higher glucose metabolism in this region compared to a control group (Baxter et al., 1987, 1988). Further, imaging studies in OCD have demonstrated a decreased metabolic activity in the caudate nuclei, both in response to treatment with SSRI and behavioural therapy (Baxter et al., 1992; Hansen et al., 2002; Saxena et al., 1999, 2002; Schwartz et al., 1996), and also an increased cerebral blood flow was found in the caudate nucleus in symptom provocation studies (McGuire et al., 1994; Rauch et al., 1994). Our data support and extend the previously reported pathology in the caudate nuclei of OCD.

Our finding of an increased distribution volume may either be caused by an increase in the regional density of 5-HT$_{2A}$ receptors or by a decrease in radioligand affinity. The latter situation could possibly be induced in the case of a decrease in the synaptic concentration of competing serotonin. However, since $[^{18}F]$altanserin binding remains unaltered during increased concentrations of synaptic serotonin (Pinborg et al., in press), we conclude that the difference is caused by an up-regulation of available 5-HT$_{2A}$ binding sites ($B_{max}$) in the caudate nuclei. Our conclusion that our findings are due to 5-HT$_{2A}$ receptor up-regulation are supported by post-mortem studies that demonstrated increased density of 5-HT$_{2A}$ receptors in the prefrontal cortex of suicide victims, and depressed subjects who died of natural causes (Arango et al., 1997; Arora and Meltzer, 1989; Hrdina et al., 1993), suggesting that 5-HT$_{2A}$ receptors up-regulate in response to a defect in 5-HT neurotransmission.

A potential confounding factor, the choice of reference region, needs to be considered with respect to DV$_3'$. It is required that the reference region represents

Figure 2. Distribution volume (DV$_3'$) for the caudate nuclei in controls and untreated OCD patients ($n = 15$). The mean DV$_3'$ value for the controls is $0.15 \pm 0.13$ (s.d.) and the mean DV$_3'$ value for the OCD patients is $0.24 \pm 0.14$ (s.d.). $p < 0.05$ indicates that the DV$_3'$ for the caudate nuclei is significantly higher than in OCD patients when compared to healthy volunteers.
non-specific binding only, or at least has negligible specific binding. In a previous paper it was found that cerebellar $5\text{-HT}_{2A}$ binding density was approximately one third of the density in the receptor-rich region prefrontal cortex (Eastwood et al., 2001). In our laboratory, the suitability of the cerebellum as a reference region was evaluated in $[^{18}\text{F}]$altanserin PET with within-scan displacement with cold ketanserin (Pinborg et al., 2003), showing a complete blockade of all ROIs binding except for the cerebellum, which remained unchanged following displacement. Based on these data, the cerebellum seems to serve as a suitable reference region in clinical $[^{18}\text{F}]$altanserin PET studies. In addition, there was no difference between the cerebellar $[^{18}\text{F}]$altanserin binding of OCD patients and control subjects.

A previous study has shown significantly higher $5\text{-HT}_{2A}$ binding potential in men than in women, especially in the frontal and cingulate cortices (Biver et al., 1996). Furthermore, it has been reported that differences in hormonal changes in women may influence $5\text{-HT}_{2A}$ receptors (Moses et al., 2000). To assess any possible interference we also conducted comparisons between males only ($n = 7$), but the exclusion of female subjects from data analysis did not reduce the variability or enhance the difference between patients and controls. The increased $5\text{-HT}_{2A}$ receptor density in the caudate nuclei suggests an altered balance in the serotonin system in OCD. This is in accordance with both the serotonin hypothesis – stating that there is an imbalance in the serotonin neurotransmitter system in OCD – and also the feedback loop between thalamus and the orbito-frontal cortex, the caudate nucleus, and globus pallidus, regions that all are known to be involved in the pathology of OCD. Thus, this loop is assumed to be crucial for the behavioural control lost in OCD patients, and down-regulation of the caudate ‘hyperactivity’ is thought to be implicated in the response to treatment with SSRIs, as serotonin is assumed to have an inhibitory effect in this feedback loop (Insel, 1992).

In conclusion, we find that in untreated OCD patients an increase in $5\text{-HT}_{2A}$ receptor binding is found in the caudate nuclei. The increase in $5\text{-HT}_{2A}$ receptors might occur as a compensation for a relative lack of serotonin in the feedback loop between the thalamus and the orbito-frontal cortex, the caudate nucleus and globus pallidus. Further studies are needed to determine whether the increase in $5\text{-HT}_{2A}$ receptor binding in the caudate nuclei is representative for other receptors of the $5\text{-HT}$ family, and to what extent our finding can be regarded as a trait or state marker.

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Statement of Interest
None.

References


