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ORIGINAL ARTICLE

Altered serotonin transporter binding potential in patients with obsessive-compulsive disorder under escitalopram treatment: [^{11}C]DASB PET study

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Background. Obsessive-compulsive disorder (OCD) is a chronic, relapsing mental illness. Selective serotonin reuptake inhibitors block serotonin transporters (SERTs) and are the mainstay of treatment for OCD. SERT abnormalities are reported in drug-free patients with OCD, but it is not known what happens to SERT levels during treatment. This is important as alterations in SERT levels in patients under treatment could underlie poor response, or relapse during or after treatment. The aim of the present study was first to validate a novel approach to measuring SERT levels in people taking treatment and then to investigate SERT binding potential (BP) using [^{11}C]DASB PET in patients with OCD currently treated with escitalopram in comparison with healthy controls.

Method. Twelve patients and age- and sex-matched healthy controls were enrolled. The patients and healthy controls underwent serial PET scans after administration of escitalopram and blood samples for drug concentrations were collected simultaneously with the scans. Drug-free BPs were obtained by using an inhibitory E_{max} model we developed previously.

Results. The inhibitory E_{max} model was able to accurately predict drug-free SERT BP in people taking drug treatment. The drug-free BP in patients with OCD currently treated with escitalopram was significantly different from those in healthy volunteers [Cohen's $d = 0.03$ (caudate), 1.16 (putamen), 1.46 (thalamus), −5.67 (dorsal raphe nucleus)].

Conclusions. This result extends previous findings showing SERT abnormalities in drug-free patients with OCD by indicating that altered SERT availability is seen in OCD despite treatment. This could account for poor response and the high risk of relapse in OCD.

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Key words: Anxiety disorder, molecular imaging, obsessive-compulsive disorder, serotonin transporter, SSRI.

Introduction

Obsessive-compulsive disorder (OCD) is a common chronic psychiatric disorder characterized by distressing intrusive thought or images (obsessions) and by repetitive or ritualistic actions (compulsions) (Karno *et al.* 1988; Weissman *et al.* 1994). The early finding that the disorder responded to clomipramine, a tricyclic antidepressant that mainly acts as a serotonin reuptake inhibitor, initiated neurobiological research into OCD (Fernandez Cordoba *et al.* 1967). The pathogenic role of the serotonergic system in OCD was first proposed on the basis of indirect pharmacological evidence that therapeutic response was specific to selective serotonin reuptake inhibitors (SSRIs) and not seen with norepinephrine reuptake inhibitors or dopamine agonists (Baumgarten *et al.* 1998; Vythilingum *et al.* 2000). However, despite treatment with SSRIs, treatment resistance and high rates of relapse still remain a major problem (Pallanti *et al.* 2002; Bech *et al.* 2010).

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging studies are able to measure the availability of

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serotonin transporters (SERTs), which represent the pharmacological target of SSRIs, and provide direct evidence for the role of the serotonergic system in OCD. The first study using SPECT performed by Pogarell *et al.* (2003) reported elevated SERT availability in the midbrain-pons in patients with OCD compared to healthy controls. However, further studies found either a reduction (Stengler-Wenzke *et al.* 2004; Hesse *et al.* 2005), or no alteration of SERT availability in the same region (van der Wee *et al.* 2004) in patients with OCD. These inconsistent results might be partly due to the lack of specificity for SERTs over other monoamine transporters shown by the tracer, [^{123}I]β-CIT, used in these studies (Innis *et al.* 1991; Laruelle *et al.* 1993; Neumeyer *et al.* 1996).

This prompted the use of highly SERT-selective radiotracers such as [^{11}C]DASB to investigate SERT availability in patients with OCD. Indeed, studies using [^{11}C]DASB are consistent in reporting significant reductions in SERT availability in key regions of interest (ROI) including the thalamus, midbrain, insular cortex, striatum, and limbic and paralimbic brain areas in patients with OCD compared to healthy controls (Reimold *et al.* 2007; Matsumoto *et al.* 2010; Hesse *et al.* 2011). The regions are mainly involving the prefrontal-basal ganglia-thalamic-prefrontal circuits, the dysfunction of which is thought to be associated with implicit processing deficits and intrusive symptoms (Rauch *et al.* 1997; Stein, 2000). All the studies were conducted in untreated patients with OCD, since the drug-free binding potentials (BPs) which represent SERT availability cannot be calculated in patients treated with SSRIs. However, it remains unclear what effect SSRI treatment has on SERT levels in OCD. This is important as alterations in SERT levels in patients under treatment could underlie poor response, or relapse during or after treatment.

We have developed and validated a method to derive drug-free BPs in patients currently treated with psychotropic drugs (Kim *et al.* 2011). This enables receptor and transporter levels to be investigated during treatment to determine if there is up- or down-regulation during the course of treatment. Based on previous studies showing SERT abnormalities in patients who have discontinued treatment (Reimold *et al.* 2007; Matsumoto *et al.* 2010), to test the hypothesis that SSRI treatment would not normalize SERT availability in patients with OCD, we sought to derive drug-free [^{11}C]DASB BPs in OCD patients treated with escitalopram and compare them to those from matched healthy controls. For this, we obtained serial [^{11}C]DASB PET scans in patients with OCD and healthy controls and collected the corresponding blood sample for determination of plasma levels of escitalopram.

Method

This study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Participants

Patients were recruited from the tertiary-care outpatient OCD clinic in the Seoul National University Hospital (http://ocd.snu.ac.kr/index.php). Healthy volunteers were recruited by advertisement from the local community. Participants (aged 19–30 years) received a full explanation of the study including the radiation dose they would be exposed to (3.7 mSv per scan and a total of 14.8 mSv in healthy volunteers and 11.1 mSv in patients) and provided written informed consent to participate. This exposure may limit the translation of this procedure to some research settings.

Twelve male patients who met the DSM-IV criteria for OCD and 12 healthy male volunteers participated in the study. For inclusion, patients had to be stable enough to follow instructions for the study. In addition they had to have received escitalopram for at least 16 weeks with no dose changes for at least 4 weeks so that treatment was at steady state.

The exclusion criteria for all subjects were history or clinical evidence of significant medical disease, or DSM-IV diagnosis (except OCD in patients); clinically significant abnormalities in laboratory tests (haematology, blood chemistry, urinalysis) and the physical examination; clinically relevant ECG abnormalities; or any psychiatric condition requiring concomitant psychotropic medication (except escitalopram in the patients). While we excluded co-morbid conditions, we did not screen for alcohol or drug use prior to participation.

Study design

Healthy volunteers received a single dose of escitalopram (5, 10, 20, and 30 mg). We selected the doses that were expected to give a wide range of BPs based on published data on SERT occupancy by escitalopram (Meyer *et al.* 2004). The dose of escitalopram was randomly assigned to healthy volunteers.

After fasting for at least 4 h, both patients and healthy volunteers received the oral dose of escitalopram, with 240 ml water, at 10:00 hours. The symptomatic severity of OCD was measured by using the Yale–Brown Obsessive Compulsive Scale (YBOCS) in patients (Goodman *et al.* 1989).

Serial [^{11}C]DASB PET scans for the measurement of SERT BPs were performed before and 3 h, 24 h

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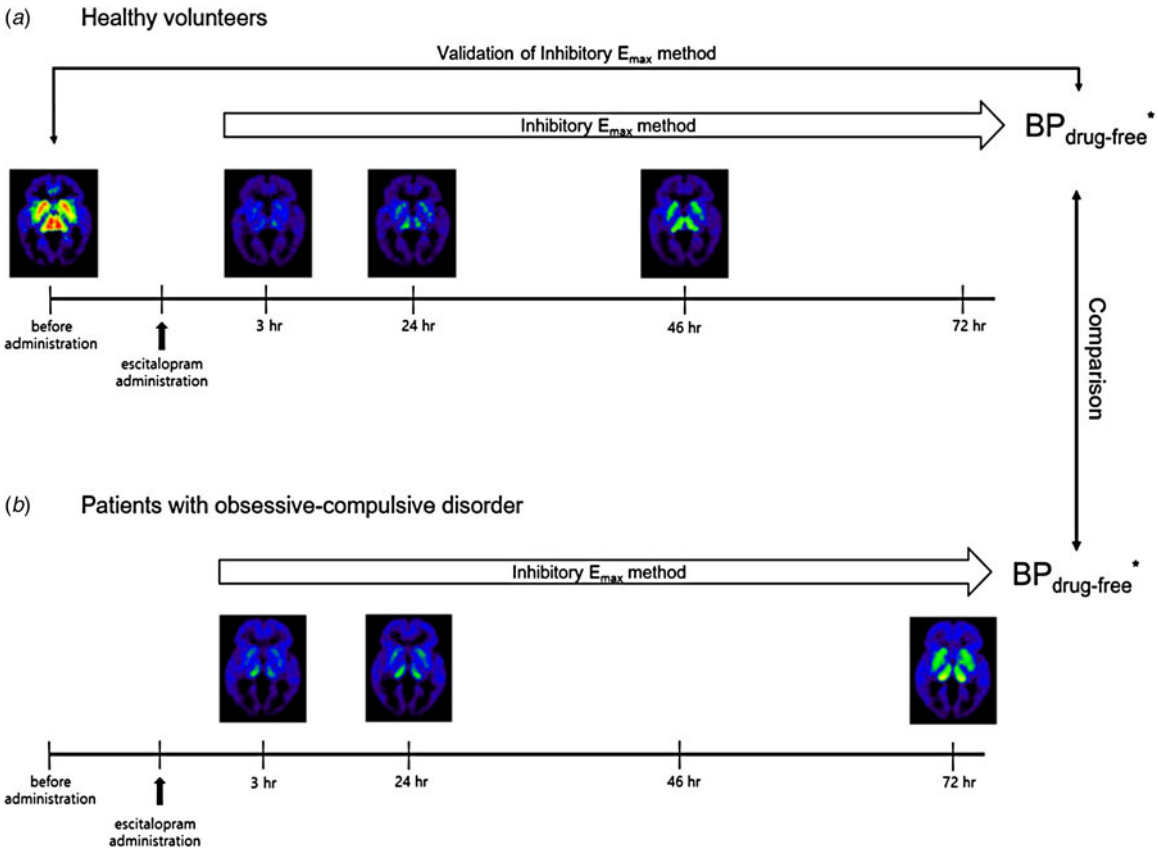

Fig. 1. Diagram illustrating the study protocol for healthy volunteers (*a*) and patients with obsessive-compulsive disorder (*b*). Parametric images for binding potentials (BPs) were from one representative healthy volunteer and one representative patient for illustrative purposes. * $BP_{drug\text{-}free}$ was derived from the inhibitory E_{max} method.

and 46 h after the single administration of escitalopram in healthy volunteers and 3 h, 24 h and 72 h after the last administration of escitalopram with the dose maintained for at least 4 weeks in patients with OCD (Fig. 1). Blood samples for the measurement of escitalopram plasma concentration were obtained 5 min before each PET scan.

Subjects were admitted to the Clinical Trial Centre, Seoul National University Hospital for the first 24 h of the study. They returned to the centre for the final measurements. All subjects were required to abstain alcohol and smoking for the duration of study.

PET scanning procedure and calculation of BPs

Subjects underwent 90-min PET imaging after an intravenous bolus injection of 555 ± 37 MBq of [^{11}C]DASB radiotracers on a Biograph 40 Truepoint PET/CT scanner (Siemens, USA). After routine corrections for uniformity, decay corrections and CT-based attenuation, the PET imaging data acquired in a list mode were reconstructed with a filtered back-projection using a Gaussian filter. Images were collected in a three-dimensional mode with 148 axial slices, an image size of 256 × 256, a pixel size of 1.3364 × 1.3364 mm^2 and a slice thickness of 3 mm. The dynamic volumetric images were sequenced using the following framing: 1 × 7.5 s, 7 × 15 s, 1 × 22.5 s, 15 × 30 s, 1 × 45 s, 9 × 60 s, 1 × 150 s, 9 × 240 s, 1 × 270 s, 5 × 300 s.

The following preprocessing steps were performed for dynamic [^{11}C]DASB PET images using Statistical Parametric Mapping 8 (SPM8, http://fil.ion.ac.uk/spm) implemented in MATLAB 2009b (http://mathworks.com). The mean of dynamic frames was co-registered to subject's T1-weighted image using normalized mutual information method, and then the dynamic frames were co-registered in alignments to the mean image. The co-registered PET images were spatially normalized to a standard MNI space.

Three ROIs – caudate, putamen and thalamus, were defined using population-based probability maps (Kang *et al.* 2001; Lee *et al.* 2005), and time-activity curves for the ROIs were acquired to calculate BPs of [^{11}C]DASB by multilinear reference tissue model with two parameters (MRTM2) using the cerebellum as a reference region (Ichise *et al.* 2003). The dorsal raphe

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nucleus (DRN) was drawn manually on averaged [^{11}C]DASB images across subjects as previously described (Selvaraj *et al.* 2012), and the investigator who outlined the DRN was blind to the participant diagnostic status. BPs in the DRN were calculated using the same procedures outlined above for the other regions.

Determination of escitalopram plasma concentration

Escitalopram plasma concentrations were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS; Agilent 1260 series and Agilent 6460 Quadrupole; Agilent Technologies Inc., USA). Sample preparation was performed by liquid-liquid extraction using methyl tertiary-butyl ether. Escitalopram-d6 was used as an internal standard for the quantification of escitalopram. Chromatographic separation was conducted on a Luna C18 (Phenomenex Inc., USA) with a mobile phase consisting of 10 mM ammonium acetate in distilled water and 0.2% formic acid in acetonitrile. The lower limit of quantitation for escitalopram was 0.05 ng/ml, with calibration curves ranging from 0.05 to 50 ng/ml. The intra-day and inter-day accuracies from 96.53% to 103.0%, and the intra-day and inter-day precisions (%CV) were both <5.4%. These results were judged to indicate that the serum concentration analysis was reliable over the given range.

Estimation of BP free of the escitalopram effect

We estimated BPs where escitalopram effects were removed by using an inhibitory E_{max} model with individual serial BP data (Kim *et al.* 2011). The SERT occupancy by SSRIs is usually expressed as the percentage reduction of BP as follows:

$$\text{Occupancy (\%)} = \frac{\text{BP}_{\text{drug-free}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{drug-free}}} \times 100,$$

where $\text{BP}_{\text{drug-free}}$ is the BP when SERT is not occupied by SSRIs and BP_{drug} is the BP after administration of SSRIs.

The relationship between plasma concentrations of SSRIs and their SERT occupancies follows the E_{max} model (Meyer *et al.* 2004; Takano *et al.* 2006). Thus the occupancy above can be described as follows:

$$\frac{\text{BP}_{\text{drug-free}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{drug-free}}} \times 100 = \frac{E_{max} \times \text{Conc}}{\text{EC}_{50} + \text{Conc}},$$

where E_{max} is the maximum occupancy (100% of SERT occupied by drug), EC_{50} is the plasma drug concentration associated with 50% occupancy of SERT and Conc is the plasma drug concentration.

From the equation above, we can obtain an inhibitory E_{max} model for the relationship between BP and concentration as follows:

$$\text{BP} = \text{BP}_{\text{drug-free}} - \frac{I_{max} \times \text{Conc}}{\text{IC}_{50} + \text{Conc}},$$

where I_{max} is the maximum inhibitory effect and IC_{50} is the plasma concentration associated with a 50% decrease in BP. In this model, we assume that SERT will be totally occupied by escitalopram when a supratherapeutic dose is administered and that the BP will therefore be equal to zero. Under this assumption, I_{max} was regarded as $\text{BP}_{\text{drug-free}}$. Individual $\text{BP}_{\text{drug-free}}$ values were calculated for each participant using individual serial BP data from nonlinear mixed-effects modelling.

Nonlinear mixed-effects modelling simultaneously estimates fixed effects and random effects in the inhibitory E_{max} model. The fixed effects are parameters such as I_{max} and IC_{50} which describe the relationship between the plasma drug concentration and BP in the population. The random effects consist of inter-individual variability and residual variability. The inter-individual variability is the between-subject variability of parameters which explains the difference between an individual BP and the population BP predicted from the model. Inter-individual variability of the parameter was estimated using an exponential error model:

$$P_i = \theta \cdot \exp(\eta_i),$$

where P_i is the hypothetical true parameter value for the ith individual, θ is the typical population value of the parameter, and η_i is a random inter-individual variability with zero mean and variance ω^2.

The residual variability is the within-subject variability or measurement error of the BP which results in the difference seen between the individual BPs from observation and the prediction from the model. The residual variability is modelled using a combined error model as below.

$$\text{BP}_{ij}^{\text{obs}} = \text{BP}_{ij}^{\text{pred}} \cdot (1 + \varepsilon_{ij}^{\text{P}}) + \varepsilon_{ij}^{\text{A}},$$

where $\text{BP}_{ij}^{\text{obs}}$ and $\text{BP}_{ij}^{\text{pred}}$ represent the ith subject's jth observed and predicted BP, respectively. ε_{ij} is a normally distributed random variable with zero mean and variance σ^2, and the superscripts P and A on the ε values represent the proportional and additive errors, respectively.

From the nonlinear mixed-effect modelling, we obtained individual estimates of $\text{BP}_{\text{drug-free}}$ as follows:

$$\text{BP}_{\text{drug-free}} \text{ for } i\text{th individual} = I_{max} \cdot \exp(\eta_i \text{ of } I_{max}),$$

where $\text{BP}_{\text{drug-free}}$ for the ith individual represents the BP where the escitalopram effects are removed, I_{max} is the typical population value of the maximum inhibitory effect in the inhibitory E_{max} model, and η_i of I_{max} is

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the inter-individual variability of the maximum inhibitory effect for the *i*th individual.

The calculation was performed using NONMEM v. 7.2.0 software (GloboMax, USA).

Statistical analysis

To determine the reliability of the I_{max} method for estimating $BP_{drug\text{-}free}$ and to test the correlation between $BP_{drug\text{-}free}$ and YBOCS scores, Pearson correlation analysis was applied. For normally distributed variables, Student's *t* tests were used to check for significant differences in demographic data. Differences in $BP_{drug\text{-}free}$ between healthy volunteers and patients with OCD were tested using mixed-effects models with the group (modelled as a dummy variable: 1 = patient with OCD, 2 = healthy volunteers) and the ROIs (modelled as a dummy variable: 1 = caudate, 2 = putamen, 3 = thalamus, 4 = DRN) as fixed effects and subjects as random effects. *Post-hoc* analysis for the group effect on $BP_{drug\text{-}free}$ in each ROI was conducted using Student's *t* test.

Results

All subjects who participated in the study were male Koreans. Mean age (±S.D.), height and body weight of healthy volunteers was 23.0 ± 2.7 years, 173.1 ± 6.9 cm and 69.4 ± 7.9 kg, respectively. The average age (±S.D.), body weight and height of patients were 25.1 ± 5.2 years, 75.0 ± 11.4 kg and 174.5 ± 4.9 cm, respectively.

The single dose of escitalopram was 5 mg for four healthy volunteers, 10 mg for four healthy volunteers, 20 mg for one healthy volunteers and 30 mg for three healthy volunteers. The average maintenance dose (±S.D.) of escitalopram for patients with OCD was 40.8 ± 19.8 mg and the mean corresponding period for the maintenance dose was 60.6 ± 52.3 days. The mean total YBOCS score (±S.D.) in patients was 18.2 ± 4.3 (Table 1).

The plasma concentrations (±S.D.) of escitalopram in healthy volunteers were 10.8 ± 6.8 ng/ml, 5.2 ± 3.2 ng/ml and 2.3 ± 1.1 ng/ml at 3 h, 24 h and 46 h after drug administration, respectively. The concentrations in patients were 71.3 ± 38.0 ng/ml, 47.6 ± 27.8 ng/ml and 20.9 ± 15.2 ng/ml at 3 h, 24 h and 72 h after drug administration, respectively.

The individual $BP_{drug\text{-}free}$ estimated by the inhibitory E_{max} model in healthy volunteers were significantly correlated with the measured $BP_{drug\text{-}free}$ in all ROIs [caudate: Pearson's correlation coefficient (r) = 0.829, $p < 0.001$; putamen: $r = 0.829$, $p < 0.001$; thalamus: $r = 0.678$, $p = 0.015$; DRN: $r = 0.649$, $p = 0.022$; Fig. 2].

There was a significant effect of group and ROI on $BP_{drug\text{-}free}$ and a significant interaction between group and ROI [group: degrees of freedom (df) = 1,41.879, $F = 83.714$, $p < 0.001$; ROI: df = 3,46.527, $F = 171.453$, $p < 0.001$; group × ROI: df = 3,46.527, $F = 66.409$, $p < 0.001$; Fig. 3]. *Post-hoc* analysis revealed significantly lower $BP_{drug\text{-}free}$ in the putamen and the thalamus but higher in the DRN in patients with OCD than in healthy volunteers (caudate: df = 22, $t = 0.0729$, $p = 0.943$; putamen: df = 22, $t = 3.750$, $p = 0.001$; thalamus: df = 22, $t = 3.433$, $p = 0.002$; DRN: df = 22, $t = -13.297$, $p < 0.001$; Fig. 3) [Cohen's $d = 0.03$ (caudate), 1.16 (putamen), 1.46 (thalamus), −5.67 (DRN)] [percentage difference = 0.95% (caudate), 15.31% (putamen), 21.69% (thalamus), 85.79% (DRN)].

The $BP_{drug\text{-}free}$ in each ROI was not significantly correlated with YBOCS scores (caudate: $r = 0.231$, $p = 0.470$; putamen: $r = 0.096$, $p = 0.764$; thalamus: $r = 0.375$, $p = 0.229$; DRN: $r = 0.098$, $p = 0.761$).

Table 1. *Demographic data* (±S.D.)

	Patients	Healthy volunteers	*p* value[a]
Age, yr	25.1 ± 5.2	23.0 ± 2.7	0.235
Sex (male/female)	12/0	12/0	
Height (cm)	174.5 ± 4.9	173.1 ± 6.9	0.597
Weight (kg)	75.0 ± 11.4	69.4 ± 7.9	0.179
Dose[b] (mg)	40.8 ± 19.8	14.2 ± 10.4	<0.001
YBOCS scores			
Total	18.2 ± 4.3		
Obsession	10.1 ± 3.0		
Compulsion	8.1 ± 3.6		

YBOCS, Yale–Brown Obsessive Compulsive Scale.
[a] Student's *t* test.
[b] Single dose of escitalopram for healthy volunteers and maintenance dose for patients.

Discussion

Our main finding in the healthy volunteer study is that the inhibitory E_{max} approach is able to accurately predict SERT $BP_{drug\text{-}free}$ in people taking drug treatment. This approach may thus be used to index baseline SERT levels in patients during treatment without the ethical and clinical challenges of drug withdrawal. This approach enables longitudinal studies of SERT availability in patients on treatment, and the evaluation of SERT density as a predictor of relapse, for example. Even if it were possible to discontinue drug treatment in patients to determine SERT density it would still not be possible to know if changes were due to treatment, or were caused by discontinuation (e.g. serotonergic rebound). The inhibitory E_{max} model was developed using healthy volunteers.

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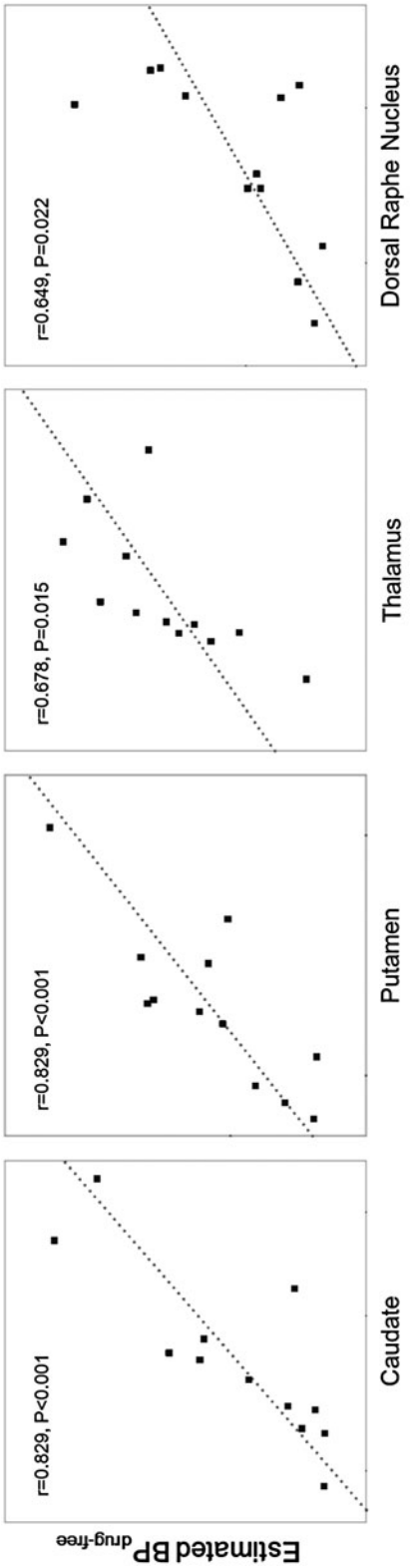

Fig. 2. The correlations between drug-free binding potentials (BPs) estimated by using the inhibitory E_{max} model (estimated $BP_{drug\text{-}free}$) and truly measured in healthy volunteers (measured $BP_{drug\text{-}free}$).

While there are no reasons to think that the model would be different in patients, an important next step is to test this in patients. This would require withholding treatment from patients but we believe that this is justified by our findings here.

Our main finding applying this approach in patients with OCD is that the $BP_{drug\text{-}free}$ in key brain regions in patients during treatment are significantly different from those in healthy volunteers. This result extends previous findings showing SERT abnormalities in drug-free patients with OCD by indicating that altered SERT availability in OCD is seen despite treatment.

Clinical implications

Our finding that patients with OCD exhibited significantly lower $BP_{drug\text{-}free}$ than healthy volunteers in the putamen and the thalamus is consistent with previous findings in drug-free patients in the striatum and thalamus (Stengler-Wenzke *et al.* 2004; Reimold *et al.* 2007; Hesse *et al.* 2011) [the magnitude of the difference in SERT availability was larger than the previous studies, e.g. effect size in thalamus: Cohen's d (d) = 1.46 (our result), d = 1.03 (Reimold *et al.* 2007), d = 0.72 (Stengler-Wenzke *et al.* 2004)]. However, in contrast to the previous studies performed in drug-naive or drug-free patients, our study was conducted in patients currently treated with escitalopram. Thus our finding indicates that altered SERT availability in the putamen and the thalamus is still seen in OCD despite long-term treatment with escitalopram. This may explain the high risk of relapse seen in OCD when SSRI treatment is stopped (Bech *et al.* 2010) because the underlying SERT abnormalities are unmasked when patients stop treatment. For example, Fineberg *et al.* (2007) reported that the relapse rate of OCD for patients stopping escitalopram was 52%, significantly higher than the 23% relapse rate seen in patients who continued escitalopram treatment for the same period. However, an alternative implication is that SERT dysfunction is not intrinsic to the pathoaetiology of OCD. Furthermore, the lack of correlation between the clinical scale of OC severity and SERT availability (Pogarell *et al.* 2003; Matsumoto *et al.* 2010; Hesse *et al.* 2011), which is similar to our result, raises the question about a singular role of the serotonergic system in OCD. Growing evidence indicates that dopaminergic augmentation of SSRIs is useful in the treatment of refractory patients with OCD (McDougle *et al.* 1994). Furthermore, there is evidence that SSRIs like paroxetine, fluoxetine and citalopram (which is a racemic mixture containing escitalopram and whose pharmacodynamic effect is primarily due to the S enantiomer, escitalopram) modulate other neurochemical systems in the brain including noradrenaline and

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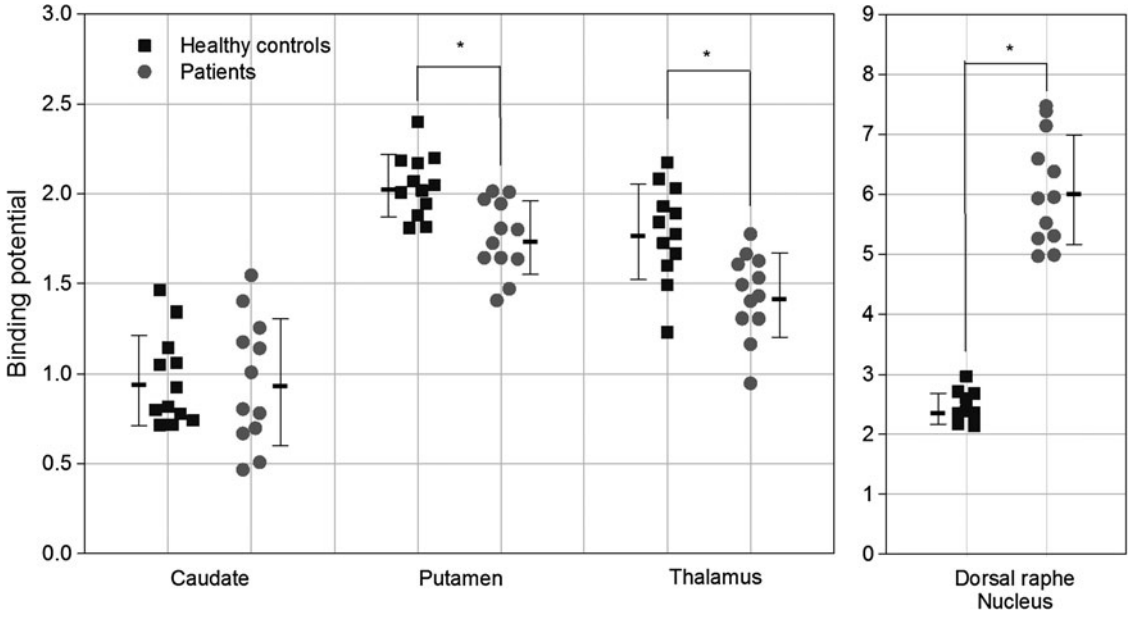

Fig. 3. Drug-free binding potentials estimated from the inhibitory E_{max} model in healthy controls and patients with obsessive-compulsive disorder. Each dot represents an individual binding potential and each vertical bar indicates the mean and the standard deviation for the corresponding group. * Statistically significant in *post-hoc* analysis using Student's *t* test ($p < 0.005$).

dopamine (Collu *et al.* 1997; Hajos-Korcsok *et al.* 2000; Dziedzicka-Wasylewska *et al.* 2002; Cadeddu *et al.* 2014). This suggests that investigation into the role of other neurochemical systems than serotonin may be warranted in OCD.

Although there is theoretical support (Kwon *et al.* 2009) for structural or functional abnormalities of the caudate having a role in OCD, we did not find any $BP_{drug\text{-}free}$ difference in the caudate. A meta-analysis of neuroimaging literature also did not demonstrate a consistent abnormality of the caudate (Aylward *et al.* 1996). This could be due to the heterogeneous nature of this disorder (Pauls *et al.* 1995) and the degree of caudate nucleus abnormality might differ between subgroups. For example, reduced caudate volume and activity were evident in patients with involuntary tic behaviours (Aylward *et al.* 1996; Wang *et al.* 2011) while patients in the current study were free from the neurological symptoms.

Contrary to the putamen and the thalamus, the DRN exhibited significantly higher $BP_{drug\text{-}free}$ in patients with OCD than in healthy controls (Fig. 3). Pogarell *et al.* (2003) also reported a 25% increase in SERT availability in the midbrain which is consistent with our findings. The study conducted by Pogarell *et al.* (2003) used [^{123}I]β-CIT for measuring SERT availability. Thus our study is the first to report higher SERT availability in patients with OCD using a high selective SERT tracer, [^{11}C]DASB. However, Hesse *et al.* (2011) and Matsumoto *et al.* (2010) observed no significant difference in raphe nucleus in patients with OCD relative to controls, and Reimold *et al.* (2007) reported reduced SERT in the midbrain. The inconsistency may relate to the lack of specificity for SERT shown by the radiotracers used in the studies (Innis *et al.* 1991; Laruelle *et al.* 1993; Neumeyer *et al.* 1996). The raphe nucleus is the origin of serotonin neurons where SSRIs are primarily acting on (Bel *et al.* 1992; Gartside *et al.* 1995; Malagie *et al.* 1995) and altering serotonergic function in the raphe influences of serotonin neurotransmission across the brain (Giovacchini *et al.* 2005; Selvaraj *et al.* 2012). Thus, the higher $BP_{drug\text{-}free}$ in patients could reflect an adaptation to long-term exposure to escitalopram, which, by blocking available SERT binding sites, may induce SERT expression in the raphe to compensate, or may be intrinsic to the pathophysiology of OCD.

Another explanation for the higher $BP_{drug\text{-}free}$ in the putamen and the thalamus and lower $BP_{drug\text{-}free}$ in the raphe nucleus observed in healthy volunteers could come from the differences in the dosing between the healthy volunteers (i.e. single administration) and the patients with OCD (i.e. chronic administration) and the mechanism of action of escitalopram. The measurement of BP is primarily based on the ligand displacement and the BP of radiotracers could theoretically be affected by the concentration of the endogenous neurotransmitter (Egerton *et al.* 2009); serotonin in this case. Although the effect of medication was removed in determining the $BP_{drug\text{-}free}$ by using the

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inhibitory E_{max} model, endogenous serotonin might have affected the determination of $BP_{drug\text{-}free}$. SSRIs are generally assumed to increase endogenous serotonin concentrations in serotonergic nerve terminals. However, acute administration of SSRIs can influence the concentration of endogenous serotonin in a different way depending on the brain regions. The increases in extracellular serotonin after acute administration of SSRI are largest in the raphe nucleus (Bel & Artigas, 1992; Gartside *et al.* 1995; Malagie *et al.* 1995) and the stimulation of inhibitory serotonergic autoreceptors by increased endogenous serotonin in the raphe nucleus, where inhibitory serotonergic autoreceptors are presynaptically located on cell bodies, may reduce neuronal cell firing leading to a decrease of endogenous serotonin in the serotonergic projection areas like the cortex where the autoreceptors are postsynaptically located (Barnes *et al.* 1999). Indeed, recent molecular imaging studies showed decreased BP in the raphe nucleus and increased BP in the serotonergic projection areas after a single administration of escitalopram (Nord *et al.* 2013) and intravenous injection of citalopram (Selvaraj *et al.* 2012). The acute effect of escitalopram on the endogenous serotonin might lead to different comparison results across the brain regions between healthy volunteers with a single administration and patients with chronic administration of escitalopram. However, [^{11}C]DASB seems insensitive to change in endogenous serotonin concentrations in human subjects. Two studies, conducted in human subjects, did not show any effect of serotonin manipulation such as tryptophan depletion on [^{11}C]DASB BP (Praschak-Rieder *et al.* 2005; Talbot *et al.* 2005). It is indicated that [^{11}C]DASB can determine regional SERT densities in which the value of BPs are not affected by confounding effects of endogenous serotonin. For this reason, differences in endogenous serotonin across the brain regions are unlikely to have a major effect on our results.

When interpreting the results, some limitations need to be taken into consideration. First, this is a cross-sectional study and we did not measure BP and symptomatic severity in the drug-naive state in patients. Second, we did not measure anxiety and depressive symptoms. We expected the patients enrolled might not have clinically significant depressive and/or anxiety symptoms, since for inclusion patients had to be stable after long-term administration of escitalopram and we excluded patients with depressive and anxiety disorder. However, It has been reported that anxiety and depressive symptoms are prevalent as co-morbidities in OCD (Overbeek *et al.* 2002). Thus, the measurement of the symptoms would have provided more insight into the current results. Last, the dose of escitalopram in patients was higher than in the healthy control study, which resulted in higher plasma concentration of escitalopram in patients. This may lead to an overestimate of $BP_{drug\text{-}free}$ in patients due to extrapolation error in the application of the inhibitory E_{max} model. However, we conducted PET scans at longer time intervals (3 h, 24 h and 72 h after the last administration of escitalopram) in patients relative to controls (3 h, 24 h and 46 h) to obtain reliable trajectories for $BP_{drug\text{-}free}$ (Fig. 1). Furthermore, we found $BP_{drug\text{-}free}$ was lower in the putamen and the thalamus but higher in the DRN in patients than in controls. It is unlikely that a systematic bias in the method that resulted in a $BP_{drug\text{-}free}$ overestimate in patients would explain both an increase in one region and a decrease in other regions.

In conclusion, this is the first study to measure SERT availability conducted in OCD patients currently treated with escitalopram. In spite of the long-term treatment with escitalopram, the abnormality in drug-free SERT availability was similar to the abnormality observed in drug-naive patients (Hesse *et al.* 2005, 2011). This could account for the high risk of relapse in OCD when the medication is discontinued.

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Declaration of Interest

None.

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