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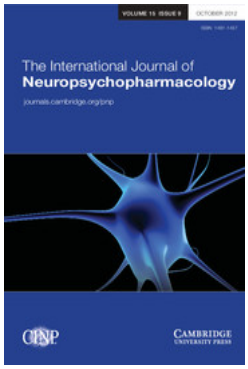

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ARTICLE

Gender-specific abnormalities in the serotonin transporter system in panic disorder

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Abstract

The central serotonergic system has been implicated in the pathophysiology of panic disorder (PD) by evidence of abnormally elevated serotonin-turnover, reduced pre- and post-synaptic 5-HT_{1A} receptor sensitivity and binding and clinical improvement during administration of agents that enhance serotonergic transmission. Polymorphisms in genes that putatively influence serotonergic neurotransmission increase the vulnerability for developing PD specifically in males. We tested the hypotheses that serotonin transporter (5-HTT) binding is elevated in PD subjects *vs.* healthy controls in regions where *in vivo* evidence exists for both elevated 5-HTT and 5-HT_{1A} receptor levels in PD and investigated whether the extent of this difference depends upon gender. Volunteers were out-patients with current PD ($n=24$) and healthy controls ($n=24$). The non-displaceable component of 5-HTT binding-potential (BP_{ND}) was measured using positron emission tomography and the 5-HTT selective radioligand, [^{11}C]DASB. PD severity was assessed using the PD Severity Scale. The 5-HTT-BP_{ND} was increased in males with PD relative to male controls in the anterior cingulate cortex ($F=8.96$, $p_{FDR}=0.01$) and midbrain ($F=5.09$, $p_{FDR}=0.03$). In contrast, BP_{ND} did not differ between females with PD and female controls in any region examined. The finding that 5-HTT-binding is elevated in males but not in females with PD converges with other evidence suggesting that dysfunction within the central serotonergic system exists in PD, and also indicates that such abnormalities are influenced by gender. These findings conceivably may reflect a sexual dimorphism that underlies the greater efficacy of serotonin reuptake inhibitor treatment in females *vs.* males with PD.

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Key words: Cingulate cortex, [C-11]DASB, insula, positron emission tomography, 5-HTT.

Introduction

Panic disorder (PD) is characterized by recurrent episodes of intense fear, thoughts of impending death, accelerated and more forceful heartbeat, shortness of breath, chest discomfort, dizziness, sweating and tremulousness, which arise in the absence of identifiable precipitants and generally are followed by persistent anticipatory anxiety. While the pathophysiology underlying PD remains unclear, alterations in the function of the serotonergic system appear to play roles both in the predisposition to panic attacks and in the mechanisms of anti-panic pharmacotherapy. For example, unmedicated subjects with PD show evidence of elevated serotonin (5-HT) turnover (Esler *et al.* 2007), reduced raphe and cingulate cortex 5-HT_{1A} receptor binding (Nash *et al.* 2008; Neumeister *et al.* 2004) and increased sensitivity to panic following tryptophan depletion in individuals with PD (Miller *et al.* 2000). Moreover, chronic administration of selective serotonin reuptake inhibitors (SSRIs) is a clinically effective treatment for PD that reduces the panic attack severity and frequency (Clayton *et al.* 2006).

These data appear compatible with preclinical evidence in experimental animals indicating that the physiological elevation of 5-HT release and turnover acutely following exposure to threats or stressors plays an adaptive role in the modulation of stress responses (reviewed by Charney & Drevets, 2002). In untreated individuals with PD, Esler *et al.* (2007) reported that cerebral 5-HT turnover is increased during the absence of a panic attack. These data raise questions as to whether

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this elevation in 5-HT turnover in unmedicated PD subjects is associated with altered serotonergic neurotransmission. Although intrasynaptic 5-HT concentrations and release cannot be assessed directly in humans, these parameters may be reflected indirectly by the concentration of serotonin transporters (5-HTT) expressed on serotonergic neurons, an index of which can be measured *in vivo* using positron emission tomography (PET). Regional 5-HTT function plays a major role in modulating local serotonergic neurotransmission and 5-HTT expression is modulated by 5-HT release (Blakely *et al.* 1998). Moreover, the trafficking of 5-HTT protein to the cell membrane rapidly responds to acute changes in 5-HT concentrations, such that greater intrasynaptic 5-HT levels result in increased trafficking of cytosolic 5-HTT protein to the cell surface (Blakely *et al.* 1998). Nevertheless, it remains unclear how chronic changes in serotonergic transmission would affect 5-HTT protein expression. In monoamine oxidase type A (MAOA) genetic knock-out mice the chronic absence of 5-HT metabolism is associated with higher 5-HT concentrations in the dorsal raphe nucleus (DRN), hippocampus and frontal cortex, reduced firing rates and 5-HT_{1A} autoreceptor concentrations in the DRN, yet lower 5-HTT density. The effect of chronically elevated intrasynaptic 5-HT levels on 5-HTT expression thus may differ from that associated with acutely elevated 5-HT levels (Evrard *et al.* 2002). Little evidence exists regarding the effects of chronically altered 5-HT concentrations on 5-HTT expression in other paradigms, however, making it difficult to predict the effect of persistent abnormalities in 5-HT levels on 5-HTT expression. Following treatment with the SSRI, citalopram, for 3 months, the mean 5-HT turnover decreased toward normative levels in a sample of PD subjects who manifested elevated 5-HT prior to treatment (Esler *et al.* 2007).

Notably, the anti-panic efficacy of SSRI agents appears greater in women than in men (Clayton *et al.* 2006) and the prevalence of PD is more than twice as high in women than in men (Kessler *et al.* 1994), suggesting the hypothesis that gender differences in serotonergic function are relevant to the pathophysiology and treatment of PD. Potentially compatible with this hypothesis, genetic variation in systems that participate in 5-HT synthesis influence the risk for developing PD specifically in men (Nakamura *et al.* 1999). Taken together, these data suggest that serotonergic neurotransmission may be differentially altered in males *vs.* females with PD.

A previous PET study of 5-HTT availability in patients with PD supported this hypothesis in finding significant gender × diagnosis interactions in the absence of a main effect of diagnosis. Males with PD ($n=5$) showed a higher 5-HTT binding potential (BP_{ND}) than controls in the raphe, temporal gyri and the anterior cingulate, insular, orbitofrontal, prefrontal and frontal cortices, but lower 5-HTT availability in the hippocampus. In contrast, females with PD ($n=6$) showed no significant difference in any region (Maron *et al.* 2011). While the results of this study were considered preliminary because of the small sample sizes involved, they suggested clear hypotheses for the current study. Thus, to establish in a larger sample size the significance of the male-specific 5-HTT binding abnormalities in PD, we used PET and [^{11}C]DASB to compare 5-HTT BP_{ND} between PD subjects and healthy controls and to assess the significance of gender × diagnosis interactions on BP_{ND}. To preserve statistical sensitivity, the *a priori* hypothesis testing was performed in regions of interest (ROI) implicated by Maron *et al.* (2011), where we also had identified abnormalities in 5-HT_{1A} receptor binding in PD (Neumeister *et al.* 2004); both sets of abnormalities involved the anterior cingulate cortex (ACC) and midbrain raphe.

Method

Subjects

The experimental group consisted of out-patients aged 18–55 yr who met DSM-IV-TR criteria for PD ($n=24$, 13 female; mean age $=29.3\pm8.6$ yr; APA, 2000). Healthy controls, who denied having a history of a major psychiatric disorder and were matched for age and gender to the PD sample, were also selected ($n=24$, 13 female; mean age $=29.7\pm8.0$ yr). The psychiatric diagnosis was established using both an unstructured interview with a psychiatrist and the Structured Clinical Interview for DSM-IV-TR Disorders (APA, 1994, 2000; Gould *et al.* 2000). Exclusion criteria included exposure to psychotropic drugs (including herbal preparations with reported serotonergic effects) within the 3 wk prior to scanning (eight for fluoxetine), pregnancy, major medical or neurological illnesses, lifetime history of substance dependence, substance abuse within 1 yr and recent suicidal behaviour or serious suicidal ideation. Additional exclusions included age-at-illness onset later than 40 yr for the PD subjects and having a first-degree relative with a mood or anxiety disorder for the controls. Severity and frequency of panic attacks were rated using the PD Severity Scale (PDSS; Shear *et al.* 1997). Depression severity was rated using the Montgomery–Asberg Depression Rating Scale (Montgomery & Asberg, 1979) and the Inventory of Depressive Symptoms Clinician Version. Anxiety symptoms were rated using the Hamilton Anxiety Rating Scale (Hamilton, 1959). The PD and control subjects were recruited from the greater Washington, DC metropolitan area through the NIMH Central Office for Recruitment and Evaluation and provided written informed consent as approved by the NIMH Institutional Review Board.

Data acquisition and processing

PET scans were acquired using a GE Advance Scanner in 3D-mode [3D-resolution = 6 mm full-width at half-maximum (FWHM); GE Healthcare, UK], a 90 min

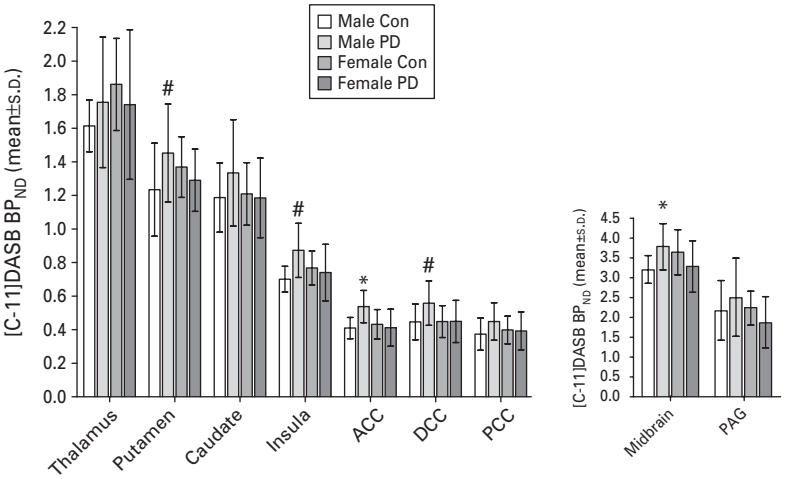

Fig. 1. Mean regional serotonin transporter binding in the male and female subgroups of the panic disorder (PD) and healthy control (Con) samples. *A group × gender interaction significant at $p_{FDR} < 0.05$. #A group × gender interaction that trended toward significance p (uncorrected) < 0.05 in *post hoc* tests. FDR, False discovery rate; ACC, anterior cingulate cortex; DCC, dorsal cingulate cortex; PCC, posterior cingulate cortex; PAG, periaqueductal grey.

dynamic emission scan and measured attenuation correction as described previously (Cannon *et al.* 2007). Head position was stabilized during scanning using a thermoplastic mask fixed to the scanner table. [^{11}C]DASB was synthesized as described previously (Wilson *et al.* 2000). Following i.v. bolus administration of a mean injected dose = 19 ± 2 mCi [^{11}C]DASB and a mean specific activity of 1987 ± 754 mCi/μmol, the emission scan was acquired as 33 frames of increasing length [number × frame duration (min): 6 × 0.5; 3 × 1; 2 × 2; 22 × 5]. Neither the dose ($p = 0.95$) nor specific activity ($p = 0.13$) differed significantly across the four diagnosis by gender groups.

PET data were corrected for head motion by aligning later frames (11–33) to a summed image of all frames using AIR (Woods *et al.* 1992) as implemented by SPM2 (FIL Methods Group, 1994) and Medx software (Sensor Systems Inc., USA). The realigned image frames were co-registered to the anatomical magnetic resonance imaging (MRI) scan for each subject using the Oxford Center for Functional Magnetic Resonance Imaging of the Brain Linear Image Registration Tool and a mutual information cost-function (Jenkinson & Smith, 2001). MRI scans were acquired using a GE 1.5 or 3.0 T scanner and a T_1-weighted pulse sequence (voxel size = 0.86 × 0.86 × 1.2 mm).

Data processing

The co-registered PET and MRI data were spatially normalized to a common stereotaxic array using SPM2 (FIL Methods Group, 1994) and resampled into 2 × 2 × 2 mm voxels, such that each voxel was stereotaxically located relative to the anterior commissure. The 5-HTT binding parameter estimates were computed voxel-wise using the modified reference tissue model (MRTM2) of Ichise *et al.* (2003), as described more fully in Cannon *et al.* (2006). Parametric images were generated using PMOD 2.6 (PMOD Technologies Ltd., Switzerland; Mikolajczyk *et al.* 1998). This model provided the non-displaceable component of the BP_{ND}, a transporter density parameter that is independent of the effects of blood flow (K_1) or radiotracer delivery (Ichise *et al.* 2003). BP_{ND} is proportional to transporter density (B_{max}) and expressed as:

$$BP_{ND} = f_{ND} B_{max} / K_D$$

where f_{ND} and K_D are the free tissue fraction and dissociation constant of the tracer, respectively. The MRTM2 estimates the BP_{ND} in target regions using the reference region time activity data as the input function. The reference region was defined in the cerebellar grey matter, which contains negligible 5-HTT concentrations. This ROI spanned nine axial slices, total volume = 7.04 ml, with the dorsal-most slice situated at least one FWHM ventral to the occipital and temporal cortex and specifically excluded the cerebellar vermis and voxels situated on or adjacent to the edge of the cerebellum. The MRTM2 requires *a priori* estimation by the MRTM of the cerebellum tissue clearance rate constant k_2'[25]. The weighted mean tracer clearance time (fixed k'_2) obtained from the thalamus, midbrain and striatum did not differ ($t = -0.23$, $p = 0.82$) between the control (0.057 ± 0.012 min^{-1}) and PD groups (0.058 ± 0.014 min^{-1}).

To compare the 5-HTT binding of [^{11}C]DASB across groups, the mean 5-HTT BP_{ND} was obtained in MRI-based ROI in the thalamus, caudate, putamen, anterior insular cortex, ACC, dorsal cingulate cortex (DCC), posterior cingulate cortex (PCC), midbrain raphe and midbrain periaqueductal grey (PAG). These regions were selected based on their moderate to high concentration of 5-HTT sites (Varnas *et al.* 2004) in addition to their established involvement in anxiety behaviours (Drevets *et al.* 2008; Graeff *et al.* 1993; Owens & Nemeroff, 1994). The ACC and midbrain were selected as the ROI

Table 1. Demographic and clinical characteristics of the study samples

	Con ($n=24$)	PD ($n=24$)	Con *vs.* PD (χ^2/T, p)
Percent female (n)	54 (13)	54 (13)	n.s.
Age (mean yr ± S.D.)	29 ± 8.6	30 ± 8.0	−0.16, 0.87
Age males (mean yr ± S.D.)	32 ± 10	33 ± 9	−0.23, 0.82
Age females (mean yr ± S.D.)	27 ± 7	27 ± 6	0.03, 0.98
PDSS score (mean ± S.D.)	n/a	7 ± 4	n.a
MADRS score (mean ± S.D.)	0.3 ± 0.6	8.4 ± 8.9	−4.3, $3.0e^{-4}$
HAMA score (mean ± S.D.)	0.1 ± 0.3	6.4 ± 5.7	−5.2, $3.8e^{-5}$
IDS-C score (mean ± S.D.)	0.1 ± 0.3	12 ± 11	−4.9, $7.6e^{-5}$
Age-at-illness onset (mean yr ± S.D.)	n/a	24 ± 8.3	n.a
Range		12–39	
Illness duration (mean yr ± S.D.)	n/a	6.7 ± 5.1	n.a
Range		0.3–16	
Weeks since last panic attack (mean ± S.D.)	n/a	4 ± 5	n.a
Range		0–20	
Treatment naive	24	11	18, $2.6e^{-5}$
Duration medication free			
Mean no. months ± S.D.	n/a	16 ± 14	n.a
Range	n/a	1–42	n.a
Subjects with prior suicide attempts (n)	0	2	n.a
History of co-morbid major depression (n)	0	13	n.a
History of exposure to antidepressants (n)	0	8	n.a
History of exposure to mood stabilizers (n)	0	0	n.a
Prior exposure to antipsychotic drug (n)	0	1	n.a
Remote history of alcohol abuse (n)*	0	1	n.a
History of exposure to MDMA (n)	0	0	n.a
Current cigarette smoking (n)	10	11	n.a

Con, Healthy control group; PD, panic disorder group; PDSS, Panic Disorder Severity Scale; MADRS, Montgomery–Asberg Depression Rating Scale; HAMA, Hamilton Rating Scale for Anxiety; IDS-C, Inventory of Depressive Symptoms-Clinician Rated.
* Lifetime dependence of alcohol or abuse within 1 yr of the study were exclusion criteria, so this column refers to alcohol abuse occurring prior to 1 yr from scanning.

of primary interest based on having *in vivo* evidence for elevated 5-HTT levels (Maron *et al.* 2011) and reduced $5HT_{1A}$ receptor levels in PD (Neumeister *et al.* 2004).

Regional templates were defined on a mean MRI image generated by summing the spatially normalized MRI images from all subjects. Anatomical boundaries for the thalamus, caudate, putamen, ACC, DCC, PCC and midbrain raphe are described in Cannon *et al.* (2006, Fig. 1). The anterior insular (3.7 cm^3) and midbrain-PAG ROI (0.10 cm^3) were defined as described in Cannon *et al.* (2007, Fig. 4).

Statistical analysis

The normality of the data distribution was tested using the Shapiro–Wilk test. Mean voxel BP_{ND} values in each ROI were extracted from the parametric BP_{ND} images and compared across groups using a multivariate analysis of covariance (MANCOVA) with multiple dependent variables (two regions of primary interest: ACC; midbrain), in which diagnosis and gender were entered as factors of interest. Factors for which the multivariate test (Wilk's λ) was significant were further corrected at the level of each region to reduce the likelihood of type I error using the false discovery rate (FDR) with initial significance set at $p<0.05$, two-tailed (Benjamini & Hochberg, 1995). Regions of secondary interest were explored *post-hoc* using MANCOVA with gender and diagnosis as factors and, where the multivariate test was significant, results were further corrected for multiple comparisons using FDR for the number of regions examined (six: thalamus; caudate; putamen; DCC; PCC; anterior insula; PAG). FDR-corrected p values are given as p_{FDR} and uncorrected as p, throughout. Season was defined astronomically using the equinoxes as the cut-off between autumn/winter and spring/summer seasons, consistent with the previous literature. Because an age effect on thalamic 5HTT BP_{ND} has been reported (Cannon *et al.* 2006), the BP_{ND} values were co-varied for age in all analyses. Follow-up contrasts were performed using t tests.

Additional *post-hoc* analyses were performed to examine relationships between BP_{ND}, PD symptom severity and age-at-illness onset using Spearman's correlation coefficients (a non-parametric test was applied because

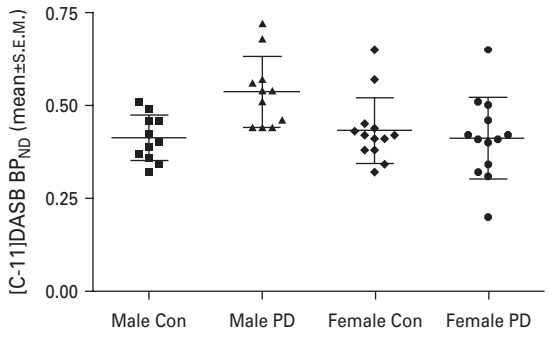

Fig. 2. Serotonin transporter binding (BP_{ND}) in the anterior cingulate cortex. Con, Healthy control group; PD, panic disorder group.

of the small n in each subgroup). These correlations were performed in regions where a significant between-group difference was identified in the ROI analysis. In these regions, the relationships between BP_{ND} and the presence of co-morbid depression, family history of a mood disorder or past exposure to psychotropic medications were assessed using Mann–Whitney U tests.

Results

The mean age and gender distribution did not differ significantly across groups (Table 1). The injected mass of [^{11}C]DASB did not differ significantly across diagnostic [healthy controls (Con) 11.5±3.5 nmol, PD 9.5±4.8 nmol, $F=2.5$, $p=0.1$] or gender groups (males 11.0±4.7 nmol, females 10.1±4.0 nmol, $F=0.6$, $p=0.4$) and did not show a diagnosis × gender interaction ($F=0.3$, $p=0.6$).

After controlling for age, there was a significant main effect of gender ($F=3.75$, $p=0.03$), no main effect of diagnosis ($F=2.26$, $p=0.12$, Fig. 1) and a significant diagnosis × gender interaction ($F=5.01$, $p=0.01$, Fig. 2) on the BP_{ND} values. The main effect of gender on BP_{ND} was significant in the ACC ($F=7.62$, $p_{FDR}=0.02$) but not in the midbrain raphe ($F=1.82$, $p=0.18$). A diagnosis × gender interaction (Fig. 1) was significant in both the ACC ($F=8.96$, $p_{FDR}=0.01$, Fig. 2) and the midbrain raphe ($F=5.09$, $p_{FDR}=0.03$). The significant interaction effects were accounted for by greater [^{11}C]DASB BP_{ND} in the PD males relative to the Con males in each region (Fig. 1, Table 2). The PD males showed greater binding than the Con males in the ACC (32%, $t=-3.64$, $p=0.002$) and in the midbrain raphe (14%, $t=-2.22$, $p=0.038$, Fig. 1). In contrast, the mean [^{11}C]DASB BP_{ND} did not differ significantly between female PD subjects and female Con in any region. No outliers were identified in any region where a significant group difference is reported using a conservative outliers threshold [$y>3$(S.D.)].

The season in which scanning was performed was not differentially distributed across the four diagnosis by gender subgroups ($\chi=4.29$, $p=0.23$, spring/summer: three Con male, seven Con female, six PD male, nine PD female; autumn/winter: eight Con male, six Con female, five PD male, four PD female). After co-varying for season the main effect of gender remained significant overall ($F=3.51$, $p=0.04$) and in the ACC ($F=7.13$, $p=0.01$), but not in the midbrain ($F=1.67$, $p=0.12$). In addition, after co-varying for season the gender × diagnosis interaction remained significant overall ($F=5.31$, $p=0.01$) and in the ACC ($F=9.59$, $p=0.003$) and midbrain ($F=5.18$, $p=0.03$). In addition, the main effect of diagnosis reached a non-significant trend level overall ($F=2.79$, $p=0.07$), became significant in the ACC ($F=5.71$, $p_{FDR}=0.04$), but remained non-significant in the midbrain ($F=0.72$, $p=0.40$).

In the *post-hoc* analyses involving regions of secondary interest (thalamus, caudate, putamen, DCC, PCC, anterior insula, PAG) after co-varying for age, a main effect of gender was evident in the DCC ($F-=5.19$, $p=0.03$). Within individual regions, trends were evident at p (uncorrected) <0.05 for the diagnosis and the gender × diagnosis interaction in the insula ($F=4.13$, $p=0.048$; $F=6.58$, $p=0.014$) and the DCC ($F=5.21$, $p=0.03$; $F=4.50$, $p=0.04$) and for the gender × diagnosis interaction in the putamen ($F=4.45$, $p=0.04$). The diagnosis × gender interaction showed non-significant trends in the same direction in the PCC ($F=3.11$, $p=0.09$) and the PAG ($F=3.31$, $p=0.08$). After co-varying for season, the trends persisted at p (uncorrected) <0.05 for the main effect of gender in the DCC ($F=4.92$, $p=0.03$), the main effect of diagnosis and the gender × diagnosis interaction in the insula ($F=5.80$, $p=0.02$; $F=7.49$, $p=0.009$) and the DCC ($F=5.22$, $p=0.03$; $F=4.51$, $p=0.04$) and the diagnosis × gender interaction in the putamen ($F=4.90$, $p=0.03$). None of the results in the regions assessed *post hoc* would remain significant after applying corrections for multiple comparisons.

The *post-hoc* exploratory analyses showed no relationship between [^{11}C]DASB BP_{ND} in the male PD subjects and age of onset, panic symptom severity (PDSS total score), family history of mood disorder or past exposure to psychotropic medications. The distribution frequency of PD subjects with co-morbid major depressive disorder (MDD) did not differ significantly between gender [females (PD alone, $n=4$; PD+MDD, $n=9$), males (PD alone, $n=7$; PD+MDD, $n=4$), $\chi^2=2.59$, $p=0.11$]. After removing the PD subjects with co-morbid depression, the diagnosis × gender interactions remained significant in the ACC ($F=13.8$, $p=0.001$) and midbrain raphe ($F=9.08$, $p=0.005$). The main effect of gender ($F=7.90$, $p=0.002$) and the diagnosis × gender interaction ($F=9.37$, $p=0.001$) remained significant in the ACC and the midbrain after excluding PD subjects with co-morbid depression; this did not reveal a main effect of diagnosis ($F=1.65$, $p=0.21$).

With regard to past exposure to SSRI drugs, eight of the 24 PD subjects had remote exposure to a SSRI

Table 2. Mean regional serotonin transporter binding ($BP_{ND} \pm$ S.D.)

	Con Males	Con Females	PD Males	PD Females
Primary regions of interest				
ACC	0.41 ± 0.06	0.43 ± 0.09	0.54 ± 0.10*	0.41 ± 0.11
Midbrain raphe	3.32 ± 0.39	3.51 ± 0.47	3.77 ± 0.54*	3.28 ± 0.66
Secondary regions of interest (assessed *post hoc*)				
Thalamus	1.60 ± 0.15	1.85 ± 0.19	1.76 ± 0.39	1.74 ± 0.45
Putamen	1.24 ± 0.28	1.37 ± 0.18	1.45 ± 0.29#	1.29 ± 0.18
Caudate	1.18 ± 0.20	1.20 ± 0.18	1.34 ± 0.32	1.19 ± 0.24
Insula	0.70 ± 0.08	0.77 ± 0.10	0.87 ± 0.17#	0.74 ± 0.17
DCC	0.42 ± 0.10	0.44 ± 0.11	0.56 ± 0.13#	0.45 ± 0.13
PCC	0.36 ± 0.09	0.40 ± 0.10	0.45 ± 0.11	0.39 ± 0.11
Midbrain PAG	2.14 ± 0.77	2.18 ± 0.42	2.58 ± 0.93	1.86 ± 0.69

Con, Healthy control group; PD, panic disorder group; ACC, anterior cingulate cortex; DCC, dorsal cingulate cortex; PCC, posterior cingulate cortex; PAG, periaqueductal grey; FDR, false discovery rate.

* A group × gender interaction $p_{FDR} < 0.05$.

A group × gender interaction that trended toward significance p (uncorrected) < 0.05 in *post hoc* tests.

(i.e. >8 wk for fluoxetine, or >3 wk prior to scanning for other SSRIs). Within the PD sample, the distribution of these cases did not differ significantly across gender, as nine of the 11 males and seven of the 13 females were naive to SSRI medications ($\chi^2 = 2.01$, $p = 0.15$).

Discussion

The mean 5-HTT binding is elevated in PD in the ACC and midbrain where 5-HT_{1A} receptors are additionally reduced (Neumeister *et al.* 2004). These abnormalities appear to be influenced by gender effects, however, as the 5-HTT specific binding parameter, BP_{ND}, significantly differed between groups only in the males with PD in both the ACC and the midbrain. These data confirm in a large sample size the preliminary observations of Maron *et al.* (2011), who demonstrated a diagnosis × gender interaction with the same pattern in the raphe and several cortical areas, where males with PD ($n = 5$) showed abnormally elevated 5-HTT binding (BP_{ND}) levels measured using PET and [^{11}C]MADAM (Maron *et al.* 2011). Moreover, the results of both studies suggest that the abnormal increase in BP_{ND} may be relatively widespread (see Table 2 and Maron *et al.* 2011). In our study the diagnosis × gender interactions showed the same trend in other regions assessed *post hoc*, namely, the insula, DCC and putamen.

Epidemiological studies have shown that both the lifetime and 12 month prevalence of PD is 2.5 times greater in females than males (Kessler *et al.* 1994) and that females with PD experience a higher frequency of attacks and a more debilitating form of illness than males with PD (Maier & Buller, 1988; Yonkers *et al.* 1998). Moreover, 'anxiety sensitivity', a risk factor for developing PD (Schmidt *et al.* 1997), reportedly is heritable in women (Stein *et al.* 1999) but not in men (Jang *et al.* 1999). The anxiety sensitivity components of experiencing fear of somatic sensations, cognitive dyscontrol and publicly observable anxiety accounted for 37–48% of the total variance in heritability in women, but none of the variance in men in whom environmental factors accounted for the variability (Jang *et al.* 1999). Finally, a genome-wide gene expression study detected abnormal transcription rates for several genes, including three members of the solute carrier family SLC 1, 25 (down-regulated) and 39 (up-regulated) in males, but not in females, with PD (Philibert *et al.* 2007). These genes code for the high affinity glutamate and neutral amino acid transporter, mitochondrial carrier and metal ion transporter families, respectively. However, the rate of transcription of the 5-HTT mRNA transcript SLC6A4 did not feature in the top 30 up-regulated or down-regulated transcripts reported (Philibert *et al.* 2007).

In relation to the serotonergic system, gender differences have been reported for genetic variants that increase vulnerability to PD (Gorwood *et al.* 1999; Haghighi *et al.* 1999; Maron & Shlik, 2006; van den Heuvel *et al.* 2000). Two polymorphisms in the gene coding for the human homologue (21q22.3) of the Drosphilia ABC-transporter gene, which putatively participates in the cellular uptake of the 5-HT precursor tryptophan, reportedly increase the risk for PD in males but not females (Nakamura *et al.* 1999). In healthy humans, pre-administration of 5-hydroxytryptophan (5-HTP) lowered the likelihood of developing panic and the intensity of cognitive panic symptoms in females, as well as the intensity of somatic symptoms in males, in response to the panicogenic agent, cholecystokinin tetrapeptide (CCK-4; Maron *et al.* 2004*a*, *b*). Furthermore, among 34 healthy controls studied, females with MAOA longer allelic variants or 5-HTT promoter gene polymorphic region short allelic variants experienced a greater reduction in

CCK-4-induced panic symptoms in response to 5-HTP pre-administration (Maron *et al.* 2004*a*, *b*).

In females but not males with PD, an excess of high activity MAOA gene promoter alleles (3a, 4 or 5) has been detected relative to controls (Deckert *et al.* 1999). Notably, the gene coding for MAOA is located on the X chromosome (Xp11.3). These data suggest a hypothesis that may account for our findings, namely, that the serotonergic system of females may respond to the elevated 5-HT turnover associated with PD by increasing 5-HT metabolism through higher MAOA activity, whereas males may do so by relying more on increasing 5-HT reuptake via higher 5-HTT expression. In addition, in females but not males, a single nucleotide polymorphism in the gene coding for tryptophan hydroxylase (TPH2, rs1386494) showed a gender-specific association in PD (Maron *et al.* 2007). This association with PD conceivably may have influenced 5-HTT binding in females but not males with PD through the regulation of expression of the rate limiting enzyme in 5-HT synthesis.

The finding that regional 5-HTT binding is increased in males with PD also may relate to the observation that females show greater therapeutic benefit than males from 5-HTT-inhibiting drugs (Clayton *et al.* 2006). Pooled data from four independent studies involving 335 males and 338 females with PD who were treated with the SSRI, sertraline, showed that the females experienced greater anti-panic efficacy than males (Clayton *et al.* 2006). Our data suggest the hypothesis that, at comparable sertraline plasma concentrations, this SSRI would prove less effective in males than in females with PD because the PD males would have a greater number of 5-HTT proteins remaining unoccupied by SSRI than the PD females. That is, a relatively higher proportion of 5-HTT sites would need to be occupied in males than in females to obtain the same clinical efficacy. Thus, it is conceivable that sertraline doses that are effective in females with PD may need to be increased to achieve comparable therapeutic efficacy in males with PD. Evidence has accumulated linking ~80% (Meyer *et al.* 2004) 5-HTT occupancy with clinical efficacy in MDD (Lundberg *et al.* 2012; Meyer *et al.* 2001; Parsey *et al.* 2006; Voineskos *et al.* 2007), although it remains unclear whether a similar occupancy–efficacy relationship exists for SSRI treatment of PD.

An alternative hypothesis based upon our findings that may account for the differential anti-panic efficacy of sertraline in males *vs.* females is that the higher [^{11}C]DASB binding in males with PD reflects a more effective compensatory enhancement of serotonergic neurotransmission produced in response to the acute episodic nature of the fear and stress associated with PD. Serotonin turnover reportedly was increased in unmedicated PD subjects, as assessed via the 5-HT metabolite overflow in internal jugular venous samples from subjects with PD (Esler *et al.* 2007). Although the effect of gender on 5-HT turnover in PD has not been assessed, the elevated 5-HTT BP_{ND} that we observed in untreated male PD subjects is consistent with elevated 5-HT turnover (see Introduction). In preclinical studies, the enhancement of 5-HT release in structures such as the ACC, insula and striatum under conditions of stress and anxiety is thought to play an adaptive role by modulating or compensating for the effects of stress. Clinical evidence supports this conclusion, as pharmacological manipulations that enhance 5-HT neurotransmission, such as 5-HTP (den Boer & Westenberg, 1990), D-fenfluramine (Solyom, 1994) and SSRIs exert anti-panic effects. Thus, if the higher basal levels of 5-HTT binding in males with PD reflect enhanced 5-HT release and transmission, this finding conceivably may explain the lower incidence of PD and the lower frequency of panic attacks in males *vs.* females with PD (Kessler *et al.* 1994). An important corollary of this hypothesis is that women with PD may benefit more than males from enhancing 5-HT transmission via SSRI administration because they have lower 5-HT transmission than men during the pretreatment baseline.

In PD subjects, two studies reported that the 5-HT_{1A} receptor binding is decreased in the raphe relative to healthy controls, although neither study examined gender effects (Nash *et al.* 2008; Neumeister *et al.* 2004). Stimulation of the raphe somatodendritic 5-HT_{1A} autoreceptors inhibits 5-HT synthesis and release and a reduction in this inhibitory tone would putatively increase serotonergic neurotransmission. A consequent elevation of 5-HTT levels in this region that contains the cell bodies of serotoninergic neurons thus may reflect a compensatory response to the presence of reduced inhibitory tone by somatodendritic 5-HT_{1A} receptors. To further understand the relationship between 5-HTT binding and the course of illness or treatment in PD, future studies are needed that incorporate longitudinal and multi-tracer approaches. For example, one potentially useful future approach may be to examine diagnosis and gender effects on regional 5-HTT binding and 5-HT synthesis using both PET-[^{11}C]DASB and PET-α-[^{11}C]methyl-L-tryptophan.

Notably, the regions where 5-HTT binding was abnormally increased in males with PD previously have been implicated in the neurobiology of PD by physiological and receptor pharmacological data. For example, the cerebral blood flow and glucose metabolism have been shown to increase in the anterior insula and the ACC during panic attacks induced by a variety of panicogens in healthy volunteers (Benkelfat *et al.* 1995; Cameron *et al.* 2000; Dieler *et al.* 2008; Javanmard *et al.* 1999; Schunck *et al.* 2006) and patients with PD (Reiman *et al.* 1989). Moreover, the haemodynamic activity in the insula and the ACC increased during augmentation of the subjective salience of interoceptive stimuli associated with panic and dread (reviewed in Charney & Drevets, 2002) and direct stimulation of the ACC reportedly elicits panic-like response in humans and experimental animals (reviewed in Price *et al.* 1996). Finally, reduced

benzodiazepine (BZD) receptor binding in insula and the ACC has been reported in PD (Cameron *et al.* 2007; Hasler *et al.* 2008; Talbot, 2004); the co-occurrence of 5-HTT and BZD receptor binding abnormalities in this region is noteworthy because expression of the γ-amino-butyric-BZD receptor complex is influenced by serotonergic transmission. For example, reduced 5-HT_{1A} receptor concentrations result in reduced BZD receptor levels, insensitivity to BZD receptor agonists and increased anxiety (Sibille *et al.* 2000).

An alternative hypothesis regarding the involvement of the serotonergic system in PD proposes a deficit in 5-HT neurotransmission (Maron & Shlik, 2006), leading from the proposal that 5-HT plays a restraining role on anxiety and panic (Deakin & Graeff, 1991). The present findings are potentially compatible with a role for 5-HT in restraining anxiety and panic. Reduced 5-HT_{1A} receptors have been suggested to be a trait abnormality in PD as reducing 5-HT levels experimentally does not alter 5-HT_{1A} levels in the cerebral cortex or hippocampus and normalization of 5-HT_{1A} receptors binding in the amygdala and frontal cortex is anxiolytic (Maron & Shlik, 2006). In contrast, 5-HTT capacity or activity (V_{max}) is under rapid regulation by phosphorylation through silencing/activation or endocytosis/insertion (Blakely *et al.* 1998) to maintain the homeostasis of 5-HT neurotransmission and may be more tightly coupled to react dynamically to 5-HT levels in their responses to aversive stimuli. This hypothesis is supported by the normalization of elevated 5-HT turnover observed in PD following treatment with SSRIs (Esler *et al.* 2007). In PD, it is possible that elevated 5-HTT and 5-HT turnover are consequences of a primary trait-like factor such as reduced 5-HT_{1A} levels and associated increases in the firing rate of serotonergic neurons and of 5-HT release. Behavioural responses to aversive stimuli potentially restrained by 5-HT release could therefore be impaired in at least a subgroup of individuals with PD having elevated 5-HTT levels.

Limitations of our study include the relatively small sample size of 11 males and 13 females in each diagnostic category. These samples provided particularly low power when subdivided further to assess the effects of co-morbid depression and the relationship between BP_{ND} and illness severity among males. Thus, while the male and female PD samples did not differ significantly with regard to rates of depression, larger samples are needed to clarify the influence of co-morbid depression on 5-HTT binding in PD. Similarly, our study may have been underpowered and the sample may not have manifested a sufficiently wide range of symptom severity to detect clinically relevant relationships between [^{11}C]DASB BP_{ND} and illness severity. For example, the range of severity (0–23, overall and male only mean of 15) was greater in the study by Maron *et al.*, who detected a relationship between 5-HTT binding and the severity of the disorder, relative to the present data (0–16, overall mean of 7 and mean among males of 5). Although previous studies have detected a gender-based difference in 5-HTT binding among healthy controls, these have not been consistent in the direction of the finding. Jovanovic *et al.* (2008) used PET and [^{11}C]MADAM in a smaller, younger subject sample and reported higher binding in males ($n=10$, age 21–37, mean = 26 yr) relative to females ($n=8$, age 23–39, mean = 28 yr) in the dorsal raphe and caudate. Kalbitzer *et al.* (2009) reported, however, that BP_{ND} values were lower in healthy males ($n=35$, mean age $\pm$ S.D. 32 ± 15) relative to healthy females ($n=15$, 38 ± 18, $p=0.03$) using PET and [^{11}C]DASB. We did not detect any significant difference in binding between healthy males and females; however, a *post-hoc* independent *t* test with no correction applied to adjust for multiple comparisons revealed a similar direction to the latter study (females > males) in the midbrain ($t=-2.27$, $p=0.03$) and the thalamus ($t=-2.65$, $p=0.01$) and no significant difference in the ACC ($p=0.49$) or any other region examined ($p>0.08$). It thus may still be informative to examine the effect of PD on the 5-HTT BP_{ND} specifically in women in future studies involving larger sample sizes.

A limitation of the model used to derive [^{11}C]DASB BP_{ND} assumed no difference between groups in the cerebellar distribution volume in order to avoid the use of arterial blood sampling. Furthermore, methods to measure intrasynaptic 5-HT concentrations in humans are not currently available (e.g. [^{11}C]DASB is insensitive to displacement by endogenous 5-HT at the 5-HT concentrations that can be non-invasively studied in humans; Talbot *et al.* 2005). Thus, the relationship between [^{11}C]DASB BP_{ND} and 5-HT neurotransmission could not be tested directly. The BP_{ND} parameter is proportional to both 5-HTT density and affinity, so, while unlikely, we cannot rule out the possibility that the elevation in BP_{ND} observed in PD males reflects increased affinity of [^{11}C]DASB for 5-HTT sites as opposed to increased 5-HTT density. In female primates, the 5-HTT concentrations are influenced by ovarian steroids (Bethea *et al.* 2002; Pecins-Thompson *et al.* 1996) and our study design did not test whether difference in 5-HTT binding in females with PD may be limited to a particular menstrual phase. Finally, although our subjects had not been medicated recently, and 16 of the 24 PD subjects were naive to SSRI agents, we cannot exclude the possibility that our findings were influenced by differences in the remote exposure to other medication types.

In conclusion, the mean 5-HTT BP_{ND} was increased significantly in males with PD, compatible with other data implicating gender differences in the serotonergic system associated with PD. It is conceivable that the long-term compensatory mechanisms or consequences of acute elevations in 5-HT concentrations following panic attacks may differ in men *vs.* women. Finally these data may hold implications for the mechanism(s) underlying the lower anti-panic efficacy of SSRIs in males *vs.* females with PD.

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Statement of Interest

The authors declare that over the past 12 months W.C.D. has received compensation from Pfizer, Johnson and Johnson, Eisai Inc. and Myriad/Rules Based Medicine Inc.

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