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ORIGINAL INVESTIGATION

Diminished brain 5-HT transporter binding in major depression: a positron emission tomography study with [^{11}C]DASB

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Abstract
Background The serotonin transporter (5-HTT) plays a critical role in the regulation of serotonin neurotransmission and has been implicated in the pathophysiology of major depression. In a previous positron emission tomography study, we found no difference in brain 5-HTT binding between unmedicated recovered depressed patients and healthy controls.
Aim This study aims to assess brain 5-HTT binding in a group of unmedicated acutely depressed patients in comparison to healthy controls.
Methods We studied 5-HTT binding using [^{11}C]DASB in conjunction with positron emission tomography in 12 medication-free depressed patients with a mean duration of illness of about 1 year and 24 healthy controls.
Results The depressed patients had lowered 5-HTT binding in several brain regions including brain stem, thalamus, caudate, putamen, anterior cingulate cortex and frontal cortex.
Conclusions These results suggest that diminished availability of the 5-HTT in the brain may be a state marker of acute depression. Alternatively, low 5-HTT binding may delineate a group of depressed patients with a poor long-term prognosis.

Keywords Serotonin · Serotonin transporter · [^{11}C]DASB · Depression · Mood disorder · PET

Introduction

For over three decades, there has been intense interest in the pathophysiological role of the serotonin transporter (5-HTT) in depressive disorders. The methods available to study the 5-HTT in humans have steadily become more sophisticated, and with the use of selective radioligands, it is now possible to image the 5-HTT directly in the living human brain using positron and single photon emission tomography (PET and SPET).

The SPET radioligand most commonly used to study depressed patients has been [^{123}I]β-CIT, a cocaine derivative that binds with high affinity to both 5-HTT and dopamine transporters (Laruelle et al. 1993). Apart from lack of selectivity, accurate quantification of [^{123}I]β-CIT SPET is made difficult by relatively low image resolution and the derivation of binding parameters from tissue ratios rather than kinetic analysis. A number of PET ligands are also now available to image the 5-HTT, in particular [^{11}C]

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(+)McN5652 and [^{11}C]DASB. Both these radiotracers are highly selective for the 5-HTT; however, [^{11}C]DASB has advantages in terms of a higher ratio of specific binding to nonspecific binding and a measurable free fraction in plasma (Frankle et al. 2004; Szabo et al. 2002).

The majority of 5-HTT imaging studies have found decreased 5-HTT binding, particularly in thalamus and brain stem, in acutely depressed patients (Joensuu et al. 2007; Lehto et al. 2006; Malison et al. 1998; Newberg et al. 2005; Oquendo et al. 2007; Parsey et al. 2006a; Reimold et al. 2008; Staley et al. 2006; Willeit et al. 2000); however, there are some notable inconsistencies with some groups reporting either increased (Cannon et al. 2006; Cannon et al. 2007; Dahlstrom et al. 2000; Ichimiya et al. 2002) or no change in 5-HTT binding in the same areas (Herold et al. 2006; Meyer et al. 2004; Reivich et al. 2004).

The reasons for these inconsistent findings are unclear and may be due to a number of different factors, including patient characteristics and effects of previous antidepressant drug treatment. Another potential confound relates to SPET and PET methodology, in particular the use of a reference region that itself contains displaceable 5-HTT binding and may therefore differ in 5-HTT binding between patient and control group. The latter problem can be resolved only by the use of a quantitative approach using arterial input modelling (Innis et al. 2007).

In a previous PET investigationm, we used an arterial input model in conjunction with [^{11}C]DASB to measure 5-HTT binding potential in unmedicated recovered depressed patients (Bhagwagar et al. 2007). We found no difference in 5-HTT binding between recovered patients and controls. The aim of the present study was to use [^{11}C]DASB to assess 5-HTT binding in a group of unmedicated acutely depressed patients, again using an arterial input model to provide a fully quantitative approach to the measurement of binding potential.

Methods and materials

Participants

The study was approved by the Research Ethics Committee at Hammersmith Hospital, London, and the Administration of Radioactive Substances Advisory Committee, UK. All participants gave written informed consent for the study. We recruited 12 men (mean±SD age 42.1±11.6 years) who were currently depressed based on clinical interview and Structured Clinical Interview for DSM-IV Disorders criteria for major depressive disorder. Except for one participant who used nitrazepam occasionally, the depressed patients were free of antidepressant and other psychotropic medication for a mean duration of 41 months (range 4–240 months); four patients had never received any antidepressant medication. Overall, the mean duration of the current depressive episode was 12.4 months (range 4–20 months). Eight patients had a family history of mood disorder. Six of the depressed patients had comorbid illnesses; four had dysthymia, one had a past history of panic disorder, two had current generalised anxiety disorder symptoms and one subject had a history of alcohol dependence (in remission for 18 years).

Twenty-four age-matched healthy men (mean age 42.4±11.4 years) were both specifically recruited for this study (n=9) and randomly selected from our [^{11}C]DASB healthy volunteer database (n=15). The healthy volunteers did not have any current or past psychiatric history or history of substance misuse. All the patients and controls were scanned during the same period. The details of participant characteristics are shown in Table 1. Patients and healthy subjects with any major medical or neurological (current or past) illness were excluded from the investigation. All subjects were administered the Hamilton Rating Scale for Depression (HAM-D; 17 items) and the Beck Depression Inventory (BDI). We restricted our study to male subjects only because of lack of nonclinical reproductive toxicology data (effect of [^{11}C]DASB on pregnancy and on foetus for [^{11}C]DASB).

PET scanning

PET scans were carried out as previously detailed (Bhagwagar et al. 2007; Hinz et al. 2008). Briefly, all participants underwent a 90-min dynamic 3D emission scan in list mode on the ECAT EXACT3D tomograph. The data were rebinned postacquisition into a sequence of 28 frames and reconstructed with the reprojection algorithm. The metabolite-corrected arterial plasma input functions for each scan were derived from continuous online whole blood activity-monitoring and ten discrete blood samples, in eight of which the fraction of the unchanged parent compound in plasma was determined. The plasma-free fraction (fp) was determined by mixing 1 ml cell-free plasma (collected before DASB injection) with radiotracer. A known aliquot of 10–20 µl was then transferred into ultrafiltration unit (Microcon YM-30 (millipore), centrifuged at room temperature for 15 min at 14,000 rpm; activities from the plasma and ultrafiltrate were then counted (corrected for nonspecfic binding). The fp was estimated from the ratio of activity in the ultrafiltrate to total activity (Frankle et al. 2004). Note, however, that due to accidental loss of some of the blood samples, the plasma-free fraction was only determined in a subgroup of seven controls and six patients, respectively.

The radiotracer [^{11}C]DASB (3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile) was synthesised as

Table 1 Clinical characteristics of the participants and radiochemical parameters

Clinical characteristics	Healthy controls (n=24)	Acute depressed (n=12)
Age (years; mean±SD)	41.9±11.4	43.8±13.6
HDRS	0.4±0.8	20.3±5.3
BDI	2.3±3	33.3±10.3
Mean age at onset of depression (range)	–	33.6 (26–62)
Mean number of episodes (range)	–	2.2 (1–4)
Mean months duration of current episode (range)	–	12.4 (4–24)
Mean months medication free since last episode (range)	–	41 (4–240)
Medication naïve (n)	–	4
Melancholic depression (n)	–	2
Suicide attempts (n)	–	2
Family history of depression (n)	–	8
Pet parameters		
Injected radioactivity (MBq/μmol)	537.3±20.2	529±35.9
Specific activity (MBq/μmol)	57852±39560	49129±19681
Injected stable "COLD" ligand (μg)	3.7±2.2	3.8±2
Plasma-free fraction fp (unitless)	0.043±0.01 (n=7)	0.05±0.02 (n=6)

previously described (Wilson et al. 2000). The standard DASB and the precursor desmethyl DASB were obtained from Target Molecules Ltd., Southampton (UK). [^{11}C]DASB was injected into an antecubital vein, as a smooth bolus over 30 s. There was no significant difference between the participants' groups in the amount of radioactivity, in the specific activity, the amount of cold ligand injected and the free fraction in plasma (Table 1).

MR scans and definition of volumes of interest

All volunteers had a structural T1 MRI scan performed on either 0.5 T (0.5 Apollo system, Marconi Medical Systems, Cleveland, OH, USA), 1.5 T (1.5 Eclipse system, Marconi Medical Systems, Cleveland, OH, USA) [TR=30 s, TE=3 s, flip angle=30, NSA=1, voxel dimensions 0.98×1.6×1.6 mm, acquisition time=13 min] or 3 T (3 T Intera Philips Medical Systems) MR scanners [TR=9.6 s, TE=4.6 s, flip angle=8, NSA=1, voxel dimensions 0.94×0.94×1.2 mm]. The scans were inspected by an independent clinical radiologist and found to be normal.

MRIs were resliced to a voxel size of 1×1×1 mm, centred on Anterior Commissure (AC) and aligned to the AC–PC line. MRIs were coregistered to the individual summated PET images using SPM2, which adopts a rigid body transformation using a normalised mutual information method. Based on our previous investigation in recovered depressed patients (Bhagwagar et al. 2007), we studied nine regions of interest, namely, amygdala, anterior cingulate cortex (ACC), brain stem, caudate, frontal cortex, hippocampus, insula, putamen and thalamus. Regions of interests (ROIs) were defined on the coregistered MRI with the help of an automated probabilistic brain atlas template (Hammers et al. 2002). The brain stem regional definition included midbrain, pons and medulla oblongata. Standard MNI T1 template (available in the SPM2) was normalised to the coregistered individual MRI, and the deformation parameters were applied to the probabilistic atlas. This normalised brain atlas was resliced to PET space and segmented to obtain data from grey matter only. Dynamic PET scans were sampled by applying the individual ROI object maps. Cerebellar grey matter was used as a reference region because of the reported negligible density of 5-HTT in the cerebellum (Kish et al. 2005). We obtained data from cerebellar grey matter up to five to eight slices carefully avoiding spill over from occipital cortex by placing the volume of interest well within the cerebellar cortex area leaving a margin of several millimetres around to the outer borders of the cerebellum as defined in Hammers et al. (2003).

Quantification of DASB binding

For the quantitation of the [^{11}C]DASB binding in brain tissue relative to the radioligand concentration in arterial plasma, we used graphical analysis together with the metabolite-corrected plasma input function (Logan et al. 1990) to obtain regional estimates of the total volume of distribution V_T from the slope of the linear part of the plot. Parameters such as the threshold for the graphical analysis t^*=35 min were set as previously optimised for [^{11}C]DASB (Hinz et al. 2008).

The main study outcome was the in vivo binding potential $BP=B_{max}/K_D$ as the ratio of the serotonin transporter density B_{max} and the radioligand equilibrium dissociation constant K_D (Innis et al., 2007). The binding potential of the specifically bound radioligand relative to the parent radioligand in plasma $BP_P = f_P \cdot BP = f_P \cdot B_{max}/K_D$, with f_p being the fraction of free radioligand in plasma was calculated as follows:

$$BP_P = V_T - V_{ND},$$

where V_T is the distribution volume of total ligand uptake in tissue relative to total concentration of ligand in plasma, and V_{ND} is the distribution volume of nondisplaceable compartment relative to total concentration of ligand in plasma (Innis et al. 2007). V_{ND} is derived from the V_T of the cerebellar reference region. Therefore, the value of BP_P in a particular ROI is given by: V_T (ROI)–V_T (cerebellum).

Alternatively, the binding potential of the specifically bound radioligand relative to the nondisplaceable radioligand in tissue $BP_{ND} = f_{ND} \cdot BP = f_{ND} \cdot B_{max}/K_D$, with f_{ND} being the fraction of free radioligand in the nondisplaceable tissue compartment was calculated as follows:

$$BP_{ND} = (V_T - V_{ND})/V_{ND}.$$

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 12. Demographic data were analysed using independent sample *t* tests. We tested our hypothesis that regional 5-HTT receptor binding potential would be reduced in acutely depressed patients compared with healthy control subjects using a repeated measures ANOVA with "group" (acute depressed vs controls) as a between subjects factor, and "region" (nine ROIs as described above) as a within-subjects factor, with Huynh–Feldt correction. Significant effects on the repeated measures ANOVA were followed up with post hoc independent *t* tests. Correlations (duration of symptoms, BDI and HAM-D vs ROI BP_P) were analysed using Pearson's correlation coefficient *r* and nonparametric rank order data (age of onset and number of episodes vs ROI BP_P) using Spearman's correlation coefficient ρ. The significance was set at $p<0.05$.

Results

The clinical characteristics of the depressed patients are shown in Table 1. There were no statistically significant differences in age (t=0.43; df=34; p=0.7) or the plasma-free fraction of [^{11}C]DASB in a subgroup (t=-0.92; df=11; p=0.48) between the two groups. There was significant positive correlation of age on BP_P values in healthy controls in five out of nine regions using Pearson's *r* (all $p<0.05$; amygdala, caudate, frontal cortex, insula and putamen); in patients, age positively correlated only with amygdala BP_P. However, none of these correlations survived multiple comparison tests. Age was used as a covariate in the further analysis.

The cerebellar total volume of distribution V_T (cerebellar V_T) was significantly less in acute depressed patients than controls (9.1±1.9 ml cm^{-3} vs 10.5±1.6 ml cm^{-3}; t=2.34; df=34; p=0.03).

The repeated measures ANOVA of the BP_P data showed a significant effect of group (F=16.79; df=1, 33; $p<0.001$); there were also significant main effects of region (F=5.27; df=8, 264; $p<0.001$) and a significant region by group interaction (F=8.04; df=8, 264; $p<0.001$). There was a significant effect of age (F=6.74; df=1, 33; p=0.01) and a significant region by age interaction (F=3.55; df=8, 264; $p<0.01$).

Post hoc independent *t* tests (uncorrected) showed that BP_P of the 5-HTT in acute depressed patients were significantly less than the controls in six of the nine ROIs (brainstem, thalamus, caudate, putamen, ACC and frontal cortex, (Fig. 1 and Table 2)). In acutely depressed subjects, BP_{ND} data showed more restricted changes with significantly reduced 5-HTT BP_{ND} only in brain stem and thalamus; however, there was also a trend level decrease in caudate (Table 2).

In the acutely depressed patients group, there was a significant negative correlation between BDI total score and BP_P value in the frontal cortex (Pearson's $r_{uncorrected}$=−0.65; p=0.02). Trend level negative correlations were seen between BDI total score and BP_P values in caudate (r=−0.55; p=0.06); ACC (r=−0.55; p=0.07) and Insula (r=−0.51; p=0.09). However there was no significant correlation between HAM-D score and BP_P in any of the ROIs.

There was a statistically significant positive correlation between age of onset of illness and BP_P values in caudate (Spearman $\rho_{uncorrected}$ =0.57; p=0.05) and frontal cortex ($\rho_{uncorrected}$ =0.57; p=0.05) and a trend level correlation in putamen (ρ=0.54; p=0.07; the earlier the age of onset of illness the lower BP_P). There was also a significant negative correlation in the acutely depressed patients between the number of episodes of depression and BP_P in the thalamus ($\rho_{uncorrected}$=−0.68; p=0.02), and putamen ($\rho_{uncorrected}$=−0.62; p=0.03). However, none of the reported correlations survived multiple comparison correction. There was no correlation between duration of current episode and BP_P in any of the ROIs (all $p>0.1$).

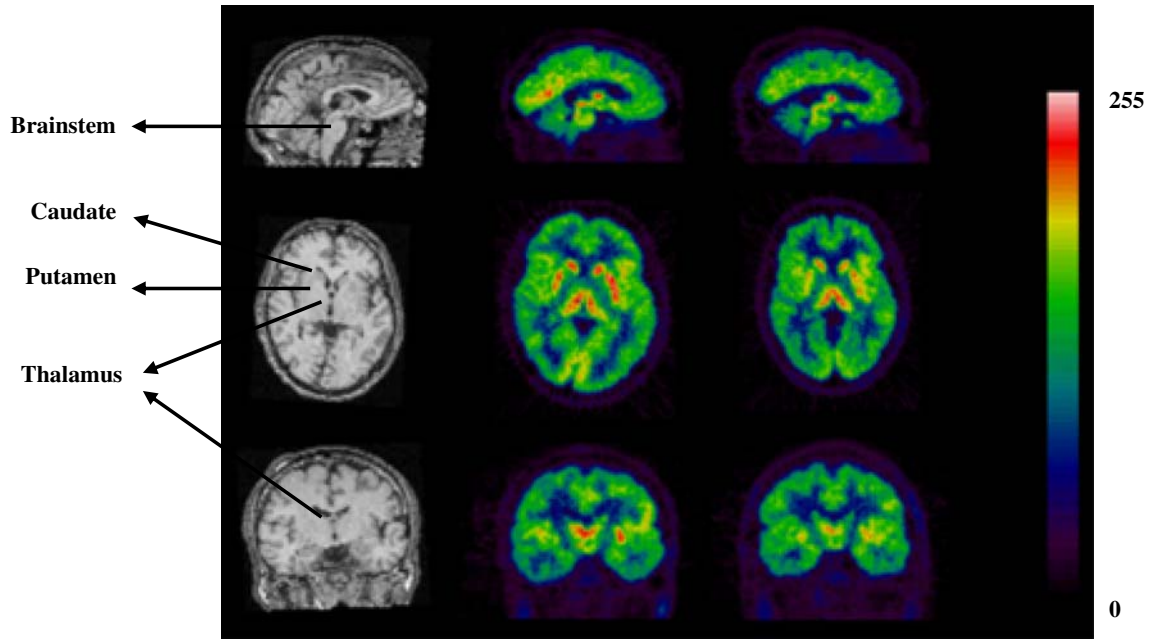

Fig. 1 A representative magnetic resonance image and [^{11}C]DASB summated image from a control subject in the *left* and *middle column*, respectively, and [^{11}C]DASB summated image (1–28 frames) from a acute depressed (*right most column*). Subjects were chosen such that their brain stem and thalamus BP_P were closest to the respective group mean. From *top row* to *bottom*: sagittal, axial and coronal view, respectively

Discussion

Our data suggest that unmedicated acutely depressed patients have statistically significant decreases in binding potential (BP_P) of the 5-HTT in multiple brain regions including brain stem, thalamus, striatum, ACC and frontal cortex. Decreases in brain stem and thalamus remained significant even if BP_{ND} values were used as a quantitative measure. While these findings appear relatively clear-cut, it must be acknowledged that imaging studies of the 5-HTT in acute depression have yielded conflicting results (Cannon et al. 2006; Cannon et al. 2007; Dahlstrom et al. 2000; Herold et al. 2006; Ichimiya et al. 2002; Joensuu et al. 2007; Lehto et al. 2006; Malison et al. 1998; Meyer et al. 2004; Newberg et al. 2005; Oquendo et al. 2007; Parsey et al. 2006a; Reimold et al. 2008; Reivich et al. 2004; Staley et al. 2006; Willeit et al. 2000). Why this should be the case is not easy to explain. Technical factors may well be involved, particularly the use of different ligands to label the 5-HTT binding site. However, even studies using [^{11}C]DASB,

Table 2 Binding potential (BP_P; millilitre cubic centimtre) and BP_{ND} of [^{11}C]DASB in control subjects and medication-free acute depressed patients

Regions of interest (ROI)	Controls (n=24) BP_P mean (SD)	Patients (n=12) BP_P mean (SD)	P value (independent t test)	Controls (n=24) BP_{ND} mean (SD)	Patients (n=12) BP_{ND} Mean (SD)	p value (independent t test)
Amygdala	19.3 (4.3)	16.8 (4.9)	0.12	1.84 (0.3)	1.84 (0.3)	0.99
Anterior cingulate cortex	8.0 (1.8)	6.2 (2.5)	0.02*	0.77 (0.2)	0.69 (0.3)	0.31
Brain stem	19.4 (3.1)	13.1 (4.6)	<0.001***	1.87 (0.3)	1.44 (0.4)	<0.0001***
Caudate	15.2 (4.2)	9.7 (5.4)	0.002**	1.46 (0.4)	1.12 (0.7)	0.06
Frontal cortex	4.8 (1.0)	3.6 (1.9)	0.02*	0.46 (0.1)	0.40 (0.2)	0.3
Hippocampus	9.6 (2.5)	8.7 (3.3)	0.33	0.92 (0.2)	0.98 (0.4)	0.58
Insula	11.5 (2.9)	9.5 (3.5)	0.08	1.10 (0.3)	1.06 (0.3)	0.67
Putamen	20.6 (4.8)	15.2 (4.3)	0.002**	1.99 (0.5)	1.72 (0.5)	0.11
Thalamus	18.4 (4.2)	11.9 (4.7)	<0.001***	1.77 (0.4)	1.35 (0.6)	0.01*

*p<0.05; **p<0.01; ***p<0.001, uncorrected for multiple comparison

often regarded as the best currently available radiotracer, are quite inconsistent (Cannon et al. 2007; Meyer 2007; Meyer et al. 2004; Reimold et al. 2008).

Modelling methods in ligand-imaging

As noted in the Introduction, reference tissue modelling methods can be confounded if the reference region itself possesses specific displaceable binding of the radioligand under investigation. The cerebellum is often used as a reference region in studies of the 5-HTT; however, it is now well-established that treatment of subjects with selective serotonin reuptake inhibitors displaces 5-HTT binding in the cerebellum, suggesting that this brain region does possess displaceable 5-HTT binding (Kent et al. 2002; Parsey et al. 2006b). Indeed our study like that of Oquendo et al. (2007) revealed low 5-HTT binding in cerebellum in depressed participants relative to controls, though the patients in the latter study suffered from bipolar illness. In these circumstances, it is unlikely that total volume of distribution V_T of [^{11}C]DASB in the cerebellum is an accurate reflection of the volume of distribution of the nondisplaceable tissue uptake (VD_{ND}) required to obtain valid estimates of in vivo binding potential using a reference tissue model (Hinz et al. 2008; Innis et al. 2007).

Where there is evidence of displaceable 5-HTT binding in the reference region, the outcome measure BP_P should be more sensitive than the more usually employed BP_{ND} to detect changes in specific binding of the 5-HTT in patient groups (Innis et al. 2007; Oquendo et al. 2007). However, to assess whether the cerebellar V_T in an investigational group is different from controls, and to calculate BP_P, it is necessary to quantify radioligand binding in tissue relative to the concentration of parent radiolabelled compound in the plasma input function. Therefore, investigations using simplified scanning protocols without measurement of the plasma input function cannot assess changes in the cerebellar V_T or obtain BP_P (Hinz et al. 2008; Innis et al. 2007).

In our study, the decrease in cerebellar V_T in the depressed patients was small relative to the decrease of V_T in most of the ROIs, and therefore, significant decreases in BP_P in several ROIs were still apparent (though may be underestimated because of the reduction in cerebellar V_T). However, had the decrease of V_T in cerebellum been greater, then reductions in 5-HTT in other brain regions could have been missed. It is possible therefore that the presence of displaceable binding of 5-HTT in the cerebellum, with undetected differences in V_T between patients and controls, may have contributed to the inconsistent findings of imaging studies of the 5-HTT in depression (Oquendo et al. 2007). However, this explanation may not apply to studies that have reported increased binding in depression using DASB. In addition, the BP_{ND} analysis revealed significant differences in brain stem and thalamus regions only and in not in other regions. It may be possible that these brain areas are the ones most likely to reveal changes in acute depression, perhaps because the high density of 5-HTT binding sites in this brain area provides a strong imaging signal.

Relationship of low 5-HTT binding to clinical symptomatology

Interestingly, in our previous study, using an identical methodology, we found no differences in 5-HTT binding in unmedicated recovered depressed participants compared to controls. This suggests that lowered 5-HTT binding might be a state marker of acute depression which remits with clinical recovery. However, this conclusion must remain tentative at present because there were some potentially important clinical differences between the acutely depressed patients in the present study and the recovered depressed subjects who participated in our previous investigation.

For example, in comparison to the recovered subjects, the acutely depressed patients had greater levels of psychiatric comorbidity and higher rates of affected first degree relatives. It is therefore possible that this particular group of acutely depressed patients might have lowered 5-HTT binding even after recovery. It is also possible that low 5-HTT binding might delineate a group of acutely depressed patients less likely to recover. Indeed, we found in several brain regions that low 5-HTT binding was correlated with greater number of depressive episodes and early age of onset, both factors associated with a poorer long-term prognosis (Gelder et al. 2006). However, caution must be applied to interpretation of our correlational findings because they did not survive correction for multiple comparisons. Interestingly, a recent prospective study by Miller et al. (2008) reported that lower pretreatment 5-HTT binding was associated with a decreased likelihood of remission during the following year (Miller et al. 2008). Meyer (2007) has suggested that low 5-HTT binding in depressed patients may be associated particularly with psychiatric comorbidity, which is also regarded as negative prognostic factor (Meyer 2007).

Recently, using [^{11}C]DASB, Praschak-Rieder et al. (2008) reported that 5-HT transporter binding potential values were significantly higher in healthy subjects when scanned in the fall and winter compared with those in the spring and summer (Praschak-Rieder et al. 2008). In our sample, we did the analysis using the same seasonal cut-off period as reported in the Praschak-Rieder et al. (2008). We found the BP_P results were still significant if a subgroup of patients (n=6) vs controls (n=15) scanned in winter and

fall were compared (eight out nine regions, $p<0.05$). Similarly thalamus and brain stem BP_P ($p<0.05$) remained significantly different if the subgroup of patients ($n=6$) vs controls ($n=9$) scanned in spring and summer were analysed. We also did not find significant effect of seasonality on ROI BP_P in healthy controls. Based on the above results, we conclude that seasonal variation in 5-HTT binding is unlikely to account for the serotonin transporter differences between patients and controls seen in this study.

Limitations

The current study was restricted to male participants and further work is needed to delineate brain 5-HTT binding with [^{11}C]DASB in acutely depressed and recovered depressed women. Recently, Ruhe et al. (2009) reported low *BP* in midbrain and diencephalon in a subgroup of male depressed subjects using SPET ligand [^{123}I]β-CIT, although there was no overall difference between the patients and controls (Ruhe et al. 2009). However, Staley et al. found lowered diencephalic 5-HTT binding specifically in acutely depressed women (Staley et al. 2006) making it unlikely that abnormal 5-HTT binding in acute depression is restricted to men.

The number of depressed patients in our study was relatively small, mainly because of our wish to recruit participants who had been medication free for significant periods of time. While, to some extent, this avoids problems in interpretation of binding data posed by recent antidepressant use, it may mean that our findings are not generalisable to the broad range of depressed patients.

In summary, this study suggests that the use of [^{11}C]DASB in conjunction with arterial input modelling reveals widespread decreases in 5-HTT availability in acutely depressed patients. Based on our findings in recovered depressed patients, we suggest that decreased 5-HTT binding may be a state marker of depression which resolves with clinical recovery. It is also possible, however, that low 5-HTT binding identifies a subgroup of depressed patients with a relatively poor prognosis. Follow-up imaging studies will be required to distinguish between these possibilities.

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