Increased Seasonal Variation in Serotonin Transporter Binding in Seasonal Affective Disorder

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Seasonal affective disorder (SAD) is highly prevalent with rates of 1–6% and greater prevalence at more extreme latitudes; however, there are almost no direct brain investigations of this disorder. In health, serotonin transporter binding potential (5-HTT BP$_{ND}$), an index of 5-HTT levels, is greater throughout the brain in fall-winter compared with spring-summer. We hypothesized that in SAD, this seasonal variation would be greater in brain regions containing structures that regulate affect such as the prefrontal and anterior cingulate cortices (PFC and ACC). Furthermore, given the dimensional nature of SAD symptoms, it was hypothesized that seasonal fluctuation of 5-HTT BP$_{ND}$ in the PFC and ACC would be greatest in severe SAD. Twenty SAD and twenty healthy participants underwent [^{11}C]DASB positron emission tomography scans in summer and winter to measure seasonal variation in [^{11}C]DASB 5-HTT BP$_{ND}$. Seasonal increases in [^{11}C]DASB 5-HTT BP$_{ND}$ were greater in SAD compared with healthy in the PFC and ACC, primarily due to differences between severe SAD and healthy (severe SAD vs healthy; Mann–Whitney U, $U = 42.5$ and 37.0, $p = 0.005$ and 0.003, respectively; greater magnitude in severe SAD of 35.10 and 14.23%, respectively), with similar findings observed in other regions ($U = 40.0$–62.0, $p = 0.004$–0.048; greater magnitude in severe SAD of 13.16–17.49%). To our knowledge, this is the first brain biomarker identified in SAD. This creates a new opportunity for phase 0 studies to target this phenotype and optimize novel prevention/treatment strategies for SAD.
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INTRODUCTION

Seasonal affective disorder (SAD), a subtype of major depressive disorder (MDD) characterized by recurrent winter major depressive episodes (MDEs) with full remission in spring-summer, poses a heavy burden: among the subtypes of MDD, it has the highest frequency of MDEs being almost yearly, and 40% of cases progress to non-seasonal MDEs (Faedda *et al*, 1993; Rosenthal *et al*, 1984a; Schwartz *et al*, 1996). The annual prevalence of SAD is typically 1–6%, with the highest rates occurring at more extreme latitudes (Magnusson, 2000). Although information about SAD is accumulating, a critical gap is the lack of direct brain investigations of this illness. Biological abnormalities of SAD include, in winter, increased duration and/or delay of nocturnal melatonin secretion, blunted norepinephrine, cortisol, and prolactin response to challenge with the non-selective serotonin receptor agonist m-CPP, and decreased rod sensitivity to light as measured by flash

electroretinography (Lavoie *et al*, 2009; Levitan *et al*, 1998; Schwartz *et al*, 1997; Wehr *et al*, 2001). Vulnerability markers include altered polymorphism frequencies for clock genes NPAS and PER3, as well as a variable number tandem repeat on exon 3 of the DRD4 gene (Johansson *et al*, 2003; Levitan *et al*, 2006). Given the substantial burden of SAD, its high prevalence, and the lack of knowledge regarding the brain phenotypes of this disorder, there is a clear need to identify neurochemical and neuropathological markers in the central nervous system of SAD.

One strategy for selecting a target to investigate in SAD is to choose a functionally relevant brain marker that is sensitive to seasonal effects in healthy humans. Brain markers influenced by season in health include greater striatal L-Dopa uptake in the fall-winter, decreased 5-HT$_{1A}$ receptor binding in limbic regions in winter, altered whole-brain serotonin turnover, and greater 5-HTT BP$_{ND}$, an index related to 5-HTT density, in the fall-winter as compared with spring-summer (Buchert *et al*, 2006; Eisenberg *et al*, 2010; Kalbitzer *et al*, 2010; Lambert *et al*, 2002; Ruhé *et al*, 2009; Spindelegger *et al*, 2012; Praschak-Rieder *et al*, 2008). In regards to seasonal variation of 5-HTT BP$_{ND}$, neuroimaging studies of reasonably large sample size consistently report this finding in a high proportion of brain regions sampled: a [^{11}C]DASB PET study of 88 subjects at Toronto, Canada, found seasonal variation in [^{11}C]DASB 5-HTT BP$_{ND}$ across

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all examined brain regions including the prefrontal cortex, anterior cingulate cortex (PFC and ACC, respectively), and hippocampus (Praschak-Rieder et al, 2008). Similarly, an independent [11C]DASB PET study of 57 participants at Copenhagen, Denmark found evidence of seasonal fluctuation in [11C]DASB 5-HTT BP_{ND} in 3 of 4 brain regions with significant change in the caudate and putamen, a trend in the thalamus and no effect in the midbrain (Kalbitzer et al, 2010). Two additional studies in Amsterdam, Netherlands and Hamberg Germany, applying [123I]β-CIT SPECT and [11C]McN5652 PET, respectively, investigated the thalamus and midbrain and both found seasonal variation in 5-HTT BP_{ND} in the midbrain (the Amsterdam study included both healthy and non-SAD MDD patients) (Buchert et al, 2006; Ruhé et al, 2009). The 5-HTT is also an important target for controlling affect given that polymorphisms in the 5-HTT promoter are associated with risk toward MDD, medications that affect the 5-HTT influence both cognitive recall of emotionally valent material and negative cognitive interpretations of life events; and that overexpression of 5-HTT in regions controlling affect are associated with depressive behaviors in rodents (Caspi et al, 2010; Harmer et al, 2004; Line et al, 2014; Meyer et al, 2003; Mouri et al, 2012).

Given the consistency of seasonal change in 5-HTT BP_{ND} in health and the importance of the role of 5-HTT in affect regulation, the aim of the present study was to investigate seasonal fluctuation in [11C]DASB 5-HTT BP_{ND} in SAD as compared to health. We prioritized the PFC and ACC as these regions had considerable seasonal variation in previous study, contain structures with key roles in mood regulation and cognitive processing of emotion, and are the regions for which 5-HTT overexpression is associated with depressive behaviors (Line et al, 2014; Mouri et al, 2012; Praschak-Rieder et al, 2008; Ressler and Mayberg, 2007).

The first hypothesis was that the magnitude of seasonal variation in [11C]DASB 5-HTT BP_{ND} in the PFC and ACC would be greater in SAD as compared to health. The second hypothesis was that seasonal variation in [11C]DASB 5-HTT BP_{ND} in the PFC and ACC would be associated with severity of SAD symptoms. The rationale for the second hypothesis is that SAD is well known to be a dimensional illness with a continuous distribution within health (such that 25% of

healthy individuals experience mild seasonal symptoms) through to SAD of moderate to high severity (Bartko and Kasper, 1989; Kasper et al, 1989; Rohan et al, 2011); and in MDD the magnitude of brain biomarker abnormalities is often correlated with severity, reflecting that MDD is a complex neuropsychiatric illness for which any individual pathology is more likely to present when MDD is more severe (Chiuccariello et al, 2014; Deschwanden et al, 2011; Fujita et al, 2012; Meyer, 2012; Sanacora et al, 2004; Setiawan et al, 2015).

MATERIALS AND METHODS

Study Participants

Twenty SAD participants (14 women and 6 men; mean [SD] age: 31.3 [4.8] years; age range: 24–39) and twenty healthy volunteers (13 women and 7 men; mean [SD] age: 30.5 [4.2] years; age range: 24–39) were recruited from the Greater Toronto Area between June 2012 and July 2015. Healthy subjects were age-matched to SAD subjects within 3 years. Demographics are listed in Table 1.

Criteria for all included being age 18–40, non-smoking and in good physical health, no history of alcohol or substance abuse, no antidepressant use within the past 6 months, and no use of prescription medications or herbal supplements within the past 2 months. In addition, as light exposure has been found to reduce [11C]DASB 5-HTT BP_{ND} during the winter months and [11C]DASB 5-HTT BP_{ND} has been shown to be inversely correlated with duration of daily sunshine, both use of light therapy within the past 3 months and travel to more southern latitudes during the study period were exclusionary (Harrison et al, 2015; Praschak-Rieder et al, 2008). Exclusion criteria for female subjects included the use of oral contraceptives, current pregnancy, postpartum or recent abortion (within one year), and in perimenopause or menopause. Subjects were asked not to take over the counter medications 1 week before scanning, to avoid alcohol 4 days before scanning, and not to consume caffeinated beverages within 2 days of the PET scan. In addition, lifetime history of Axis I or Axis II disorders was exclusionary for healthy subjects, and comorbid Axis I or Axis II disorders were exclusionary for SAD subjects. Screening instruments

Table 1 Demographic Characteristics

	SAD (n = 20)	Healthy (n = 20)	Statistical comparison
Age, mean (SD)	31.3 (4.8)	30.5 (4.2)	$t_{38} = 0.56$ ($p = 0.58$)
Female to male ratio	14 : 6	13 : 7	$\chi^2_{(1)} = 0.11$ ($p = 0.74$)[a]
Body mass index (SD)	24.6 (3.5)	23.2 (3.2)	$t_{38} = 1.37$ ($p = 0.18$)
Years in climatic area	22.3 (12.2)	22.2 (11.4)	$t_{38} = 0.013$ ($p = 0.99$)
Age of SAD onset	21.9 (5.4)	N/A	N/A
No. of seasonal MDE	9.2 (5.2)[b]	N/A	N/A
Winter SIGH-SAD (HDRS-29)	26.0 (9.93)	1.56 (1.9)	$t_{20} = 10.79$ ($p < 0.0001$)[c]
Summer SIGH-SAD (HDRS-29)	2.7 (3.05)	1.3 (1.8)	$t_{30} = 1.78$ ($p = 0.09$)[c]
SPAQ (Global Seasonality Score)	16.8 (3.2)	2.1 (1.7)	$t_{28} = 18.15$ ($p < 0.0001$)[c]

[a]Chi-Square test for association. [b]No. of seasonal MDEs consistent with reports from the literature (Lam et al, 2006; Modell et al, 2005). [c]Welch's independent samples t-test for unequal variances.

included the Structured Clinical Interview for DSM-IV-TR (SCID-I/II); and all SAD subjects received a consultation with a psychiatrist (JHM or RDL) to verify diagnosis. Urine drug screening was performed at initial assessment and on each PET scanning day to rule out recent drug and medication use. Urine toxicology was performed using gas chromatography–mass spectrometry (GC-MS) at the CAMH clinical laboratory (drug screening sensitive to ethanol, drugs of abuse, all classes of antidepressants, antipsychotics, anticonvulsants, benzodiazepines, narcotics, NSAIDs, anthelmintics, statins, β-blockers, muscle relaxants, and anti-allergy medications).

Participants also completed the Seasonal Pattern Assessment Questionnaire (SPAQ) from which a summed global seasonality score (SPAQ GSS) was calculated to determine the degree of seasonality (ie, seasonal change in sleep, mood, energy, appetite, weight, and social activity) (Rosenthal et al, 1984b). The SPAQ was administered in randomized order in accordance with the season in which subjects were scanned (summer: 10 healthy, 8 SAD; winter: 10 healthy, 12 SAD; $\chi^2_{(1)} = 0.40$, $p = 0.53$). Subjects were defined categorically as healthy (with no seasonality) for SPAQ scores below 12, moderate SAD for scores between 12 and 16, and severe SAD for scores equal to or greater than 16 (Bartko and Kasper, 1989). All healthy participants had SPAQ scores of less than 7 (mean [SD]: 2.1 [1.7], range 0–6, Table 1). On each scan day, the Structured Interview Guide for the Hamilton Depression Rating Scale with Seasonal Affective Disorder Supplement (SIGH-SAD) was also administered. For each participant, written informed consent was obtained after the procedures were fully explained. The study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto.

Scanning

All participants underwent two [11C]DASB PET and MRI scans: one in spring-summer and the other in fall-winter, in randomized order, to measure the seasonal percent change in [11C]DASB 5-HTT BP_{ND}. Scan dates of healthy controls were matched to SAD participants within 4 weeks. To minimize any potential effects of circadian rhythm, all scans were scheduled in the morning and took place at either 0930 h or 1130 h. All participants were non-smoking and on each PET scan day underwent laboratory tests (plasma sampling for cotinine, calcium and thyroid hormones, complete blood cell count) to verify non-smoking status and ensure physical health.

Synthesis of [11C]DASB has been described previously (Ginovart et al, 2001; Wilson et al, 2000). Briefly, [11C]-CH3I was trapped in a high-performance liquid chromatography sample loop coated with a solution of the N-normethyl precursor (1 mg) in dimethylformamide (80 μl). After 5 min at ambient temperature, the contents of the sample loop were injected onto a reverse-phase high-performance liquid chromatography column, and the fraction containing the product was collected, evaporated to dryness, formulated in saline, and filtered through a 0.2-μ filter. Before each scan, an intravenous bolus of 10 mCi (370 MBq) of [11C]DASB was injected. The [11C]DASB was of high radiochemical purity (98.10 ± 5.16%) and high specific activity (65.62 ± 26.36 GBq/μmol) at the time of injection. PET images were obtained using a high-resolution PET/CT Siemens-Biograph HiRez XVI scanner (81 axial sections of 2 mm; Siemens Molecular Imaging, Knoxville, TN). The emission scan was reconstructed in 15 frames of 1 min, followed by 15 frames of 5 min, totaling to a scan duration of 90 min in length. The images were corrected for attenuation using a germanium 68-labeled transmission scan and reconstructed using 2D filtered back projection algorithms with a ramp filter. Subsequent to the initial PET scan, each participant also underwent a magnetic resonance imaging scan (GE 3.0-T scanner, fast spin echo –XL sequence, proton density-weighted image, x, y, and z voxel dimensions; 0.37, 0.37, and 0.90 mm, GE Medical Systems, Milwaukee, WI).

Regions of interest (ROIs) on the MRI were determined using a semi-automated method in which regions of a template MRI are transformed onto the individual MRI based on a series of transformations and deformations that matched the template image to the individual co-registered MRI, as well as segmentation of the individual MRI to select gray-matter voxels as previously described (Meyer et al, 2009; Rusjan et al, 2006). ROIs on the MRI were subsequently located on the PET image using the rigid body transformations from co-registration of the MRI to PET image via a mutual information algorithm. ROIs included the prefrontal cortex, anterior cingulate cortex, ventral striatum, dorsal caudate, dorsal putamen, thalamus, hippocampus, midbrain, and cerebellar cortex. The location of the ROIs was verified by visual assessment of their display on the integral [11C]DASB PET image. The cerebellar cortex reference region was defined as the posterior half of the cerebellar cortex, excluding the vermis and cerebellar white matter. Reference tissue methods have been validated for [11C]DASB to calculate [11C]DASB 5-HTT BP_{ND} (Ginovart et al, 2001; Ichise et al, 2003). We applied the non-invasive Logan method, which has a modest underestimate, but the advantage of having the lowest coefficient of variation (ie, standard deviation/mean) of calculated BP_{ND} values (Logan et al, 1996). An additional analysis was conducted using the simplified reference tissue method 2 (SRTM2), which has a negligible underestimate but a higher coefficient of variation (Wu and Carson, 2002). Test-retest variability of [11C]DASB 5-HTT BP_{ND} values using the non-invasive Logan method have been reported to have a mean regional change of 0% with a standard deviation of ± 4.75% in the prefrontal cortex, ± 3.7% in the anterior cingulate cortex, ± 1.6% in the bilateral caudate, ± 2.6% in the bilateral putamen, ± 2.5% in the thalamus, and ± 0.3% in the midbrain/superior raphe nuclei, with similar results obtained using the SRTM2 method (Praschak-Rieder et al, 2005).

Statistical Analyses

Seasonal percent change in [11C]DASB 5-HTT BP_{ND} (% Δ [11C]DASB 5-HTT BP_{ND}) was calculated in each region for each subject [(winter [11C]DASB 5-HTT BP_{ND} – summer [11C]DASB 5-HTT BP_{ND})/summer [11C]DASB 5-HTT BP_{ND}] and, as one value pertaining to a severe SAD case was slightly outside the normal distribution, non-parametric tests were used for all statistical analyses. The % Δ [11C]DASB 5-HTT BP_{ND} in the PFC and ACC was compared between SAD and healthy groups using the Mann–Whitney U-test. To assess the relationship of % Δ [11C]DASB 5-HTT

Table 2 Group Differences in Seasonal Change in 5-HTT BP_{ND}

Brain region	Mann–Whitney U-test	
	SAD vs Healthy	
	U^a	p-Value
Prefrontal cortex	126.5	0.046
Anterior cingulate cortex	114.0	0.02
Dorsal putamen	140.0	0.10
Thalamus	147.0	0.15
Dorsal caudate	154.0	0.22
Ventral striatum	154.0	0.21
Midbrain	96.0	0.005
Hippocampus	153.0	0.20

5-HTT BP_{ND}, serotonin transporter binding potential (non-displaceable); Mann–Whitney U, non-parametric equivalent of an independent samples t-test.
[a]Mann–Whitney U-test statistic.

BP_{ND} to severity, the primary method was to categorize SAD into two groups, moderate and severe, applying a cutoff of greater than 16 on the SPAQ as previously described (Bartko and Kasper, 1989). To determine whether a difference was present among healthy, moderate SAD, and severe SAD groups, the Kruskal–Wallis H-test was applied to assess % Δ [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC, and then the Mann–Whitney U-test was used to compare healthy with severe SAD. As a secondary approach, all analyses were applied in the other ROIs.

As an additional analysis, the effect of severity of SAD upon seasonal change in [^{11}C]DASB 5-HTT BP_{ND} was investigated. Seasonal change in [^{11}C]DASB 5-HTT BP_{ND} (Δ [^{11}C]DASB 5-HTT BP_{ND}) was calculated for each participant (winter [^{11}C]DASB 5-HTT BP_{ND} – summer [^{11}C]DASB 5-HTT BP_{ND}). To determine whether a difference was present among healthy, moderate SAD, and severe SAD groups, the Kruskal–Wallis H-test was applied to assess Δ [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC and other examined regions, followed by the Mann–Whitney U-test to compare healthy with severe and moderate SAD groups. The Kruskal–Wallis H-test was also used to determine whether [^{11}C]DASB 5-HTT BP_{ND} values differed across groups in winter and in summer.

Further assessments of the relationship to severity of symptoms were to determine the Spearman correlation coefficients between % Δ [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC and seasonal depressive symptoms, as measured by the SPAQ. These correlations were also determined for other ROIs. Finally, as an exploratory analysis, Spearman correlation coefficients were used to assess the relationship between Δ [^{11}C]DASB 5-HTT BP_{ND} and seasonal depressive symptoms in all brain regions assayed.

RESULTS

Effect of SAD and Severity Category on % Δ [^{11}C]DASB 5-HTT BP_{ND}

Seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} was greater in SAD as compared to health in the PFC and ACC

(Mann–Whitney U, $U = 126.5$ and 114.0, $p = 0.046$ and 0.02, respectively, Table 2). However, the strongest finding was a main effect of group (healthy, moderate SAD, and severe SAD) upon seasonal fluctuation in [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC (Kruskal–Wallis H-test, $\chi^2_{(2)} = 8.82$, $p = 0.01$ and $\chi^2_{(2)} = 9.62$, $p = 0.008$, respectively, Figure 1 and Table 3). Similar findings were present across other ROIs ($\chi^2_{(2)} = 7.01$–10.45, $p = 0.005$–0.03, Figure 1 and Table 3), excepting the hippocampus in which a trend-level effect was observed ($\chi^2_{(2)} = 5.26$, $p = 0.07$; Figure 1 and Table 3). These findings were primarily explained by greater seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC of severe SAD cases relative to healthy volunteers (Mann–Whitney U, $U = 42.5$ and 37.0, $p = 0.005$ and 0.003, respectively, Figure 1 and Table 3), an effect also observed in other ROIs ($U = 40.0$–62.0, $p = 0.004$–0.048, Figure 1 and Table 3), excepting the midbrain for which seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} was significantly greater across all SAD participants relative to healthy volunteers ($U = 96.0$, $p = 0.005$; Figure 1 and Table 2). In contrast, seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} in moderate SAD subjects was similar to healthy individuals with no difference in any ROI (Mann–Whitney U, $U = 51.0$–106.0, $p = 0.07$–0.96; Figure 1 and Table 3). Seasonal % Δ 5-HTT BP_{ND} was consistent within individuals across brain regions (Cronbach's alpha, $\alpha = 0.89$).
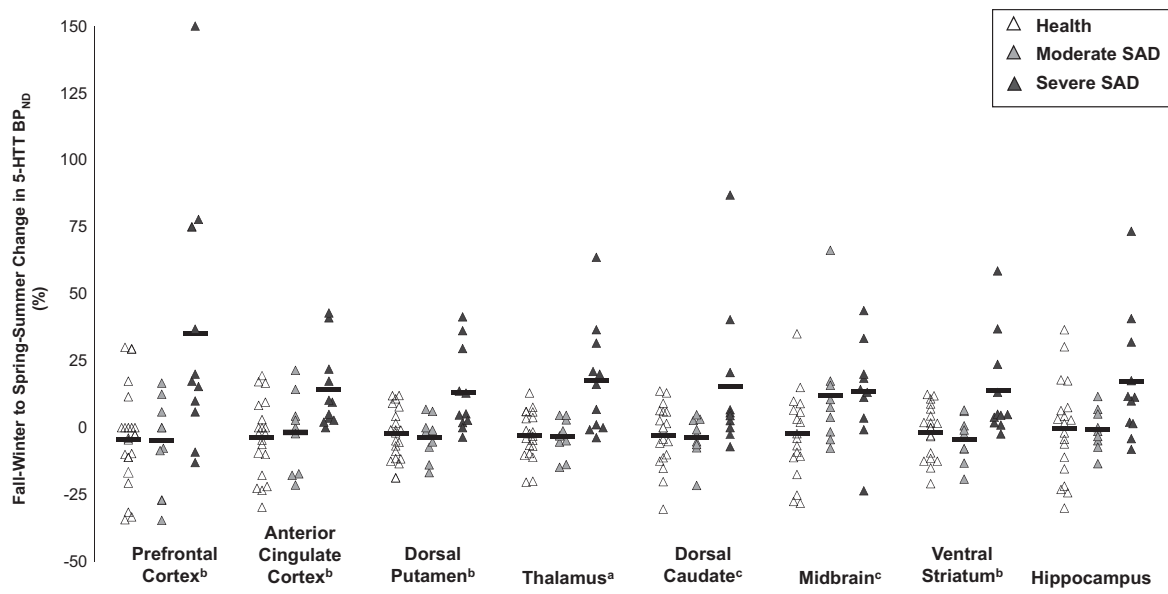
Seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} measured applying the SRTM2 was similar to that of the non-invasive Logan method (Pearson's correlation coefficient, $r = 0.93$–0.98, $p < 0.0001$, across regions) and yielded similarly consistent main results.

Effect of SAD and Severity Category on Δ [^{11}C]DASB 5-HTT BP_{ND}

The effects of SAD and severity on Δ [^{11}C]DASB 5-HTT BP_{ND} were consistent with those on % Δ [^{11}C]DASB 5-HTT BP_{ND}. A main effect of group was observed on Δ [^{11}C]DASB 5-HTT BP_{ND} in the PFC and ACC (Kruskal–Wallis H-test, $\chi^2_{(2)} = 8.32$, $p = 0.016$ and $\chi^2_{(2)} = 9.00$, $p = 0.01$, respectively) and across other ROIs ($\chi^2_{(2)} = 6.45$–10.56, $p = 0.005$–0.04). These findings were similarly driven by increased Δ [^{11}C]DASB 5-HTT BP_{ND} of severe SAD cases relative to healthy volunteers (Mann–Whitney U, $U = 38.5$–52.5, $p = 0.003$–0.018) with no difference in Δ [^{11}C]DASB 5-HTT BP_{ND} values upon comparison of moderate SAD and healthy groups ($U = 60.0$–108.0, $p = 0.16$–0.91, Supplementary Figure S2). In comparison with healthy and moderate SAD groups, mean regional [^{11}C]DASB 5-HTT BP_{ND} values of severe SAD cases were greater in fall-winter by 0–7.89% and lower in spring-summer by 11.47–20.83%; however, these differences were not statistically significant (Kruskal–Wallis H-test, fall-winter: $\chi^2_{(2)} = 0.01$–1.59, $p = 0.45$–0.99; spring-summer: $\chi^2_{(2)} = 1.61$–5.11, $p = 0.08$–0.45, Supplementary Table S5).

Relationship of Symptoms to % Δ [^{11}C]DASB 5-HTT BP_{ND}

In SAD participants, a positive correlation was also observed between magnitude of seasonal % Δ [^{11}C]DASB 5-HTT BP_{ND} and SAD severity, as measured by the SPAQ GSS

Figure 1 Seasonal percent change in serotonin transporter binding potential (% Δ 5-HTT BP$_{ND}$) as measured in eight brain regions of interest (ROIs). Open (healthy, $n = 20$) and grey (moderate SAD, $n = 9$) and black (severe SAD, $n = 11$) triangles represent individual subject % Δ 5-HTT BP$_{ND}$. Black bars represent mean % Δ 5-HTT BP$_{ND}$ for each group. % Δ 5-HTT BP$_{ND}$ was significantly greater in the prefrontal and anterior cingulate cortices (severe SAD vs healthy; Mann–Whitney U, $U = 42.5$ and 37.0, $p = 0.005$ and 0.003, respectively; greater magnitude in severe SAD of 35.10% and 14.23%, respectively) with similar findings observed in other regions ($U = 40.0$–62.0, $p = 0.004$–0.048; greater magnitude in severe SAD of 13.16–17.49%). To compare groups, the Kruskal–Wallis H-test was also applied at each region of interest. [a]p-value $\leqslant 0.005$; [b]p-value $\leqslant 0.01$; [c]p-value $\leqslant 0.05$. Seasonal % Δ 5-HTT BP$_{ND}$ was consistent within individuals across brain regions (Cronbach's alpha, $\alpha = 0.89$).

(Table 4), which was significant in the PFC (Spearman's rank correlation coefficient, $\rho = 0.52$, $p = 0.018$) and trend level in the ACC (Spearman's rank correlation coefficient, $\rho = 0.42$, $p = 0.07$). Similar relationships were observed in other examined brain regions ($\rho = 0.44$–0.55, $p = 0.012$–0.055), with the exception of the midbrain, in which no significant correlation was observed ($\rho = 0.25$, $p = 0.29$; Table 4). However, in healthy participants, for which the range in SPAQ GSS scores was narrow, no significant correlations were observed between seasonal % Δ [^{11}C]DASB 5-HTT BP$_{ND}$ and SPAQ GSS in any brain region ($\rho = -0.18$–0.36, $p = 0.12$–0.92; Table 4). Correlations between SPAQ scores and Δ [^{11}C]DASB 5-HTT BP$_{ND}$ in SAD and healthy groups were comparable to those of seasonal % Δ [^{11}C]DASB 5-HTT BP$_{ND}$ in all examined brain regions (Supplementary Figure S3, Supplementary Table S6).

In SAD, *post hoc* exploratory analyses comparing a breakdown of the GSS by mood, energy, and appetite clusters found that the strongest correlations between seasonal change in mood symptoms and seasonal % Δ [^{11}C]DASB 5-HTT BP$_{ND}$ occurred in the PFC ($\rho = 0.53$, $p = 0.015$), dorsal putamen ($\rho = 0.66$, $p = 0.002$), dorsal caudate ($\rho = 0.62$, $p = 0.004$), ventral striatum ($\rho = 0.63$, $p = 0.003$) and thalamus ($\rho = 0.59$, $p = 0.006$; Supplementary Table S6). In terms of seasonal changes in energy levels, the strongest correlations were also observed in the PFC ($\rho = 0.52$, $p = 0.019$), dorsal putamen ($\rho = 0.70$, $p = 0.001$), dorsal caudate ($\rho = 0.68$, $p = 0.001$), ventral striatum ($\rho = 0.71$, $p < 0.0001$) and thalamus ($\rho = 0.66$, $p = 0.001$; Supplementary Table S7). Similar relationships were found between changes in appetitive behaviors and

seasonal % Δ [^{11}C]DASB 5-HTT BP$_{ND}$ with the strongest correlations observed in the PFC ($\rho = 0.50$, $p = 0.024$), thalamus ($\rho = 0.54$, $p = 0.014$), dorsal putamen ($\rho = 0.48$, $p = 0.03$), hippocampus ($\rho = 0.48$, $p = 0.033$) and ventral striatum ($\rho = 0.42$, $p = 0.069$; Supplementary Table S7).

DISCUSSION

This is the first study to investigate seasonal change in [^{11}C] DASB 5-HTT BP$_{ND}$ in SAD. Although greater seasonal variation of [^{11}C]DASB 5-HTT BP$_{ND}$ in SAD as compared to health was observed across most brain regions sampled, including the PFC and ACC, the strongest finding was a more pronounced seasonal fluctuation of [^{11}C]DASB 5-HTT BP$_{ND}$ in severe SAD across all brain regions relative to moderate SAD and asymptomatic healthy groups. These results indicate that 5-HTT BP$_{ND}$, as measured with [^{11}C] DASB PET, is detecting a brain phenotype of SAD and suggests new opportunities for applying this neuroimaging method in biomarker-based approaches to develop new strategies for both prevention and treatment.

Greater seasonal fluctuation of [^{11}C]DASB 5-HTT BP$_{ND}$ across all examined brain regions, including the PFC and ACC, has important implications for SAD pathophysiology, particularly in regards to severe SAD. [^{11}C]DASB has a strong preferential binding to 5-HTT on the cell surface where functional 5-HTT are located and the binding of [^{11}C] DASB is insensitive to competition by endogenous serotonin as demonstrated by the lack of effect of physiologically tolerable serotonergic manipulations in humans, such as

Table 3 Group Differences in Seasonal Change in 5-HTT BP$_{ND}$ in Participants Undergoing [^{11}C]DASB PET in Spring-Summer and Fall-Winter

Brain region	Kruskal–Wallis *H*-test		Mann–Whitney *U*-test			
			Moderate SAD *vs* Healthy[a]		Severe SAD *vs* Healthy[b]	
	$\chi^2_{(2)}$[c]	*p*-Value	*U*[d]	*p*-Value	*U*[d]	*p*-Value
Prefrontal cortex	8.82	0.01	84.0	0.78	42.5	0.005
Anterior cingulate cortex	9.62	0.008	77.0	0.54	37.0	0.003
Dorsal putamen	10.09	0.006	100.0	0.64	40.0	0.004
Thalamus	10.45	0.005	106.0	0.45	41.0	0.004
Dorsal caudate	7.01	0.03	99.0	0.67	55.5	0.02
Ventral striatum	8.84	0.01	106.0	0.45	48.0	0.01
Midbrain	8.71	0.013	51.0	0.07	45.0	0.007
Hippocampus	5.26	0.07	91.0	0.96	62.0	0.048

5-HTT BP$_{ND}$, serotonin transporter binding potential (non-displaceable); Kruskal–Wallis *H*, non-parametric test; Mann–Whitney *U*, non-parametric equivalent of an independent samples *t*-test.
[a]*n* = 9 (Moderate SAD) *vs* *n* = 20 (Healthy).
[b]*n* = 11 (Severe SAD) *vs* *n* = 20 (Healthy).
[c]Kruskal–Wallis test statistic.
[d]Mann–Whitney *U*-test statistic.

Table 4 Correlations Between Seasonal Change in 5-HTT BP$_{ND}$ and Seasonal Pattern Assessment Questionnaire Global Seasonality Score

Brain region	SAD (*n* = 20)		Healthy (*n* = 20)	
	ρ[a]	*p*-Value	ρ[a]	*p*-Value
Prefrontal cortex	0.52	0.018	0.21	0.38
Anterior cingulate cortex	0.42	0.07	0.20	0.39
Dorsal putamen	0.55	0.012	0.08	0.74
Thalamus	0.54	0.014	0.11	0.65
Dorsal caudate	0.46	0.04	0.36	0.12
Ventral striatum	0.51	0.02	0.12	0.60
Midbrain	0.25	0.29	− 0.18	0.44
Hippocampus	0.44	0.055	0.03	0.92

[a]Spearman's rank correlation coefficient.

acute tryptophan depletion (Praschak-Rieder *et al*, 2005; Quelch *et al*, 2012; Talbot *et al*, 2005). As such, the changes in 5-HTT BP$_{ND}$ observed *in vivo* using [^{11}C]DASB PET reflect greater availability of the 5-HTT to clear serotonin from extracellular space in the winter, thereby lowering levels of extracellular serotonin. This is a key issue, given that overexpression of 5-HTT in the PFC is associated with decreased stimulation-induced release of 5-HT from serotonergic neurons and differential expression of the 5-HTT is associated with magnitude of response to anxiogenic stimuli (Jennings *et al*, 2010; Lesch *et al*, 1996; Mouri *et al*, 2012). In addition, while greater seasonal variation in [^{11}C]DASB 5-HTT BP$_{ND}$ was observed in severe SAD relative to health, [^{11}C]DASB 5-HTT BP$_{ND}$ values did not differ across groups in summer and winter seasons, suggesting that, in SAD, change across seasons is more relevant than the [^{11}C]DASB 5-HTT BP$_{ND}$ levels, themselves. Taken together, these findings suggest that across the shift from summer to winter, seasonal change in 5-HTT levels and/or affinity may alter the dynamics of extracellular 5-HT release within the PFC, ACC, and subcortical structures thereby dysregulating systems adversely affected in severe SAD, including mood, energy, and appetite.

Identifying a new brain biomarker in SAD is critical for therapeutic advances because brain biomarkers are an essential guide for developing treatments of complex neuropsychiatric illnesses with multiple phenotypes. Although it is well accepted that novel therapeutics require target engagement, it is a newer direction in therapeutic development to assess the effects of treatment on the target biomarker itself. Knowledge that the biomarker has been engaged then allows for assessment of whether an adequate

number of phenotypes have been targeted in clinical trials with symptom burden as the primary outcome. In the present investigation, the biomarker identified provides opportunities to create novel prevention methods for SAD: it is clear that the environmental combination of seasonal variables, including light, temperature, and humidity, influence [^{11}C]DASB 5-HTT BP$_{ND}$ in those with severe SAD. Future studies could identify combinations of specific environmental factors and their exposure thresholds that induce seasonal change in [^{11}C]DASB 5-HTT BP$_{ND}$ so that by staying below such thresholds or adding other preventative interventions, such as light therapy, the winter elevation in [^{11}C]DASB 5-HTT BP$_{ND}$ could be avoided. Avoidance of environmental qualities and exposure thresholds that induce seasonal fluctuations in [^{11}C]DASB 5-HTT BP$_{ND}$ could then be incorporated into larger scale clinical studies as prevention strategies in high-risk communities, such as those in Northern latitudes where SAD prevalence exceeds 6% (Magnusson, 2000).

There are some limitations of this study typical of SAD investigations and human brain studies of neuropsychiatric disease. First, it was not always possible to scan SAD participants when their winter MDE was at its most severe. As such, completion of winter scanning before the symptomatic nadir may have underestimated the strength of the relationship between severity of SAD and seasonal % Δ [^{11}C]DASB 5-HTT BP$_{ND}$. Second, exposure to different seasonal environmental influences are highly inter-correlated and does not allow for the differentiation of their individual effects. Third, while [^{11}C]DASB has strong preferential binding to 5-HTT on outer cell membranes, 5-HTT BP$_{ND}$ is a measure of both 5-HTT density and its affinity for [^{11}C]DASB (Quelch *et al*, 2012). Thus, it is not possible to differentiate between changes in 5-HTT density and affinity, although it would be expected that when the affinity of the serotonin transporter is altered there is a similar functional effect on the dynamics of extracellular serotonin concentrations. Finally, as our approach was to determine whether the severity of SAD was related to seasonal change in [^{11}C]DASB 5-HTT BP$_{ND}$, we deliberately chose healthy volunteers with negligible severity of seasonal symptoms for comparison

with SAD subjects with more extreme symptoms, and this may have reduced our ability to detect seasonal differences in healthy subjects (see Supplementary Discussion for further explanation). Since 25% of healthy people experience seasonal variation in mood symptoms, an additional interesting future direction would be to assess % Δ [^{11}C]DASB 5-HTT BP_{ND} in a group of healthy subjects with substantial seasonality to further characterize this brain phenotype (Kasper et al, 1989).

In summary, this is the first investigation to compare seasonal variation in [^{11}C]DASB 5-HTT BP_{ND} in SAD participants, across a spectrum of illness severity, to a group of healthy volunteers, asymptomatic for seasonal changes in mood and behavior. The primary finding is that, across brain regions sampled, including the PFC and ACC, [^{11}C]DASB 5-HTT BP_{ND} was significantly elevated in winter as compared to summer in SAD, particularly in severe SAD. Given that [^{11}C]DASB binds preferentially to the 5-HTT on the cell surface, this has important pathophysiological implications for the dynamics of serotonin release and is best interpreted as reflecting a key phenotype of SAD (Quelch et al, 2012). As a brain biomarker, greater seasonal % change in [^{11}C]DASB 5-HTT BP_{ND} is an important breakthrough because it can be applied to develop interventions to reduce environmental impact on this target and create very specific prevention strategies for SAD.

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