doi:10.1093/brain/aww043 BRAIN 2016: 139; 1605–1614 | 1605

BRAIN
A JOURNAL OF NEUROLOGY

Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder

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Cross-sectional neuroimaging studies in non-depressed individuals have demonstrated an inverse relationship between daylight minutes and cerebral serotonin transporter; this relationship is modified by serotonin-transporter-linked polymorphic region short allele carrier status. We here present data from the first longitudinal investigation of seasonal serotonin transporter fluctuations in both patients with seasonal affective disorder and in healthy individuals. Eighty ^{11}C-DASB positron emission tomography scans were conducted to quantify cerebral serotonin transporter binding; 23 healthy controls with low seasonality scores and 17 patients diagnosed with seasonal affective disorder were scanned in both summer and winter to investigate differences in cerebral serotonin transporter binding across groups and across seasons. The two groups had similar cerebral serotonin transporter binding in the summer but in their symptomatic phase during winter, patients with seasonal affective disorder had higher serotonin transporter than the healthy control subjects ($P = 0.01$). Compared to the healthy controls, patients with seasonal affective disorder changed their serotonin transporter significantly less between summer and winter ($P < 0.001$). Further, the change in serotonin transporter was sex- ($P = 0.02$) and genotype- ($P = 0.04$) dependent. In the patients with seasonal affective disorder, the seasonal change in serotonin transporter binding was positively associated with change in depressive symptom severity, as indexed by Hamilton Rating Scale for Depression – Seasonal Affective Disorder version scores ($P = 0.01$). Our findings suggest that the development of depressive symptoms in winter is associated with a failure to downregulate serotonin transporter levels appropriately during exposure to the environmental stress of winter, especially in individuals with high predisposition to affective disorders.

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Keywords: seasonal affective disorder; serotonin; serotonin transporter; serotonin transporter linked polymorphic region; PET

Received October 08, 2015. Revised December 26, 2015. Accepted January 28, 2016. Advance Access publication March 19, 2016

Abbreviations: 5-HTTLPR = serotonin-transporter-linked polymorphic region; BMI = body mass index; BP_{ND} = non-displaceable binding potential; MDI = Major Depression Inventory; PSQI = Pittsburgh Sleep Quality Index; SIGH-SAD = Hamilton Rating Scale for Depression – Seasonal Affective Disorder version; SPAQ = Seasonal Pattern Assessment Questionnaire

Introduction

In Scandinavia as well as other countries at Northern latitudes, people are subjected to long and dark winters. Although well tolerated by most inhabitants, ~5% of the Copenhagen population experience symptoms consistent with seasonal affective disorder and an additional 10% suffer from sub-syndromal seasonal affective disorder (Dam *et al.*, 1998), a more moderate condition where diagnostic criteria for depression are not met. Seasonal affective disorder is characterized by season-triggered depression and encompasses feelings of hopelessness and blameworthiness, loss of energy, impaired concentration, hyperphagia and hypersomnia (Rosenthal *et al.*, 1984). Risk factors for developing seasonal affective disorder include being female, with females being afflicted between 2–40 times more often than males (Partonen, 1995), young age (Magnusson and Partonen, 2005) and being a serotonin-transporter-linked polymorphic region (5-HTTLPR) short allele carrier (S-carrier) (Rosenthal *et al.*, 1998). There is additional evidence for seasonal affective disorder being related to serotonin dysfunction: the disorder can be effectively treated with either bright light therapy or with a serotonin transporter reuptake inhibitor (Thaler *et al.*, 2011), the effects of bright light can be reversed by lowering cerebral serotonin levels by tryptophan depletion (Lam *et al.*, 1996; Neumeister *et al.*, 1997*a*, *b*, 1998), and dietary (Miller, 2005) or pharmacological (O'Rourke *et al.*, 1989; Dilsaver and Jaeckle, 1990; Partonen and Lonnqvist, 1996) enhancement of serotonin transmission alleviates seasonal affective disorder symptoms. Further, serotonin transporter function in platelets is enhanced in seasonal affective disorder (Willeit *et al.*, 2008). Intriguingly, these risk factors are also uniquely associated with differences in cerebral serotonin transporter levels: healthy females have higher serotonin transporter density in the midbrain than males (Erritzoe *et al.*, 2010), cerebral serotonin transporter density declines with age (Buchert *et al.*, 2006; Kalbitzer *et al.*, 2009; Erritzoe *et al.*, 2010), and several studies suggest that the 5-HTTLPR genotype is related to cerebral serotonin transporter density (Willeit and Praschak-Rieder, 2010).

The season-dependent fluctuation in cerebral serotonin transporter has been examined in a number of neuroimaging studies conducted in healthy volunteers. Early single photon emission computerized tomography (SPECT) studies using fewer serotonin transporter-specific radioligands, were inconclusive (Neumeister *et al.*, 2000; Koskela *et al.*, 2008; Cheng *et al.*, 2011). However, PET studies of healthy males and females consistently found higher serotonin transporter binding in certain brain regions in the winter than in the summer. One study examined 29 Germans with ^{11}C-McN5652 PET (Buchert *et al.*, 2006) and two studies used the selective serotonin transporter radiotracer ^{11}C-DASB [^{11}C-labelled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)benzonitrile] and PET in 88 Canadians (Praschak-Rieder *et al.*, 2008) and in 57 Danes (Kalbitzer *et al.*, 2010). In the latter study, a significant gene × environment interaction effect was found, with S-carriers displaying larger seasonal serotonin transporter fluctuations in putamen as compared to long allele (L_A/L_A) carriers, with the peak in serotonin transporter levels around winter solstice (Kalbitzer *et al.*, 2010). By contrast, two later PET studies reported no effect of season on cerebral serotonin transporter binding, one using ^{11}C-DASB in 63 male UK citizens (Murthy *et al.*, 2010) and another using ^{11}C-MADAM (^{11}C-labelled-*N,N*-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine) in 40 male Swedes (Matheson *et al.*, 2015). In general, these cross-sectional studies did not take relevant factors, such as S-carriers status, sex, traveling habits, night shift work, seasonality and mood, into account.

Surprisingly, in spite of seasonal affective disorder representing a unique model for investigating the relationship between serotonin transporter availability in the brain and season-related mood variations, no studies have so far examined patients with seasonal affective disorder both in their asymptomatic and in their symptomatic phases. A single study investigated 11 patients with seasonal affective disorder in their symptomatic phase and 11 non-depressed healthy volunteers with the non-selective dopamine transporter and serotonin transporter radioligand ^{123}I-β-CIT and SPECT and reported lower thalamic-hypothalamic serotonin transporter binding in patients with seasonal affective disorder compared to healthy controls (Willeit *et al.*, 2000).

In the present study we aimed, for the first time in a longitudinal study design, to characterize how patients with seasonal affective disorder regulate the serotonin transporter across seasons, if gender and S-carrier status modifies this regulation, and to what extent serotonin transporter changes can predict symptom severity. We hypothesized that in the winter, patients with seasonal affective disorder have higher cerebral serotonin transporter levels than healthy controls, whereas the levels are comparable in the summer. Moreover, we expected a positive association between change in serotonin transporter and in seasonal affective disorder symptom severity.

Materials and methods

Participants

Healthy volunteers and potential patients with seasonal affective disorder were recruited through advertisements posted on the internet and in newspapers. The exclusion criteria were smoking, past or present neurological or psychiatric (ICD-10) disorders, use of drugs with known effects on the serotonin system, use of recreational illegal drugs including cannabis within the last week or more than 10 times in total (cannabis was allowed up to 50 times in total), significant medical history, known retinal pathology, use of photosensitizing medications, travelling to destinations with a different climate 6 months prior to any of the scans, or night shift work. Individuals with seasonal affective disorder were required not to have received bright light therapy or psychotropic drugs as treatment of their seasonal affective disorder in the past year. All participants were within a body mass index (BMI) of 19–28 kg/m^2. Subjects that met the initial screening criteria were asked to fill in the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal *et al.*, 1984), a self-assessment questionnaire that evaluates seasonal variations in sleep, social activity, mood, body weight, appetite and energy. The score on each item is summed to obtain a global seasonality score, which indexes the degree of seasonality symptoms [range: 0–24, global seasonality score (GSS) >10 consistent with seasonal affective disorder] (Kasper *et al.*, 1989). Healthy volunteers were required to have a maximum GSS of 10, reporting no problems with seasonality, whereas those with seasonal affective disorder were required to have a GSS $\geqslant 11$ and state that seasonality was a least a moderate problem. Seasonal affective disorder candidates were assessed by trained psychiatrists both in summer and winter. The seasonal affective disorder diagnosis was established when subjects met the ICD-10 diagnostic criteria for major depression and the seasonal affective disorder criteria described by Rosenthal *et al.* (1984). All referred candidates underwent a Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing *et al.*, 1990) to exclude any other axis I or axis II disorders before final inclusion. The Hamilton Rating Scale for Depression – Seasonal Affective Disorder version (SIGH-SAD) (Williams *et al.*, 1988) was used to quantify symptom severity both in summer and winter.

In total 36 patients were referred for psychiatric assessment. Of these, 12 were excluded due to co-morbidity or failure to meet diagnostic seasonal affective disorder criteria. All eligible subjects were screened with respect to S-carrier/L_A/L_A carrier status prior to inclusion. As previous data suggest a larger seasonal change in serotonin transporter availability in healthy S-carriers compared to L_A/L_A homozygotes, we chose to include only S-carriers in the healthy control group. To investigate genotype effects in seasonal affective disorder cohorts we included an L_A/L_A group of six individuals in the seasonal affective disorder group.

The scan sequence was randomized so that half of the individuals were scanned for the first time in the summer, the other half for the first time in winter; defined as a 12-week interval centred around the winter or summer solstice. All participants underwent a medical and neurological examination before each PET scan and were found to be normal. They all had normal findings on routine blood tests and their cerebral MRI scans were without any pathological findings. To measure seasonal fluctuations in mood and sleep, participants filled out online versions of the Major Depression Inventory (MDI) (range: 0–50, >21 indicates depressed mood) (Bech *et al.*, 2001; Olsen *et al.*, 2003) and the Pittsburgh Sleep Quality Index (PSQI) (global scores range: 0–21, >5 indicates sleep disturbances) (Buysse *et al.*, 1989). Information regarding menstrual cycle length, timing of current cycle and use of hormonal contraceptives were obtained from female participants on the day of the PET scan. There were no drop-outs in the healthy control group, but one subject was excluded due to technical problems with the PET image. Seven patients with seasonal affective disorder were lost to follow-up: one individual failed to go into spontaneous summer remission and six individuals decided to leave the study before follow-up for various personal reasons; none of them left the study because of the treatment restriction.

The final sample included 23 healthy S-carriers with low seasonality scores (13 females, GSS: 4.8 ± 2.1, age: 26 ± 6 years) and 17 patients with seasonal affective disorder (nine females, 11 S-carriers, GSS: 14.1 ± 2.2, age: 27 ± 9 years), all values given as mean $\pm$ standard deviation (SD). The groups were comparable with respect to age {unpaired *t*-test of mean age [$(age_{winter} + age_{summer})/2$], $P = 0.55$}, sex (Fishers exact test, $P > 0.99$) and BMI (unpaired *t*-test summer: $P = 0.15$ and winter: $P = 0.32$). Detailed sample characteristics are included in Table 1.

The study was approved by The Copenhagen Region Ethics Committee (H-1-2010-085 with amendments and KF-01-2006-20 with amendment 21971/220225, H-1-2010-91 and H-2-2010-108) and performed in accordance with the Declaration of Helsinki II. All subjects gave informed written consent prior to participation

Genotyping, plasma amino acids and hormone data

Analysis of the serotonin transporter length polymorphism carrier status was performed on DNA purified from saliva, as described in the Supplementary material. Immediately before all PET scans, blood was drawn for determination of plasma tryptophan as well as the tryptophan load relative to its amino acid carrier competitors (Knudsen *et al.*, 1990). In a subsample of females, oestradiol and progesterone levels were measured in serum collected on the day of the PET scan and analysed as detailed previously (Frokjaer *et al.*, 2015).

MRI data acquisition

Participants were scanned on a 3 T Siemens Magnetom Trio ($n = 31$) or Verio MR scanner ($n = 9$). High-resolution 3D T_1-weighted magnetization prepared rapid gradient echo was used for tissue classification and T_2-weighted turbo spin echostructural images were used for brain-masking. Images were acquired as previously described (Madsen *et al.*, 2011).

PET imaging

All PET scans were conducted using a Siemens ECAT High-Resolution Research Tomography scanner operating in 3D list-

mode. Following a 6-min transmission scan, dynamic PET scans were acquired over 90 min after injection of ^{11}C-DASB [593 ± 13 (range 536–612) MBq] over 20 s into the antecubital vein. PET acquisition and quantification were performed as previous described (Frokjaer *et al.*, 2009, 2015) and ^{11}C-DASB radiosynthesis as described elsewhere (Lehel *et al.*, 2009).

The quantification of ^{11}C-DASB was done using the Multilinear Reference Tissue Model with a fixed k_2' (MRTM2) (Ichise *et al.*, 2003), to generate the BP_{ND} (non-displaceable binding potential) using cerebellum as a reference region. We chose whole brain serotonin transporter binding as our primary outcome measure because (i) ^{11}C-DASB binding potentials are highly correlated across brain regions suggesting that serotonin transporter is regulated globally putatively through raphe nuclei serotonergic firing (Erritzoe *et al.*, 2010); and (ii) seasonal serotonin transporter changes have been described in various brain regions (Willeit *et al.*, 2000, 2008; Reimold *et al.*, 2007; Praschak-Rieder *et al.*, 2008; Kalbitzer *et al.*, 2010). A volume-weighted average of whole brain ^{11}C-DASB binding potential (global BP_{ND}) was calculated based on 17 volume-weighted grey matter segmented brain regions (amygdala, anterior cingulate gyrus, caudate, entorhinal cortex, hippocampus, insula cortex, medial inferior frontal gyrus, medial inferior temporal gyrus, occipital cortex, orbitofrontal cortex, parietal cortex, posterior cingulated gyrus, putamen, sensorimotor cortex, superior frontal gyrus, superior temporal gyrus, and thalamus):

$$Global\ BP_{ND} = \left(\sum (BP_{NDx} * volume_x)\right) / \sum volume_x \quad (1)$$

Statistical analysis

Based on previous test-retest studies ^{11}C-DASB BP_{ND} has a variability of 3.7% and a reliability of 0.89 (Kim *et al.*, 2006), thus eight subjects are needed to detect a 20% deference in BP_{ND}.

Group and seasonal differences in oestradiol and progesterone levels (females only), psychometric scores, BMI, plasma tryptophan load, k_2', non-displaceable binding (as proxy: $AUC_{cerebellum}$) and injected DASB mass/kg were tested with paired or unpaired Students *t*-tests, as appropriate, two-tailed *P*-values were adopted throughout all analyses. The correlation between the psychometric scores (PSQI global score versus MDI and SIGH-SAD versus MDI) was tested by linear correlation regression. Multicollinerity between continuous variables in multiple regression analysis was tested by calculation of the variance inflation factor (VIF) ($1 / 1 - R_2$) with a R^2 threshold of 0.75. A significance level of $P = 0.05$ was adopted throughout all analyses. All results are expressed as means ± SD.

Seasonal changes in global serotonin transporter BP_{ND} were analysed in multiple regression models of various complexities to investigate:

(i) If global BP_{ND} differs between groups in either summer or winter, using absolute global BP_{ND} as outcome variable and parameters known to affect SERT binding BMI (Erritzoe *et al.*, 2010), age (Frokjaer *et al.*, 2009; Erritzoe *et al.*, 2010), genotype (Willeit and Praschak-Rieder, 2010) and sex (Kalbitzer *et al.*, 2009) as covariates: Global $BP_{ND\ season} \sim$ group × BMI_{season} × age_{season} × genotype × sex.

(ii) If change in serotonin transporter across seasons (i.e. $\Delta BP_{ND} = BP_{ND}$ winter − BP_{ND} summer) differs between patients with seasonal affective disorder and healthy controls, using ΔBP_{ND} as an outcome variable and group as variable of interest. As seasonal affective disorder is more common in young individuals (Magnusson, 2000), females (Magnusson, 2000), and possibly in S-carriers (Rosenthal *et al.*, 1998) we included age, sex, genotype, and group × sex interaction (but not BMI, as BMI changes is part of the seasonal affective disorder symptomatology) as covariates: Global $\Delta BP_{ND} \sim$ group × sex × sex by group × mean age × genotype. In a *post hoc* analysis, we also examined three additional brain regions of relevance for depression: the raphe nuclei, hippocampus and anterior cingulate cortex.

(iii) If the relative ΔBP_{ND} (rel $\Delta BP_{ND} = \Delta BP_{ND}$ / winter BP_{ND}) adjusted for sex and genotype predicts seasonal symptom devolvement in seasonal affective disorder, defining the outcome variable as the relative difference in SIGH-SAD score [rel ΔSIGH-SAD = (winter score − summer score) / winter score]: rel $\Delta BP_{ND} \sim$ rel ΔSIGH-SAD × sex × genotype.

Statistical data analyses were carried out in GraphPad Prism version 6, GraphPad Instat version 3 and R version 3.1.

Results

Sample characteristics

Objective ratings evaluated by the psychiatrists and subjective mood ratings reported by the participants were highly correlated. SIGH-SAD scores and MDI scores correlated positively for both summer: $n = 17$, estimate = 0.89 SIGH-SAD scores per MDI score, $r^2 = 0.23$, $P = 0.05$, and winter: $n = 17$, estimate = 0.52 SIGH-SAD scores per MDI score, $r^2 = 0.34$, $P = 0.01$. As expected, individuals with seasonal affective disorder had significantly higher MDI, PSQI and SIGH-SAD scores in the winter compared to the summer (Table 1), but similar MDI and PSQI scores as the healthy controls in the summer ($P = 0.20$ and 0.41, respectively). The group difference in winter for PSQI and MDI scores was large ($P < 0.0001$ for both scores). Across all participants, winter and summer MDI and PSQI global score were highly correlated, $n = 40$, summer: estimate = 1.2 PSQI global score per MDI score, $r^2 = 0.37$, $P < 0.0001$ and winter: estimate = 3.0 PSQI global score per MDI score, $r^2 = 0.55$, $P < 0.0001$.

We did not observe seasonal differences in BMI, plasma tryptophan, k_2' or non-displaceable binding in any of the two groups (Table 1). In a subset of the female participants, we showed that serum oestradiol and progesterone were similar across seasons, suggesting no significant difference in timing of menstrual cycle in summer and winter (Table 1).

Coincidently, the healthy control group received a lower injected DASB mass/kg bodyweight in the summer (Table 1); the maximal dose given was 0.05 μg/kg whereas the maximal dose given in the winter was 0.13 μg/kg. However, when tested as a covariate in the statistical models, injected DASB mass/kg did not change the outcome

Table 1 Sample characteristics and radioligand variables

	Summer	Winter	Paired *t*-test *P*-value
Healthy controls, *n* = 23			
Clinical data			
MDI score	5.4 ± 3.6	5.0 ± 3.5	0.49
PSQI GS	3.7 ± 2.1	3.6 ± 1.8	0.79
BMI (kg/m^2)	23.1 ± 2.1	22.9 ± 2.1	0.42
Biochemistry			
Tryptophan load **(*n* = 14)**	0.13 ± 0.02	0.13 ± 0.02	0.86
Oestradiol (nmol/l) (*n* = 10)	0.13 ± 0.07	0.24 ± 0.14	0.06
Progesterone (nmol/l) (*n* = 11)	1.56 ± 1.00	4.1 ± 7.93	0.32
Radioligand variables			
Non-displaceable binding (Bq/ml)[a]	18634 ± 2650	18489 ± 3351	0.77
k_2' (per min)	0.07 ± 0.01	0.07 ± 0.001	0.57
Injected mass (μg/kg)	0.02 ± 0.01	0.04 ± 0.03	0.001
Seasonal affective disorder patients, *n* = 17			
Clinical data			
MDI score	6.4 ± 4.2	21.4 ± 7.9	<0.001
PSQI GS	4.5 ± 1.8	6.5 ± 2.3	0.02
SIGH-SAD score	2.1 ± 2.3	23.1 ± 8.8	<0.001
BMI (kg/m^2)	22.3 ± 2.5	22.1 ± 2.5	0.29
Biochemistry			
Tryptophan load	0.14 ± 0.03	0.13 ± 0.02	0.07
Oestradiol (nmol/l) (*n* = 8)	0.19 ± 0.20	0.18 ± 0.18	0.81
Progesterone (nmol/l) (*n* = 7)	6.6 ± 13.7	0.84 ± 0.40	0.32
Radioligand			
Non-displaceable binding (Bq/ml)[a]	18516 ± 3747	17600 ± 3684	0.07
k_2' (per min)	0.07 ± 0.01	0.07 ± 0.01	0.48
Injected mass (μg/kg)	0.02 ± 0.03	0.03 ± 0.06	0.68

Data are shown as mean ± SD.
[a]As evaluated by $AUC_{cerebellum}$.

of group differences and therefore this variable was not included in any of the final models.

Serotonin transporter binding

Seasonal effects across groups

In the summer, subjects with seasonal affective disorder and healthy controls had comparable global BP_{ND} levels [$n = 40$, estimate = −0.02 BP_{ND}, 95% confidence interval (CI) = −0.073 to 0.033, $R^2 = 0.20$, df = 34, $P = 0.45$].

In the winter, patients with seasonal affective disorder had higher global BP_{ND} compared to the healthy controls ($n = 40$, estimate = 0.06 BP_{ND}, 95% CI = 0.013 to 0.101, $R^2 = 0.27$, df = 34, $P = 0.01$) (Fig. 1).

Group effects across seasons (ΔBP_{ND})

An example of a seasonal affective disorder patient's ^{11}C-DASB PET image in summer and winter is shown in Fig. 2. We found a significant group effect when comparing seasonal change, ΔBP_{ND} (=BP_{ND} winter − BP_{ND} summer), adjusted for genotype, sex, age and sex × group interaction ($n = 40$, estimate = 0.10 ΔBP_{ND}, $P < 0.001$) (Fig. 3A). We found a significant effect of genotype (S-carriers > L_AL_A, $P = 0.04$) and of sex (females > males: $P = 0.02$), whereas we did not see any effect of age ($P = 0.21$). The group difference in ΔBP_{ND} was driven by the female participants, with a significant sex × group interaction effect ($P = 0.03$); in the winter, females with seasonal affective disorder upregulate whereas healthy females downregulate the serotonin transporter (sex-contrasts: seasonal affective disorder females versus healthy control females, estimate = 0.10 ΔBP_{ND}, $P < 0.001$, adjusted for all pair-wise comparisons by the Tukey *post hoc* test procedure) (Supplementary Table 1).

In a *post hoc* analysis, we examined if the differences in global ΔBP_{ND} could be replicated in brain regions of relevance for depression and this generated results similar to the global ΔBP_{ND}: raphe nuclei ($P = 0.004$), hippocampus ($P = 0.03$), anterior cingulate ($P = 0.0001$).

Relation between change in relative symptom severity and seasonal binding potential

In the seasonal affective disorder group, the relative change in binding potential was positively correlated to relative change in depressive symptom severity, as indexed by SIGH-SAD scores: relative seasonal serotonin transporter change predicted relative seasonal SIGH-SAD change

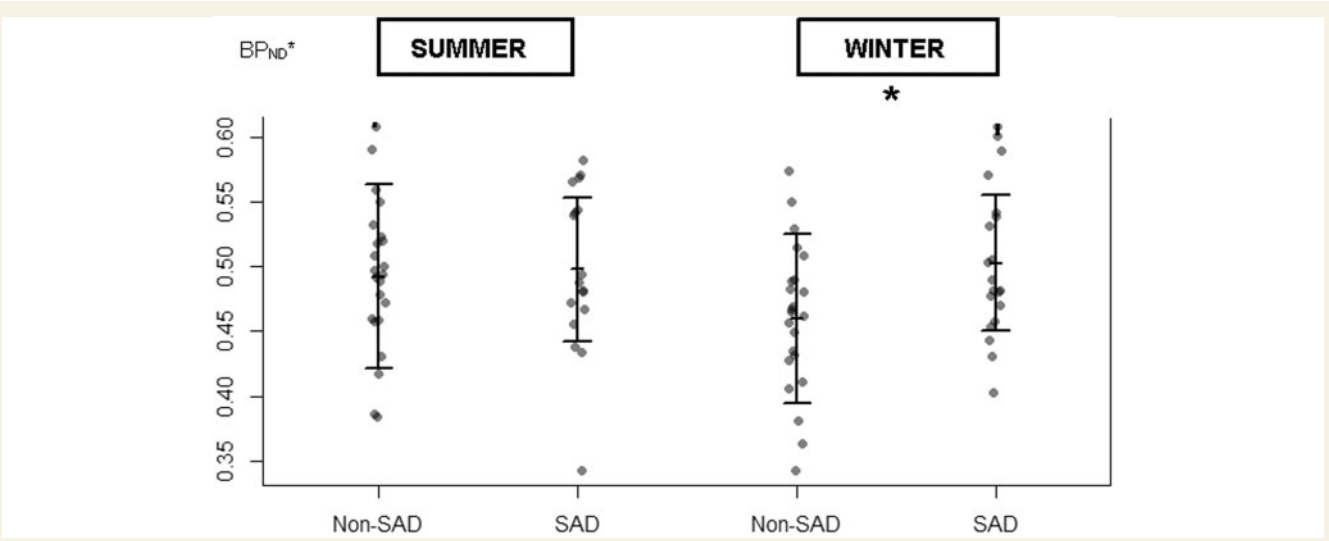

Figure 1 Seasonal effects across groups. No difference in serotonin transporter BP_{ND} was found across groups in summer ($P = 0.45$), whereas patients with seasonal affective disorder had higher serotonin transporter compared to healthy controls in the winter ($P = 0.01$). Binding potential values are adjusted for differences in age, BMI, sex and 5-HTTLPR genotype. SAD = seasonal affective disorder.

($n = 17$, estimate = 0.83 SIGH-SAD score per BP_{ND}, 95% CI = 0.28 to 1.38, $R^2 = 0.47$, df = 13, $P = 0.01$) (adjusted for genotype and sex) (Fig. 3B). In the correlation analysis, genotype constituted a significant covariate ($P_{S\text{-}carriers} = 0.04$), whereas sex did not ($P = 0.94$).

Discussion

This is the first study to compare seasonal fluctuations in cerebral serotonin transporter binding in people with and without seasonal affective disorder. First, we found that in the winter, but not in the summer, individuals with seasonal affective disorder have higher cerebral serotonin transporter binding than people without seasonality symptoms. Second, seasonal affective disorder individuals regulate their cerebral serotonin transporter binding differently than individuals without seasonality symptoms: there is a significant group effect when comparing ΔBP_{ND} in seasonal affective disorder individuals versus in healthy individuals; having seasonality symptoms, S-carrier status and being female makes you less likely to reduce your cerebral serotonin transporter binding in the winter. Third, among the seasonal affective disorder individuals, a relative larger change in serotonin transporter binding from winter to summer is associated with relatively more depressive symptoms. Overall, our findings suggest that seasonal affective disorder-prone individuals are unable to appropriately adjust their serotonin transporter binding levels to accommodate the environmental stressor of winter, thereby eliciting the symptoms of seasonal affective disorder. We can of course not rule out that the changes in serotonin transporter are appropriate adjustments to a the depressive condition, but this does not seem to be a sensible interpretation, given that higher serotonin transporter density generally is associated with lower serotonin levels (Jennings *et al.*, 2006) and given that blocking of the serotonin transporter often is used to treat seasonal affective disorder (Pjrek *et al.*, 2009).

As mentioned above, the only small study in patients with seasonal affective disorder that was carried out in the winter only, reported that as measured with a non-selective SPECT radioligand, patients had lower thalamic serotonin transporter binding compared to non-depressed individuals (Willeit *et al.*, 2000). In a subsequent *post hoc* analysis, we investigated this and found significantly higher thalamic binding potential in patients with seasonal affective disorder in the winter compared to healthy controls.

By DSM-IV definition, seasonal affective disorder is considered a sub-specifier of major depressive disorder and therefore, it makes sense to relate our findings to the outcome from studies in patients with major depressive disorder. In a recent review of cross-sectional molecular neuroimaging studies of major depressive disorder patients and healthy controls, it was established that in various brain regions, serotonin transporter binding was higher in patients with major depressive disorder (Savitz and Drevets, 2013). As an explanation, the authors suggest that a chronically higher expression of serotonin transporter leads to lower serotonin levels and decreased serotonergic neurotransmission. Another two PET studies investigated symptom severity versus serotonin transporter binding: Meyer *et al.* (2004) reported a positive association between serotonin transporter binding in various brain regions and scores of the Dysfunctional Attitudes Scale in depressed subjects, but not in healthy controls, while Cannon and co-workers (2007) found, in patients with bipolar disorder, that serotonin transporter binding correlated positively with anxiety ratings in insular cortex and dorsal cingulate cortex. In the

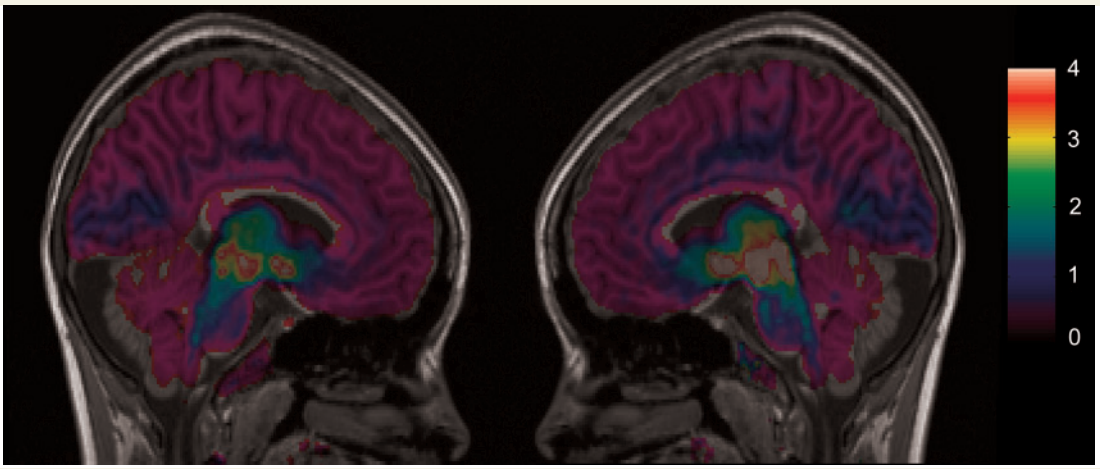

Figure 2 The ^{11}C-DASB PET image of a patient with seasonal affective disorder in summer and in winter. Cerebral serotonin transporter binding in a 22-year-old female S-carrier scanned symptom-free in the summer (*left*) and during winter when she had severe depressive symptoms and a SIGH-SAD score of 27 (*right*). The quantified ^{11}C-DASB PET image is overlaid on a T_1-weighted structural magnetic resonance image. The patient had the highest cerebral serotonin transporter in the winter.

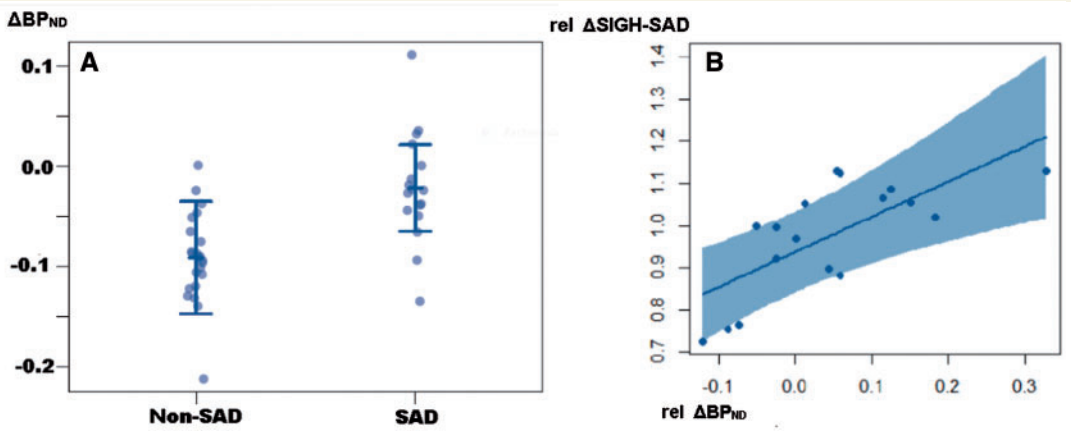

Figure 3 Group effects across seasons and correlation to seasonal affective disorder symptoms. (**A**) Cerebral serotonin transporter change across seasons between patients with seasonal affective disorder and healthy controls adjusted for sex, age, genotype and sex × group interaction effects. The ΔBP_{ND} (BP_{ND} winter − BP_{ND} summer) was significantly different between groups ($P < 0.0001$). (**B**) Relative change in symptom severity [SIGH-SAD scores winter-summer difference relative to winter (rel ΔSIGH-SAD)] was significantly associated with relative difference in global cerebral serotonin transporter binding (rel ΔBP_{ND} = $\Delta BP_{ND}/BP_{ND}$ winter) (n = 17, estimate = 0.83, R^2 = 0.47, P = 0.01).

latter study, no correlations were found between serotonin transporter binding and ratings derived from the Hamilton, the Montgomery–Åsberg Depression Rating Scale or the Inventory of Depressive Symptomatology. Notably, these cross-sectional studies do not involve comparisons to serotonin transporter binding in the patients' symptom-free phase and accordingly, the findings cannot be directly compared to our longitudinal design study.

In accordance with observations from our population-based study (Kalbitzer *et al.*, 2010), we found a gene × environment interaction effect in this sample of patients with seasonal affective disorder; the S-carriers had a significantly larger seasonal serotonin transporter binding change compared to the L_A/L_A-carriers. A limitation of the study is that it was designed to include only S-carriers in the control group, which means that we cannot conclude anything about L_AL_A carriers in this group. In continuation of this, the control group was carefully selected to include only subjects without seasonality symptoms, which means that the control group cannot be taken as representative for the population as a whole.

Irrespective of group, our data show that seasonal serotonin transporter fluctuations are particularly prominent in the female participants, in accordance with their higher frequency of affective disorders, compared to males (Magnusson and Partonen, 2005). Differences in sex

hormone profiles are likely to shape differences in susceptibility to affective disorders (Patton *et al.*, 2014) and oestradiol fluctuations are known to augment the risk of depression (Munk-Olsen *et al.*, 2006; Freeman *et al.*, 2014; Frokjaer *et al.*, 2015). Further, oestradiol levels have, in a large Norwegian study, been reported to show small, but significant seasonal fluctuation with a peak in June and a nadir in October (Bjornerem *et al.*, 2006). Thus, seasonal fluctuations in oestradiol levels may add to the vulnerability to depression. Finally, in a randomized clinical trial where 60 healthy females underwent intervention with either placebo or a gonadotrophin-releasing hormone agonist, it was found that the combination of large oestradiol decreases and higher serotonin transporter binding at follow-up relative to baseline interacted in predicting depressive responses to gonadotrophin-releasing hormone agonist manipulation (Frokjaer *et al.*, 2015). This increase in serotonin transporter binding may represent a mechanism by which sex hormone manipulation triggers depressive symptoms.

Notably, for all participants low mood and poor sleep quality were highly correlated both in summer and winter. We suggest that this may be due to a common regulation of mood and sleep mediated by serotonin (Murillo-Rodriguez *et al.*, 2012) or through interaction between mood and sleep. Sleep disturbances often coincide with a diagnosis of depression (Vandeputte and de Weerd, 2003; BaHammam *et al.*, 2015) and disruptions of circadian rhythms are common in depression, in particular seasonal affective disorder, and vice versa, depressive mood causes rumination and increased latency to sleep.

In conclusion, we find evidence that the development of depressive symptoms in winter is due to a failure to down-regulate serotonin transporter appropriately during exposure to the environmental stress of winter, especially in individuals with high risk profiles for affective disorders. We suggest that the increased serotonin transporter causes low levels of endogenous serotonin and thus facilitates symptoms of seasonal affective disorder. However, to confidently establish whether changes in serotonin transporter binding represent a primary event or a secondary compensatory regulation prompted by changes in serotonin levels, it is necessary to conduct a biannual assessment of serotonin levels in a seasonal affective disorder cohort, e.g. by quantification of serotonin 4 receptors (Haahr *et al.*, 2014). Our data suggest that intervention with selective serotonin re-uptake inhibitors may be particularly effective in female or S-carrier patients with seasonal affective disorder and that stratification according to sex and genotype may be warranted in a reanalysis of previously conducted trials in seasonal affective disorder.

Acknowledgements

We wish to thank all the participants for kindly joining the research project. We thank the John and Birthe Meyer Foundation for the donation of the cyclotron and PET scanner. The excellent technical assistance of Lone Ibsgaard Freyr, Agnete Dyssegaard, Bente Dall, Sussi Larsen, Gerda Thomsen and Svitlana Olsen is gratefully acknowledged. Part of the data was presented on a poster at the 27th European College of Neuropsychopharmacology Congress in October 2014.

Funding

This project was funded by the Lundbeck Foundation, the Danish Council for Independent Research (D.F.F.), a scholar from Dr Ejlif Trier-Hansen and wife Ane Trier-Hansen and a Brain Mind and Medicines scholar. G.M.K. serves as consultant for H. Lundbeck A/S.

Supplementary material

Supplementary material is available at *Brain* online.

References

BaHammam AS, Kendzerska T, Gupta R, Ramasubramanian C, Neubauer DN, Narasimhan M, et al. Comorbid depression in obstructive sleep apnea: an under-recognized association. Sleep Breath 2015.

Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W. The sensitivity and specificity of the major depression inventory, using the present state examination as the index of diagnostic validity. J Affect Disord 2001; 66: 159–64.

Bjornerem A, Straume B, Oian P, Berntsen GK. Seasonal variation of estradiol, follicle stimulating hormone, and dehydroepiandrosterone sulfate in women and men. J Clin Endocrinol Metab 2006; 91: 3798–802.

Buchert R, Schulze O, Wilke F, Berding G, Thomasius R, Petersen K, et al. Is correction for age necessary in SPECT or PET of the central serotonin transporter in young, healthy adults? J Nucl Med 2006; 47: 38–42.

Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193–213.

Cannon DM, Ichise M, Rollis D, Klaver JM, Gandhi SK, Charney DS, et al. Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and [11C]DASB; comparison with bipolar disorder. Biol Psychiatry 2007; 62: 870–7.

Cheng YS, Chen KC, Yang YK, Chen PS, Yeh TL, Lee IH, et al. No seasonal variation in human midbrain serotonin transporter availability in Taiwan. Psychiatry Res 2011; 194: 396–9.

Dam H, Jakobsen K, Mellerup E. Prevalence of winter depression in Denmark. Acta Psychiatr Scand 1998; 97: 1–4.

Dilsaver SC, Jaeckle RS. Winter depression responds to an open trial of tranylcypromine. J Clin Psychiatry 1990; 51: 326–9.

Erritzoe D, Frokjaer VG, Haahr MT, Kalbitzer J, Svarer C, Holst KK, et al. Cerebral serotonin transporter binding is inversely related to body mass index. Neuroimage 2010; 52: 284–9.

Freeman EW, Sammel MD, Boorman DW, Zhang R. Longitudinal pattern of depressive symptoms around natural menopause. JAMA Psychiatry 2014; 71: 36–43.

Frokjaer VG, Pinborg A, Holst KK, Overgaard A, Henningsson S, Heede M, et al. Role of serotonin transporter changes in depressive

responses to sex-steroid hormone manipulation: a positron emission tomography study. Biol Psychiatry 2015; 78: 534–43.
Frokjaer VG, Vinberg M, Erritzoe D, Svarer C, Baare W, Budtz-Joergensen E, et al. High familial risk for mood disorder is associated with low dorsolateral prefrontal cortex serotonin transporter binding. Neuroimage 2009; 46: 360–6.
Haahr ME, Fisher PM, Jensen CG, Frokjaer VG, Mahon BM, Madsen K, et al. Central 5-HT4 receptor binding as biomarker of serotonergic tonus in humans: a [11C]SB207145 PET study. 2014; 19: 427–32.
Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 2003; 23: 1096–112.
Jennings KA, Loder MK, Sheward WJ, Pei Q, Deacon RM, Benson MA, et al. Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. J Neurosci 2006; 26: 8955–64.
Kalbitzer J, Erritzoe D, Holst KK, Nielsen FA, Marner L, Lehel S, et al. Seasonal changes in brain serotonin transporter binding in short serotonin transporter linked polymorphic region-allele carriers but not in long-allele homozygotes. Biol Psychiatry 2010; 67: 1033–9.
Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FA, et al. The personality trait openness is related to cerebral 5-HTT levels. Neuroimage 2009; 45: 280–5.
Kasper S, Wehr TA, Bartko JJ, Gaist PA, Rosenthal NE. Epidemiological findings of seasonal changes in mood and behavior. A telephone survey of Montgomery County, Maryland. Arch Gen Psychiatry 1989; 46: 823–33.
Kim JS, Ichise M, Sangare J, Innis RB. PET imaging of serotonin transporters with [11C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. Journal of nuclear medicine: official publication. Soc Nucl Med 2006; 47: 208–14.
Knudsen GM, Pettigrew KD, Patlak CS, Hertz MM, Paulson OB. Asymmetrical transport of amino acids across the blood-brain barrier in humans. J Cereb Blood Flow Metab 1990; 10: 698–706.
Koskela A, Kauppinen T, Keski-Rahkonen A, Sihvola E, Kaprio J, Rissanen A, et al. Brain serotonin transporter binding of [123I]ADAM: within-subject variation between summer and winter data. Chronobiol Int 2008; 25: 657–65.
Lam RW, Zis AP, Grewal A, Delgado PL, Charney DS, Krystal JH. Effects of rapid tryptophan depletion in patients with seasonal affective disorder in remission after light therapy. Arch Gen Psychiatry 1996; 53: 41–4.
Lehel S, Madsen J, Gillings NC. HPLC methods for the purification of [11C]-labelled radiopharmaceuticals: reversal of the retention order of products and precursors. J Labelled Comp Radiopharm 2009; 52: 6
Madsen K, Haahr MT, Marner L, Keller SH, Baare WF, Svarer C, et al. Age and sex effects on 5-HT(4) receptors in the human brain: a [(11)C]SB207145 PET study. J Cereb Blood Flow Metabo 2011; 31: 1475–81.
Magnusson A. An overview of epidemiological studies on seasonal affective disorder. Acta Psychiatr Scand 2000; 101: 176–84.
Magnusson A, Partonen T. The diagnosis, symptomatology, and epidemiology of seasonal affective disorder. CNS Spectrums 2005; 10: 625–34. quiz 1–14.
Matheson GJ, Schain M, Almeida R, Lundberg J, Cselenyi Z, Borg J, et al. Diurnal and seasonal variation of the brain serotonin system in healthy male subjects. Neuroimage 2015; 112: 225–31.
Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, et al. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. Arch Gen Psychiatry 2004; 61: 1271–9.
Miller AL. Epidemiology, etiology, and natural treatment of seasonal affective disorder. Altern Med Rev 2005; 10: 5–13.
Munk-Olsen T, Laursen TM, Pedersen CB, Mors O, Mortensen PB. New parents and mental disorders: a population-based register study. JAMA 2006; 296: 2582–9.
Murillo-Rodriguez E, Arias-Carrion O, Zavala-Garcia A, Sarro-Ramirez A, Huitron-Resendiz S, Arankowsky-Sandoval G. Basic sleep mechanisms: an integrative review. Cent Nerv Syst Agents Med Chem 2012; 12: 38–54.
Murthy NV, Selvaraj S, Cowen PJ, Bhagwagar Z, Riedel WJ, Peers P, et al. Serotonin transporter polymorphisms (SLC6A4 insertion/deletion and rs25531) do not affect the availability of 5-HTT to [11C] DASB binding in the living human brain. Neuroimage 2010; 52: 50–4.
Neumeister A, Pirker W, Willeit M, Praschak-Rieder N, Asenbaum S, Brucke T, et al. Seasonal variation of availability of serotonin transporter binding sites in healthy female subjects as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. Biol Psychiatry 2000; 47: 158–60.
Neumeister A, Praschak-Rieder N, Besselmann B, Rao ML, Gluck J, Kasper S. Effects of tryptophan depletion on drug-free patients with seasonal affective disorder during a stable response to bright light therapy. Arch Gen Psychiatry 1997a; 54: 133–8.
Neumeister A, Praschak-Rieder N, Hesselmann B, Vitouch O, Rauh M, Barocka A, et al. Rapid tryptophan depletion in drug-free depressed patients with seasonal affective disorder. Am J Psychiatry 1997b; 154: 1153–5.
Neumeister A, Turner EH, Matthews JR, Postolache TT, Barnett RL, Rauh M, et al. Effects of tryptophan depletion vs catecholamine depletion in patients with seasonal affective disorder in remission with light therapy. Arch Gen Psychiatry 1998; 55: 524–30.
O'Rourke D, Wurtman JJ, Wurtman RJ, Chebli R, Gleason R. Treatment of seasonal depression with d-fenfluramine. J Clin Psychiatry 1989; 50: 343–7.
Olsen LR, Jensen DV, Noerholm V, Martiny K, Bech P. The internal and external validity of the Major Depression Inventory in measuring severity of depressive states. Psychol Med 2003; 33: 351–6.
Partonen T. Estrogen could control photoperiodic adjustment in seasonal affective disorder. Med Hypotheses 1995; 45: 35–6.
Partonen T, Lonnqvist J. Moclobemide and fluoxetine in treatment of seasonal affective disorder. J Affect Disord 1996; 41: 93–9.
Patton GC, Coffey C, Romaniuk H, Mackinnon A, Carlin JB, Degenhardt L, et al. The prognosis of common mental disorders in adolescents: a 14-year prospective cohort study. Lancet 2014; 383: 1404–11.
Pjrek E, Konstantinidis A, Assem-Hilger E, Praschak-Rieder N, Willeit M, Kasper S, et al. Therapeutic effects of escitalopram and reboxetine in seasonal affective disorder: a pooled analysis. J Psychiatr Res 2009; 43: 792–7.
Praschak-Rieder N, Willeit M, Wilson AA, Houle S, Meyer JH. Seasonal variation in human brain serotonin transporter binding. Arch Gen Psychiatry 2008; 65: 1072–8.
Reimold M, Smolka MN, Schumann G, Zimmer A, Wrase J, Mann K, et al. Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. J Neural Transm (Vienna) 2007; 114: 635–9.
Rosenthal NE, Mazzanti CM, Barnett RL, Hardin TA, Turner EH, Lam GK, et al. Role of serotonin transporter promoter repeat length polymorphism (5-HTTLPR) in seasonality and seasonal affective disorder. Mol Psychiatry 1998; 3: 175–7.
Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, et al. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 1984; 41: 72–80.
Savitz JB, Drevets WC. Neuroreceptor imaging in depression. Neurobiol Dis 2013; 52: 49–65.

Thaler K, Delivuk M, Chapman A, Gaynes BN, Kaminski A, Gartlehner G. Second-generation antidepressants for seasonal affective disorder. Cochrane Database Syst Rev 2011; Cd008591

Vandeputte M, de Weerd A. Sleep disorders and depressive feelings: a global survey with the Beck depression scale. Sleep Med 2003; 4: 343–5.

Willeit M, Praschak-Rieder N. Imaging the effects of genetic polymorphisms on radioligand binding in the living human brain: A review on genetic neuroreceptor imaging of monoaminergic systems in psychiatry. Neuroimage 2010; 53: 878–92.

Willeit M, Praschak-Rieder N, Neumeister A, Pirker W, Asenbaum S, Vitouch O, et al. [123I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. Biol Psychiatry 2000; 47: 482–9.

Willeit M, Sitte HH, Thierry N, Michalek K, Praschak-Rieder N, Zill P, et al. Enhanced serotonin transporter function during depression in seasonal affective disorder. Neuropsychopharmacology 2008; 33: 1503–13.

Williams JBLM, Rosenthal NE, Amira L, Terman M. Structured interview guide for the Hamilton depression rating scale - seasonal affective disorder version. In: Institute NYSP, editor, New York, New York State Psychiatric Institute, 1988.

Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, et al. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry 1990; 47: 589–93.