European Neuropsychopharmacology (2018) 000, 1–10

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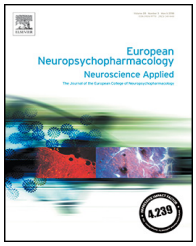

Seasonality-resilient individuals downregulate their cerebral 5-HT transporter binding in winter – A longitudinal combined ^{11}C-DASB and ^{11}C-SB207145 PET study

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Received 13 October 2017; received in revised form 17 May 2018; accepted 25 June 2018
Available online xxx

KEYWORDS
Resilience;
PET imaging;
Seasonal affective disorder;
Serotonin transporter;
Serotonin 4 receptor

Abstract
We have recently shown that the emergence and severity of seasonal affective disorder (SAD) symptoms in the winter is associated with an increase in cerebral serotonin (5-HT) transporter (SERT) binding. Intriguingly, we also found that individuals *resilient* to SAD downregulate their cerebral SERT binding in the winter. In the present paper, we provide an analysis of the SERT- and 5-HT dynamics as indexed by 5-HT$_4$ receptor (5-HT4R) binding related to successful stress coping. We included 46 ^{11}C-DASB positron emission tomography (PET) scans ($N = 23$, 13 women, age: 26 ± 6 years) and 14 ^{11}C-SB207145 PET scans (7 participants, 3 women, age: 25 ± 3 years) from 23 SAD-resilient Danes. Data was collected longitudinally in summer and winter.

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https://doi.org/10.1016/j.euroneuro.2018.06.004
0924-977X/

Please cite this article as: B. Mc Mahon et al., Seasonality-resilient individuals downregulate their cerebral 5-HT transporter binding in winter – A longitudinal combined 11C-DASB and 11C-SB207145 PET study, European Neuropsychopharmacology (2018), https://doi.org/10.1016/j.euroneuro.2018.06.004

We found that compared to the summer, raphe nuclei and global brain SERT binding decreased significantly in the winter ($p_{raphe} = 0.003$ and $p_{global} = 0.003$) and the two measures were positively correlated across seasons (summer: $R^2 = 0.33$, $p = .004$, winter: $R^2 = 0.24$, $p = .018$). A voxel-based analysis revealed prominent changes in SERT in clusters covering both angular gyri ($0.0005 < p_{corrected} < 0.0016$), prefrontal cortices ($0.00087 < p_{corrected} < 0.0039$) and the posterior temporal and adjacent occipital cortices ($0.0001 < p_{corrected} < 0.0066$). We did not observe changes in 5-HT4R binding, suggesting that 5-HT levels remained stable across seasons. We conclude that resilience to SAD is associated with a global downregulation of SERT levels in winter which serves to keep 5-HT levels across seasons.

1. Introduction

Stress can have a negative influence on the human brain, and much attention has been directed towards identifying disease-specific changes in the depressed brain. However, it has been argued that resilience, i.e., the ability to withstand severe stress deserves much more attention (King, 2016). We believe that identifications of factors that determine resilience is a viable research strategy in the search for novel therapeutic targets. Accordingly, we here propose to use seasonal changes in daylight as a naturalistic model to examine brain changes in the serotonin (5-HT) system related to resilience to affective disorders.

Seasonal fluctuations in mood and physiology are particularly frequent at latitudes with pronounced season-associated variation in daylight. At the latitude of Copenhagen (55.7°N), the day is twice as long at summer solstice (> 1000 min) as it is at winter solstice (<500 min). The circadian system is entrained by the daylength and the seasonal transitions are associated with marked changes in neurobiology and behavior, which can ultimately lead to seasonal affective disorder (SAD). SAD is defined as a subspecifier of major depressive disorder (MDD) or bipolar disorder (BD) where season-dependent emergence and remission of affective episodes has been present for at least two consecutive years and outnumber any non-seasonal episodes. Characteristic features of SAD include vegetative symptoms such as hyperphagia, increased appetite, weight gain and depressive- or euphoric symptoms (American Psychiatric Association, 2013; Rosenthal et al., 1984). A cross-sectional survey found that as many as 85% of Copenhagen inhabitants experienced low mood, fatigue and/or increased appetite in winter, 5% fulfilled the diagnostic criteria of a depressive episode and only 10% were unaffected by the adversity of winter (Dam et al., 1998). Thus, deprivation of daylight is a potent trigger of depression and only a minority of the population manage to withstand this distress, i.e., are resilient to depression.

SAD is more prevalent in young adults, in females (Magnusson and Partonen, 2005), and in carriers of the short allele of the 5-HT transporter linked polymorphic region (5-HTTLPR) (Johansson et al., 2003; Praschak-Rieder et al., 2002; Rosenthal et al., 1998; Willeit et al., 2003). Accordingly, several neuroimaging studies have in population-based samples addressed whether 5-HT transporter (SERT) levels differ between seasons (Buchert et al., 2006; Cheng et al., 2011; Kalbitzer et al., 2010; Koskela et al., 2008; Matheson et al., 2015; Murthy et al., 2010; Neumeister et al., 2000; Praschak-Rieder et al., 2008). The outcome of cross-sectional studies that used highly selective SERT positron emission tomography (PET) radioligands suggests that cerebral SERT is higher in the winter than in the summer, at least in mixed-sex healthy populations investigated at high latitudes. We have previously in a longitudinal design compared seasonal SERT changes in individuals with and without SAD. We found that SAD patients upregulate their cerebral SERT in winter, and that the upregulation was positively correlated with the emergence of depressive symptoms (Mc Mahon et al, 2016). Our finding was subsequently corroborated by a Canadian study (Tyrer et al., 2016). These studies suggest that depressive symptoms are associated with a decrease in cerebral 5-HT levels. Although cerebral 5-HT levels currently cannot be measured in vivo there is accumulating preclinical and clinical evidence that cerebral 5-HT_4 receptor (5-HT4R) binding is inversely related to brain 5-HT levels (Ettrup et al., 2011; Haahr et al., 2014; Licht et al., 2009). Most notably, three weeks of fluoxetine intervention in healthy volunteers results in a significant reduction in cerebral 5-HT4R binding (Haahr et al, 2014).

In conclusion, at high latitudes daylight deprivation is a potent stressor that can elicit detectable changes in brain chemistry and behavior, especially in predisposed individuals. However, the stress-model is incomplete; for example, not all young female S-carriers develop SAD. In the present study, individuals resilient to SAD underwent PET imaging with ^{11}C-DASB- and ^{11}C-SB207145 both summer and winter to measure SERT and 5-HT4 R binding.

2. Experimental procedures

2.1. Participants

2.1.1. Recruitment

We recruited SAD-resilient individuals, i.e., people who in spite of being predisposed to SAD in that they were young 5-HTTLPR S-carriers living at a Northern latitude (Magnusson and Partonen, 2005; Willeit et al., 2003) were seasonally euthymic. The screening criteria (< 45 years of age, body mass index (BMI) of 19-28 kg/m^2, non-smokers, stable diurnal cycle, no past- or present use of bright light therapy, no past- or present neurological/psychiatric disorders or other significant medical history) were advertised on community websites, on bulletin boards in educational facilities and printed in the local newspaper. Subjects that met these requirements were referred to an online survey

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where detailed information regarding medical history, family history of psychiatric disorders, head trauma, alcohol consumption, lifetime use of recreational drugs, timing of menstrual cycle, use of oral contraceptives and prior participations in similar studies were acquired. Eligible candidates underwent a phone- or personal interview to ensure that they adhered to the screening criteria, to provide oral study-information, to ensure absence of overt seasonality, to make sure they had not been or were planning to travel to destinations at different latitudes and to make sure that females were not pregnant or planning to become pregnant. The volunteers that met all requirements received written information of the study and filled out an online Danish version (used in Madsen et al. (2016)) of the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al., 1984). If both SPAQ response and subjective assessment indicated absence of seasonality (Global Seasonality Score (GSS) < 10 and stating to have no problems with seasonality), saliva was sampled in a collection kit (Oragene DNA saliva kit OG-500 from DNAgenotek) for genotyping of 5-HTTLPR. In order to maximize resiliency, we included only 5-HTTLPR S-carriers in the study. Accordingly, genotyping was done prior to final inclusion. A total of 23 participants completed ^{11}C-DASB PET scans within a six-week interval centered around winter and summer solstice; half of the participants were scanned first time in winter and the other half first time in the summer. All 23 participants had unremarkable medical, neurological and biochemical findings and no pathological findings were done on the structural magnetic resonance imaging (MRI) brain scan. 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. All study participants consented to participation, in accordance with The Declaration of Helsinki II.

The cohort served as healthy control subjects in comparison to people with seasonal affective disorder (Mc Mahon et al., 2016), nine of the individuals were included in a study of affective memory (Jensen et al., 2015), in a sucrose taste sensitivity test (Andersen et al., 2014), and seven ^{11}C- SB207145 PET scans also were included in larger studies of cortisol awakening response (Jakobsen et al., 2016) and of aggression (da Cunha-Bang et al., 2016).

2.2. Psychometric assessments

The Major Depression Inventory (MDI) (range: 0–50, >21 indicates depressed mood) (Bech et al., 2001), the Pittsburgh Sleep Quality Index global scores (PSQI) (range: 0–21, >5 indicates sleep disturbances) (Buysse et al., 1989), Cohen's Perceived Stress Scale (PSS) (range: 0–40, no cut-off adapted to indicate stress) and the Stressful Life Event scale (SLE) (Roohafza et al., 2011) were administered summer and winter, in conjunction with the PET scans.

2.3. Genotyping and amino acids

The allelic status of the 5-HTTLPR was analyzed by a TaqMan 5′-exonuclease allelic discrimination assay as described previously (Mc Mahon et al., 2016). To examine if there were seasonal differences in dietary tryptophan intake, possibly changing brain 5-HT levels, plasma amino acid measurements were performed in a subset of the sample ($N = 14$). For this purpose, venous blood samples were obtained in heparinized vials immediately prior to the PET scan and kept on ice until precipitation with sulfosalicylic acid. The supernatant fluid was stored at −80 °C until the time of analysis. Norleucine was used as an internal standard and high-pressure liquid chromatography (HPLC) was used to measure the tryptophan concentrations in the samples. Tryptophan crosses the blood-brain barrier by facilitated transport, in competition with other large neutral amino acids. To take this into account, we also measured the concentrations of these and calculated both absolute plasma tryptophan values as well as the tryptophan load relative to its competitors (Knudsen et al., 1990).

2.4. Neuroimaging protocols

2.4.1. Positron emission tomography data acquisition

Cerebral SERT and 5-HT4R were measured with the highly selective radioligands ^{11}C-DASB (Wilson et al., 2002) (mean injected dose 592 ± 15 MBq) and ^{11}C-SB207145 (Marner et al., 2010) (mean injected dose 599 ± 11 MBq). Detailed information about data acquisition can be found in Frokjaer et al. (2015) and Greve et al. (2014).

Kinetic modeling was performed in FreeSurfer (Greve et al., 2014), using the Multilinear Reference Tissue Model 2 (MRTM2) (Ichise et al., 2003) to estimate SERT- and 5-HT4R non-displaceable binding potential (BP_{ND}) (Ichise et al., 2003). The cerebellum (excluding vermis) was used as reference region and thalamus, caudate, putamen and pallidum as high-binding regions for estimation of k_2'. A detailed description of the kinetic modeling can be found in the Supplemental material and methods (methods 1).

2.4.2. Magnetic resonance imaging data acquisition

All MRI scans were processed and analyzed using FreeSurfer (Fischl, 2012) version 5.3 and MATLAB R2013a (8.1.0.604) 64bit, as described in Greve et al. (2014) and in Nørgaard et al. (2015). A detailed description of the voxel-based data analysis can be found in the Supplemental material (methods 2).

2.4.3. Volume of interest delineation

An automatic and thus user-independent approach was used to delineate volumes of interest (VOIs) (Svarer et al., 2005). A detailed description of the volume delineation and co-registration of the individual PET- and MRI images can be found in Kalbitzer et al. (2009a). In brief, template VOIs sets were transferred to the individual dynamic PET image space after co-registration to create a probability map. Then time activity curves (TACs) were automatically extracted and used for kinetic modeling. A volume-weighted average of the left and right VOI was used for the bilateral regions. The rostral raphe nuclei were delineated from parametric images by a method that provides an optimal delineation as it primarily encompasses the dorsal raphe nucleus (DRN) (Kalbitzer et al., 2009b). Since this region also partly includes the median raphe nucleus, we will refer to this region as the raphe nuclei.

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A single combined outcome measure for SERT or 5-HT4R binding was generated by calculating a summed estimate of radiotracer binding across all brain regions, by averaging the left and right binding of 17 volume-weighted grey matter regions ((Global BP_{ND} = (Σ(BP_{NDx} * $volume_x$))/ $\Sigma volume_x$) (Mc Mahon et al., 2016).

2.5. Statistical analysis

2.5.1. Psychometrics, biochemistry and radioligand variables

Summer versus winter measurements of psychometric data (MDI, PSQI, PSS, recent SLE), plasma tryptophan load, BMI, k_2' and non-displaceable binding in terms of the area under the time activity curve of cerebellum ($AUC_{cerebellum}$) were compared by means of two-tailed paired *t*-tests. Eventual season-related differences in DASB and SB207145 injected mass per kg body weight was examined by means of a Wilcoxon signed rank test.

2.5.2. Region-based statistical analysis

The effects of season, sex and sex-by-season interaction on raphe nuclei and global SERT and 5-HT4R binding were investigated by means of separate single-factor repeated measures ANOVA. By design, the sample had a narrow age- and BMI span, mean ± SD (range): age 26 ± 7 (19-43) years and BMI: 23.2 ± 1.9 (19.5-27.4) kg/m^2 and therefore, we did not need to correct for these variables. Based on Marner et al. (2010), the sample sizes required to detect a 15% difference with 80% power for the tracer ^{11}C- SB207145 PET are as follows: PFC = 4, ACC = 14, amygdala = 8, and hippocampus = 5.

Because stable mood may also depend on synchronization of SERT levels in DRN with its projection areas (Hahn et al., 2014), raphe nuclei and global SERT binding summer and winter was evaluated by simple linear regression, and the slopes from the respective analyses were compared by a *t*-test to asses if the correlation changed across seasons.

2.5.3. Univariate vertex- and voxel-wise statistical analyses

The FreeSurfer generated SERT BP_{ND} images in standard surface and volume space were used in the whole-brain surface vertex- and sub-cortical voxel-wise analyses of seasonal effects using paired *t*-tests (H_0: winter-summer = 0). To control the false-positive rate, correction for multiple comparisons was executed in a clustering framework using non-parametric permutation tests as described by Nichols and Holmes (2002); a detailed description can be found in the Supplementary material (methods 3). The statistically significant cluster extent for our whole-brain search volume was 372 mm^2 in surface-space and 1475 mm^3 in volume-space. To investigate the extent to which SERT and 5-HT4R were concomitantly regulated, we used the brain clusters identified in the univariate vertex/voxel-wise SERT analysis as a mask in the whole-brain 5-HT4R BP_{ND} images and tested for seasonal differences in the included clusters using a paired *t*-test.

For all other statistical analyses, we adopted a significance level of $p = .05$. Results are reported as mean ± SD unless stated otherwise.

Statistical data analyses were carried out in GraphPad Prism version 6, GraphPad Instat version 3, R version 3.1 and MATLAB R2013a (8.1.0.604) 64 bit.

3. Results

3.1. Psychometrics, biochemistry and radioligand variables

On the day of the PET scans, both summer and winter, participants were euthymic (sample maximum MDI = 15), free of overt sleeping disturbances (sample maximum PSQI global score = 8) and felt at ease (sample maximum PSS = 22). No seasonal differences were found in sleep or mood ratings ($N = 23$, PSQI global score: summer (S): 3.7 ± 2.1 versus winter (W): 3.6 ± 1.8, $p = .79$ and MDI: S: 5.5 ± 3.6 versus W: 5.0 ± 3.5, $p = .40$). Self-reported stress levels were marginally higher in summer ($N = 23$, PSS: S: 10.3 ± 5.9 versus W: 7.9 ± 4.8, $p = .05$). No difference between summer and winter in the number of recent stress-related events ($N = 23$, recent SLE: S: 2.2 ± 2, W: 2.7 ± 2.4, $p = .40$) was found. The same applied to plasma tryptophan levels ($N = 14$, S: 0.13 ± 0.02, W: 0.13 ± 0.02, $p = .86$), BMI ($N = 23$, S: 23.1 ± 2.1 kg/m^2, W: 22.9 ± 2.1 kg/m^2, $p = .42$), k_2' ($N = 23$, S: 0.064 ± 0.010 min^{-1}, W: 0.065 ± 0.016 min^{-1}, $p = .57$), or $AUC_{cerebellum}$ ($N = 23$, S: 18.6 ± 2.7 (kBq/ml) W: 18.5 ± 3.4 (kBq/ml), $p = .77$). Coincidently, the injected ^{11}C-DASB mass/kg bodyweight was significantly lower in the summer (median = 0.01 μg/kg) than in the winter (median = 0.02 μg/kg), Wilcoxon $p = .001$. However, the mass doses given were small (max 0.05 μg/kg in summer and 0.13 μg/kg in winter) and when we in a separate cohort of 108 healthy individuals tested the effect on BP_{ND} of injected mass DASB, no correlation between BP_{ND} and injected mass/kg was found (Supplementary material).

3.2. Region based analysis

3.2.1. Sex- and season differences in SERT binding

The SERT binding was significantly lower in the winter compared to the summer for both raphe nuclei ($N = 23$, S: 4.5 ± 0.7 versus W: 4.1 ± 0.6, $F_{season} = 10.92$, $p = .003$) and its projection areas (global binding) ($N = 23$, S: 0.7 ± 0.09 versus W: 0.66 ± 0.06, $F_{season} = 11.47$, $p = .003$) (Fig. 1(A)). For the global SERT binding, we found a significant sex-by-season interaction effect ($F_{sex\text{-}by\text{-}season} = 5.54$, $p = .03$) with the female participants showing a larger decrease in global BP_{ND} from summer to winter than their male counterparts (Fig. 1(B)). We found no sex-by-season interaction the raphe nuclei SERT binding ($F_{sex\text{-}by\text{-}season} = 1.73$, $p = .20$). Within seasons, there were no sex differences in global SERT binding ($F_{sex} = 0.3740$, $p = .55$) or raphe nuclei binding ($F_{sex} = 0.56$, $p = .46$).

Both in the summer and winter, raphe nuclei SERT binding was positively correlated to global SERT binding ($N = 23$, S: $R^2 = 0.33$, $p = .004$ and W: $R^2 = 0.24$, $p = .018$) (Fig. 2). There was no significant season-related difference between the slope estimates (two-tailed, 95% confidence interval, $p = .65$).

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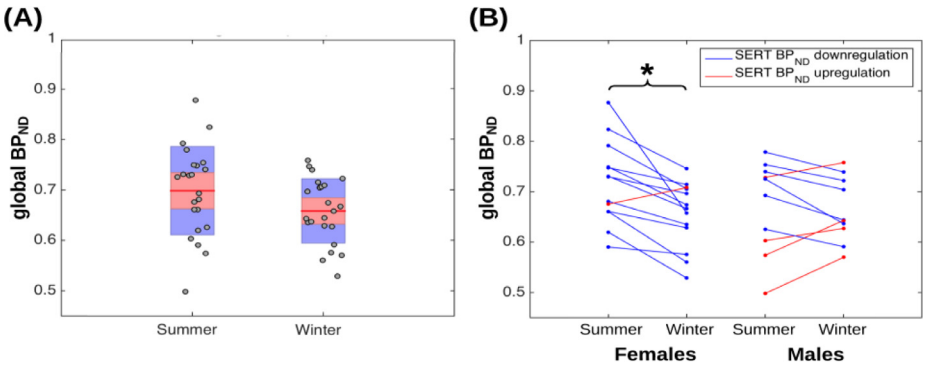

Fig. 1 (A) Global SERT binding across seasons. A single-factor repeated measures ANOVA showed a significant down-regulation of global SERT BP_{ND} in the winter for all participants, $P_{season} = 0.003$. (B) Global SERT binding across seasons in males and females. The sex-by-season interaction effect, $P_{sex\ by\ season} = 0.03$, was driven by the female participants showing a larger down-regulation in BP_{ND} from summer to winter. Individual summer to winter adjustments are indicated by the colour bars (red = up-regulation, blue = down-regulation). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

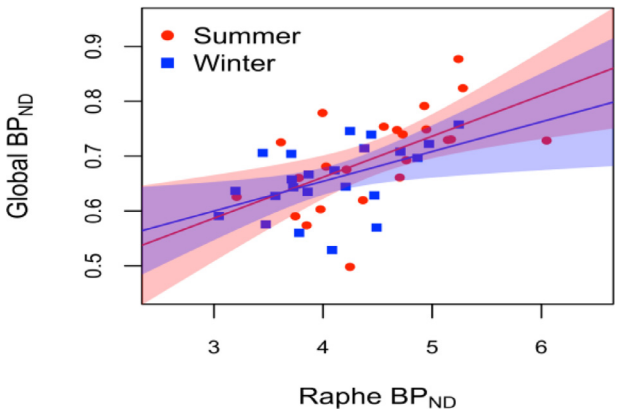

Fig. 2 Correlation between raphe nuclei SERT- and global SERT across seasons. Irrespective of season, raphe nuclei BP_{ND} was positively correlated to global BP_{ND}. There was no significant difference in slope estimates (two-tailed, 95%-confidence interval, $p = .65$). Red dot: summer, blue square: winter. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

In a post hoc analysis, we compared summer and winter SERT BP_{ND} by means of a paired *t*-test in brain regions of relevance for seasonality and affective symptoms, including the anterior cingulate cortex (ACC) (Praschak-Rieder et al., 2008), prefrontal cortex (PFC) (Praschak-Rieder et al., 2008) and the amygdala (Fisher et al., 2014). For these regions, we found significantly lower SERT binding in winter compared to summer in ACC (S: 0.91 ± 0.132, W: 0.86 ± 0.126, $p = .003$) but no difference in PFC (S: 0.52 ± 0.088, W: 0.49 ± 0.056, $p = .072$ or amygdala: S: 2.02 ± 0.34, W: 1.9 ± 0.259, $p = .08$), *p*-values are Bonferroni corrected.

3.2.2. Sex- and season differences in 5-HT4R binding

We observed no significant differences in 5-HT4R binding from summer to winter (raphe nuclei: $N = 7$, S: 0.45 ± 0.07 versus W: 0.49 ± 0.12, $F_{season} = 0.7$, $p = .44$, global: S: 0.91 ± 0.06 versus W: 0.96 ± 0.13, $F_{season} = 1.96$, $p = .22$). There were no sex-by-season interactions (raphe nuclei: $F_{sex\text{-}by\text{-}season} = 0.08$, $p = .78$, global: $F_{sex\text{-}by\text{-}season} = 0.28$, $p = .62$) and no sex-differences within seasons (raphe nuclei: ($F_{sex} = 0.26$, $p = .63$, global: $F_{sex} = 0.08$, $p = .79$) in either region.

In addition, in the post hoc analysis with paired *t*-tests, we found no significant seasonal differences in 5-HT4R binding in the depression-related brain regions (ACC: S: 0.93 ± 0.14 versus W: 0.88 ± 0.11, $p = .39$, PFC: S: 0.84 ± 0.12 versus W: 0.79 ± 0.07, $p = .57$ and amygdala: S: 0.92 ± 0.18 versus W: 0.87 ± 0.09, $p = .14$), *p*-values are Bonferroni corrected.

3.3. Univariate vertex- and voxel-based analysis

3.3.1. Voxel-based analysis of SERT binding

Several larger clusters showed a significant down-regulation of SERT binding from summer to winter, including bilateral clusters across the angular gyri (GA, also referred to as Brodmann area 39, temporoparietal junction, posterior middle temporal gyrus or temporo-parieto-occipital cortex) ($0.0005 < p_{corrected} < 0.0016$), bilateral clusters across the inferior frontal sulcus in prefrontal cortex ($0.00087 < p_{corrected} < 0.0039$) and two large clusters located bilaterally across occipital cortices and the posterior inferior temporal cortices ($0.0001 < p_{corrected} < 0.0066$). In the right hemisphere, the latter also extended to the posterior medial temporal cortex (Fig. 3 and Table 1). A post hoc analysis revealed that the detected clusters supported the sex-by-season effect found in the region-based analysis, and no significant differences were detected in any sub-cortical clusters.

3.3.2. Voxel-based 5-HT4R analysis

In the voxel-based analysis, comparing summer to winter differences in 5-HT4R binding, we found that a minor part of the clusters had voxel-wise significantly lower 5-HT4R binding in the winter (i.e., the left inferior frontal sulcus and the

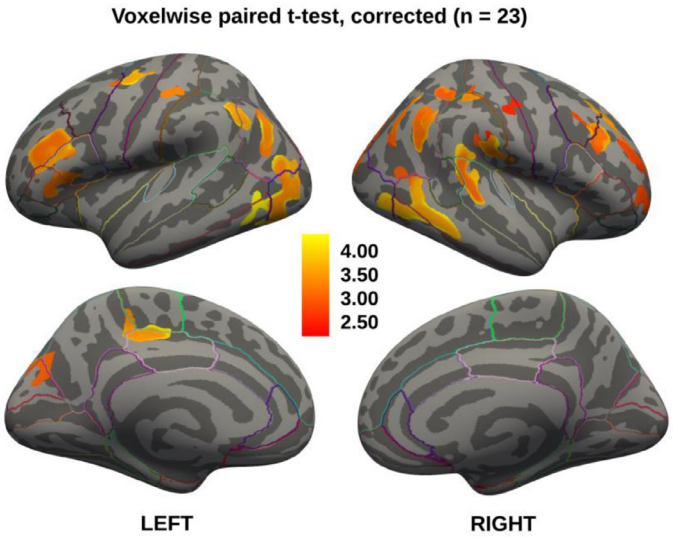

Fig. 3 Univariate surface-based SERT analysis. The univariate surface-based analysis depicted on the inflated brain. The map displays the t-values of clusters with significant changes in ^{11}C-DASB binding across seasons (summer –winter), corrected for multiple comparisons. The top row: cortical presentation, the bottom row: mesial view.

Table 1 Univariate surface-based analysis of seasonal variation in ^{11}C-DASB binding.

Region	Cluster *t*-value	Size [mm^2]	*X*	*Y*	*Z*
Left hemisphere					
Inferior temporal gyrus	4.36	1552	−44	−64	−5
Angular gyrus	4.1	445	−40	−64	34
	3.99	382	−49	−57	37
Precentral gyrus	3.97	385	−43	−6	44
Inferior frontal triangularis	3.93	875	−42	17	21
Inferior frontal sulcus	3.52	393	−39	33	−2
Occipital Cuneus	3.01	529	−21	−86	25
Right hemisphere					
Middle temporal gyrus	4.59	1236	64	−44	−5
Superior temporal gyrus	3.95	613	63	−38	13
Inferior frontal sulcus	3.85	452	36	28	37
	3.55	901	26	20	42
	3.23	434	31	48	5
Angular gyrus	3.76	367	34	−51	37
	3.7	544	46	−58	27
	3.6	445	33	−62	46
Occipital Cuneus	3.7	968	15	−87	22

Statistically significant clusters identified in the univariate surface-based analysis of seasonal variation in ^{11}C-DASB binding. Coordinates are in Montreal Neurological Institute (MNI) space.

left medial occipital cortex) whereas no clusters had significantly higher 5-HT4R binding in the winter. No seasonal difference in 5-HT4R binding was found in the majority of the clusters in the brain mask defined by the seasonal changes in SERT.

4. Discussion

We here introduce a novel approach, using the naturally occurring deprivation of daylight in winter and strict screening criteria to model resilience to seasonality. This naturalistic model allows for longitudinal measures and an ethically sound stress exposure that can be presumed to be homogeneously applied to all participants. We conducted for the first time a longitudinal investigation of cerebral SERT and 5-HT4R binding, to define brain dynamics in the 5-HT system conferring resilience to affective disorders during stress conditioning, here probed by winter season. As could be expected from the inclusion criteria, participants presented stable measures of mood and sleep quality across seasons. When exposed to the environmental stress of winter, SERT binding in the raphe nuclei and in raphe projection areas (particularly cortically) decreased significantly and

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the positive correlation between regions was preserved. We were not able to detect any significant changes in 5-HT4R binding in neither analysis, suggesting that in the SAD-resilient cohort, the cerebral 5-HT levels remained stable across seasons. To strengthen the power of our study, we included an only young seasonality-resilient S-carriers. Although this improved the power to detect differences, it also potentially makes the study less generalizable to the population as a whole. Apparently at odds with our finding, two previous ^{11}C-DASB PET studies examining seasonal SERT fluctuations cross-sectionally found that SERT binding was inversely correlated with daylight minutes in healthy males and females dwelling at high latitudes (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008). An important difference between these studies and our design is, however, that their participants were not recruited to address the specific seasonality question but from other ongoing studies. Further, they employed a cross-sectional design and the individuals were not characterized with regard to seasonality symptoms. Thus, the different outcome from these cross-sectional studies in a mixed cohort of healthy people and studies in SAD patients (Mc Mahon et al., 2016;Tyrer et al., 2016) strongly suggest that SAD-resilient individuals are characterized by unique seasonal regulation of their cerebral SERT and differ from the general population.

In agreement with Hahn et al. (2014), we found a preserved positive correlation between raphe nuclei- and global SERT binding across seasons in individuals with stable mood. Given the intimate connections between the suprachiasmatic nucleus (SCN) and the DRN (Morin, 2013), we hypothesize that raphe nuclei SERT levels are entrained to photoperiod by the SCN to orchestrate a harmonized serotonergic response across projection areas. This interpretation is supported by animal data: disconnection of the pathway between retinal ganglion cells (iRGC) (Sexton et al., 2012) and the DRN reduces raphe 5-HT levels and causes depressive-like behavior in gerbils (Luan et al., 2011), and DRN levels of 5-HT are higher under long-day conditions compared to short-day conditions (Goda et al., 2015). Nevertheless, we found that seasonal SERT regulation in the raphe nuclei and in projection areas differed in one important aspect: Females displayed larger seasonal SERT fluctuations in projection areas compared to males whereas no sex-differences were found in the raphe nuclei.

It is conceivable that differences in sex hormones and their interaction with SERT expression affect men and women differently, when it comes to vulnerability to seasonality. Thus, factors conferring risk- or resilience to SAD may be sex-specific and give rise to differences in the clinical presentation of symptoms. Accordingly, a survey of 2620 healthy Swedes (56% females) concluded that females have 1.5 times higher risk of seasonally related mood swings compared to males, and that men were more likely to experience hypersomnia whereas women were more likely to experience hyperphagia during winter (Chotai et al., 2004). We also found that compared to the male participants, females had numerically larger seasonal SERT fluctuations. Studies of the estradiol-SERT coupling suggests that estradiol affects mood via SERT signaling (Borrow and Cameron, 2014; Frokjaer et al., 2015). In addition, the risk of developing a depressive episode is markedly increased at time points where estradiol levels fluctuate in a woman's life, e.g. during puberty (Patton et al., 2014), post partum (Munk-Olsen et al., 2006) and in transition to menopause (Freeman et al., 2014). Our study suggests that a flexible SERT regulation can also serve a protective mechanism, i.e., downregulation happens during adverse experiences, and we speculate that the differences in SERT regulation stem from sex-specific neurobiological underpinnings. Females have an increased risk for developing affective disorders and may also more critically depend on a larger downregulation of cerebral SERT to stay mentally healthy in the face of seasonal stress. Thus, these observations imply presence of a sex-specific modulation of SERT expression unique to the projection areas. Similarly, a cross-sectional ^{11}C-DASB PET study found that healthy twin siblings of MDD patients had 35% lower SERT binding in the dorsolateral prefrontal cortex compared to twins without any known genetic risk (Frokjaer et al., 2009). Thus, regulation of cortical SERT seems pivotal to mental resilience in the presence of genetic- and/or environmental strain: the higher the risk load, the more SERT levels must decrease to maintain a stable mood.

Our finding of seasonal SERT changes across several cortical clusters fits well with the emotional and cognitive impairments associated with affective disorders. Accordingly, we have previously found that both SERT and 5-HT4R binding in the PFC are involved in the regulation of stress responses (Frokjaer et al., 2013; Jakobsen et al., 2016). Two large clusters were identified in the right posterior medial and left inferior temporal cortices. These regions are important for environmental awareness, i.e., object recognition and redirecting attention towards relevant auditory and visual stimuli. Significant changes were also found in the angular gyrus (AG) which is a cross-modal hub involved in higher order processing of multiple inputs including assessment of environmental salience, directing attention between the internal and the external milieu (Seghier, 2013) and modifying vigilance (Singh-Curry and Husain, 2009). Interestingly, the cyclic occurrence of mood liability, hyperphagia, and hypersomnia characteristic for Klein-Levin patients are associated with AG hypoperfusion (Geoffroy et al., 2013; Kas et al., 2014). Several clusters encompassing the occipital cortices, including the left cuneus also showed highly significant SERT changes. This may represent a reduction in retino-thalamic projections to the visual cortices, caused by diminished stimulation of the iRGC during winter.

We did not see any changes from summer to winter in the region-based analysis of raphe nuclei and global 5-HT4R binding. We have previously found, that 5-HT4R binding is inversely correlated to 5-HT levels (Haahr et al., 2014), and thus our analyses suggests that 5-HT levels remains unaltered across seasons in resilient participants.

Thus, lowering cerebral SERT levels in the winter may ensure stable synaptic 5-HT levels even during the winter and this may serve to combat lower mood during the environmental stress of the winter. Of course, we cannot that our modest sample size in terms of 5-HT4R binding could lead to a type II error and suggest that this should be replicated in larger samples and in SAD patients as well. Also, our results could potentially be biased by exposure to artificial light sources. However, we did not find it necessary to control for exposure to artificial light sources for two reasons: First, artificial light sources such as a tablet held in reading distance will illuminate with an intensity of approximately

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600 lx. In comparison, the radiation of the sun in Denmark (101 feet above sea level, latitude 55.7°), reaches an intensity of approximately 50.000 lx in summer and 2000 lx in winter. Second, our study is longitudinal, and we find it safe to assume that individual's exposure to artificial light sources would only wary slightly across seasons.

In conclusion, S-carriers who are resilient to SAD respond to the strain of winter with a significant and synchronized reduction in SERT levels, putatively stabilizing endogenous 5-HT levels and thereby protecting against depressive symptoms. We identified several cortical clusters that exhibited seasonal adjustments of SERT levels, and we propose that these sex-dependent modulations may constitute a relevant coping mechanism to avoid the emergence of depressive symptoms.

We believe that delineation of intrinsic resilience factors may turn out to be a viable research strategy in the search for novel therapeutic targets.

Acknowledgments

We gratefully acknowledge Lone Ibsgaard Freyr, Agnete Dyssegaard, Bente Dall, Sussi Larsen, Gerda Thomsen and Svitlana Olsen for excellent technical assistance and the John and Birthe Meyer Foundation for the donation of the cyclotron and PET scanner. The Lundbeck Foundation generously funded the project through a Center grant (Cimbi).

Conflict of interest

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.

The study sponsors had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

VGF has received honorarium as speaker for H. Lundbeck A/S. No other authors have any conflict of interest and no disclosures.

Contributors

Brenda Mc Mahon: Study design, draw up of protocol, ethical approval, recruitment of participants, medical investigation of participants, PET image processing, data collection, data analysis and interpretation, manuscript preparation.

Martin Nørgård: Image processing, manuscript preparation (methods) and statistics.

Claus Svarer: PET image processing and manuscript preparation (PET methods).

Sofie Bech Andersen: Recruitment of participants, medical investigation of participants and data collection, manuscript editing.

Martin Korsbak Madsen: Recruitment of participants, medical investigation of participants and data collection, manuscript editing.

William Baaré: MR image data collection and manuscript editing.

Jacob Madsen: Radiochemistry, manuscript editing.

Vibe G. Frokjaer: Study design, recruitment, data collection, PET image processing, manuscript editing.

Gitte Moos Knudsen: Study design, draw up of protocol, data analysis and interpretation, manuscript editing.

Role of the funding source

The funding sources had no influence on the study results.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2018.06.004.

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