Preface

This thesis in Neuroscience has been submitted to the Graduate school at the Faculty of Health and Medical Sciences, University of Copenhagen. It is the result of 3 years of work carried out at Neurobiology Research Unit at Rigshospitalet University Hospital, Copenhagen, from December 2012 to November 2015.

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Contents

List of papers

The thesis is based on the following papers:

I. **Mc Mahon B.**, M. Nørgard, C. Svarer, S.B. Andersen, M.K. Madsen, W. Baaré, Madsen J, V.G. Frokjaer, and G. M. Knudsen. ”Individuals resilient to seasonal affective disorder downregulate their cerebral serotonin transporter binding in winter”. In Prep

II. **Mc Mahon B.**, S.B. Andersen, M.K. Madsen, L.V. Hjordt, I. Hageman, H. Dam, C. Svarer, S. da Cunha-Bang, W. Baaré, J. Madsen, L. Hasholt, K.K. Holst, V.G. Frokjaer and G.M. Knudsen . “Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder”. Brain 2016; doi: 10.1093/brain/aww043

In addition, the thesis includes two reports

A. **Mc Mahon B.**, V.G. Frokjaer, G.M. Knudsen (2013). Motion Correction of ^{11}C-DASB PET images (appendix A)

B. **Mc Mahon B.** and G.M. Knudsen (2014).The effect of injected mass of DASB on SERT binding potential (appendix B)

The following publications are related to the work described in the thesis:

1) Fisher, P. M., M. K. Madsen, **B. Mc Mahon**, K. K. Holst, S. B. Andersen, H. R. Laursen, L. F. Hasholt, H. R. Siebner and G. M. Knudsen (2014). "Three-week bright-light intervention has dose-related effects on threat-related corticolimbic reactivity and functional coupling." Biol Psychiatry 76(4): 332-339.

2) Haahr, M. E., P. M. Fisher, C. G. Jensen, V. G. Frokjaer, **B. Mc Mahon**, K. Madsen, W. F. Baare, S. Lehel, A. Norremolle, E. A. Rabiner and G. M. Knudsen (2014). "Central 5-HT4 receptor binding as biomarker of serotonergic tonus in humans: a [11C]SB207145 PET study." Mol Psychiatry **19**(4): 427-432.

3) Andersen, S. B., **B. McMahon**, M. K. Madsen, , K. K. Holst, P. Moller, I.Hageman and G. M. Knudsen (2014). "Sweet taste sensitivity is influenced by 5-HTTLPR genotype and affected in seasonal affective disorder." Psychiatry Res **220**(1-2): 727-729.

4) Madsen, M. K., **B. Mc Mahon**, S. B. Andersen, H. R. Siebner, G. M. Knudsen and P. M. Fisher (2015). "Threat-related amygdala functional connectivity is associated with 5-HTTLPR genotype and neuroticism." Soc Cogn Affect Neurosci. Epub ahead of print.

5) Fisher, P. M., K. K. Holst, **B. Mc Mahon**, M. E. Haahr, K. Madsen, N. Gillings, W. F. Baare, P. S. Jensen and G. M. Knudsen (2012). ”5-HTTLPR status predictive of neocortical 5-HT4 binding assessed with [(11)C]SB207145 PET in humans." Neuroimage **62**(1): 130-136.

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Brenda Mc Mahon, Copenhagen, December 3th 2015

Summary

In Copenhagen, located at a Northern latitude of 55.7^{o}, the paucity of daylight in the winter impacts human physiology and mood (here termed seasonality) in up to 90% of the population. Although the winter is tolerated by most people, 5-10% meet the diagnostic criteria of Seasonal Affective Disorder (SAD); an occurrence of seasonal related depressive episodes, often with onset in the fall and spontaneous remission in the spring. Due to these relatively predictable episodes with affected mood, SAD represent a unique opportunity to study neurobiological state factors of importance for the mood swings.

The serotonergic neurotransmitter system has a clear role in the regulation of circadian rhythms and it is also involved in other aspects of SAD, including mood, sleep and appetite. Previous ^{11}C-DASB Positron Emission Tomography (PET) studies have demonstrated that in people without a diagnosis of SAD, cerebral SERT correlates inversely with daylight minutes, in particular in carriers of the short allele (S-carriers) of the serotonin transporter linked polymorphic region (5-HTTLPR). It remained to be investigated both how individuals resilient to SAD and patients with SAD regulate their cerebral SERT across seasons, if such seasonal changes were related to clinical severity of SAD, and how 5-HTTLPR genotype and sex interacted with the season-related change in SERT.

Thus, the aims of this PhD-study were:

Aim I) to characterise seasonal SERT regulation in healthy individuals resilient to SAD

Aim II) to investigate group differences in seasonal SERT regulation and the relation to symptom severity in SAD patients

Aim III) to explore the effects of 5-HTTLRP genotype on seasonal SERT regulation in SAD patients

Aim IV) to investigate gender by season effects in SAD resilient individuals and SAD patients

We enrolled 23 SAD resilient healthy controls, characterised by low seasonality ratings (all S-carriers) and 17 SAD patients (11 S-carriers) and measured cerebral SERT levels by means of ^{11}C-DASB PET twice, both summer and winter. A measure of whole brain SERT binding (global BP_{ND}) was used to examine global SERT changes across seasons, groups, sexs and 5-HTTLRP genotypes. Based on previous findings we hypothesized that global BP_{ND} would be higher in the winter than in the summer in individuals resilient to affective disorders, particularly in females. Further, assuming that high levels of cerebral SERT are associated with depressive symptoms, we hypothesized, that SAD patients would have even higher global BP_{ND} the winter compared to healthy individuals resilient to SAD. We also anticipated the increase from summer to winter would correlate with the increase in SAD ratings.

In contrast to expectations we found that SAD resilient individuals had a significant *down-regulation* of cerebral SERT levels in winter. Whereas the SAD and SAD resilient participants had comparable SERT levels in summer, SAD patients had significantly higher global SERT levels in the winter. These effects came clearly across as a highly significant group differences in seasonal SERT regulation (ΔBP_{ND}) and the relative increase in SERT correlated positively with the relative increase in depressive symptoms. In addition, we found that S-carriers had a significant larger seasonal SERT regulation compared to L_A-homozygotes.

Irrespective of group, we found more pronounced seasonal SERT regulations in female participants.

Our findings suggest that the development of SAD symptoms is associated with a failure to downregulate SERT appropriately during exposure to the environmental stress of winter, especially in individuals with high risk profiles for affective disorders, i.e. in women and in S-carriers. Our data are consistent with the interpretation that high cerebral SERT is associated with low levels of interstitial 5-HT which in turn elicits the SAD symptoms. The clinical implication is that women and S-carriers with SAD may be particularly likely to respond to pharmacological intervention with 5-HT reuptake inhibitors.

Resumé

København ligger 55.7^{o} nord for ækvator og dens befolkning udsættes dermed for betydelige svingninger i mængden af dagslys gennem året. Ni ud af ti indbyggere oplever at vinteren påvirker deres humør negativt, ligesom behovet for søvn og kulhydratrig kost forøges. Op til 5-10% opfylder de diagnostiske kriterier for en egentlig vinterdepression (Seasonal Affective Disorder, SAD). SAD er karakteriseret ved cyklisk forekomst af humør-, søvn- og appetitmæssige forandringer, oftest med forværring om efteråret og fuldkommen bedring om foråret.

Det serotonerge (5-HT) neurotransmitter-system spiller en betydelig rolle i reguleringen af vores døgn- og sæsonrytme, herunder affektregulering, søvn og appetit. Den cellemembran-bundne transporter, der bringer 5-HT tilbage i nervecellen efter en frigivelse, kaldes serotonin transporteren (SERT) og den kan afbildes hos mennesker vhja. Positron EmissionsTomografiske (PET) skanninger. Tidligere PET-skanningsstudier, hvor man har skannet raske forsøgspersoner på forskellige tidspunkter af året, har vist at SERT øges om vinteren, og især hos personer, som har S- og ikke L-variationen af et gen, som koder for SERT.

Afhandlingen havde fire delmål:

I) at karakterisere sæsonbetingede SERT-svingninger hos raske forsøgspersoner, som er særligt modstandsdygtige overfor vinterdepression.

II) at undersøge, hvordan de modstandsdygtige adskiller sig fra personer med SAD mht. reguleringen af hjernens SERT mellem sommer og vinter, og at se, om

der er sammenhæng mellem SAD patienternes SERT-regulering og sværhedsgraden af SAD.

III) at belyse, hvorledes genotype (S eller L) påvirker de sæsonbestemte SERT-svingninger hos personer med SAD.

IV) at undersøge, om de sæsonbestemte SERT-svingninger er mere udtalte hos kvinder end hos mænd.

Vi undersøgte i alt 23 raske forsøgspersoner som til trods for at de havde S-genet var karakteriseret ved at have ingen eller meget beskedne sæsonrelaterede svingninger i humør, appetit og søvn (de modstandsdygtige). Vi undersøgte også 17 personer med SAD (heraf 11 med S-gen). Begge grupper blev PET-skannet såvel sommer som vinter og fik dermed hjernens SERT binding kvantificeret (global BP_{ND}) på to årstider.

Om sommeren havde personer med SAD det samme niveau af SERT i hjernen som de modstandsdygtige. Men modsat forventningen fandt vi, at de SAD-modstandsdygtige *nedregulerede* deres SERT i hjernen om vinteren, hvorimod personer med SAD generelt *opregulerede* deres SERT i hjernen. Ikke alene havde SAD patienterne dermed højere SERT niveau end de modstandsdygtige om vinteren: Den relative stigning de udviste korrelerede positivt med den relative forværring af SAD-symptomerne. Den sæsonudløste SERT regulering var desuden mere udtalt hos personer med S-genet end med L-genet og kvinder udviste generelt større sæsonudsving end mænd i deres SERT.

Resultaterne fra denne PhD tyder på, at udviklingen af vinter-depressive symptomer kan skyldes en utilstrækkelig nedregulering af hjernens SERT, betinget af det sparsomme dagslys, som vinteren byder på ved vore længdegrader. Dette synes i særlig grad at være tilfældet hos personer med øget risiko for at udvikle SAD, nemlig hos personer med S-genet og hos kvinder. Et højt SERT-niveau i hjernen om vinteren svarer til et lavere 5-HT niveau i hjernen, hvilket kan være udløsende for SAD. PhD-afhandlingens resultat tyder derfor på at det

blandt patienter med SAD især vil være kvinder og dem med S-genet som kan drage fordel af behandling med lægemidler, der nedsætter SERT i hjernen.

Nomenclatures

Seasonal Affective Disorder, SAD: Episodes of depression with a seasonal pattern. Equivalent to the terms seasonal depression and winter depression.

Sub-syndromal seasonal affective disorder (SAD): A condition where seasonal fluctuations in mood or behaviour are subjectively impairing albeit not meeting diagnostic SAD criteria.

Seasonality: Any seasonal related change in mood, behaviour or physiology.

Non-seasonal affective disorder (non-SAD): Individuals resilient to SAD.

Major Depressive Disorder, MDD: Episodes of depression without a seasonal pattern. Equivalent to the terms unipolar depression and depression.

Abbreviations

^{11}C-DASB: ^{11}Carbon-labeled 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile
^{11}C-MADAM: 11Carbon labeled -N, N-Dimethyl-2-(2-amino-4-methylphenylthio)benzylamine
^{11}C-McN 5652: 11C-(+)-6beta-(4-Methyl-thiophenyl)-1,2,3,5,6 alpha,10beta-hexahydropyrrolo[2,1- a]isoquinoline)
^{123}I-β-CIT: ^{123}I labeled (2- β carbomethoxy-3 β -(4-iodophenyl)tropane)
5-HIAA: 5-Hydroxyindoleacetic acid
5-HT: serotonin, 5-hydroxytryptamine
5-HT$_4$R: serotonin receptor 4
5-HTTLPR: serotonin transporter linked polymorphic region
ANOVA: analysis of covariance
AUC: area under curve
AUC$_{cerebellum}$: area under the cerebellar time activity curve
BLT: bright light therapy
BMI: body mass index
BP$_{ND}$: binding potential relative to the non displaceable binding
CI: confidence interval
Cimbi: Center for Integrated Molecular Brain Imaging
df: degrees of freedom
DNA: deoxyribonukleinacid
DRN: dorsal raphe nuclei
DSM: diagnostic and statistical manual of mental disorders
Eq.: equation
fMRI: functional magnetic resonance imaging
FWHM: full width half maximum
GA: gyrus angularis
GABA: gamma-aminobutan acid
GnRH: gonadotrophine releasing hormone
HRRT: high-resolution research tomography
ICD: international classification of diseases
ipRGC: intrinsically photosensitive retinal ganglion cells
k2': reference tissue wash-out constant
L$_A$-homozygotes: homozygote carriers of the long argine allele
L$_G$-carriers: long guanine allele carriers
MAO A: monoaminoxidase A
MDD: major depressive disorder
MDI: major depression inventory
mPFC: mediale prefrontal cortex
MP-RAGE: rapid three-dimensional tradient echo
MRTM: multilinear reference tissue model
Non-SAD: non-seasonal affective disorder
***P*-value:** probability value
PET: positron emission tomography
PFC: prefrontal cortex
PMS: premenstrual syndrome
PSQI GS: Pittsburgh sleep quality index global scores
RDC: research diagnostic criteria
REM: rapid eye movement
RGC: image forming ganglion cell
ROI: region of interest
SAD: seasonal affective disorder
SCAN: schedules for clinical assessment in neuropsychiatry
S-carriers: short allele carriers
SCN: nucleus suprachiasmaticum
SD: standard deviation
SERT: serotonin transporter
SIGH-SAD: Hamilton rating scale for depression – seasonal affective disorder version
Snip: single nucleotide polymorphisms
SPAQ GSS: seasonal pattern assessment questionnaire global score
SPAQ: seasonal pattern assessment questionnaire
SPECT: single-photon emission computerized tomography
SSRI: selective serotonin reuptake inhibitor
TAC: time activity curve
TSE: turbo spin echo
VIF: variance inflation factor
VOI: volumes-of-interests
WHO: world health organizations

Background

Neurobiological Background

Today, research in biorhythms and seasonal affective disorder (SAD) can roughly be grouped in two main themes. One emphasises circadian misalignment i.e. phase-shifts of melatonin while the other emphasises changes in monoaminergic neurotransmission, in particular serotonin. SAD ultimately results from the interaction of several vulnerability factors (Levitan 2007). This thesis work focuses on the serotonergic changes only.

Introduction to the circadian system

The nucleus suprachiasmaticum

The Suprachiasmatic nucleus (SCN) is embedded in the anterior part of the hypothalamus right above the optic chiasm. The SCN is the center of a complex and widespread circadian system which act via a plethora of endocrine, immunological and neuronal pathways to entrain behavior and physiology to the light / dark cycle (Morin 2013) (figure 1). Information of the light/dark cycle is communicated by blue light-elicited excitation in the intrinsically photosensitive Retinal Ganglion Cells (ipRGC) in retina and a succeeding propagation of an excitatory signal within the retino-hypothalamic tract. Standard retrograde tracing have revealed that approximately 35 regions provide input to the SCN. The main efferent projections can be separated in to three major sources of input (Graff, Kohler et al. 2005, Morin 2013):

1) Terminus of the retino-hypothalamic tract conveying non-visual information via pituitary adenylate cyclase-activating polypeptide at glutaminergic neurons
2) Terminus of the geniculohypothalamic tract, indirectly conveying visual light information from the rodes and cones via neuropeptide-Y and GABA neurons from the intergeniculate leaflet
3) Terminus of a hub of dense serotonergic input originating from the medial median raphe nuclei and indirectly from dorsal raphe nuclei projections that emerge via the intergeniculate leaflet (figure 1)

The SCN sends afferent projections to approximately 15 brain regions across three main patterns which mainly include inter-hypothalamic projections. The first pattern is most extensive, it delivers output to the periventricular nuclei, the second and third deliver output to the septum and to thalamic paraventricular nuclei, respectively. In addition, the SCN is pivotal to endocrine entrainment to zeitgebers such as the light/dark cycle: cortisol release is regulated via the above mentioned SCN communications to the paraventricular nuclei, controlling cortico-releasing hormone and the release of gonadotrophine releasing hormone (GnRH) are under the temporal regulation by SCN inter-hypothalamic projections (rodents data) (Tonsfeldt and Chappell 2012). The communication with the pineal gland is not mediated by direct neuronal pathways, but via complex parachrine signaling that is described in detail elsewhere, e.g., (Sapede and Cau 2013). Many brain regions are included in the extended circadian system including adjacent parts of the hypothalamus, the habenula and tecmentum and via interneuronal connections across these regions whereby over 100 different brain regions are potential contributors to the circadian system (Morin 2013).

Limbic projections

A direct pathway connecting SCN to the amygdala has been described in the gold hamster (Morin, Goodless-Sanchez et al. 1994). It is, however, unclear to what extent this pathway is functional in humans and if it directly exerts seasonal effects on mood and behavior. However, the cortico-limbic circuit comprising the amygdala, the anterior cingulate gyrus and medial prefrontal cortex (mPFC) is

affected by bright light intervention (see also “Bright light therapy”) (Fisher, Madsen et al. 2013).

Retino-raphe projections

Preclinical studies conducted primarily in rodents have aided the delineation of retino-raphe projections and have not found evidence of direct ipRGC-raphe projections (Hattar, Kumar et al. 2006). However, direct projections to DRN from image forming ganglion cells (RGCs), indistinguishable from the classic image-forming alpha/Y-like RGC type, has been found across various species (summarized in (Luan, Ren et al. 2011)). One study in gerbils investigated the role of the RGC-DRN pathway by silencing the RGC input by an immunotoxine. They found a reduction in serotonin levels in DRN as well as increased depressive behavior upon intervention (Ren, Luan et al. 2013). In line with this a study of 5-HT levels and photoperiod, confirmed that DRN levels of 5-HT were higher under long-day conditions than short-day conditions in both chipmunks and nocturnal mice (Goda, Otsuka et al. 2015). As DRN is the main site of regulating global cerebral 5-HT tone these studies provides new insights into daylights lights potential to modulate mood via DRN projections.

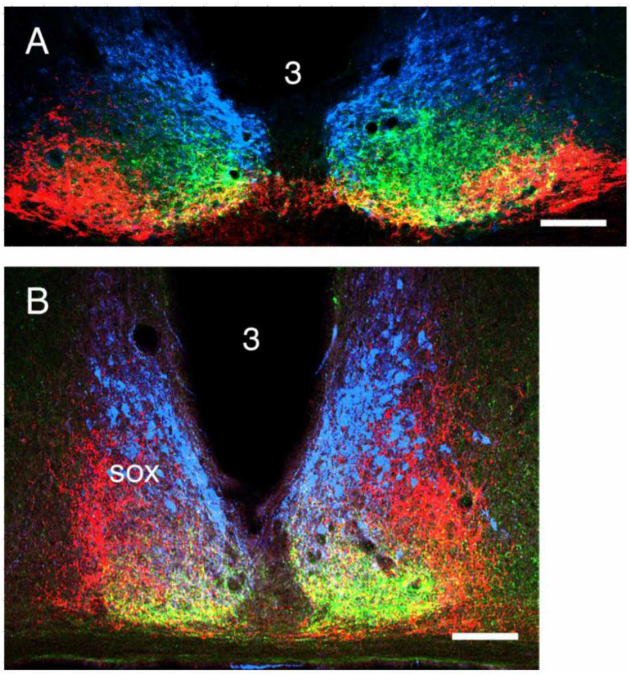


Figure 1. Triple label images of SCN anatomy in (A) the rat and (B) mouse stained for vasopressin (blue), serotonin (green) and retinal projections (red). The yellow color results from merging information from adjacent red and green pixels or because green and red items are superimposed in the original tissue. The oblate rat SCN enables the triple stain procedure to give the tissue a laminar appearance not visible in the more upright SCN of the mouse. The figure is modified after Figs. 5 and 8 in Morin et al.(2006). Printet in Exp Neurol. 2013 May ; 243: 4–20.

Introduction to the serotonin system

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic neurotransmitter that regulates both basal bodily functions including sleep, appetite, reproduction and temperature and more complex functions like mood, aggression and cognition (Jacobs and Azmitia 1992). In addition, 5-HT is involved in governing circadian rhythms by afferent and efferent communication with the SCN (Morin 2013). Serotonin is biosynthesised in a two step process, first the amino acid tryptophan is converted to 5-hydroxy-L-tryptophan by tryptophan hydroxylase then follows a conversion of the product by the enzyme aromatic L-amino acid decarboxylase to 5-HT. Serotonin can not pass the blood-brain barrier and the synthesis takes place in the soma of the serotonergic neuron located in the brain stem in the raphe nuclei. The highest concentration is found in the rostral part of the raphe nuclei (the dorsal and ventral raphe nucleus) located in the midbrain and pons and only a smaller fraction is synthesised in the caudal part located in medulla oblongata. From the raphe nuclei the serotonergic axons ascends and branches out to all cerebral regions with exception of the cerebellum which only receives a negligible amount of serotonergic fibers. The serotonergic signal transduction is initiated by an engulfment of the 5-HT vesicle in the cell membrane causing a released of 5-HT into the synaptic cleft. The 5-HT molecule can then exit the synapse in one of the following ways **a)** via binding to postsynaptic receptors (classical neuronal transmission (hard-wired) **b)** by volume neurotransmission where the molecule diffuses to remote targets located alongside the nerve terminal or **c)** by reuptake into the pre-synaptic membrane by the serotonin transporter (SERT). In addition, the 5-HT molecule can be broken down by the non-specific enzyme monoamine oxidase A (MAO A) into its metabolite 5-Hydroxyindoleacetic acid (5-HIAA) (CNS forum).

To this date 14 distinct subtypes of 5-HT receptors has been characterized. All receptors, but the 5-HT_3 receptor, that is a ligand-gated ion channel (Thompson, Lester et al. 2010), is coupled to a G-protein. The receptors can be further

categorized accordingly to their second messenger systems (Millan, Marin et al. 2008). Synaptic 5-HT levels can be reduced either by reduced synthesis and trafficking, increased degradation (increased MAO-A activity) or increased removal from active sites (increased SERT density or affinity). Low synthesis is unlikely to contribute to psychiatric disorders as the 5-HT precursor, the amino acid tryptophan, is abundantly present in various food sources. However, in experiments where a low 5-HT neurotransmission is desired, 5-HT synthesis can be manipulated by implementation of a tryptophan depletion regime e.g.(Neumeister, Praschak-Rieder et al. 1997). Reduction of 5-HT reuptake or degradation can be therapeutically exploited to treat various conditions linked to low levels of 5-HT, i.e. drugs that blocks SERT or drugs that reduce MAO-A activity are commonly used to treat depressive episodes (CNS forum).

Serotonin can not be directly measured in vivo in the human brain (Paterson, Tyacke et al. 2010). As proxies, various biomarkers have been implemented including measurements of sweet taste sensitivity (Andersen, Holst et al. 2014), measurements of 5-HIAA in cerebral spinal fluids (Luykx, Bakker et al. 2013) or venous blood (Lambert, Reid et al. 2002) or platelet 5-HT levels (Willeit, Sitte et al. 2008). However these methods are based on periphery proxy measures and therefore they are not ideal. Nevertheless, a new approach for assessing cerebral 5-HT has been implemented. Initial preclinical studies found that paroxetine administration to the Flindes sensitive rat caused a 16-47% down-regulation of 5-HT_4 receptor binding in all regions evaluated including the basal ganglia and hippocampus, while 5-HT depletion increased the 5-HT_4 receptor binding in the dorsal hippocampus, hypothalamus, and lateral globus pallidus (Licht, Marcussen et al. 2009). The finding of a possible proxy measures of 5-HT was successfully confirmed in humans by our group. In a double blinded randomized placebo controlled ^{11}C-SB207145 Positron Emission Tomography (PET) study, 40 mg of fluoxetine or placebo was administered daily to 32 healthy males for three weeks to increase 5-HT tone in the fluoxetine intervention group. After three weeks, the group receiving active compound had significantly decreased global 5-HT_4 binding (Haahr, Fisher et al. 2014), thus 5-HT_4 receptor binding can be used as a inverse proxy measure for chronic altered 5-HT levels.

The serotonin transporter linked polymorphic region

Serotonergic neurotransmission is highly depended on SERT density and manipulations of SERT efficacy can alter 5-HT levels (Torres, Gainetdinov et al. 2003). A polymorphism residing the promoter region located up stream from the transcription start site of the SLC6A4 gene (chromosome 17q11.2) encoding SERT, the 5-HT Transporter Linked Polymorphic Region (5-HTTLPR) shapes inherent differences in trait serotonergic tone. Approximately 2/3 of the population residing the Northern hemisphere carry a promoter variant that does not include 44 base pairs in the final product (the short allele referred to as S-carriers) and 1/3 are homozygote for the allele that does include the 44 base pairs (the long allele, referred to as L-carriers) (Esau, Kaur et al. 2008). In addition, a single nucleotide polymorphisms (snip, rs25531) replacing guanine with adenine has also been found to alter SERT transcription Thus, it is widely accepted that due to transcriptional commonalities of the short allele and the long guanine allele (L_G) the carriers of these genotypes should be contrasted to individuals homozygote of the long adenine allele (L_A-homozygotes) when investigating effects of 5-HTTLPR genotypes (Nakamura, Ueno et al. 2000). The majority of PET studies have found that S- and L_G-carriers have lower in vivo levels of SERT (Praschak-Rieder, Kennedy et al. 2007, Reimold, Smolka et al. 2007, Kalbitzer, Frokjaer et al. 2009) and only a single study failed to replicate this (Murthy, Selvaraj et al. 2010). In accordance, S-carriers have lower rates of mRNA transcription in lymphoblast cell lines (Lesch, Bengel et al. 1996). Based on the framework of Haahr et al., 2013, we tested the inherent differences in 5-HT profiles between S- and non S-carriers in a cohort of 47 males and females. The results were affirmative of higher levels of 5-HT, as inversely indexed by 5-HT_4 R binding, in S-carriers (Fisher, Holst et al. 2012). In addition, we implemented a sucrose sensitivity taste test to obtain a proxy for 5-HT tone (Heath, Melichar et al. 2006) and found corroborating results, as L_A-homozygotes were found to detected sucrose in the solutions at lower concentrations and have a less steep sucrose concentration detection curve relative to S-carriers (Andersen, McMahon et al. 2014). Thus stratification of 5-HTTLPR genotype can be used as a research

tool to create a model that accommodates investigations of 5-HT tone across various conditions.

Seasonal fluctuations in the serotonin system

Seasonal fluctuations in serotonin and serotonergic biomarkers

In 1980, Carlsson and coworkers published that human post-mortem levels of hypothalamic 5-HT were lower in the fall/winter samples compared to the summer samples. Two later studies were aligned with this and reported lower levels of the 5-HT metabolite 5-HIAA in plasma and CSF in winter compared to summer (Lambert, Reid et al. 2002) (Luykx, Bakker et al. 2013). The latter study also reported that seasonal 5-HIAA CSF variation was more prominent in S-carriers and that the CSF 5-HIAA variation from summer to winter correlated positively with ratings of Beck Depression Inventory-II (BDI) (Luykx, Bakker et al. 2013).

Seasonal fluctuations in serotonin transporter binding

An overview including single-photon emission computed tomography (SPECT) and PET investigations of seasonal SERT fluctuations in healthy individuals can be found in paper I. The studies of seasonal SERT fluctuations in clinical cohorts are not included. A single study contrasted SERT levels of SAD patients and healthy controls in winter (Willeit, Praschak-Rieder et al. 2000), the results of this study is elaborated in "Seasonal affective disorder, etiology". The studies are summarized in table 1. In brief, previous studies suggest that seasonal SERT fluctuations are present in cohorts of mixed genders at latitudes above 53° north of the equator, particularly in females and non L_A-homozygote individuals (figure 2). The apparent inconsistencies are thus likely to stem from **1)** small sample sizes and cross-sectional study designs **2)** the application of sub-optimal radioligands **3)** the emphasis on sub-cortical regions only **4)** differences in latitude and **5)** failure to include confounding variables such as sex and 5-HTTLPR genotype in the analysis or take into account other confounding factors such as travelling, retinal pathology or outdoor activities (paper I).

Table 1. SPECT and PET studies examining seasonal SERT fluctuations listed in chronological order. Studies exclusively in healthy individuals are included whereas studies including MDD or SAD patients are not included in the table.

PUBLICATION N (F) AREA (LATITUDE)	METHOD	SEASON- VARIABLE STASTISTICAL MODEL	RESULTS SEASONAL SERT FLUCTUATION+/-
Neumeister et al.,2000[1] 12 (12) Vienna (48° N)	Cross-sectional ^{123}I-β-CIT SPECT	dichotomised summer: May-Aug. winter: Nov.- Dec. by group comparison by Mann-Whitney tests	**+lower thalamus-hypothalamus SERT variance in the winter group 4 hours post injection** **-No difference in mesencephalon/pons**
Buchert et al., 2006[2] 29 (14) Hamburg (53° N)	Cross-sectional ^{11}C-McN5652 PET	dichotomised, cut-off at Apr. 1th and Sep. 30th by various ANOVA models., adjusted for sex age and sex by season	**+ higher SERT BP_{ND} in mesencephalon in winter** **No difference in thalamus or the voxel based anaysis** **-No sex by season effect**
Koskela et al., 2008[3] 12 (5) Helsinki (60° N)	Longitudinal ^{123}I-ADAM SPECT	season dichotomised summer: Jul.-Aug. winter: Nov.-Feb. by Wilcoxon signed-rank tests	**- No differences in thalamus or mesencephalon, irrespective of sex**
Praschack-Rieder et al.,2008[4] 88 (44) Toronto (43 ° N)	Cross-sectional ^{11}C-DASB PET	season dichotomised, cut-off at Mar. 20th-21th and Sep. 20th-21th *and* daily sunshine/day length as numerical variable By age corrected ANCOVA	**+ higher SERT BP_{ND} in ant.med. PFC, ant.cing. cortex caudate, putamen, thalamus, mesencephalon in**

		models and linear regression models	**fall/winter and + inverse correlation of SERT BP_{ND} between daily sunshine and day length**
Kalbitzer et al., 2010[5] 57 (20) Copenhagen (56°N)	Cross-sectional ^{11}C-DASB PET	daylight minutes By age, sex, gene by daylight corrected multipe harmonic regression	**+inverse correlation of SERT BP_{ND} and daylight minutes in the caudate and putamen, trend in thalamus, + gene by daylight * effect in putamen -No effect in mesencephalon -No difference in the voxel based anaysis**
Murthy et al., 2010[6] 63 (0) The United Kingdom (54° N)	Cross-sectional ^{11}C-DASB PET	Daylight minutes By repeated measures ANOVA genotype as between subject factor, region as within subject factor and age as covariable.	**- No correlation of SERT BP_{ND} and daylight minutes in raphe, mygdala, ant. Cing. Cortex, hippocampus, caudate, putamen or thalamus - No G * E* effects in any ROI**
Cheng et al.,2011[7] 66 (40) Tainan (22° N)	Cross-sectional ^{123}I-ADAM SPECT	Season measured by sunlight exposure (SE) accumulated 30 days prescan by Mann-Whitney comparisons of 3 SE groups and by linear regression of age, sex and SE	**- No difference in mesencephalon, irrespective of analysis**
Matheson et al. 2015[8] 40 (0) Stockholm (59 °N)	Cross-sectional ^{11}C-MADAM PET	daylight minutes by age corrected multiple linear regression	**-No differences in strialtal, extra striatal (thalamus, hippocampus , amygdala) or DRN**

F: females

N: latitude, defined as degree north of equator ranging from 0° to 90°

Latitudes calculated at http://www.mapsofworld.com/lat_long/

*Gene by Environment interactions effect: s a difference in amplitude of the daylight by SERT interaction between L_A-homozygotes and non-L_A-homozygotes (figure 2)

[1] (Neumeister, Pirker et al. 2000)
[2] (Buchert, Schulze et al. 2006)
[3] (Koskela, Kauppinen et al. 2008)
[4] (Praschak-Rieder, Willeit et al. 2008)
[5] (Kalbitzer, Erritzoe et al. 2010)
[6] (Cheng, Chen et al. 2011)
[7](Murthy, Selvaraj et al. 2010)
[8](Matheson, Schain et al. 2015)

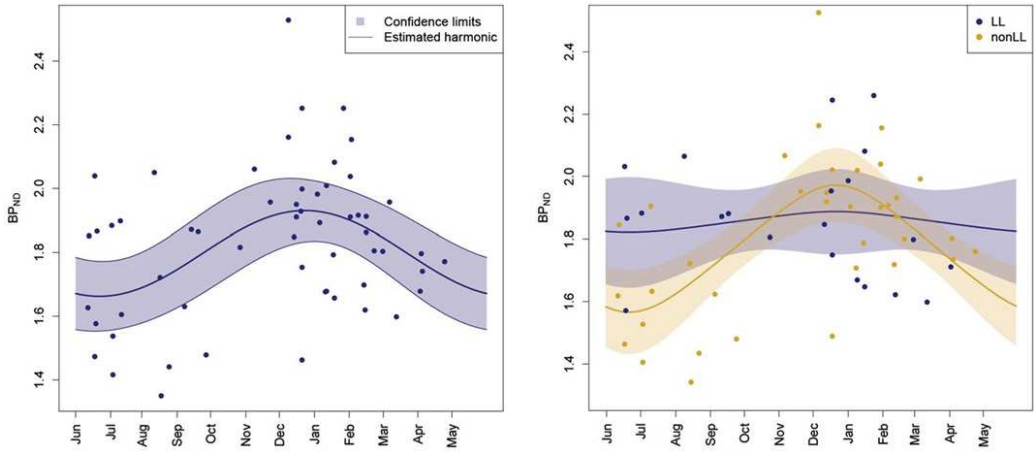


Figure 2. The seasonal SERT fluctuations in 57 healthy Danes (Kalbitzer, Erritzoe et al. 2010). Left Figure illustrates the seasonal effect on ^{11}C-DASB BP_{ND} (putamen) with pointwise confidence limits, modeled as a harmonic function with period 1 year (estimated peak in the middle of December, SE: 21 days, in good agreement with the model using daylight minutes as a predictor) adjusting for age and sex. The plotted points are the partial residuals (male of mean age). The right figure displays the interaction between number of daylight minutes and serotonin transporter linked polymorphic region-allelic status adjusting for age and sex. For comparison with the left figure the estimated linear response as a function of daylight minutes was transformed to a function of calendar time (from Kalbitzer et al. 2010, biol Psych) (Kalbitzer, Erritzoe et al. 2010).

Seasonal Affective Disorder

Etiology

SAD has been proposed to develop when the diminishing daylight superimposes strain on an individual with traits of high flexibility or instability within the serotonin system (Ciarleglio, Resuehr et al. 2011). At-risk individuals are, e.g., females, (Magnusson and Partonen 2005), S-carriers (Rosenthal, Mazzanti et al. 1998), bipolar disorder patients (Abreu and Braganca 2015) and premenstrual dysphoric disorder patients (Praschak-Rieder, Willeit et al. 2001). There is evidence suggesting that the development of SAD is linked to changes in the 5-HT system i.e. SAD symptoms can be alleviated by pharmacological enhancement of serotonergic neurotransmission (O'Rourke, Wurtman et al. 1989, Dilsaver and Jaeckle 1990, Partonen and Lonnqvist 1996) and the beneficial effects of bright light therapy can be reversed by tryptophan depletion (Neumeister, Praschak-Rieder et al. 1997, Neumeister, Turner et al. 1998). In vivo studies of cerebral changes in the 5-HT system of SAD patients are sparse, only a single study in seasonal SERT changes included a SAD sample. This study investigated 11 patients with SAD in their symptomatic phase and 11 non-depressed healthy volunteers with the non-selective dopamine transporter and SERT radioligand ^{123}I-β-CIT and SPECT and reported lower thalamic-hypothalamic variance of SERT in SAD patients compared to healthy controls (Willeit, Praschak-Rieder et al. 2000). We and others have investigated if the outcome of a sucrose taste sensitivity test - a proxy measure of 5-HT – differed in SAD and healthy controls across seasons and across groups. We found that SAD patients had a less steep sucrose concentration detection curve in the winter compared to the summer (Andersen, Holst et al. 2014). This was in accordance with previous work in this field of research (Heath, Melichar et al. 2006) (Arbisi, Levine et al. 1996) (Srivastava, Donaldson et al. 2013).

Diagnosis

The SAD diagnostic criteria were first suggested by Rosenthal et al. 1984 (Rosenthal, Sack et al. 1984). A diagnosis could be set when a patient met all of the following criteria:

1) A history of depression fulfilling RDC* criteria for major affective disorder, depressed;

2) A history of at least two consecutive years of fall/winter depressive episodes remitting in the spring or summer;

3) The absence of other major (Axis I) psychiatric disorder or psychosocial explanation for the seasonal mood changes (Rosenthal, Sack et al. 1984).

*Validated Research Diagnostic Criteria (RDC) for major depression (Spitzer, Endicott et al. 1978)

Today the diagnosis is encompassed in The Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision (DSM-V-TR) as an episode specifier for recurrent MDD (table 2). Currently the SAD diagnosis is not incorporated into the World Health Organizations (WHO) International Classification of Diseases (ICD), which is the diagnostic tool used in Denmark.

Table 2. DSM 5 diagnostic criteria for SAD (from the American Psychiatric Association; "Diagnostic and statistical manual of mental disorders 5th edition")

Criteria for MDD with a seasonal pattern (equivalent to seasonal affective disorder) (can be applied to the pattern of major depressive episode in bipolar I disorder, bipolar II disorder, or MDD, recurrent)
A. There has been a regular temporal relationship between the onset of major depressive episodes and a particular time of the year (e.g., regular appearance of the major depressive episode in the fall or winter). Note: do not include cases in which there is an obvious effect of seasonal-related psychosocial stressors (e.g., regularly being unemployed every winter)
B. Full remissions (or a change from depression to mania or hypomania) also occur at a characteristic time of the year (e.g., depression disappears in the

spring)

C. In the last 2 years, two major depressive episodes have occurred that demonstrate the temporal seasonal relationships defined in criteria A and B, and no non-seasonal major depressive episodes have occurred during the same period

D. Seasonal major depressive episodes (as described above) substantially outnumber the non-seasonal major depressive episodes that may have occurred over the individual's lifetime.

Diathesis

Female gender

Sex differences in depressive symptoms and in the serotonin system

Eighty percent of all SAD patients are of female gender (Magnusson and Partonen 2005). This exceeds the 50% overrepresentation of females suffering from MDD (Kessler, McGonagle et al. 1993). Notably, females and males do not only differ with respect to prevalence but also in clinical presentation of symptoms. A survey of 2620 healthy Swedes (56% females) concluded that females are at a 1,5 times increased risk of seasonal related mood swings compared to males and men are more likely to experience hypersomnia whereas women are more likely to experience hyperphagia during winter (Chotai, Smedh et al. 2004). Similar results have been reported with respect to MDD patients; a study found that females were more likely to suffer from hypersomnia and hyperphagia during a depressive episode that their male counterparts (Gorman 2006). Sex differences, at various levels of the 5-HT system, is well described: healthy females have lower 5-HT_4 receptor binding (corresponding to higher 5-HT) (Haahr, Fisher et al. 2014) in limbic regions compared to healthy males (Madsen, Haahr et al. 2011) and higher SERT binding in mesencephalon, but not in projection areas (Erritzoe, Frokjaer et al. 2010). Buchert et al., 2006 found season by sex interactions effect in SERT variance in mesencephalon and thalamus on a trend level (figure 3) (Buchert, Schulze et al. 2006).

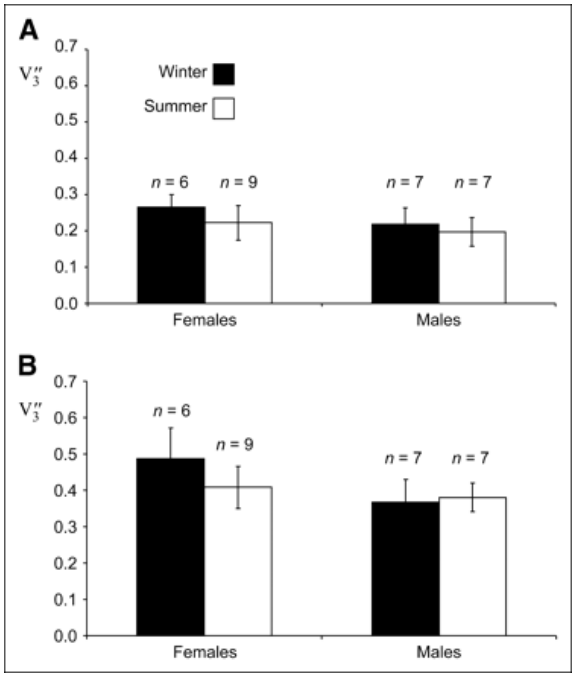


Figure 3. Season by gender interaction effect in the variance in mesencephalon and thalamus. No significant effects were deteced, allthoug a tred levels of larger seasonal SERT fluctuations in female participants were reported in both regions (*P* = .08 and .09, respectively) (from Buchert et al. 2006, Nucl Med. (Buchert, Schulze et al. 2006).

Seasonal fluctuations in estradiol levels

Findings of seasonal fluctuation in pituitary-ovarian axis output in healthy humans, namely at high latitudes is well replicated (summarised by Bjornerem el al. 2006 (Kauppila, Pakarinen et al. 1987, Bjornerem, Straume et al. 2006)) . In example, a large study of 1651 women and 1540 men from Northern Norway found that estradiol levels peak in June and trough October. However, the effect size was discrete; only 0.2–0.9% of the variation in estradiol was explained by seasonality and the effect were most notable in postmenopausal woman and men (Bjornerem, Straume et al. 2006).

Interaction of gender, estradiol and mood

The large sex difference in SAD prevalence could potentially be caused either by sex differences in the 5-HT system, differences in sex-hormone profiles and/or differences in the coupling of the sex-hormones and key features of the 5-HT system. Several studies have emphasised the close coupling of estradiol fluctuations and SERT expression, reviewed in 2014 by Borrow and Cameron (Borrow and Cameron 2014). Of note, the risk of developing a depressive episode is found to be markedly increased at time points where estadiol levels fluctuate in a woman's life, i.e. during puberty (Patton, Coffey et al. 2014), post partum (Munk-Olsen, Laursen et al. 2006) and in transition to menopause

(Freeman, Sammel et al. 2014). Moreover, mood swings across the menstrual cycle (premenstrual syndrome, PMS) can be relieved by selective serotonin re-uptake inhibitor (SSRI) treatment *or* estradiol replacement therapy (Imai, Ichigo et al. 2015). Thus, this line of evidence emphasises an association of depression and estradiol *fluctuations* which are abundant in the lifespan of females compared to males. The estradiol-SERT coupling was further elaborated in a placebo-controlled randomised ^{11}C-DASB PET trial. Sixty healthy females underwent a placebo injection or an injection of a GnRH agonist (GnRHa) to induce a biphasic ovarian hormone response that would terminate with an estradiol drop to post menopausal levels. The GnRHa provoked subclinical depressive symptoms that were associated both to an increase in neocortical SERT levels and to the magnitude of the estradiol response to treatment (Frokjaer, Pinborg et al. 2015) . The authors suggested that individuals particularly sensitive to an estradiol flare, would be more vulnerable to any superimposed biological challenges. In line with this, preclinical research have found that estradiol levels or estradiol fluctuations affect the 5-HT system, either via regulation of synthesis and degradation of endogenous 5-HT (Lokuge, Frey et al. 2011) or by increasing SERT density (Lu, Eshleman et al. 2003).

S-carrier status of the serotonin transporter repeat length polymorphism

Initially samples of SAD patients were found to have a higher prevalence of S-carriers compared to the background population (Rosenthal, Mazzanti et al. 1998, Praschak-Rieder, Willeit et al. 2002, Willeit, Praschak-Rieder et al. 2003). Thus, it was established that S-carrier status was a risk factor for developing SAD. However, these findings were later questioned by a review by Johansson et al. 2003 (Johansson, Willeit et al. 2003) (table 3). The authors pooled the samples from three studies and included yet another case-control group. They did not find any association between S-carrier status and SAD across the pooled samples, but when the SAD individuals were contrasted to a low seasonality sub-group there anticipated effects came across. Nevertheless, the full range of

studies based their analysis on the biallelic stratification (S-carriers vs. non-S-carriers) however the triallelic stratification might have yielded different results.

Table 3. Genotype frequencies of 5-HTTLPR in SAD patients and controls (from (Johansson, Willeit et al. 2003)).

Study	Group	*N*	*l/l* *N* (%)	*s/l* *N* (%)	*s/s* *N* (%)	χ^2	OR (95% CI)†
Rosenthal *et al.* (1998)	SAD	97	27 (28)	53 (55)	17 (17)	7·13*	2·38 (1·26–4·52)
	Controls	71	34 (48)	28 (39)	9 (13)		
Johansson *et al.* (2001)	SAD	82	28 (34)	43 (52)	11 (13)	0·06^{NS}	0·95 (0·50–1·80)
	Controls	82	27 (33)	43 (52)	12 (15)		
Willeit *et al.* (2003)	SAD	138	44 (32)	71 (51)	23 (17)	1·48^{NS}	1·15 (0·70–1·88)
	Controls	146	51 (35)	65 (45)	30 (20)		
New case–control study	SAD	147	51 (35)	69 (47)	27 (18)	0·34^{NS}	0·93 (0·56–1·55)
	Controls	115	38 (33)	58 (50)	19 (17)		
Combined	SAD	464	150 (32)	236 (51)	78 (17)	1·69^{NS}	1·20‡ (0·90–1·58)
	Controls	414	150 (36)	194 (47)	70 (17)		

OR: Odds ratios, assuming a dominant effect of the s-allele using the Mantel–Haenszel estimator of the common odds ratio
*P <.05;
NS: Non Significant

The clinical characteristics of seasonal affective disorder

Symptoms

Patients with SAD will present with a broad symptom profile ranging from mood swings to neurovegetative disturbances. The clinical presentation encompasses symptoms that can be divided into four main clusters: mood, cognition, energy and vegetative changes. The first three clusters are to a large extent shared with MDD whereas the neurovegetative symptoms (often denoted “atypical symptoms”) are signifying the SAD patients. The following presentation is, unless otherwise stated, based on the work of leading experts in the field (Dr. Danilenko, Dr. Levitan, dr.Rosenthal, Dr.Lam and Dr.Jacobsen ((Rosenthal, Sack et al. 1984, Jacobsen, Wehr et al. 1987, Lam and Levitan 2000, Levitan 2007, Danilenko and Levitan 2012) as well as personal communication with patients and with consultants in psychiatry Dr. Hageman

and Dr. Dam. The work of Jacobsen et al.,1987 is depicted in table 4 (Jacobsen, Wehr et al. 1987) .

Mood: The severity of the depressive mood tends to range as mild to moderate i.e. hospital admission and electroconvulsive therapy are rarely needed and suicide attempts or suicides are rare incidents. However, the SAD patients do to a large extent experience low mood, low self-esteem, rumination, blameworthiness and guild. In addition, there also seems to be some overlap with symptoms of anxiety, including experiences of derealisation.

Cognition: SAD patients often complain of impaired ability to concentrate and keep attention on a given task. This has been confirmed in research across various types of studies including surveys (Merikanto, Lahti et al. 2012), in comparison with non-seasonal depression (Sullivan and Payne 2007) and across seasons (Jensen, Hjordt et al. 2015).

Energy: Patients describe a noticeable seasonal change in social activities, in initiating projects, maintaining relationships and adhering to obligations and many take a sick leave during depressive episodes in winter, but never in summer. Changes in vigilance is a core feature of affective disorders and may be a direct effect of reduced 5-HT levels in specific brain regions important for setting the levels of arousal, e.g. the brainstem (Ferraro, Antonelli et al. 2013).

Neurovegetative symptoms

Hypersomnolence: The sleep disturbances are frequent and include both alterations in sleep timing e.g. increased latency to sleep and changes in sleep quality e.g. reduced slow wave (delta) sleep (Rosenthal, Sack et al. 1984). In spite of the long hours spent in bed patients often complain of fatigue.

Reproduction: The prevalence of SAD peaks in premenopausal young females (Magnusson and Boivin 2003) and patients report of seasonal fluctuations in libido. This may be elicited by direct changes in sex-hormone profiles and/or

the interplay of hormones and key features of the 5-HT system (elaborated in "Interaction of sex, estradiol and mood").

Hyperphagia: One of the most characteristic symptoms of SAD is the distinct craving for carbohydrate rich food elements. This often leads to overeating in an impulsive style that abates the negative self image even further. The specific carbohydrate craving has been suggested to be linked to low levels of 5-HT, as high intake of carbohydrate can increase tryptophan uptake in the brain and potentially increase 5-HT levels (Wurtman 1982). Aside from the cravings there seems to be a general increase in appetite, which contrast the anorexia often observed in MDD.

Weight gain: SAD patients often show mood associated fluctuations in body weight and they often gain 3-5 kg in the depressive state of winter. Patients often relate this to the increase in impulsive eating behaviour. However, seasonal shifts in endocrine output (e.g. cortisol- and estradiol levels) may increase anabolism and caloric need may be reduced due to lower activity of daily living.

Table 4. Clinical characterization of 156 SAD patients, adapted from Jacobsen et al 1987, AmJ Public Health 1987 (Jacobsen, Wehr et al. 1987)

TABLE 1—Symptoms Reported by SAD Patients during Winter (by per cent of patients, N = 156)

Variables	Symptom Reported	% of Patients
Activity	Decreased	96
Affect	Sadness	94
	Irritability	79
	Anxiety	84
Appetite	Incresed*	66
	Decreased	19
	Mixed	14
	No change	1
	Carbohydrate craving**	67 (N = 123)
Weight	Increased*	72
	Decreased	13
	Mixed	1
	No change	15
Libido	Decreased	62
Sleep	Increased duration*	79
	Later wakening**	69
	Earlier onset**	60
	Change in quality	77
	Daytime drowsiness**	82 (N = 123)
Other	Symptoms milder nearer equator**	74 (N = 71)
	Menstrual difficulties**	61 (N = 116)
	Work difficulties**	88
	Interpersonal difficulties**	94

*"atypical" depressive symptom.
**not diagnostic of typical or atypical depression.

Unipolar versus bipolar course of seasonal affective disorder

More patients with SAD suffer bipolar disorder, namely type II compared to patients experiencing non-seasonal depression. In the initial work by Rosenthal and colleagues 76% had a seasonal bipolar type II disorder and 17% had type I. Whereas the inventory concluded by Jacobsen found that 7% will experience a manic episode including elated mood, irritability, increased libido, elevated energy and social activity (table 4)(Jacobsen, Wehr et al. 1987).

Sub-specifiers of seasonal affective disorder

The majority of patients will experience symptoms inversely correlated to daylight minutes: as the day becomes shorter the symptoms intensify and vice versa as the light comes back on symptoms will spontaneous alleviate. The SAD winter-type covers both a condition of unipolar winter depression with full remission in summer as well as a bipolar sub-type where symptoms of hypomania will emerge in the summer. However, in rare cases symptoms will surface in the spring, intensify as the day grows longer and alleviate in the fall and winter, a condition often referred to as summer-SAD (Rosenthal, Sack et al. 1984, Partonen and Lonnqvist 1998).

Treatment

Bright light therapy

To day, the use of bright light therapy (BLT) has gained momentum and the indication has expanded well beyond its initial potentials as a cure for SAD. BLT has been tested as treatment across a range of neurological and psychiatric disorders e.g. MDD, bipolar disorder and ante-partum depression (Pail, Huf et al. 2011). A Cochrane-review concluded that SAD can be treated equally effective with SSRIs and BLT. However, treatment of choice was found to be BLT due to low risk of side effects and almost immediate response to treatment (Thaler, Delivuk et al. 2011). The optimal regime is estimated to be 5000 lux hours per day, corresponding to 30 minutes exposure to 10.000 lux in the

morning, preferably before 8 .A.M. (Levitan 2005). However, recent data suggests that not only intensity but also the emission spectrum of the bright light source should be carefully considered when choosing bright light therapy device. The ipRGC has a peak in sensitivity in the blue spectre (Hattar, Liao et al. 2002) making light emission within the blue spectrum highly potent in suppressing melatonin and delaying circadian phase (Brainard, Hanifin et al. 2015), alleviating symptoms of SAD (Glickman, Byrne et al. 2006, Strong, Marchant et al. 2009), provoking alertness (Cajochen 2007) and even in changing emotional processing (Vandewalle, Archer et al. 2011). The potency of the blue spectrum was moreover emphasised in a study by Meesters and group, where it was demonstrated that an emission 750 lux of blue light was equally effective in treating SAD as emission of a full spectre 10.000 lux (Meesters, Dekker et al. 2011). An additional effect may be obtained by inclusion of wavelengths within the green spectrum, as green emission will depolarize the image-forming retinal ganglion cells and via the geniculo-hypothalamic tract communicate the photonic information to SCN. Moreover, green emission helps restore the ipRGC upon transmission (Brainard, Hanifin et al. 2015). We conducted functional magnetic resonance imaging (fMRI) experiments and tested responses to threat by an emotional faces paradigm, before and after three weeks of BLT therapy. We found that bright light dose correlated inversely with reactivity to threat in both left and right amygdala and medial prefrontal cortex (mPFC) and positively with measures of functional connectivity between left amygdala and mPFC. In addition, bright-light dose was positively associated with intra-prefrontal functional coupling at rescan in L_G or S carriers, but not in L_A-homozygote individuals thus providing evidence of bright lights potential to modulate cortico-limbic circuits, possibly by altering the cerebral 5-HT tone (figure 4) (Fisher, Madsen et al. 2014).

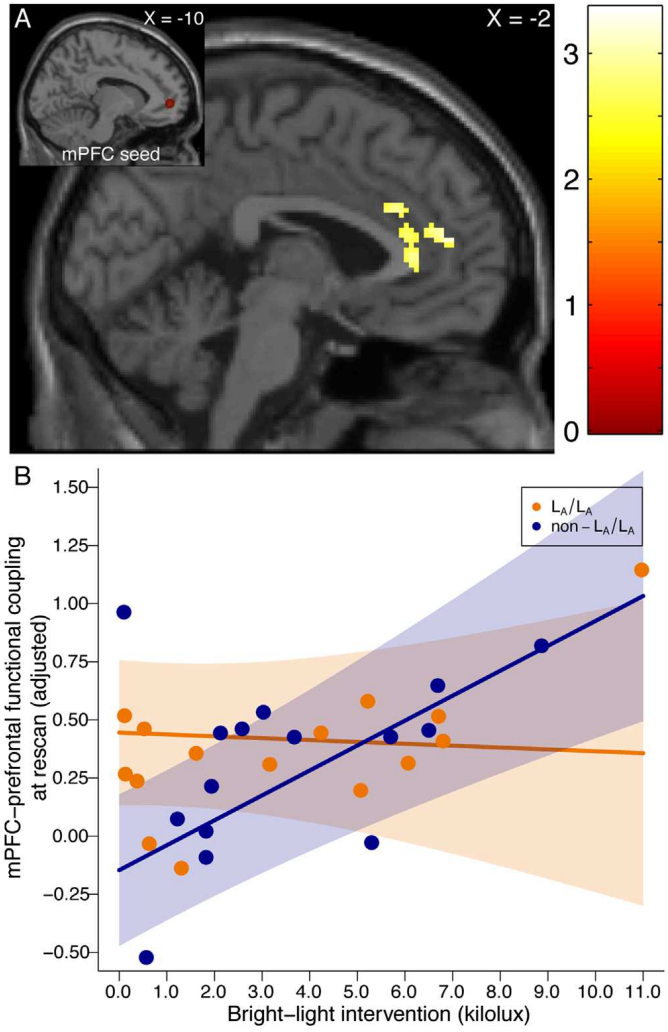


Figure 4. Serotonin transporter-linked polymerphic region (5-HTTLPR) moderates effect of bright-light intervention on medial prefrontal cortex (mPFC)-prefrontal functional coupling. (A) Statistical parametric map highlighting mPFC cluster, wherein the positive effect of bright-light intervention on functional coupling with our mPFC seed was significantly moderated by 5-HTTLPR genotype status (mPFC seed outlined within inset). Color bar represents t scores. (B) Plot of bright-light intervention by 5-HTTLPR interaction effect showing mean functional coupling estimate across 394 voxels and is intended only for visualization of interaction effect. Thirty individual data points are shown in blue or orange. Blue and orange shading represents 95% confidence limit of regression lines for L_G or S carriers (non-L_A-homozygotes) and L_A-homozygotes, respectively (from Fisher et al. 2014, biol. psych. (Fisher, Madsen et al. 2014)).

Pharmacological interventions targeting the serotonin system

As treatment for SAD, different pharmaceuticals targeting the 5-HT system at various levels have been investigated. A number of randomised placebo controlled clinical trials with SAD patients diagnosed by DSM V or research criteria's and with outcomes in terms of well validated rating tools (e.g., the SIGH-SAD) have been conducted and are included in the summary below.

SSRIs: Lam et al.1995 found that 20 mg fluoxetine alleviated symptoms in more than 50% of the patients, but the effect did not significantly differ from the effect size in the placebo group (Lam, Gorman et al. 1995). Two studies compared fluoxetine treatment to BLT and found comparable effects (approximately 50% improvement of symptom ratings in both groups in both studies) (Ruhrmann, Kasper et al. 1998, Lam, Levitt et al. 2006). Notably, concurrent with observations in MDD patients, several studies reported a time

lapse before onset of SSRI effects, whereas BLT had a faster acting onset. Moreover, one study reported that BLT was superior to fluoxetine treatment in alleviating atypical (vegetative) symptoms (Ruhrmann, Kasper et al. 1998). The effects of BLT and second generation antidepressives has been reviewed by Thaler et al in 2011 (Thaler, Delivuk et al. 2011).

MAO-A inhibitors: Several placebo-controlled studies have investigated the effects of MAO-A inhibitors, including moclobemide (Partonen and Lonnqvist 1996), tranylcypramine (Dilsaver and Jaeckle 1990) and phenelzine (Liebowitz, Quitkin et al. 1984). All the studies found significant effects of treatment and recommended the drug for treatment of SAD.

Bupropion: Inhibits monoaminergic (dopamine, 5-HT and noradrenalin) reuptake and antagonises several nicotine receptor subtypes. Placebo-controlled trials uniformly report large effect sizes with more than 70% increase in symptom ratings (Westrin and Lam 2007) (Dilsaver and Jaeckle 1990, Niemegeers, Dumont et al. 2013). Moreover, a Cochrane review; concluded that treatment with bupropion could prevent depressive relapse the following season (Gartlehner, Nussbaumer et al. 2015).

Duloxetine: Is a serotonin and noradrenalin inhibitor, one placebo-controlled trial reported effect of treatment (Pjrek, Willeit et al. 2008).

d- fenfluramin: Is a drug with a very broad recptor profile that causes a net increase in all monoamines. One placebo-controlled study found it to be very successful in promoting remission as well as inducing a significant weight loss (O'Rourke, Wurtman et al. 1989).

In conclusion, treatment response to an SSRI is comparable to BLT (Thaler, Delivuk et al. 2011). However, it is unknown if sex or 5-HTTTLRP genotype affects treatment response in SAD patients. Although it is a topic of debate, the majority of placebo controlled SSRI trials including MDD patients have found that $L_A$homozygotes and females have better response-and remission rates compared to S-carriers and males respectively (reviewed in (Porcelli, Fabbri et al. 2012) (5-HTTLPR effects) and in (Damoiseaux, Proost et al. 2014) (sex effects).

Cognitive therapy

A large study randomly assigned 89 SAD patients to six weeks of cognitive-behavioral therapy for SAD (CBT-SAD) or BLT during a depressive episode. Both groups had significant and comparable remission rates as evaluated by improvement in SIGH-SAD scores (Rohan, Mahon et al. 2015). However, mindfulness intervention during remission failed to prevent relapse (Fleer, Schroevers et al. 2014). Notable, patients own expectations to BLT predicted the speed of response (Knapen, van de Werken et al. 2014).

Investigation of SERT binding by ^{11}C-DASB PET

Data acquisition by positron emission tomography

Cerebral SERT levels can be measured in the living human brain by means of PET neuroimaging. This neuroimaging modality is based on injection of a small amount of positron emitting radioactive tracer. After injection into a peripheral vein, the radiotracer enters the brain and binds in a specific manner to the molecular target of interest e.g. a receptor on the cell surface and binds in the surrounding tissue in a non-specific manner. When decaying, the isotope emits a positron that upon encounter with an electron, will annihilate into two gamma photons carrying an energy charge of *exactly* 511 keV and travelling in diagonal directions. The gamma photons are detected by the comprehensive system of crystals, lining the PET cameras field of view. Due to the coincidence characteristics, the PET camera can separate irrelevant information and only take into account the diagonal 511 keV photons that are recorded simultaneously. During a six-minutes transmission scan, an individual attenuation map is created to correct for the radiation which is absorbed in the tissue. The emission scan then undergoes reconstruction into frames of varying time length based on count statistics, i.e. the first frames will be short and the latter longer. The data acquisition is highly sensitive to within-scan head movement and estimation of and correction for movement can be necessary.

Upon reconstruction, the image is commonly filtered and threshold to included only relevant brain voxels, this yields the input to yet another reslicing of frames before the 4D dynamic movement corrected PET image is created. To obtain sufficient structural information to delineate brain regions, the single subject PET image is co-registered to the structural MR image. Radioactivity in a given brain region can then be calculated as the volume weighted average of radioactive concentrations in left and right hemisphere regions.

Kinetic modelling of ^{11}C-DASB PET images

C-11-labeled 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile (^{11}C-DASB) has a high selectivity to the target SERT (Wilson, Ginovart et al. 2002) and is a well validated PET radiotracer (Kim, Ichise et al. 2006) that meets the requirements of an applicable radiotracer: it passes the blood brain barrier in sufficient amounts, it has a favourable ratio between tracer uptake in specific versus non-specific binding tissue, it has fast kinetics that allows quantification by kinetic modelling, it has a favourable signal to noise (Wilson, Ginovart et al. 2002) and it has a suitable reference region devoid of the target protein. In addition, the radiation burden of a ^{11}C-DASB scan is tolerable (approximately 4.8 millisievert per scan) which makes the tracer suitable for studies requiring multiple scans. Moreover the relatively short acquisition time of 90 minutes (Ginovart, Wilson et al. 2001) and the circumvention of arterial cannulation by use of a reference region leans the process and greatly opts compliance of the participants.

SERT is widely distributed within the human brain with exception of cerebellum. As cerebellum is almost fully devoid of specific SERT binding sites, any binding in cerebellum equals the non-specific tracer distribution (i.e. what sticks in tissue and vessels). Given these premises kinetic modelling of ^{11}C-DASB can be performed by reference region approaches e.g. by the Multilinear Reference Tissue Model 2 (MRTM2) (Ichise et al., 2003). By this approach, the first step is an estimation of the clearance rate constant (k2´) by the Multilinear Reference Tissue Model (MRTM). The k2´ is then fixed and used throughout modeling of all regions (MRTM2) (Ichise, Liow et al. 2003).

Motion correction of ^{11}C-DASB PET images

Within a PET scan, motion can greatly affect the accuracy of the measurement since it compromises the resolution. This issue can be handled in the reconstruction process e.g. by realigning the frames with movement. For less gross movements, a smoothing of data during preprocessing can be sufficient to make the kinetic modeling fit. Nevertheless, motion correction is a tradeoff between being able to fit data to the applied kinetic model and introducing unwarranted bias. Experimental test-retest settings are particularly sensitive to the application of different preprocessing methods, and in situations where one scan is hampered by movement it can be necessary to motion correct both image, although this approach narrows the natural variance of data and potentially can favor type II errors (rapport A).

Injection of unlabeled DASB

If the injected mass of cold compound (unlabeled tracer) is too high, it might compete with the radiotracer and cause an underestimation of the binding potential. This has been reported for other antagonist radiotracers, e.g., the 5-HT_4 receptor radiotracer ^{11}C-SB207145 (Madsen, Marner et al. 2011). The mass dose effects of DASB on SERT binding have not yet been investigated and this uncertainty is currently being managed differently from study to study e.g., by exclusion of dataset with high injected mass, by inclusion of injected mass as a covariate in statistical models or by a mathematical correction of the binding potential (Madsen, Marner et al. 2011) (rapport B).

Aims and hypothesis

Previous cross-sectional neuroimaging studies (table 1) (Kalbitzer, Erritzoe et al. 2010, Praschak-Rieder and Willeit 2012) have demonstrated that in individuals without a diagnosis of SAD, cerebral SERT correlates inversely with daylight minutes, in particular in S-carriers (Kalbitzer, Erritzoe et al. 2010). However, it remains to be investigated both how individuals resilient to SAD and patients with SAD regulate their cerebral SERT across seasons, if such seasonal changes are related to clinical severity of SAD, and how 5-HTTLPR genotype and gender interacts with the season-related change in SERT. Thus, the hypothesis and aims of this PhD-study are as follows:

Aim and hypothesis I: We aim to characterise seasonal SERT regulation in individuals resilient to SAD**.** Based on previous findings, we hypothesise that SERT levels in winter, is higher compared to summer.

Aim and hypothesis II: We aim to investigate group differences in seasonal SERT regulation and the relation to clinical severity in SAD patients. Under the assumption that depressive symptoms are manifestations of low 5-HT neurotransmission (Torres, Gainetdinov et al. 2003, Savitz and Drevets 2013), we hypothesise, that SAD patients have even higher global SERT levels in the winter compared to individuals resilient to SAD and that the increase from summer to winter correlates with clinical severity of SAD.

Aim and hypothesis III: We aim to explore the effects of 5-HTTLRP genotype on seasonal SERT regulation in SAD patients. We hypothesise, that SAD

patients that carry the S-allele will show larger seasonal SERT regulation compared to the SAD patients homozygote for the L_A-allele.

Aim and hypothesis IV: We aim to investigate gender by season interaction effects on seasonal SERT regulation in individuals resilient to SAD and in SAD patients. As female gender predisposes to seasonality (Magnusson and Partonen 2005) we hypothesise that seasonal SERT regulations will be larger in females compared to males.

Ethical Considerations

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, H-2-2010-108 and H-4-2011-103. All subjects consented to participation, in accordance with The Declaration of Helsinki II.

SAD is a highly prevalent condition in the northern hemisphere, thus many people would benefit both from the results and from less stigmatisation, as diseases with well described patophysiology are often more accepted in society. The individual risk and discomfort each participant underwent in conjunction with the study should be weighted against the burden of disease that follow depression and the importance of understanding neurobiological underpinning in order to keep progressing with respect to both pharmacological as well as psychosocial treatment strategies. During the experiment subjects receive radiation equivalent to three years of background radiation, all participants were informed of this prior to entering the study. SAD patients were required not to receive treatment for their condition during the experiment, but they were informed of these premises prior to inclusion and no one left the study due to this requirement.

Finally, the 5-HT system is involved in numerous severe psychiatric as well a neurological diseases i.e. major depressive disorder, schizophrenia and dementia and knowledge regarding key features of the 5-HT system may constitute an important contribution in these areas of research as well.

Material and Methods

Methodological details can be found in the paper I and paper II.

Study Participants

Subjects that met the initial screening criteria were asked to fill out The Seasonal Pattern Assessment Questionnaire (SPAQ)(Rosenthal, Sack et al. 1984), a self assessment questionnaire that evaluates seasonal variations in sleep, social activity, mood, weight, appetite and energy. The score on each item is summed to obtain a Global Seasonality Score (GSS), which indexes the degree of seasonality symptoms (range: 0-24, GSS>10 indicates SAD)(Kasper, Wehr et al. 1989).

Inclusion criteria

- Age 18-45 years
- BMI < 25
- Non-smokers
- Normal medical and neurological examination
- Copenhagen residency

Exclusion criteria

- Past or present neurological or psychiatric (ICD-10) disorders
- Use of drugs with effects on the 5-HT system
- Use of recreational illegal drugs more than 10 times, use of cannabis more than 50 times

- Significant medical history
- Retinal pathology
- Use of photosensitizing medications
- Travelling to destinations at a different latitude 3 months prior to any of the scans or unstable diurnal cycle
- Planned or current pregnancy
- Pathological findings on medical examinations, routine blood tests or MRI scans

Individuals resilient to seasonal affective disorder (Non-SAD group)

Additional inclusion criteria

- S- or L_G carriers of the 5-HTTLPR genotype

All subjects were interviewed by one of the investigators prior to inclusion to assure adherence to the screening criteria, to assure absence of overt seasonality and to confirm absence of overt psychiatric illness.

The group was matched to the SAD group with respect to sex distribution, age and BMI.
A total of 112 healthy subjects were genotyped with respect to 5-HTTLPR genotype to include 24 S-carriers. There were no drop-outs but one subjected was not included due to poor quality of the PET image. Thus 23 longitudinal data sets were included in the study.

Psychometric assessments

A panel of state questionnaires including the Major Depression Inventory (MDI) (range: 0-50, > 21 indicates depressed mood) (Bech, Rasmussen et al. 2001, Olsen, Jensen et al. 2003), The Pittsburgh Sleep Quality Index global scores (PSQI GS) (range: 0-21, > 5 indicates sleep difficulties) (Buysse, Reynolds et al. 1989) and Cohens Perceived Stress scale (PSS) (range: 0-40, no

fixed cut-off formally applied to indicate stress) were implemented to assure that all subjects had a stable mood and sleeping pattern across the year. Trait measures related to risk and resilience (The Family History Assessment Module screener (FHAM) (Rice, Reich et al. 1995)and The Stressfull Life Events (SLE) (Roohafza, Ramezani et al. 2011), personality (NEO-PI-R) (P.T. Costa 1992), intelligence (Reynolds Intellectual Screening Test (RIST)) (C.R. Reynolds 1998), educational level, marital status and ethnical origin were also registered.

Seasonal affective disorder patients (SAD group)

Additional exclusion criteria

- Bright light therapy within the past year
- Psychotropic drug therapy within the past year
- Significant neuropsychiatric co-morbidity (axis I or axis II disorders)
- Failure to achieve spontaneous remission or depression the succeeding season

Additional inclusion criteria

- GSS $\geq$ 11 and stating seasonality to be at least a moderate problem
- Meet the ICD 10 diagnostic criteria for MDD *and* the SAD criteria described by Rosenthal (Rosenthal, Sack et al. 1984) .

Psychiatric assessment

SAD candidates were biannually assessed by consultants in psychiatry to confirm the SAD diagnosis. All referred candidates underwent The Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing, Babor et al. 1990) to exclude any other axis I or axis II disorders before final inclusion. The Structured Interview Guide for the Hamilton Rating Scale for Depression – Seasonal Affective Disorder version (SIGH-SAD)(Williams JB 1988) was used to index symptom severity both summer and winter.

A total of 36 patients were referred to psychiatric interview, of those 12 were excluded due to co-morbidity or failure to meet diagnostic SAD criteria. Seven subjects 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. As follows, a longitudinal data sets of 17 patients were included in the study.

Group characteristics

The study included 23 healthy S-carriers with low seasonality scores (13 females, GSS: 4.8±2.1, age: 26±6 years) and 17 SAD patients (9 females, 11 S-carriers, GSS: 14.1±2.2, age: 27±9 years), all values given as mean±SD. The groups were comparable with respect to age (un-paired t-test of mean age ((age $_{winter}$ + age $_{summer}$)/2), P = .55), sex (Fishers exact test, P >.99) and BMI (un-paired t-test summer: P = 0.15 and winter: P = .32). Detailed sample characteristics and radioligand variables can be found in table 5.

Table 5. Sample characteristics and radioligand variables (from paper II)

	Summer	Winter	*P*-value
Non-SAD, N=23			
Clinical data			
MDI score	5.4 ± 3.6	5.0 ± 3.5	.49
PSQI GS	3.7 ± 2.1	3.6 ± 1.8	.79
BMI (kg/m^2)	23.1± 2.1	22.9 ± 2.1	.42
Biochemistry			
Tryptophan load[1](n=14)	0.13 ± 0.02	0.13 ± 0.02	.86
Estradiol (nmol/L) (n=10)	0.13 ± 0.07	0.24 ± 0.14	.06
Progesterone (nmol/L)(n=11)	1.56 ± 1.00	4.1 ± 7.93	.32
Radioligand variables			
Non-displaceablebinding (Bq/ml)[1]	18634 ± 2650	18489 ± 3351	.77
k_2' (per min)	0.07 ± 0.01	0.07 ± 0.001	.57
Injected mass (µg/kg)	0.02 ± 0.01	0.04 ± 0.03	.001
SAD patients, N=17			
Clinical data			
MDI score	6.4 ± 4.2	21.4 ± 7.9	<.001
PSQI GS	4.5 ± 1.8	6.5 ± 2.3	.02
SIGH-SAD score	2.1 ± 2.3	23.1 ± 8.8	<.001
BMI (kg/m^2)	22.3 ± 2.5	22.1 ± 2.5	.29
Biochemistry			
Tryptophan load[1]	0.14 ± 0.03	0.13 ± 0.02	.07
Estradiol (nmol/L) (n=8)	0.19 ± 0.20	0.18 ± 0.18	.81
Progesterone (nmol/L)(n=7)	6.6 ± 13.7	0.84 ± 0.40	.32
Radioligand			
Non-displaceable binding (Bq/ml)[*]	18516 ± 3747	17600 ± 3684	.07
k_2' (per min)	0.07 ± 0.01	0.07 ± 0.01	.48
Injected mass (µg/kg)	0.02 ± 0.03	0.03 ± 0.06	.68

[*]As evaluated by $AUC_{cerebellum.}$

Genotyping, plasma amino acids and sex-hormone data

Analysis of the 5-HTTLPR genotype was performed on DNA purified from saliva. Immediately before all PET scans, blood was drawn for determination of plasma tryptophan (Knudsen, Pettigrew et al. 1990) and estradiol and progesterone levels (in a subset of females) (Frokjaer, Pinborg et al. 2015).

Study Design

Participants in the SAD and Non-SAD samples were assessed summer *and* winter with ^{11}C-DASB PET and structural MRI acquisitions. The scan

sequence was randomized so that half of the individuals were scanned first time in the summer, the other half first time in the winter; defined as a 12 week interval centred around winter or summer solstice (figure 5).

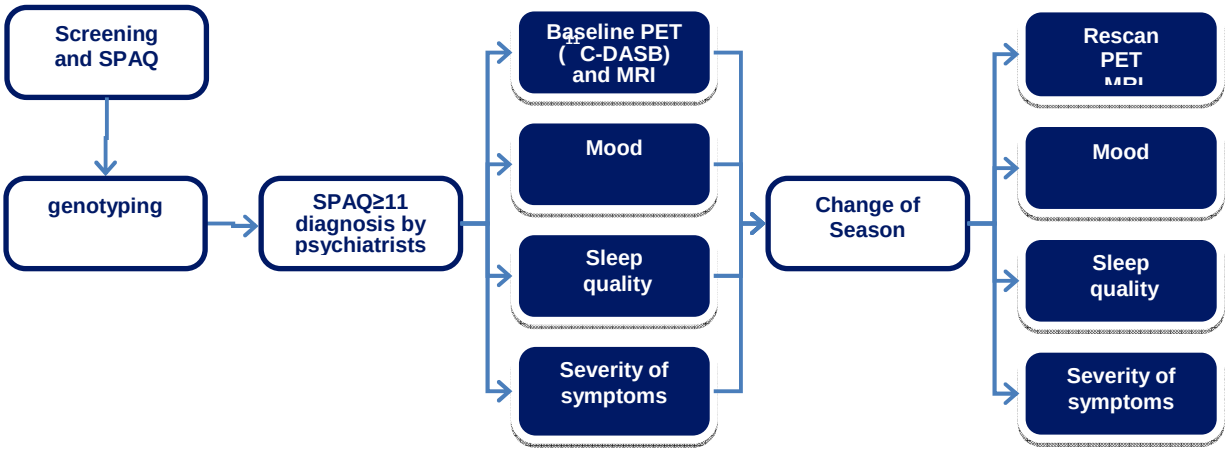


Figure 5. The study design and assessments. Only SAD patients were referred to psychiatric evaluation.

Neuroimaging Protocols

^{11}C-DASB PET acquisition

PET acquisition and quantification were performed as described in the papers and in Frokjaer et al. 2009 et 2015 (Frokjaer, Vinberg et al. 2009, Frokjaer, Pinborg et al. 2015). In brief, all subjects were scanned using a Siemens ECAT High-Resolution Research Tomography (HRRT) scanner operating in 3D list-mode with the highly selective radioligand ^{11}C-DASB. The 90 minutes of data acquisition were preceded by a 6 minutes transmission scan to obtain individual attenuation maps. To created coherence in the longitudinal data sets, all scans were in-scan motion corrected using AIR 5.2.5 (Woods, Cherry et al. 1992). The mean PET image was aligned to individual structural MR images. The quantification of ^{11}C-DASB non-displaceable binding potential (BP_{ND}) was determined using a reference tissue model (MRTM2) (Ichise, Liow et al. 2003).

Magnetic resonance imaging data acquisition and co-registration

All participants had a high-resolution structural Magnetic Resonance (MR) image on a Siemens Magnetom Trio 3T MR scanner or a Siemens 3T Verio MR scanner. A 3D T1-weighted Rapid three-dimensional Tradient Echo (MP-RAGE) and a T2-weighted Turbo Spin Echo (TSE) structural image were acquired and used for segmentation (MP-RAGE) and brain-masking as previously described (Madsen, Haahr et al. 2011).

Paper I: MR scans were processed and analyzed using FreeSurfer. All Single-subject PET time activity curves (TAC) were initially summed and averaged over all time frames in order to estimate a weighted 3D Image for co-registration. A rigid intra-subject multimodal registration was used for co-registration (Greve and Fischl 2009). Cortical BP_{ND} maps were smoothed (averaging over neighboring vertices) with a Gaussian 2D filter using a full width half maximum (FWHM) of 10 mm.

Paper II: Co-registration of the high-resolution MR and PET images was performed in SPM8 using the mean of the first 20 minutes of the PET scan, corresponding to a flow-weighted image. A fully user independent automatic delineation of volumes-of-interests (VOI) was performed using probability maps (Svarer, Madsen et al. 2005) based on ten high-resolution MR templates were VOI's have been delineated manually. Radioactivity in regions was calculated as the volume weighted average of radioactive concentrations in left and right hemisphere regions

A priori outcome measures

As seasonal SERT fluctuations has been found in a range of brain regions (table 1) and as there is substantial inter-regional correlation of SERT binding (Erritzoe, Frokjaer et al. 2010) and an extensive neuronal communication between the raphe nuclei and the SCN (Morin 2013), we assume that cerebral

seasonal SERT adjustments are orchestrated within the raphe nuclei. Thus we implemented a global measure of cerebral SERT binding.

Calculation of global SERT binding potential

A volume weighted average of whole brain ^{11}C-DASB BP_{ND} (global BP_{ND}) was estimated based on 17 grey matter segmented brain regions (X = 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 cingulate gyrus, putamen, sensori-motor cortex, superior frontal gyrus, superior temporal gyrus, and thalamus):

Equation (Eq.)1

$$\text{Global } BP_{ND} = (\sum(BP_{NDx} * \text{volume}_x))/ \sum \text{volumen}_x$$

Calculation of seasonal SERT regulation

The longitudinal information was integrated into a single variable that described seasonal SERT regulation:

Eq.2

$$\Delta\ BP_{ND} = BP_{ND}\ \text{winter} - BP_{ND}\ \text{summer}$$

Calculation of relative seasonal SERT regulation

Information of relative SERT changes across seasons was integrated into a single variable that describes seasonal SERT regulation relative to winter:

Eq. 3

$$\text{rel } \Delta BP_{ND} = \Delta\ BP_{ND} / \text{winter } BP_{ND}$$

Calculation of relative change in SIGH-SAD scores

Information of *relative* mood changes across seasons was integrated into a single variable that describes seasonal SIGH-SAD regulation relative to winter (equivalent to Eq. 2 and 3):

Eq.4

$$\text{rel } \Delta\text{SIGH-SAD} = (\text{winter score-summer score})/\text{winter score})$$

Statistical analysis

Hypothesis testing

The null-hypothesis (H_0) was specified as no differences between measures:

H_0 is true when:

$$\text{Summer } BP_{ND} = \text{winter } BP_{ND} \quad \text{or} \quad \text{SAD } BP_{ND} = \text{Non-SAD } BP_{ND}$$

H_0 is rejected when:

$$\text{Summer } BP_{ND} \neq \text{winter } BP_{ND} \quad \text{or} \quad \text{SAD } BP_{ND} \neq \text{Non-SAD } BP_{ND}$$

Power calculations

Based on previous test-retest studies ^{11}C-DASB BP_{ND} has a variability of 3.7% and a reliability of 0.89 in high-binding regions (Kim, Ichise et al. 2006). Based on this 8 subjects are needed to detect a 20% deference in BP_{ND}.

Management of covariates

Covariates known to affect BP_{ND} (age (Kalbitzer, Frokjaer et al. 2009), 5-HTTLPR genotype (Willeit and Praschak-Rieder 2010), sex and BMI (Erritzoe, Frokjaer et al. 2010)) were included as covariates in multiple regression analysis of absolute BP_{ND} levels. Prior to analysis, multicollinerity between continuous variables was tested by calculation of the variance inflation factor (VIF = 1/1-R^2) with a R^2 threshold of 0.75. In addition, differences in these covariates and in parameters that could potentially affect BP_{ND} (radioligand variables, tryptophan level, estradiol- and progesterone levels in females) were compared across seasons and across groups by means of paired or un-paired students t-tests respectively for numerical variables and by means of Fischers exact test for dichotome variables. As SAD is more common in females and young individuals (Magnusson and Partonen 2005) and possibly in S-carriers (Johansson, Willeit et al. 2003), age, 5-HTTLPR genotype, sex and sex by group interactions were included in the longitudinal SAD data analysis of as covariates. Data was not adjusted for BMI as BMI changes is part of the SAD symptomatology. As injected mass DASB do not affect SERT BP_{ND} with in the

range given in the studies (rapport B), injected DASB was not included as a covariable in the statistical models.

Statistical models, paper I

Longitudinal analysis of global SERT binding across seasons

Global seasonal SERT regulation and sex by season interaction effects were investigated by means of a single-factor repeated measures analysis of covariance (ANOVA).

Longitudinal univariate voxel-wise analysis of SERT binding across seasons

Parametric maps of estimated BP_{ND} at a voxel level were compared across seasons by means of a two tailed students paired t-test. Correction for multiple comparisons was executed in a clustering framework using non-parametric permutations tests.

Statistical models, paper II

Cross-sectional group comparisons of SERT binding in winter and in summer

Group differences in global SERT binding summer and winter were assessed in separate multiple regression analysis (A1 and A2):

$$\text{A1.global } BP_{NDsummer} \sim \text{group} * BMI_{summer} * age_{season} * \text{genotype} * \text{sex}$$

and

$$\text{A2. global } BP_{NDwinter} \sim \text{group} * BMI_{winter} * age_{winter} * \text{genotype} * \text{sex}$$

Comparison of seasonal SERT regulation across groups

The difference in global seasonal SERT regulation across groups was assessed in a multiple regression analysis employing ΔBP_{ND} (Eq. 2) as outcome parameter (A3):

$$\text{A3.global } \Delta BP_{ND} \sim \text{group} * \text{sex} * \text{sex by group} * \text{mean age} * \text{genotype}$$

Correlation of relative change in SERT levels and relation to relative change in SIGH-SAD scores

A multiple regression analysis was applied to investigate if relative global SERT regulation (Eq.3) predicted relative change in SIGH-SAD scores (Eq.4) (A4):

$$\text{A4.rel } \Delta BP_{ND} \sim \text{rel } \Delta\text{SIGH-SAD} * \text{sex} * \text{genotype}$$

A probability (P) of 0.05 was considered statistical significant in all analysis. The statistical analyses were carried out in GraphPad Prism v.6, GraphPad Instat v.3, R v.3.1 and MATLAB R2013a (8.1.0.604) 64 bit.

Results and Discussion

The main results are summarised and put into perspective whereas a more detailed reporting and discussion of individual findings can be found in paper I and paper II. Multiple regression analysis (A1-4) and equations used to compute outcome parameters (Eq.1-4) are detailed in Materials and Methods. All results are reported as mean ± SD.

Aim I

Characterisation of seasonal SERT regulation in SAD resilient individuals

Summer and winter global BP_{ND} values (Eq.1) and single voxel values were compared by two-tailed students paired t-test (corrected for multiple comparisons). The results are reported in paper I.

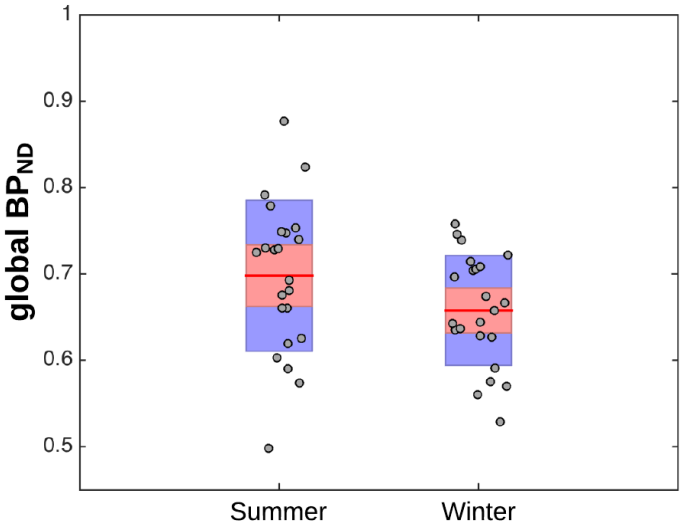


Figure 5. Global SERT BP_{ND} summer and winter in Non-SAD (paper I). Non-SADs have significantly lower global SERT BP_{ND} in summer (0.7 ± 0.09) compared to winter (0.66 ± 0.06), two tailed paired t-test P = .003.

We hypothesised that our healthy participants would have higher global SERT BP_{ND} levels in winter compared to summer. Instead, we found that they had significantly *lower* global SERT BP_{ND} in the winter compared to summer (figure 6). At first glance, this is puzzling because several large PET studies of seasonal SERT fluctuations have found highest levels of SERT in winter (table 1). A possible explanation for this is that the healthy controls enrolled in those studies were not included with the particular aim to investigate seasonality. This means that the cohorts were not investigated closely for absence of seasonality symptoms, likely to be present in a large fraction of the population living at high latitudes (50°- 62° N) where seasonal mood swings are frequent (Magnusson, 2000.) Thus, the contrasting findings may be a consequence of the deliberate bias of low seasonality in our sample. When 11 SAD resilient healthy controls (GSS: 1.9±1.6) and 11 SAD patients were scanned in the winter with ^{123}I-β-CIT SPECT it was found that thalamic-hypothalamic SERT variance was lower in SAD patients compared to the SAD healthy controls when measured 24, but not 4 hours post injection (Willeit, Praschak-Rieder et al. 2000). However, the discrepancies may stem from use of a less optimal tracer or the lack of longitudinal information in the ^{123}I-β-CIT SPECT study. Nevertheless, several studies support that individuals maintaining mental health in spite of an increased risk-load are characterized by *trait* markers of low SERT levels or high 5-HT levels, in particular in regions important to affect regulation, that is **1)** limbic and striatal levels of 5-HT_4 receptors, as quantified by ^{11}C-SB207145

PET, were found to correspond inversely with the number of depressed first degree relatives across three groups of risk-staged healthy participants, suggesting a positive "dose-response" correlation of 5-HT levels (Haahr, Fisher et al. 2014) and risk-load (Madsen et al., 2014) **2)** SERT levels, as quantified by ^{11}C-DASB PET, in the dorsolateral prefrontal cortex were found to be higher in subjects with a depressed co-twin compared to subjects with a healthy co-twin (Frokjaer, Vinberg et al. 2009) and **3)** the majority of neuroimaging studies have reported lower levels of SERT in high binding regions of S and L_G-carriers compared to L_A-homozygotes, as reviewed by (Willeit and Praschak-Rieder 2010). Although a later ^{11}C-DASB PET study could not confirm that (Murthy, Selvaraj et al. 2010)) and **4)** by means of ^{11}C-SB207145 PET scans, we found lower neocortical availability of 5-HT_4 receptors in S-carriers compared to L_A-homozygotes (Fisher et al., 2012). In line with these indices of permanent increased 5-HT levels in individuals with high genetic liability, our healthy subjects responded to increased environmental liability by lowering SERT level and presumably increasing synaptic levels of 5-HT (Nagayasu, Kitaichi et al. 2010). Thus our study and previous literature imparts that sustaining an euthymic mood in the context of genetic or environmental pressure is not biological effortless, rather it seems to demand an active adaptation in key features of the 5-HT system.

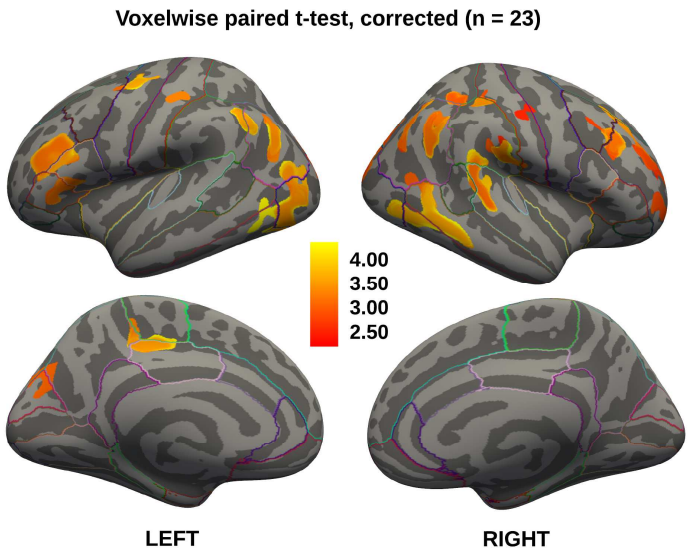


Figure7.The univari-ate voxel-wise analysis depicted on the inflated brain (paper I). The map displays the t-values of clusters with significant changes in ^{11}C-DASB binding across seasons (summer - winter), corrected for multiple comparisons. The top row: Cortical presentation, the bottom row: Mid-sagittal presentation.

Table 6. A selection of significant clusters with significant higher SERT BP_{ND} in summer compared to winter (paper I).

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

As illustrated in figure 7 and table 6 the whole brain univariate voxel-wise analysis revealed several larger cortical clusters (e.g. five clusters exceeds 900 voxels) with a significant down-regulation of SERT in winter. Previous

neuroimaging studies have primarily found seasonal SERT changes in sub-cortical regions (table 1). However, two PET studies using ^{11}C-McN5652 in 29 Germans (Buchert, Schulze et al. 2006) or ^{11}C-DASB in 57 Danes (Kalbitzer, Erritzoe et al. 2010), included post hoc whole brain voxel based analysis. Both studies had reported higher SERT binding in high-binding regions in winter, but neither of them was able to extend these findings to cortical brain areas. However, in the setting of an optimal radiotracer and a paired design, we find substantial evidence of cortical involvement in seasonal SERT regulation (of note, all cluster displayed decreased levels of SERT in winter, but none of the sub-cortical clusters survived corrections for multiple comparisons). Several clusters were detected across the prefrontal cortices ($.0039 < P_{corrected} < .0009$). One previous ^{11}C-DASB PET study found higher SERT in winter in the anterior medial prefrontal cortex and the anterior cingulated gyrus in 88 citizens of Toronto . We conducted fMRI experiments in 30 healthy males before and after three weeks of BLT, and found a negative dose-response effect in threat-related cortical reactivity and, in L_G- and S-carries, we found increased inter-prefrontal coupling (Fisher, Madsen et al. 2014). Thus our findings are complementarily to the Toronto study implying that the prefrontal cortex is highly engaged in directing mood and vigilance according to environmental cues. As the winter is perceived as a more hostile environment (Kalbitzer, Kalbitzer et al. 2013) seasonal SERT fluctuations in prefrontal cortex may represent seasonal modifications of prefrontal engagement in limbic top-down control.

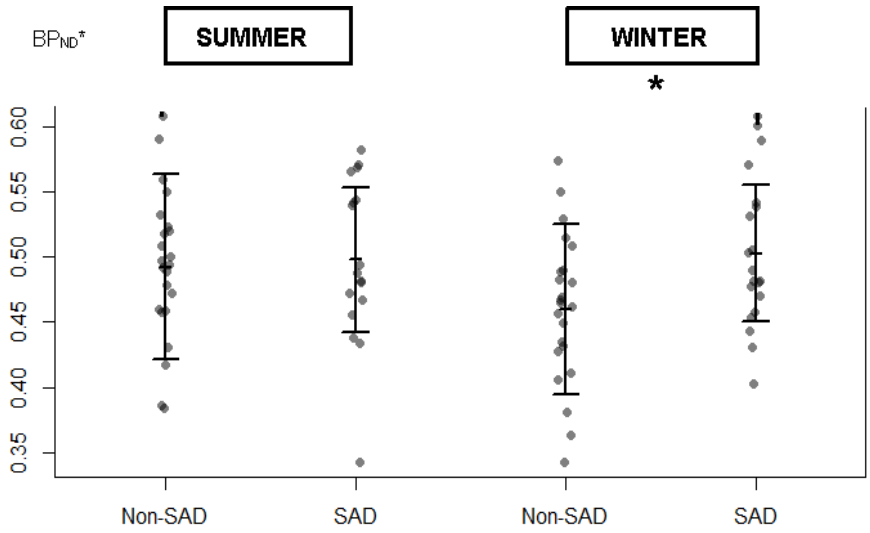
In addition, we recorded large winter down-regulations of SERT binding across the posterior lateral medial/inferior parts of the temporal cortices with extensions to the adjacent parts of the occipital cortices (cuneus) ($.0066 < P_{corrected} < .0001$). These regions receive substantially retinal input from the rods and cones via thalamic-cortico projections and they are crucial to object recognition and visual image formation (e.g. contains secondary visual cortex). No previous studies have investigated seasonal fluctuations of SERT or other features of the 5-HT system in these regions. Nevertheless, it is feasible that the low levels of SERT in winter is linked to a reduction in excitatory input, due to

less illuminative stimulation of the image-forming retinal ganglion cells in winter.

Aim II

Investigation of group differences in seasonal SERT regulation and the relation to symptom severity in SAD patients

Separate multiple regression analysis was applied to data from summer (A1) and winter (A2) to investigate cross-sectional group differences in absolute BP_{ND} values in the two seasons. Difference in seasonal SERT regulation (ΔBP_{ND}, Eq.2) across groups was investigated in a multiple regression analysis (A3). In the SAD group, the relative change in ΔBP_{ND} (rel ΔBP_{ND}, Eq.3) was correlated to the relative change in symptom severity (relΔSIGH-SAD, Eq.4) in a multiple regression analysis (A4). The results are reported in paper II.



*adjusted for differences in age, BMI, sex and 5-HTTLPR genotype

Figure 8. Seasonal effects across groups (paper II). No difference in SERT BP_{ND} was found across groups in summer (A1, N= 40, estimate = -0.02 BP_{ND}, 95% CI = -0.073 to 0.033, R^2 = .20, df= 34, *P* = .45), whereas SAD individuals had higher SERT compared to Non-SAD in the winter (A2, N = 40, estimate = 0.06 BP_{ND}, 95% CI = 0.013 to 0.101, R^2 = .27, df = 34, *P* = .01)

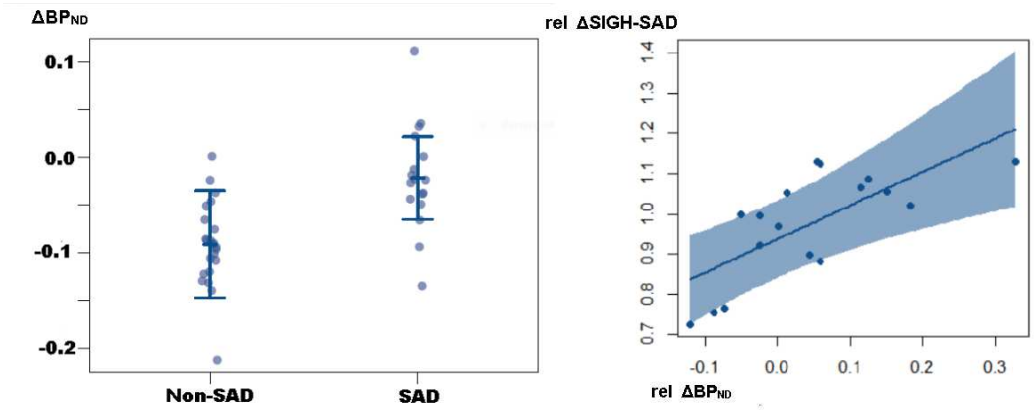


Figure 9. Seasonal effects across seasons and correlation to SAD symptoms (paper II). Figure 9A: Global ΔBP_{ND} was significantly different between groups (A3, N = 40, estimate = .10 ΔBP_{ND} Non-SAD > SAD), $P < 0.001$). **Figure 9B:** Relative change in symptom severity was significantly associated with relative difference in global cerebral SERT binding (A4, N = 17, estimate = .83 rel SIGH-SAD, R^2 =.47, P =.006).

By analysis A1-3 (figure 8 and 9), we have, in agreement with our hypothesis, found higher SERT in winter but not in summer in SAD patients and a significant difference in seasonal SERT regulation across groups. A ^{123}I-β-CIT SPECT study (detailed above) (Willeit, Praschak-Rieder et al. 2000) found *lower* thalamic binding in SAD patients compared to healthy controls in winter. Thus, we subsequently addressed seasonal effects in thalamus but, in accordance with the outcome of the global analysis, we found significantly higher thalamic BP_{ND} in SAD's in the winter compared to Non-SAD's. Nevertheless, as SERT actively clears 5-HT from the synaptic cleft (Torres, Gainetdinov et al. 2003) and SAD symptoms can be alleviated by SSRI treatment (Thaler, Delivuk et al. 2011) it is likely that SERT levels are raised during the depressive stages. Albeit this matter is continuously being debated, the majority of neuroimaging studies including MDD patients are in agreement with this observation, as reviewed in (Savitz and Drevets 2013). The authors of this review suggest that a primary increase in SERT levels facilitate decreased synaptic levels of 5-HT that manifests as depressive symptoms. Our data are in agreement with this concept, extending the model to include individuals with SAD. The group difference in seasonal SERT regulation (A3) was driven by a

marked drop in global SERT levels in the shift to winter in the Non-SAD group. This observation indicates that SAD patients are unable to downregulate their cerebral SERT levels in winter, at least to a sufficient extent. The SAD S-carriers mostly responded to the environmental stressor by an upregulation of SERT. However, this effect was only borderline significant (P = .056) and the understanding of these processes would greatly benefit from an independent replication. It also suggests that those individuals that fail most to downregulate SERT may in fact be those who would benefit most from SSRI intervention. Nevertheless, the results of analysis A1-3 uniformly imply that the SAD depressive state in winter is linked to an increase in global cerebral SERT levels. The high levels of SERT may prompt a rapid removal of endogenous 5-HT; depriving the brain of 5-HT and facilitating manifestations of depression. Albeit, we cannot conclude from the data if the association between changes in SERT levels and the depressive condition is a causal relation, but given that blocking of SERT by SSRI is use therapeutically to treat depression it may be present as a causal relationship. Moreover, our data does not inform us to what extent the seasonal regulation of SERT levels is a primary event linked directly to changes in daylight minutes or if it represents a secondary mechanism initiated to keep of 5-HT homeostasis under circumstances of seasonal changes in 5-HT synthesis or vesicle trafficking. The causality of these events could ideally be explored by a biannual assessments of 5-HT levels (e.g. by quantification of 5-HT_4 receptors (Haahr, Fisher et al. 2014) preferably in a longitudinal setting of individuals staged according to seasonality.

We found a robust positive association between relative increase in SIGH-SAD scores (rel ΔSIGH-SAD) and relative increase in SERT BP_{ND} (rel ΔBP_{ND}) (figure 9B) (e.g. inclusion of age or exclusion of the sex in the statistical model did not change the outcome). Moreover, the SIGH-SAD (derived from the Hamilton Depression Rating Scale (HDRS)) is a well validated psychiatric instrument that takes into account both core symptoms of depression i.e. low energy, anhedonia and low mood as well as the atypical vegetative symptoms often described in SAD (Williams JB 1988). Three other molecular neuroimaging studies have investigated ratings of symptom severity versus

SERT binding during depressive stages of uni- or bipolar disorders **1)** Meyer et al. 2004, reported that SERT binding across various brain regions correlated positively with anxiety ratings, as indexed by the Dysfunctional Attitudes Scale (DAS), in MDD patients, but not in healthy controls (Meyer, Houle et al. 2004) **2)** Cannon et al. 2006 found a positive correlation of SERT binding in insular cortex and dorsal cingulated cortex and anxiety ratings (Beck Anxiety Inventory (BAI) during depression in 18 unmedicated patients with bipolar disorder whereas no correlations was found between SERT binding and HDRS ratings, the Montgomery–Åsberg depression rating scale (MADRS) or the inventory of Depressive Symptomatology-Clinician Rated (IDS-C) in mesencephalon, striatum, thalamus or selected parts of the anterior cingulate cortex (Cannon, Ichise et al. 2007) and **3)** Ruhe et al., 2009 (^{123}I-β-CIT SPECT) correlated HRDS ratings of 49 MDD patients and SERT binding in mesencephalon and thalamus (Ruhe, Booij et al. 2009) but did not find any correlation. In addition, Frokjaer et al., 2015 investigated the correlation of sub-clinical symptoms and SERT binding in *healthy* individuals by a placebo-controlled randomised ^{11}C-DASB PET using a GnRHa intervention to trigger symptoms. They found that HDRS ratings was positively associated with higher cortical SERT binding post intervention in the active group (Frokjaer, Pinborg et al. 2015). Nonetheless, diversity in examined cohorts, neuroimaging tools and psychometric instruments makes it is difficult to draw a mutual conclusion across studies. The clear association of SERT changes and increase in depressive symptoms in our study implies that SERT changes are part of the SAD aetiology, although this analysis cannot establish causality.

Aim III

Exploration of 5-HTTLRP genotype effects on seasonal SERT regulation in SAD patients

The SAD group was divided into two samples of 6 L_A-homozygotes and 11 S-carriers and seasonal differences in global BP_{ND} was analysed in separate paired

t-tests (two-tailed). The non-SAD sample was included in the multiple regression analysis of genotype effects on seasonal SERT regulation (A3, N S-carriers = 34, N = L_A-homozygote, ΔBP_{ND} adjusted for age, sex and group effects). The effect of genotype on the association of seasonal SERT regulations and symptom severity in SAD patients was tested by analysis A4.
The results of analysis A3 and A4 are reported in paper II.

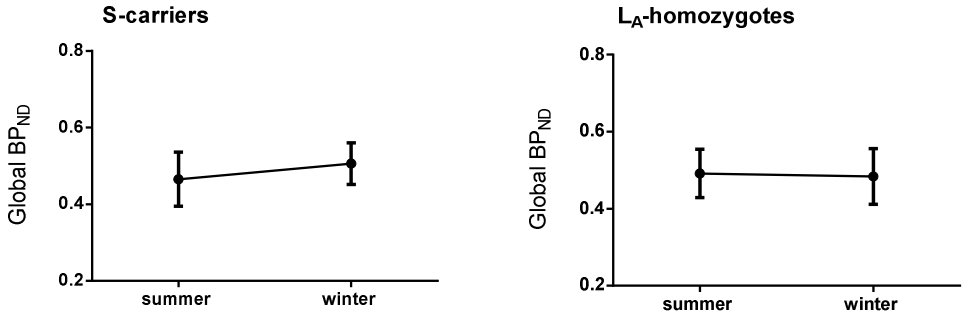


Figure 10. Summer and winter SERT BP_{ND} in S-carriers and L_A-homozygote SAD patients. SAD S-carriers had a borderline significant up-regulation of global SERT in winter (n = 11, t(10)= 2.173, *P* =.0549), whereas L_A-homozygote SAD patients had no difference (n= 6, t(5) = 0. 73, *P* =.50).

A previous ^{11}C-DASB PET study in an independent sample of 57 citizens from the Copenhagen community described increased seasonal SERT fluctuations in putamen in non-L_A-homozygotes compared L_A-homozygote males and females (table 1, figure 2) (Kalbitzer, Erritzoe et al. 2010)). The authors suggested that the S-allele imparts lability of 5-HTT expression (Kalbitzer, Erritzoe et al. 2010); this is in agreement with S-carriers' increased propensity to develop MDD in the context of stressful life events previously described (Caspi, Hariri et al. 2010). Thus, we aimed to examine if these observations could be extended to a SAD sample. We hypothesised that S-carrying SAD patients would exhibit a more pronounced winter up-regulation in SERT compared to L_A-homozygote SAD patients. We found, on a trend level, that SAD S-carriers had higher global SERT levels in winter compared to summer, whereas the 6 L_A-homozygote SAD patients had stable SERT across the year (figure 10). When including both SAD and Non-SAD participants and adjusting data for sex, age and group we

detected a significant larger seasonal SERT regulation in S-carriers compared to L_A-homozygote SAD patients (A3, estimate = 0.05 ΔBP_{ND}, P = .04). As genotype was a significant variable in analysis A4 (P = .04), we tested if the rel ΔBP_{ND} - rel ΔSIGH-SAD association was significantly different across genotypes by spitting the sample, repeating the analysis and comparing the slope estimates. This revealed that the association was only present in S-carriers (S-carriers: N = 11, df = 8, estimate = 0.87, P = .04, L_A-homozygotes: N = 6, df = 4, estimate = -0.05, P = .85, comparison of slopes, N = 17, P = .009). Due to the modest sample size of L_A-homozygotes, the analysis was repeated without adjusting for sex to increase the degrees of freedom (as this variable was insignificant across the full range of analysis) but this did not change the outcome. Nevertheless, this post hoc analysis was not well powered and we emphasize that an independent replication in a larger data set is required to confirm this preliminary finding. However, the range of analysis of genotype effects imply that in S-carriers, the SAD symptoms are elicited by a failure to downregulate or even upregulate SERT levels in winter whereas these events does not seems to occur in the L_A-homozygotes SAD patients. By design, we did not include L_A-homozygote individuals in our SAD resilient group. Thus, we are not aware to what extent these effects operate in low seasonality samples. Initially it was reported that the prevalence of S-carriers was higher among SAD patients (Rosenthal, Mazzanti et al. 1998), but a later meta analysis of three samples only found an allelic differences across samples, when the SAD sample was contrasted to a selected samples of low seasonality individuals (table 3))(Johansson, Willeit et al. 2003). However, the studies were all based on biallelic genotype stratifications. In our recruitment procedure we performed triallelic genotype testing (Nakamura, Ueno et al. 2000) to 112 healthy subjects (GSS > 11) and 73 potential SAD candidates (GSS ≥11) but we did not observe any differences in non L_A-homozygote allele frequency across the samples (P = .12) (table 7).

Table 7. Genotyping of 112 healthy controls (GGS < 11) and 73 potential SAD candidates GSS ≥11)

	112 healthy controls percent (N)	73 potential cases percent (N)
L-homozygotes total	38% (43)	23% (17)
L_AL_A	29% (32)	18% (13)
L_AL_G	10% (11)	6% (4)
S-allele carriers	62% (69)	77% (56)
SL_A	34% (38)	37% (27)
SL_G	9% (10)	7% (5)
SS	19% (21)	33% (24)
Non-L_A homozygotes	71% (80)	82% (60)

Taken together, our analysis suggests that the coupling between depressive symptoms and SERT changes primarily applies to S-carriers. The differences in transcription capacity across the alleles shapes profound differences in 5-HT signalling, by two temporally separated modes of action **1)** during brain development, neuronal migration and maturation are directed by disparate embryonic 5-HT settings (Azmitia 2001, Vitalis, Cases et al. 2007) which may shape differences in limbic fear responses (Fisher, Grady et al. 2015) and **2)** in the matured brain, allelic differences in cellular SERT expression elicit trait differences in endogenous 5-HT resources (Fisher, Holst et al. 2012) which seems to predispose S-carriers towards increased flexibility in SERT expression (Caspi, Hariri et al. 2010, Kalbitzer, Kalbitzer et al. 2013). Data from fMRI fear-conditioning studies suggest that these events conform to an intermediate phenotype expressing high traits of neuroticism and an elevated susceptibility to cyclic mood disorders (Hamilton, Etkin et al. 2012, Madsen, Mc Mahon et al. 2015). In essence, differences in SERT expression may cause L_A-homozygotes to be more sensitive to subtle SERT changes (to small to detect in our sample) or cause key components of the 5-HT system to operate differently i.e. the symptoms of inadequate 5-HT neurotransmission in (presumably) SERT stable participants may render from decreased synthesis/trafficking of 5-HT vesicles or reduced post synaptic 5-HT reception. Moreover, we adopted the summed SIGH-SAD score in our analysis however, it is plausible that some items are more susceptible to SERT changes than others. One study investigated

differences in depressive subtypes and found female L_A-homozygotes were more likely to experience melancholic depression whereas female S-carriers were more likely to experience atypical depression. Thus, we might see a different pattern of correlation if the sample was divided with respect to depression sub-types. Such an analysis was, however, restrained by the modest L_A-homozygote sample size.

Aim IV

Investigation of gender by season interaction effects on seasonal SERT regulation in individuals resilient to SAD and in SAD patients

Gender by season effects on global seasonal SERT levels were investigated in the Non-SAD group by a single factor repeated measures ANOVA. Effects of gender on seasonal SERT regulation (ΔBP_{ND}) in Non-SAD and SAD groups was examined by inclusion of a categorical gender variable and a second order gender by group interaction term in the statistical model (A3). Results are reported in paper I and paper II.

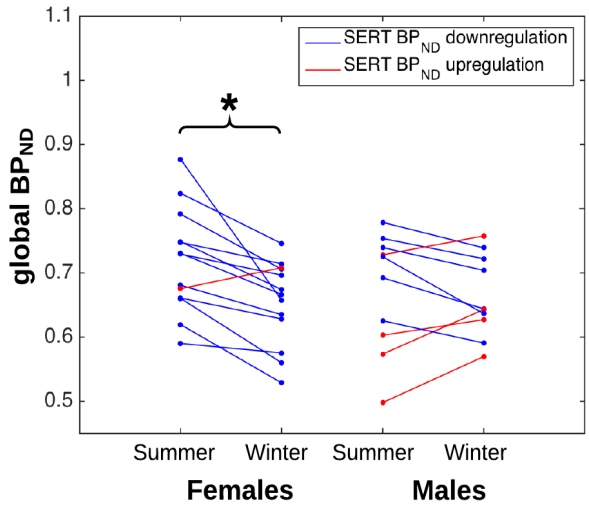


Figure 11. Female and male global SERT BP_{ND} values across seasons (paper I). A single factor repeated measures ANOVA showed a significant increase in global SERT BP_{ND} in the summer (0.7 ± 0.09) compared to the winter (0.66 ± 0.06) (F_{season} = 11.47, *P* = .003). With a significant gender by season interaction effect ($F_{season\_x\_sex}$ = 5.54, *P* = .029); females increased global BP_{ND} in the summer whereas males expressed stable SERT levels across the year. Males and females had comparable SERT levels when season was not taken into account (F_{sex} = 0.37, *P* = .55). Summer and winter BP_{ND} from individual observations are paired by the colour bars (red: up-regulation, blue: down-regulation).

We hypothesised that female and male healthy participants would have higher SERT levels in winter, but in contrast to our expectations, we found that the females had a significant larger down-regulation of global SERT in winter compared to males (figure 11). Due to a female preponderance to SAD (Magnusson and Boivin 2003) and the lack of seasonal SERT fluctuations in ^{11}C-DASB PET studies of males only (Murthy, Selvaraj et al. 2010, Matheson, Schain et al. 2015), we had hypothesised that SAD females would have a larger up-regulation of SERT in winter compared to males and this statement was confirmed by our data analysis (A3, N = 40, $estimate_{sex}$ = 0.050 ΔBP_{ND} (females > males), $estimate_{sex\ by\ group}$ = -0.07 ΔBP_{ND} (Non-SAD > SAD), group contrasts based on estimates from A3: females, n =22, *P* <.001 and males, n=18, *P*=.64). Two previous neuroimaging studies investigated gender by season effects in mesencephalon and thalamus but neither found any significant effects: Buchert et al., 2006 (^{11}C-McN5652 PET) adopted a gender by season interaction term and Koskela et al. 2008, explored sex differences in a longitudinal ^{123}I-ADAM SPECT study of 5 females and 7 males by means of paired t-tests in gender separated groups. Neither of the existing ^{11}C-DASB PET season studies applied sex focused investigations (e.g., included a second order gender by daylight minutes term) but data was corrected for sex differences in SERT BP_{ND} (Praschak-Rieder, Willeit et al. 2008, Kalbitzer, Erritzoe et al. 2010).

We suggest that sex differences in sex-hormone profiles may contribute to the gender specific SERT adjustment observed in our dataset. In particular, fluctuations in estradiol levels has been described to affect mood via SERT signaling (reviewed by (Borrow and Cameron 2014), investigated in (Frokjaer, Pinborg et al. 2015) and summarized in “Interaction of gender, estradiol and mood”. Notably, these studies have put great emphasis on females, whereas the SERT-estradiol coupling in males is relatively unknown. However, differences in sex-hormone profiles may promote gender specific expressions of the SERT-estradiol coupling and SERT dynamics in general (Borrow and Cameron 2014). Thus, we cannot exclude that males react differently to light induced SERT

manipulations and seasonal estradiol fluctuations (Bjornerem, Straume et al. 2006).

We found the most pronounced seasonal SERT adjustments in our cohort of SAD resilient females and not, as expected, in the SAD cohort. However, it is possible that the "stress-intervention" (deprivation of daylight) may be perceived with disparate valence across the genders. As seasonality is far more common among females (Magnusson 2000) our resilient females are indeed different from the females of the background population, whereas the male contrast presumably is more modest. In any instance, these results highlight the importance of meticulous handling of gender effects in particular when the prevalence across genders is not balanced.

Perspectives

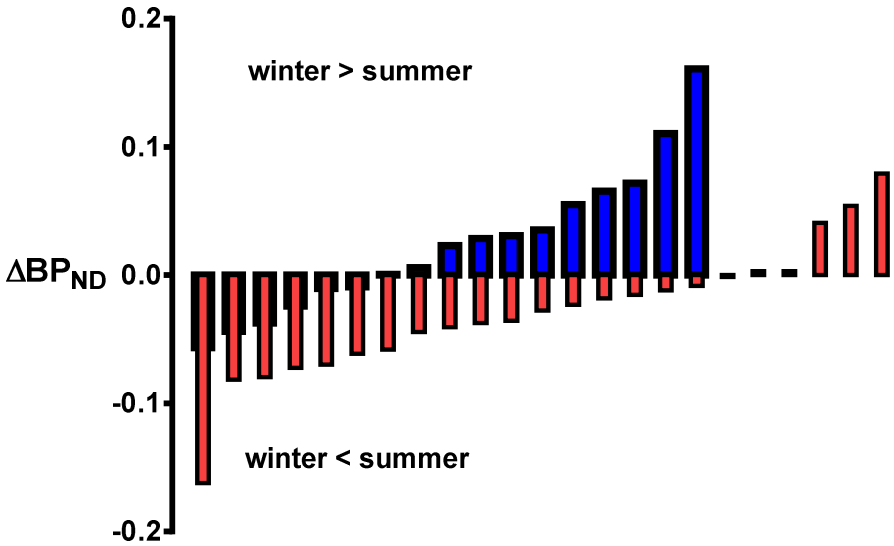


Figure 12. A graphical represent-tation of seasonal SERT regula-tion in SAD and Non-SAD par-ticipants (ΔBP_{ND}). The difference in ΔBP_{ND} is illustrated by an overlaying of the two datasets. Each participant is represented by a colour bar. The bars are sorted according to numerical ΔBP_{ND} values. Red: Non-SAD and blue: SAD

Our data, as depicted in figure 12, suggests that the observed high levels of SERT in winter found in previous cross-sectional ^{11}C-DASB PET studies (table 1) (Matheson, Schain et al. 2015)((Praschak-Rieder and Willeit 2012) is the

underlying neurobiological explanation for the high rates of seasonality, winter blues and SAD, reported at these high latitudes (Dam, Jakobsen et al. 1998, Magnusson and Partonen 2005). As our study participants in paper II represent two opposite outcomes in response to the environmental stress of winter and thus widens our understanding of seasonal SERT changes in SAD resilient individuals and in SAD patients. The link of unique SERT-regulation profiles and clinical expression of seasonality supports the understanding of seasonality as a dimensional entity with varying degree of penetration across populations (Levitan 2007). The current work converges with previous studies on the topic, demonstrating that the amplitude of seasonal SERT fluctuations increases with latitude and frequency of females and S-carriers within the investigated sample, whereas the presence of SAD resilient individuals will cause the amplitude to decrease, due to an opposite positioning of the peak and trough (paper I) (see also figure 2). Our finding underscores the importance of a thorough assessment of seasonality within a cohort, to avoid inclusion of individuals with subclinical expression of SAD features. This is in particularly important in a longitudinal setting as SERT regulations in opposite directions can result in a failure to detect even large inter-individual differences.

Methodological Considerations

Rapport A

The effect of motion correction

We applied MC to all PET datasets to assure that any detected difference was not caused by different degree of head motion across seasons or groups (type I error).

We investigated the effects of MC on absolute SERT binding from HRRT PET scans in a sample of 115 C ^{11}C-DASB datasets and on test-retest difference in SERT binding in 28 ^{11}C-DASB dataset pairs (not independent samples). We found that applying MC shifts data into a more narrow distribution curve with a bias towards a lower median. However, a low median voxel movement (less than 1 mm) was not associated with any significant change after MC neither on

a BP_{ND} level nor in the covariance of data points. Theses results suggests that MC can be applied by default in the pre-processing of data, as it does not induce unacceptable noise to the datasets that does not need it. Detailed results and discussion can be found in rapport A.

Rapport B

The effect of injected mass of DASB on SERT BP_{ND}

^{11}C-DASB PET is the best available neuroimaging tool for quantification of cerebral SERT (Wilson, Ginovart et al. 2002) and kinetic modeling can reliably be performed by use of a reference tissue models (Ginovart, Wilson, Meyer, Hussey, Houle, 2001; Ichise et al., 2003). Nevertheless, some considerations must be taken into account i.e. the injection of unlabelled compound will way across productions and potentially this could potentially bias the BP_{ND} estimation. Thus we investigated the effects of injected mass DASB across a sample of 108 ^{11}C-DASB PET HRRT scans conducted on healthy subjects below the age of 35 years and with a BMI less that 30 (n=108, 78 females). The correlation of injected mass DASB and BP_{ND} values from 3 regions of interest (a pooled subcortical high-binding region (highbinding), amygdala and a pooled cortical region (neocortex)) where investigated with simple linier regression to test if injected DASB predicted BP_{ND}. In addition, a sub-analysis was performed in 19 of the 108 datasets where the amount of injected DASB exceeded 5 μg (10 females). Across all ROIs we found that neither the total amount of injected mass DASB nor the amount of injected mass corrected for body weight correlated with $BP_{ND.}$ Detailed results and discussion can be found in rapport B

The implementation of a global BP_{ND} measure

Both region-based and voxel-based methods are reliable and accepted methods. Nevertheless, we are the first to implement a global region based on volume weighted BP_{ND} estimates of single regions. This method has the advantage of being based on time activity curves extracted from larger regions in native space

with less noise, as opposed to voxel based analysis that are computed from normalized and smoothed maps based on more noisy time activity curves necessitating rigorous corrections for multiple comparisons which can potentially discard true differences. However, some precaution should be taken upon the implementation of a global BP_{ND} in analysis. For one, the approach can conceal unsuccessful kinetic modeling in single regions. Thus, to assure that gross misspecifications of the BP_{ND} estimates were not included in the computation, the data fitting of data points for every single region was manually inspected. An alternative and perhaps more correct way to compute global SERT could be to extract a single time activity curve averaged across the whole brain with subsequent kinetic modeling (observation of C.Svarer). This approach would circumvent the somewhat counterintuitive extraction of a mean computed from values that range-six fold, but this approach does not allow for inspection of local fitting to the kinetic model and it hardly makes in major difference, as long as regions with poor fitting outcome are excluded, as is the case for our routine handling of the PET data.

The limitations of the studies included in the thesis

As SERT expression might be affected by menstrual phase, estradiol and progesterone levels were assessed for female participants across seasons and no difference was found. However, an inclusion according to timing of menstrual cycle would have been more ideal. Moreover, we selected a special cohort of resilient individuals to be included as healthy controls; they were relatively young and all of them were S-carries. Thus our findings do not inform on seasonal SERT changes in the general Danish population.

The Non-SAD group had received a lower injected DASB mass/kg body weight in the summer (0.02±0.01 (0.005-0.05 μg/kg), (mean±SD (range)) than in the winter (0.04±0.03 (0.005-0.13 μg/kg)), paired t-test: P =.001, 95% CI = -0.05 to -0.01. However, there was no correlation between global SERT and injected dose per kg and the impact of injected dose DASB was investigated in an

independent sample, where no correlation of DASB and BP_{ND} was found (rapport B). Thus we find it unlikely that this have affected our results.

The SERT quantification from cerebellar references tissue models are not suited for contrasted comparisons in the case of systematic difference in non-specific binding across groups or conditions. To investigate this, we compared $AUC_{cerebellum}$, as proxy for the non-specific binding, across conditions and groups and found no difference (paper II).

Due to participant drop-outs the power in the SAD genotype analysis (6 L_A-homozygotes) was not optimal but yet it was acceptable due to the high test-retest of ^{11}C-DASB PET (Kim, Ichise et al. 2006) and the longitudinal study design.

Other methodological factors such as temperature and humidity were not taken into account. However, they were found to be of marginal importance at comparable latitudes (Praschak-Rieder, Willeit et al. 2008).

Furthermore, we cannot fully exclude that our investigations have been influenced by an unusual variation in global light radiation (an hour where the intensity of the sun exceeds 50.000 (Nielsen 2005)). Amount of sunlight hours have previously been shown to affect the 5-HT system via 5-HT_{1A} binding in healthy subjects (Spindelegger, Stein et al. 2012). Notably, the two seasons where we conducted the experiments were characterized by winters that had well above average amount of sunlight hours (2010/11 and 2011/12: 18 and 39%, respectively) and summers with slightly fewer hours of sunlight than normal (http://www.dmi.dk/vejr/arkiver/maanedsaesonaar/).

Conclusion

In summary, our main findings are that

I) Individuals with high predisposition to affective disorders (5-HTTLPR S-carriers, females) but who nevertheless manage to stay free of SAD during the winter are able to downregulate their cerebral SERT.

II) In the winter, patients with SAD fail to downregulate their cerebral SERT to the same extent as mentally healthy people and the more they upregulate their SERT, the more severe are their SAD symptoms.

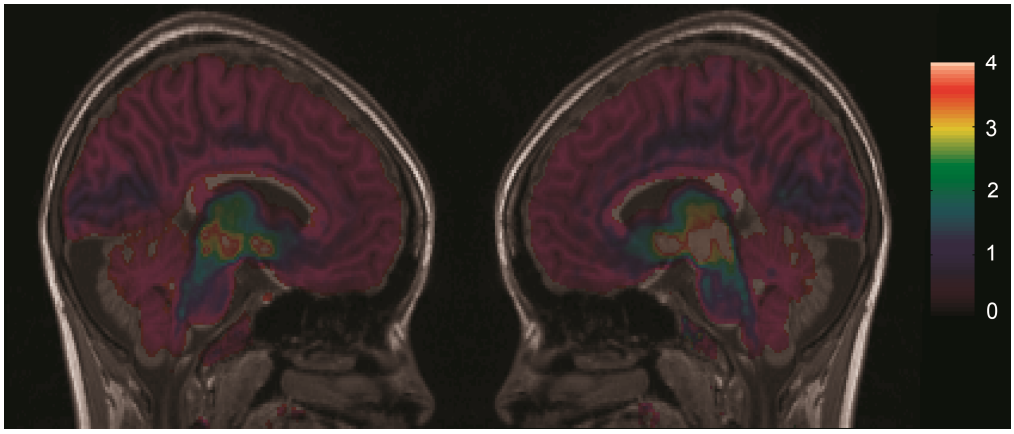


Figure 13. A ^{11}C-DASB PET image of a SAD patient in summer and in winter. Cerebral SERT binding in a 22-year old female S-carrier scanned symptom-free in the summer (left) and during winter where she presented with severe SAD, a SIGH-SAD score of 27 (right). The quantified ^{11}C-DASB PET image is overlaid on a T1-weighted structural MR-image showing the highest cerebral SERT in the winter (from Paper II).

In study I, we find that Danes who in spite of their genetic and sex-defined predisposition to SAD manage to remain mentally healthy also in the winter show a *down-regulation* of global cerebral SERT levels in winter compared to summer-levels. At first glance, this may seem to contrast to findings in previous studies, notably (Kalbitzer, Erritzoe et al. 2010, Praschak-Rieder and Willeit 2012) who describe higher subcortical SERT in healthy people scanned in the winter compared to the summer, or (Koskela, Kauppinen et al. 2008), (Murthy, Selvaraj et al. 2010, Cheng, Chen et al. 2011) or (Matheson, Schain et al. 2015) who found no change in SERT. However, we argue that those previous studies may have failed to attend to strict inclusion criteria ensuring absence of seasonality symptoms, they were not conducted in the same individuals both summer and winter, and some of them did not include women. In study II where we oversampled for S-carriers, the seasonal contrast in cerebral SERT regulation between SAD and Non-SAD was most pronounced in women. The SAD patients with most severe symptoms also happened more often to meet these two criteria. Taken together, our research suggests that female SAD patients – and possibly also S-carriers - are more likely to respond to selective serotonin reuptake inhibitor intervention. The unique pattern of inverse SERT regulations that signifies the Non-SAD group suggests that maintaining euthymic mood in the context of environmental liability is coupled to an active adaptation in key components of the 5-HT system. In contrast, the SAD group was characterised by failure of such SERT adjustments; in fact, the depressive response to winter was robust and positively correlated to a global upregulation of SERT levels. Given that blocking of SERT is an effective treatment of SAD, we find it plausible that the increase in SERT levels elicit depressive symptoms via accelerated removal of synaptic 5-HT levels. Moreover, presence of known risk factors for development of SAD, that is, female gender (Magnusson 2000) and 5-HTTLPR S-carrier status (Johansson, Willeit et al. 2003) significantly increased the likelihood of increased SERT levels in the winter, and by visual inspection of data female S-carriers were also clinically more affected by their SAD. In agreement with this observation, the majority of clinical SSRI experiments have reported better response- and remission rates in females,

summarised in (Damoiseaux, Proost et al. 2014). In continuation of the study by Kalbitzer et al 2010, we found that the augmented seasonal SERT fluctuations in S-carriers extended to individuals with SAD. Although this result should be replicated, the identification of increased seasonal SERT regulation across predisposed individuals complementary imparts that the development of affective symptoms is above all linked to flexibility in SERT expression. Our sample size did not, however, allow us to explore third order interaction effects; thus, the elucidation of gender by genotype by season effects on seasonal SERT regulation awaits future research. Although we advocate that independent replications are warranted, our work suggests that seasonal and non-seasonal depression share fundamental neurobiological changes in the form of elevated levels of SERT (Savitz and Drevets 2013). In this regard, change of season could be applied as an experimental model, to study implications of risk and resilience to affective disorders across a broader range of clinical conditions.

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Individuals Resilient to Seasonal Affective Disorder Downregulate their Cerebral Serotonin Transporter Binding in Winter

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Abstract

Objective: At the Northern latitudes, the majority of the population perceives the diminished daylight in winter as a substantial environmental stressor. Women in particular display seasonality, with behavioral changes in serotonergic controlled domains, i.e., low mood, reduced vigilance and increased appetite. We undertook a longitudinal study of healthy individuals who in spite of being genetically predisposed did not have seasonality-associated symptoms, i.e., they were particularly *resilient* to seasonality-associated symptoms and we investigated their cerebral serotonin transporter (SERT) binding summer and in the winter.

Methods: The sample included 23 (13 women, age: 26±6 years) carefully selected healthy short allele carriers of the SERT linked polymorphic region (5-HTTLPR) with low seasonality ratings. in In a randomized counterbalanced fashion, participants underwent ^{11}C-DASB PET scans both summer and winter to investigate seasonal SERT fluctuations and sex-by-season interaction effects.

Results: Global- and raphé nuclei SERT binding was lower in winter compared to summer; this effect was particularly driven by the women. In an exploratory analysis, we found that the seasonal SERT fluctuations were most prominent across the angular gyrus, the prefrontal cortices, the posterior temporal cortices and adjacent occipital cortices.

Conclusion: Our study suggests that resilience to seasonal affective disorder is associated with a global down-regulation of SERT levels in winter, possibly causing an increased serotonergic tone.

1. Introduction

Seasonal fluctuations in mood and physiology are particularly frequent at latitudes with pronounced season-associated variation in daylight. For example, in a large community based survey, 90% of inhabitants in Copenhagen, Denmark (latitude 55.7^{o}) reported seasonal changes in mood, sleep and/or eating behavior (Dam, Jakobsen, & Mellerup, 1998), a condition often referred to as seasonality. The importance of environmental adaptation is emphasized by the fine-tuned circadian system in the human brain. Upon light exposure, specialized non-visual forming intrinsically photosensitive retinal ganglion cells (iRGC) (Sexton, Buhr, & Van Gelder, 2012) propagate a signal through the retino-hypothalamic tract that terminates within the suprachiasmatic nuclei (SCN). Two preclinical studies suggest that interruption of the iRGC-dorsal raphe nuclei (DRN) pathway leads to photoperiodic adjustments to DRN serotonin (5-HT) levels: One study found that disconnection of the iRGC-DRN pathway reduced raphé 5-HT levels and caused depressive like behavior in gerbils (Luan et al., 2011), while another study in chipmunks and nocturnal mice found that DRN levels of 5-HT were higher under long-day conditions than under short-day conditions (Goda et al., 2015).

The 5-HT level in the brain is tightly regulated by the serotonin transporter (SERT) and accordingly, several studies have addressed whether SERT levels differ between seasons. The first neuroimaging investigation of seasonal serotonin transporter (SERT) fluctuations in healthy individuals was conducted in Vienna (latitude: 48°N) where healthy women were examined with the non-selective SERT tracer ^{123}I-β-CIT and single-photon emission computed tomography (SPECT) (Neumeister et al., 2000). Five women were scanned in the winter and another 7 in the summer and the authors found a lower ^{123}I-β-CIT ratio in the thalamus/hypothalamus in the group scanned in winter. Buchert et al. investigated 29 Hamburg citizens (53°N) with ^{11}C-McN5652 positron emission tomography (PET), mainly to address sex and age effects on SERT binding (Buchert et al., 2006). They included a dichotomised season variable and found significantly *higher* mesencephalon SERT binding in the winter scans compared to the summer scans, but no difference in the thalamus. A sex-by-season effect was detected on a trend level (P = .09 and .08, respectively) with women displaying larger seasonal SERT change. When 66 healthy individuals from Tainan (22° N) were examined with ^{123}I-ADAM SPECT, no correlation between radioligand binding in mesencephalon and

sun exposure was seen (Cheng et al., 2011), suggesting that daylight duration has a stronger influence on SERT than does sunlight. The first and so far only longitudinal investigation of cerebral SERT across seasons was carried out in 12 healthy men and women from Helsinki (60°N) who were biannually assessed with ^{123}I-ADAM SPECT (Koskela et al., 2008); no significant difference between summer and winter was detected in this small study. The first large-scale study of its kind with the superior positron emission tomography (PET) radioligand ^{11}C-DASB was done in 88 healthy individuals recruited from Toronto (44° N) (Praschak-Rieder, Willeit, Wilson, Houle, & Meyer, 2008) and in this cross-sectional study, they found a significant inverse correlation between daylight minutes and SERT binding across different brain regions of interest (ROIs), consistent with the observations of (Buchert et al., 2006). Then followed another large (N = 57) cross-sectional ^{11}C-DASB PET study of healthy Copenhagen citizens (56°N)(Kalbitzer et al., 2010); not only did they corroborate the inverse relationship between daylight minutes- and SERT binding in striatum but they also showed that short allele carriers (S-carriers) of the 5-HT transporter linked polymorphic region (5-HTTLPR) genotype displayed significantly larger seasonal SERT amplitude compared to participants homozygote of the long allele (L_A-homozygotes) (Kalbitzer et al., 2010). Shortly after, another ^{11}C-DASB PET study in 63 British (51°N) men investigated the effects of triallelic 5-HTTLPR on cerebral SERT binding, but amount of daylight was not a significant covariate (Murthy et al., 2010). Finally, a Stockholm (59°N) based ^{11}C-MADAM PET study in 40 men did not detect any significant correlation between radioligand binding and daylight minutes in sub-cortical regions, and the majority of the scans were conducted in the winter, since the average day length was 6.5 hours (Matheson et al., 2015). Notably, in the two negative large PET studies that also used suitable SERT radioligands,(Matheson et al., 2015; Murthy et al., 2010) only men were included.

Taken together, cross-sectional decently sized studies using highly selective SERT PET radioligands and scanning throughout the year, allowing for a maximal range of daylight hours to be encountered, suggest that cerebral SERT is higher in the winter than in the summer, at least in mixed-gender healthy populations free of overt depression. Importantly, none of the latter studies was specifically designed to address the within-subject seasonal SERT fluctuations and the volunteers were not specifically assessed for their resilience towards seasonality. 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. Increasingly, however, the ability to withstand severe stress is the focus of research (King, 2016).
In the present study, we made use of a longitudinal design where we in randomized order PET-scanned the same individual both summer and winter, thereby maximizing contrast to our analysis to capture intra-individual across seasons variations in cerebral SERT binding. Given the known high inter-regional correlation of SERT binding (Erritzoe et al., 2010; Hahn et al., 2014) and the extensive neuronal communication between the raphé nuclei and the SCN (Morin, 2013), we considered it likely that the cerebral seasonal SERT regulation is orchestrated by the raphé nuclei. Therefore, both raphé nuclei and a global measure of cerebral SERT binding in raphé projection areas were primary outcome regions of interest.
In addition, in order to gain information about brain regions of relevance for staying mentally healthy during seasonal transitions, i.e., resilience to SAD, we assessed the participants for their resilience towards seasonality and scaled the study to allow for a voxel-wise analysis. All participants were carefully screened to ascertain that they exhibited absence or only minimal symptoms of seasonality. Moreover, to optimize sample homogeneity and maximize power (Kalbitzer et al., 2010; Kalbitzer, Kalbitzer, Knudsen, Cumming, & Heinz, 2013) we chose a priori to include only S-carriers. We hypothesized that we would replicate earlier findings of higher SERT-binding in the winter compared to summer (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008). Due to female preponderance to seasonality (Magnusson & Boivin, 2003) and failure to identify seasonal SERT changes in studies exclusively in males (Matheson et al., 2015; Murthy et al., 2010), we anticipated to find larger effects in females than in males.

2. Methods and Material

2.1 Participants

2.1.1. Recruitment

The screening criteria (< 45 years of age, body mass index (BMI) of 19-28 kg/m^2, non-smokers, stable diurnal cycle, Danish-speaking Copenhagen citizen) 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 where detailed information regarding past or present

neurological/psychiatric disorders, family history of psychiatric disorders, head trauma, alcohol consumption, lifetime use of recreational drugs, timing of menstrual cycle and oral contraceptives and prior participations in similar studies were acquired. Eligible candidates underwent a phone or personal interview to assure that they adhered to the screening criteria, to provide oral study-information, to assure absence of overt seasonality, to make sure they had not been or were planning to travel to destinations at different latitudes and to assure no female subjects were pregnant or planning pregnancy. The volunteers that met all requirements received written information of the study and an online Danish version (as used in (H. O. Madsen, Dam, & Hageman, 2016)) of the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al., 1984). If the SPAQ response indicated absence of seasonality (Global Seasonality Score (GSS) < 10 and stating to have no problems with seasonality) and the volunteer consented to participation, a saliva collection kit (Oragene DNA saliva kit OG-500 from DNAgenotek) for genotyping of 5-HTTLPR genotype was sent by postal service and only S-carriers were finally invited for participation. PET scans were conducted within a six-week interval centered around winter or summer solstice; half of the study participants were first scanned in winter (442±19 daylight minutes, mean±SD) and the other half in the summer (1025±32 daylight minutes). All participants had unremarkable medical, neurological and biochemical investigations and no pathological findings on the structural magnetic resonance image (MRI) brain scan. Twenty-four subjects were enrolled in the study; there were no drop-outs however one ^{11}C-DASB data set was lost for analysis due to unexplained low radiotracer brain up-take (10% of the expected). Thus, 23 subjects (13 females, age: 26±6 years) were included in the final data analysis.

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 resilient control subjects in comparison to people with seasonal affective disorder (Mc Mahon et al., 2016) and 9 of the individuals were also included in a study of affective memory(Jensen et al., 2015) and in a sucrose taste sensitivity test (Andersen et al., 2014).

2.1.3 Psychometric assessments

The Major Depression Inventory (MDI) (range: 0-50, > 21 indicates depressed mood)(Bech, Rasmussen, Olsen, Noerholm, & Abildgaard, 2001), the Pittsburgh Sleep Quality Index global scores (PSQI) (range: 0-21, > 5 indicates sleep disturbances) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), Cohens Perceived Stress Scale (PSS) (range: 0-40, no cut-off adapted to indicate stress) and the Stress-full Life Event scale (SLE) recent events item (Roohafza et al., 2011) were administered summer and winter in proximity to the PET scan.

2.1.3 Genotyping and amino acids

The allelic status of the 5-HTTLPR was analysed by a TaqMan 5′-exonuclease allelic discrimination assay as described previously (Mc Mahon et al., 2016). To examine if there were seasonal difference in dietary tryptophan intake, possibly changing brain serotonin levels, plasma amino acid measurements was done in a subset of the sample (N= 14). For this purpose, venous blood samples were taken in heparinized vials immediately prior to the PET scan and kept on ice until precipitation with sulfosalicylic acid. The supernatant fluid was then stored at -80 ^{o}C, until analyzed. 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, Pettigrew, Patlak, Hertz, & Paulson, 1990).

2.2 Neuroimaging protocols

2.2.1 PET data acquisition

For imaging the cerebral SERT, we used the highly selective radioligand ^{11}C-DASB. After intravenous injection of 592 ± 15 MBq, the volunteers were scanned 90 minutes with a Siemens ECAT High-Resolution Research Tomography (HRRT) scanner operating in 3D list-mode. Data acquisition was performed as previously described (Frokjaer et al., 2015).

The kinetic modeling by the Multilinear Reference Tissue Model 2 MRTM2 (Ichise et al., 2003) was performed using FreeSurfer (Greve et al., 2014) with cerebellum as reference region, and thalamus, caudate, putamen and pallidum as high-binding regions for estimation of k_2'. High resolution anatomical 3D T1-weighted MP-RAGE scans were additionally acquired for all subjects using a Siemens Magnetom Trio 3T MR scanner (1 mm^3 isotropic voxels) (n = 14) or a Siemens 3T Verio MR scanner (0.9 mm^3 isotropic voxels) (n = 9). All MP-RAGE scans were corrected for spatial distortions due to scanner specific gradient non-linearities (Jovicich et al., 2006), before further analyses, in order to achieve optimal PET-MR co-registration.

2.2.2 Image analysis

All MR 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 (Nørgaard, 2015). For each individual, all PET time activity curves (TAC) were summed and averaged over all time frames in order to estimate a weighted mean 3D PET image for co-registration. The resulting mean PET image was aligned to the individual structural MRI using a rigid intra-subject multimodal registration utilizing a boundary-based cost function with 6 degrees of freedom (Greve & Fischl, 2009). The individual cortical PET surfaces were registered to a cortical surface atlas, which is the surface-based equivalent to MNI space (Greve et al., 2014). The PET surfaces were sampled half way between the white and pial surface to minimize partial volume effects. The anatomical volume was registered to the MNI305 atlas, as previously described (Beliveau et al., 2015) and the volume-based group analysis of subcortical structures was performed in this space. TACs on the surface and in the volume space were smoothed with respectively a Gaussian 2D and 3D filter using a full width half maximum (FWHM) of 10 mm, prior to estimating the SERT binding (BP_{ND}) using MRTM2. The raphe nuclei was defined as described in (Kalbitzer, Svarer, et al., 2009).

2.3 Statistical analysis

2.3.1 Psychometrics, biochemistry and radioligand variables

Summer versus winter measurements of psychometric data (MDI, PSQI, PSS, recent SLE), plasma tryptophan load, BMI, k2' 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 students t-tests. Eventual season-related differences in injected mass DASB/ kg body weight was examined by means of a Wilcoxons signed rank test.

2.3.2 Region based statistical analysis

Two ROIs were chosen for the region-based ^{11}C-DASB BP_{ND} analyses: The raphé nuclei and its projection areas. The raphé ROI was defined, as described in (Kalbitzer, Frokjaer, et al., 2009). The projection (global) ROI BP_{ND} was calculated by averaging left and right, grey matter volume weighted BP_{ND} including thalamus, putamen, caudate, hippocampus, amygdala, anterior and posterior cingulate cortex, entorhinal cortex, insula cortex, orbitofrontal cortex, sensorimotor cortex, occipital cortex, medial inferior temporal gyrus, medial inferior frontal gyrus, parietal cortex, and superior frontal gyrus projection areas.

$$\text{Global } BP_{ND} = (\sum(BP_{NDx} * \text{volxume}_x))/ \sum \text{volume}_x)$$

The effects of season, sex and sex-by-season interaction on raphé and global BP_{ND} were investigated by means of a single-factor repeated measures ANOVA. By design, the sample had a narrow age- and BMI span at baseline: mean ± SD (range): age 26 ± 7 (19 -43) years and BMI: 23.2 ± 1.9 (19.5 – 27.4) kg/m^2. Thus we did not correct for these variables.

As a stable mood may also depend on the synchronisation of SERT levels between DRN and projection areas (Hahn et al., 2014) we examined if season changed the correlation of raphé nuclei SERT and global SERT levels by comparing the slope estimates from simple linear regression analysis.

2.3.3 Univariate vertex- and voxel-wise statistical analyses

The FreeSurfer generated BP_{ND} maps in surface and volume space were used in the whole-brain surface vertex- and subcortical voxel-wise analyses of seasonal season effects using paired t-tests (H_0: winter – summer = 0). Correction for multiple comparisons was executed in a clustering framework using non-parametric

permutation tests as described by Nichols and Holmes (Nichols & Holmes, 2002) in order to sufficiently control the false-positive rate. Due to the randomized study design (summer and winter), we defined the exchangeability within the summer and winter scans, respectively. However, imaging data sets with less than 20 degrees of freedom tend to have biased variance estimates, which appears as high frequency noise or sharpness in the variance image (variance across all scans). We therefore smoothed the variance image using an 8 mm FWHM, replacing variance estimates at the single voxel-level with a weighted average of its neighbors. A new statistical parametric map (SPM) was thereby obtained, and in this context we refer to this map as a pseudo t-statistic map. In order to capture a single overall feature providing evidence against H_0 at each resampling we utilized the maximum thresholded cluster size. Under the null-hypothesis H_0, statistically assuming no underlying BP_{ND} difference between summer and winter, significantly large clusters would therefore indicate the presence of a seasonal variation in BP_{ND}. We resampled the data 1000 times (permutations), and recorded the maximum thresholded clusters for each of the resamples using a predefined pseudo t-threshold of 2.5 ($p < 0.01$) (Nichols & Holmes, 2002). 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.

For all other statistical analyses we adopted a significance level of $P = .05$. 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) 64bit.

3. Results

Results are reported as mean ± SD.

3.1 Psychometrics, biochemistry and radioligand variables

When assessed on the day of the PET scan, 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: **PSQI global score:** summer (S): 3.7 ± 2.1, winter (W): 3.6 ± 1.8, $P = .79$, **MDI**: S: 5.5 ± 3.6, W: 5.0 ± 3.5, $P = .40$). The level of stress was found to be marginally higher in

summer: **PSS:** S: 10.3 ± 5.9, W: 7.9 ± 4.8, P = .05. No difference was found in the number of recent stress-related events from summer to winter: **recent SLE:** S: 2.2 ± 2, W: 2.7 ± 2.4, P = .40.

No differences were found across seasons in plasma tryptophan (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 = 0.42), k2´ (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 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 =.000. However, the mass doses given were small (max 0.05 μg/kg in summer and 0.13 μg/kg in winter). To investigate if the injected mass DASB could potentially bias our result we conducted an analysis in a separate cohort of 108 healthy individuals (78 females, injected mass/kg 0.0284±0.035 μg (range 0.0045 – 0.25 μg) from the Cimbi database (Knudsen et al., 2016). Simple linear regression was used to test if injected mass DASB/kg predicted ^{11}C-DASB BP_{ND} in a pooled subcortical high-binding region and in neocortex. In this large sample, we found no correlation between BP_{ND} and injected mass/kg; slope estimates ranged between -0.34 to - 0.28 injected μg DASB/kg per BP_{ND} (.12 < P <.59).

3.2 Region based analysis

BP_{ND} was significantly higher in the summer compared to the winter for both the raphé nuclei: S: 4.5 ± 0.7, W: 4.1 ± 0.6, F_{season} = 10.92, P = .003 and global binding: S: 0.7 ± 0.09, W: 0.66 ± 0.06, F_{season} = 11.47, P = .003) (figure 1A). A significant sex-by-season interaction effect in global SERT binding: $F_{sex\text{-}by\text{-}season}$ = 5.54, P = .03 with females showing a larger decrease in global SERT binding in the winter compared to summer (figure 1B).

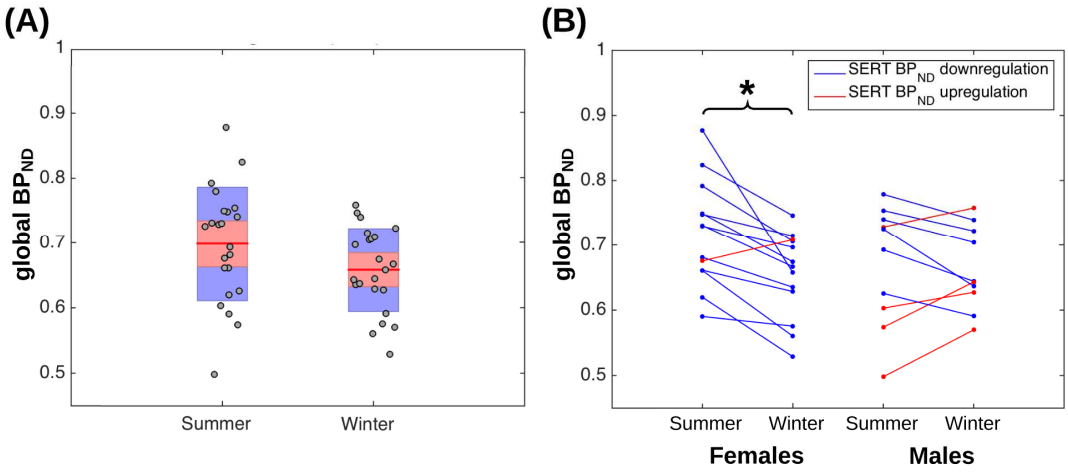

Figure 1A. Global SERT binding across seasons. A single-factor repeated measures ANOVA showed a significant down-regulation of global SERT BP_{ND} in the winter for all participants, P_{season} = .003.

Figure 1B. **Global SERT binding across seasons in males and females.** The sex-by-season interaction effect, $P_{sex\ by\ season}$ = .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).

No sex-by-season interaction was found in raphé nuclei ($F_{sex\text{-}by\text{-}season}$ = 1.73, *P* = .20). Within seasons, there were no sex differences (raphé nuclei ROI: F_{sex} = 0.56, *P* = .46 and global ROI: F_{sex} = 0.3740, *P* = .55).

Both in the summer and winter, raphé nuclei BP_{ND} was positively correlated to global BP_{ND} (figure 2). S: R^2 = 0.33, *P* = .004, and W: R^2= 0.24, *P* = .018. There was no significant season-related difference between the slope estimates (two-tailed, 95%-confidence interval, *P* = .65).

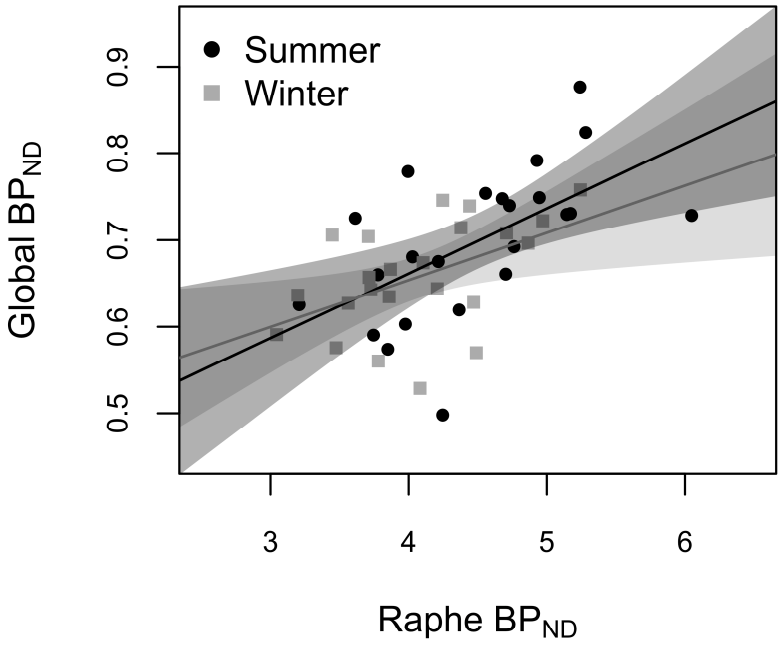

Figure 2 . Correlation between raphé nuclei SERT- and global SERT across seasons. Irrespective of season, raphé nuclei BP_{ND} was positively correlated to global BP_{ND}. There was no significant difference in slope estimates (two-tailed, 95%-confidence interval, P = .65)**.** Black: summer, grey: winter.

3.3 Univariate vertex- and voxel-wise analysis

Several larger clusters showed a significant down-regulation of SERT binding from summer to winter, including bilateral clusters across the angular gyrus (GA, also referred to as Brodmann area 39, temporoparietal junction, posterior middle temporal gyrus or temporo-parieto-occipital cortex) ($.0016 < P_{corrected} < .00047$), bilateral clusters across the middle frontal gyrus in prefrontal cortex ($.0039 < P_{corrected} < .00087$) and two large clusters located bilaterally across occipital cortices and the posterior inferior temporal cortices ($.0066 < P_{corrected} < .0001$) . In the right hemisphere, the latter extended to the posterior medial temporal cortex as well (figure 3, table I). A post-hoc analysis revealed that the detected clusters supported the sex by season effect found in the region-based analysis. No significant differences were detected in subcortical regions.

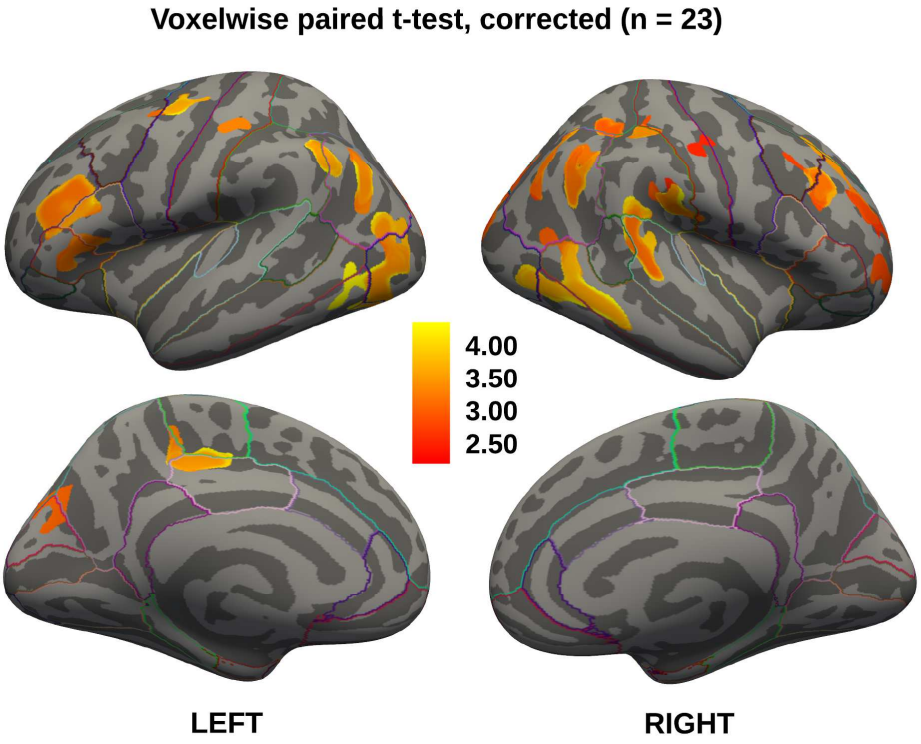

Figure 3. Univariate surface-based analysis. The univariate surface-based analysis depicted on the inflated brain. The map displays the t-values of clusters with significant changes in ^{11}C-DASB binding across seasons (summer - winter), corrected for multiple comparisons. The top row: Cortical presentation,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

Table I. Significant clusters reflecting a seasonal variation in BP_{ND}

Region	Cluster t-value	Size [mm^2]	MNI X	MNI Y	MNI Z
Left hemisphere					
Inferior temporal gyrus	4.36	1552	-44	-64	-5
Angular gyrus	4.1 3.99	445 382	-40 -49	-64 -57	34 37
Precentral gyrus	3.97	385	-43	-6	44
Inferior frontal triangularis	3.93	875	-42	17	21
Middle frontal gyrus	3.52	393	-39	33	-2
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
Middle frontal gyrus	3.85 3.55 3.23	452 901 434	36 26 31	28 20 48	37 42 5

Angular gyrus	3.76 3.7 3.6	367 544 445	34 46 33	-51 -58 -62	37 27 46
Cuneus	3.7	968	15	-87	22

4. Discussion

For the first time we here present evidence that individuals particularly resilient to seasonal fluctuations in mood and behaviour, i.e., 5-HTTLPR S-carrier woman without seasonality symptoms exhibit a pronounced down-regulation of cerebral SERT levels in winter compared to summer. The finding was observed both in the raphé nuclei and its global projection area. Our sample was carefully selected and screened for seasonality symptoms prior to inclusion, which is likely to explain why we see opposite effects compared to previous studies, notably Praschak-Rieder et al. (Praschak-Rieder et al., 2008) and our own previous study (Kalbitzer et al., 2010) both describing higher subcortical SERT binding in healthy people scanned in the winter compared to the summer. However, in those two cross-sectional studies, volunteers were primarily enrolled as healthy controls from other ongoing studies. The deliberate selection bias in the present study comes clearly across; our healthy participants had an average GSS of 4.8±2.0 as compared to the general Danish population of 6.2±4.5(Dam et al., 1998) and they had stable mood and sleeping patterns across seasons.

From a neurobiological point of view, our observation makes perfect sense: Lowering cerebral SERT levels in the winter may constitute a mechanism to increase or perhaps just maintain synaptic 5-HT levels and thereby enable higher serotonergic neurotransmission (Nagayasu, Kitaichi, Shirakawa, Nakagawa, & Kaneko, 2010; Torres, Gainetdinov, & Caron, 2003) which would serve to combat lower mood during the environmental stressor of the winter.

To our knowledge, the current study is the first neuroimaging study to couple SERT dynamics to affective resilience in the context of environmental stress. Other investigators have primarily indexed genetic liability to model *trait* features conferring risk or resilience to affective disorders. For example, one ^{11}C-SB207145 PET study by our group showed a positive dose-response correlation with risk-load, as indexed by the number of first degree relatives with major depressive disorder (MDD), in (yet) unaffected participants and striatal 5-HT levels, as indexed by 5-HT_4 receptor binding (K. Madsen et al., 2015). This suggests that maintaining

mental health in spite of an increased risk load, may be linked to elevated 5-HT levels. In agreement with this observation, we also shown in an independent cohort of healthy twin siblings to depressed co-twins that dorsolateral prefrontal SERT binding, as imaged by ^{11}C-DASB PET is lower (Frokjaer et al., 2009). Thus, we speculate that the ability to raise 5-HT levels through down-regulation of SERT could constitute an important mechanism to combat emergence of depressive symptoms in the context of environmental stress. In favor of this model, multiple studies have reported gene by environment interaction effects in the development of depression, i.e., S-carriers that are characterized by a lower SERT expression (Willeit & Praschak-Rieder, 2010) and higher levels of 5-HT (Fisher et al., 2012), are more prone to develop MDD in the context of environmental stress (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010), suggesting that when 5-HT levels are permanently raised, the flexibility and compensatory capacity of the system is compromised such that the ability to launch a further increase in endogenous 5-HT levels during stress is reduced and in consequence a major depressive episode may develop.

A previous study that examined MDD patients with ^{11}C-DASB PET found a decreased correlation between DRN and ventral striatal SERT in depressed patients compared to healthy controls (Hahn et al., 2014). In agreement with our sample having stable mood across the year, we did not find any difference in global SERT-raphé nuclei SERT regression slopes across seasons.

The global down-regulation in SERT levels observed across all participants was particularly driven by the women. Previous neuroimaging studies investigated sex-by-season effects in mesencephalon and thalamus but neither demonstrated significant effects: Buchert et al. (^{11}C-McN5652 PET, N=29) investigated second order sex-by-season interaction effects and Koskela et al., explored sex differences in a longitudinal ^{123}I-ADAM SPECT study of 5 females and 7 males. Thus, the finding of a sex-by-season interaction effect in healthy individuals is novel and we interpret this with caution; there are multiple mechanisms by which this observation may occur, i.e., sex-differences in early brain development or interactions of SERT expression and sex hormones, as reviewed in (Borrow & Cameron, 2014). Nonetheless, our observation strongly suggests that females require a larger adjustment of SERT levels in order to withstand the environmental stress of winter.

In contrast to previous seasonality studies, which mainly focused on sub-cortical regions, our explorative vertex- and voxel-based whole brain analysis revealed significant clusters of season-related differences, across several cortical areas. The angular gyrus (AG) is a cross-modal hub involved in higher order processing of multiple inputs including assessment of environmental salience, directing attention between internal and the external milieu (Seghier, 2013) and modifying vigilance (Singh-Curry & Husain, 2009). Interestingly, the cyclic reoccurrence of mood liability, hyperphagia, and hypersomnia in Klein-Levine patients has been found to be associated with AG hypoperfusion (Geoffroy, Arnulf, Etain, & Henry, 2013; Kas, Lavault, Habert, & Arnulf, 2014). We also observed a winter-associated decrease in SERT levels in several clusters across the prefrontal cortex, in particular in the middle frontal cortex. In line with this, when we investigated prefrontal and limbic responses to aversive faces in 30 healthy males before and after three weeks of bright light therapy, we found that the intervention successfully reduced amygdala reactivity and increased amygdala-medial prefrontal cortex (mPFC) connectivity in a light-dose dependent manner. Moreover, the increase in intra-prefrontal coupling upon intervention was larger in S-carriers, supporting a neuromodulatory role of 5-HT in this particular region (Fisher et al., 2014). The study was, however, designed to investigate specific effects in the amygdala-mPFC circuits and the analysis was not extended to the middle frontal cortex. Nevertheless, these findings are complementarily supporting entailing prefrontal engagement in directing mood and behavior according to environmental cues. We also identified two large clusters across the posterior medial (lateralized to the right) and inferior (lateralized to the left) temporal cortices. We are not aware of any studies addressing these specific regions in the context of seasonality, seasonal or non-seasonal related depression. But with regard to functional aspects, these regions are important to environmental awareness, i.e., object recognition and redirecting attention towards relevant auditory and visual stimuli are orchestrated within the these brain areas (Paulson, Gjerris, & Solberg, 2004) . Interestingly, the clusters extended into the occipital cortices (cuneus). Thus it is possible, that the augmented signal is caused by seasonal contrasts in illuminative stimuli as the image forming retinal ganglion cells are indirectly providing extensive input to these regions via thalamic-cortico projections (Morin, 2013). Moreover, the expression of SERT may be linked to the proportion of projecting fibers, and thus in winter it is plausible that the sustained reduction in daylight reduces projection to visual cortices.

In contrast to our data, the two previous studies that included post hoc whole brain voxel based analysis did not find any clusters with cross-seasonal differences (Buchert et al., 2006; Kalbitzer et al., 2010). However, a strength of our study is the longitudinal study design that effectively eliminates inter-individual differences, the strict inclusion criteria to ensure absence of seasonality and inclusion of S-carriers only. While arguably the sample is not representative of the population as a whole, not the least because of the genotype selection prior to inclusion, it allowed us to study disease resilience as opposed to disease itself.

In conclusion, we find evidence of a winter-associated down-regulation of global SERT levels in individuals resilient to seasonality symptoms, especially in females. As our cohort was selected to be resilient to seasonality, the SERT down-regulation in winter may reflect a compensatory mechanism to balance or raise 5-HT levels to accommodate the stress of lack of daylight. Additionally, we found augmented effects in regions that deal with environmental safety; seasonal adjustments in SERT levels of these regions may be pivotal to a successful long-term stress response.

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Disclosure/Conflict of interest

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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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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.

Methods and materials

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

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 $AUC_{cerebellum}$.

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 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 (Δ BP_{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,

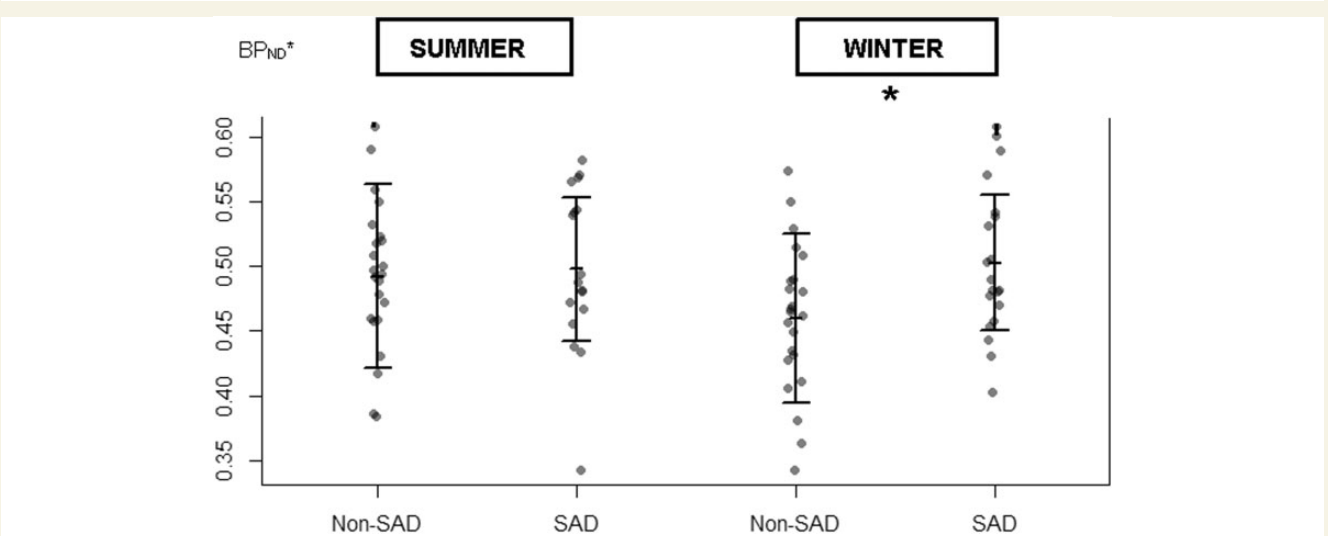

Figure 1 Seasonal effects across groups. No difference in serotonin transporter BP_{ND} was found across groups in summer ($P = 0.45$), whereas patients with seasonal affective disorder had higher serotonin transporter compared to healthy controls in the winter ($P = 0.01$). Binding potential values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

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 ($n = 17$, 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.

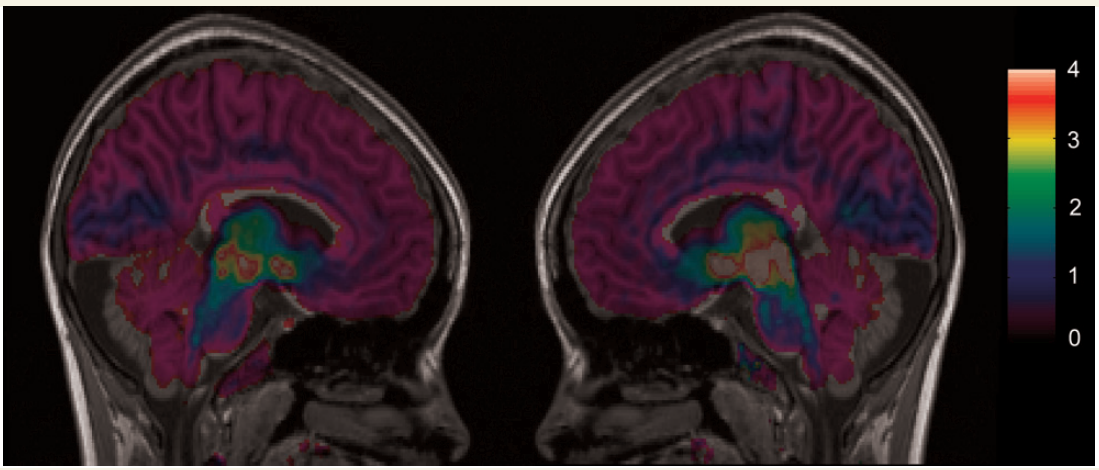

Figure 2 The ^{11}C-DASB PET image of a patient with seasonal affective disorder in summer and in winter. Cerebral serotonin transporter binding in a 22-year-old female S-carrier scanned symptom-free in the summer (*left*) and during winter when she had severe depressive symptoms and a SIGH-SAD score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

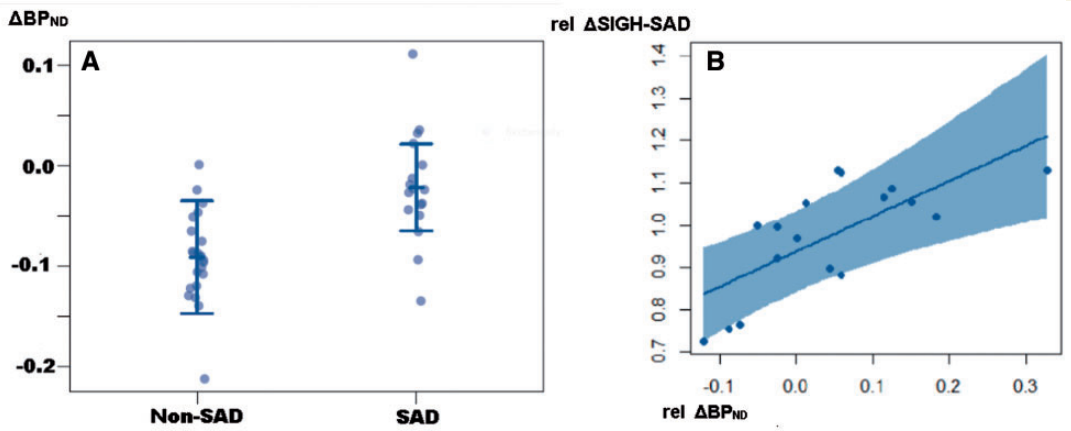

Figure 3 Group effects across seasons and correlation to seasonal affective disorder symptoms. (**A**) Cerebral serotonin transporter change across seasons between patients with seasonal affective disorder and healthy controls adjusted for sex, age, genotype and sex × group interaction effects. The ΔBP_{ND} (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).

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 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.

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Supplementary material

Supplementary material is available at *Brain* online.

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Motion Correction of ^{11}C-DASB PET images

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Aim

This report aim to investigate how pre-processing motion correction (MC) using AIR 5.2.5 [Woods et al., 1992] to PET ^{11}C-DASB images (kinetic modelled with MRTM2 (Ichise et al., 2003)) introduces bias or noise to the outcome parameter BP_{ND} and to what extent the noise is determined by median movement.

Background

Previously, MC was applied routinely when the estimated median voxel movement exceeded 3 mm (investigators (Haahr, Madsen, Svarer) decision spring 2009) as the align frames program, AIR 5.2.5, has a precision of approximately 2.3 mm and thus applying MC to images with a lesser movement was thought to induce unwarranted noise. Later on it was discovered that some datasets had a large change in BP_{ND} due to MC (as high as 30%) while others barely changed at all. The BP_{ND} change induced by MC could not be explained solely by a high median voxel movement and the arbitrary cut-off limit of 3 mm was challenged. Furthermore, in contrast to i.e. ^{11}C-SB207145 PET images ^{11}C DASB PET images show brain distribution patterns consistent with a bias in the alignment between frames (Svarers observation).

One of the main concerns with the fixed (somewhat arbitrary) threshold for MC regarded longitudinal observations as applying MC to i.e. the baseline while not doing so at rescan could lead to gross misinterpretations of data. Nevertheless, some images have a legitimate need for MC to make data fit to the kinetic model and we cannot discard MC altogether. As follows, this issue could only be circumvented by applying MC to all images. However this approach will inevitably introduce noise and perhaps bias the outcomes. The following report was initiated to thoroughly investigate the impact of MC on the outcome BP_{ND} across 3 different levels of movement.

Materials

All scans conducted on a Siemens HHRT PET scanner

Data sets were drawn from the Cimbi database (Knudsen et al., 2015).

Inclusion criteria

- Healthy individuals
- Non-smokers
- < 45 years of age
- Normal BMI

Exclusion criteria

Data sets with considerable movement (median movement >3 mm) were required to undergo reconstruction to correct the attenuation map thus only datasets with a median movement below 3 mm was included in the analysis.

This yielded a total of 117 ^{11}C-DASB PET datasets.

Detection of outliers

Prior to the analysis all the data sets were inspected for outliers (any dataset that had a large change in BP_{ND} when MC was applied) using a ROUT (Q=1%) test on ΔBP_{ND}

Six datasets were identified as outliers. Prior to data analysis, each dataset was evaluated for inclusion by BM. The evaluation was based on three parameters: Median movement, fitting of data points to MRTM2 and comments in investigators data process files (i.e. regarding data acquisition)
In a subset of datasets (n=3) the processing was repeated to assure that differences were not caused by i.e. use of incorrect files (checking that the correct kinpar files has been used to extract the BP_{ND} and that all the correct files (extracted data and sif files) are used to generate time activity curves (TACs)). No mistakes were detected.

Included data sets, N = 4

DASB211

- Median voxel movement 1.9 mm
- One larger movement, but fitting to MRTM2 acceptable.

- No comments in investigators files

DASB236:

- Median voxel movement 1.5 mm,
- Good fitting of MRTM2
- No comments in data process files

DASB338

- Median voxel movement 1.8 mm
- Good fitting of MRTM2
- Comment made about pituitary hotspot in data process files.

DASB348:

- Median voxel movement 1.9mm
- Acceptable fitting of MRTM2
- No comments in investigators files

Excluded datasets, N = 2

DASB 369:

- Median voxel movement 2 mm, but one large movement in frame 31-34 and the TAC curve revealed a sharp decline in data points from 2000-4000 sec.
- Considerable improvement of fitting in highbinding regions to MRTM 2 when MC is applied, but no considerable variation in k_2' (0.050 and 0.059)
- Comments of subjects movement in investigators files

Conclusion: The dataset acquires a new reconstruction

DASB 349:

- Median voxel movement 2 mm

- Large variation in k_2' (0.0878 before MC and 0.055 after MC, cross checked by redoing the kinetic modelling)
- Large changes in sub-cortical regions, but only a 3% change in neocortex.
- Nice TACs
- Fitting to MRTM2 acceptable, slightly better with MC (aside from one data point in highbinding regions, however toggling the point did not change the outcome).

Conclusion: Dataset is excluded from the analysis since it is unclear why k_2' vary so much

Stratification of datasets

The 115 datasets that entered the cross-sectional analysis (sample A and B) were stratified according to maximum median movement (fig 1).

Low movement : Max median voxel movement 1 mm: 19 datasets
Intermediate movement: Max median voxel movement 1-2 mm: 80 datasets
High movement: Max median voxel movement 2-3 mm: 16 datasets

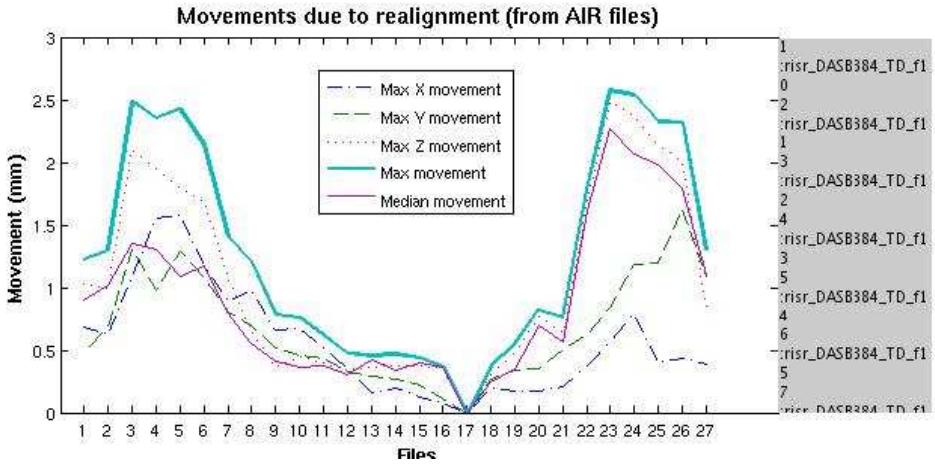

Figure 1. Example of the realignment from AIR files, frames realigned to frame 17, the max median movement is 2.3 mm.

Cross-sectional samples

Sample A:

N = 113

Sample A was used to investigate if there was a significant change in BP_{ND} in when applying MC

To cover specific effect in subcortical highbinding and cortical low binding regions, pallido-striatum and global neocortex were chosen as regions of interest (ROIs) .

Low movement: 19 datasets
Intermediate movement: 78 datasets
High movement: 16 datasets

Sample B

N = 63

Sample B was used to investigate the change in covariation (COV = STDEV/median) before and after MC.

Low movement: 11 datasets
Intermediate movement: 41 datasets
High movement: 11 datasets

Longitudinal sample

Sample C

N = 28 pairs/56 datasets

Sample C was used to investigate a) the covariance of the relative percentage change in BP_{ND} before and after MC was investigated using the same 17 regions as stated above and b) the effects on change in COV difference (ΔCOV = COV test-retest) in 17 ROIs (including the pooled regions i.e. highbinding, pallidostriatum and neocortex) were investigated

Low movement: 5 datasets
Intermediate movement: 42 datasets

High movement: 9 datasets

The majority (21 pairs) had both a test and a retest median voxel movement of 1-2-mm.

Methods and statistics

Cross sectional data analysis:

Comparison of BP_{ND}

Sample A

BP_{ND} difference before and after MC in pallidostriatum and neocortex was investigated with a parametric students paired t-test.

Comparison of COV

Sample B

The COV was calculated for 17 brain regions (no overlapping regions, not: Raphe, med.post.tegm., hypothalamus, enth.cortex, cerebellum and no functional regions). The COV (N= 17) before and after MC was compared with a non parametric students paired t-test.

MC in a test-retest setting

(Longitudinal data analysis)

Sample C, N = 26 pairs

The BP_{ND} test-retest information was incorporated into a single measure of relative percentage change in BP_{ND} (BP_{ND} %) for each region that was used as output to compared data before and after MC.

$$BP_{ND}\ \%_{region} = (|test\text{-}retest|/ ((test + retest)/2))*100\%)$$

Analysis 1: Regional BP_{ND} % (17 pair of regions) before and after MC was compared with a students paired t-test.

Analysis 2: The difference in BP_{ND}% COV before and after MC for the 17 regions was subsequently compared with a students paired t-test.

All statistics were performed in Graphpad prism 6. All comparisons between groups are done using a parametric two tailed paired t-test. A significance level of 0.05 is adopted through out.

Results

Motion Correction of low movement datasets

Sample A, comparison of BP_{ND}, low movement, n = 19 data sets

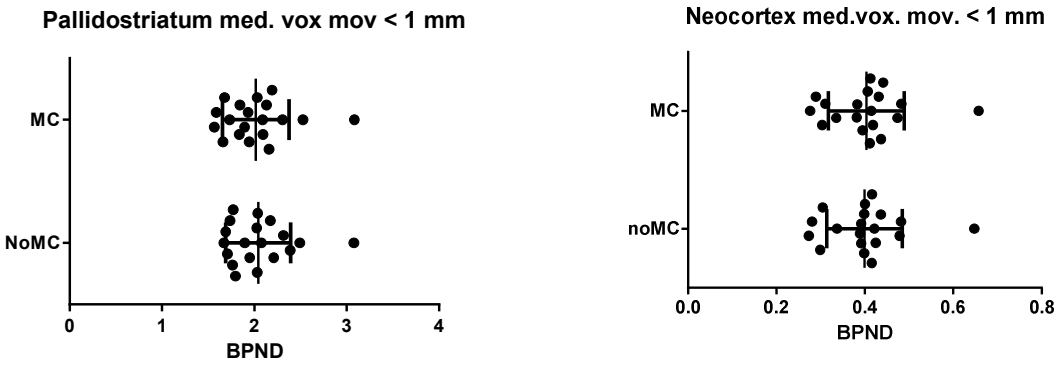

Figure 1 and 2. No significant difference was found in BP_{ND} values in 17 regions (*P* = .21 and *P* = .22, respectively).

Sample B, comparison of COV, low movement, n = 11 datasets

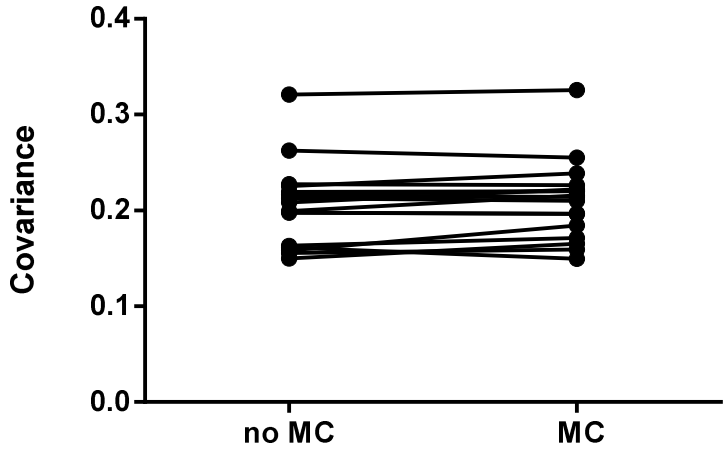

Figure 3. MC did not cause a significant difference in covariance (median covariance no MC= 0.205 and median covariance MC= 0.210, *P*= .068).

Motion Correction of scans with intermediate movement:

Sample A, comparison of $BP_{ND,}$ intermediate movement, n = 78 data sets

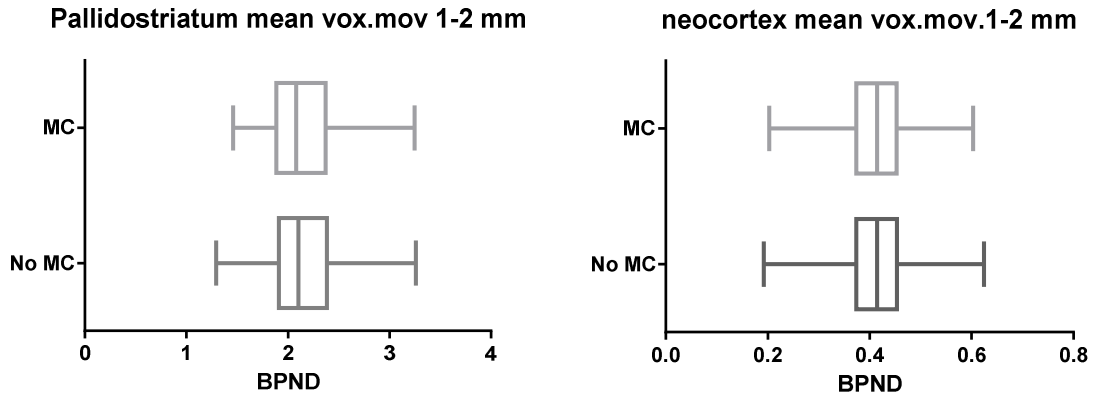

Figure 4 and 5. No significant difference was found in pallidostriatum (*P* = .96); a trend was detected in neocortex (median BP_{ND} no MC=0.417 and median BP_{ND} MC=0.420, *P* = .06).

Sample B, comparison of COV, intermediate movement, n = 41 datasets

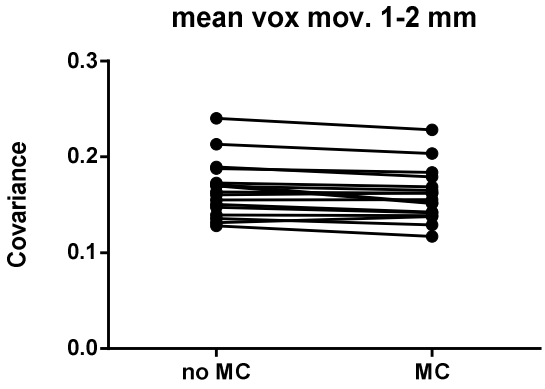

Figure 6. MC causes a significant decrease in the BP_{ND} COV (median COVno MC=0.17 and median COV MC=0.16, P=0.0016).

Motion Correction of scans with high movement:

Sample A, comparison of BP_{ND}, high movement, n = 16 data sets

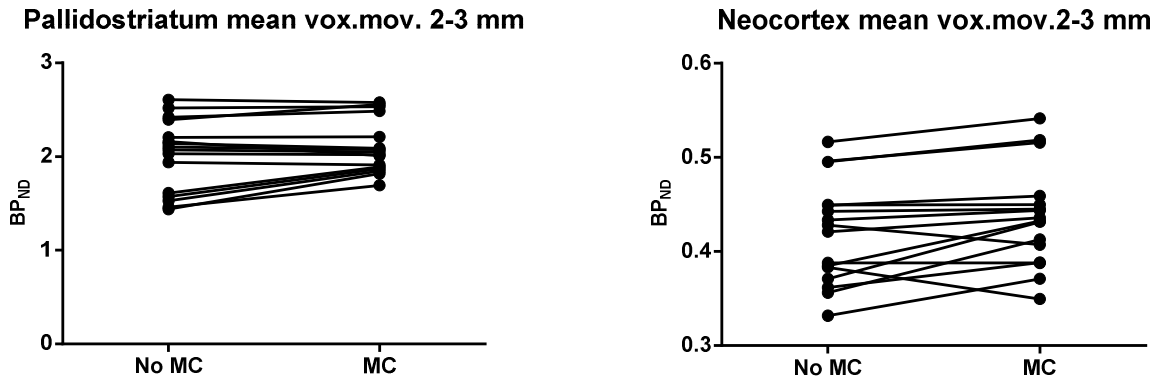

Fig. 7 and 8: In both regions MC increased BP_{ND} significantly (pali.stri.: mean BP_{ND} No MC = 2.01, mean BP_{ND} MC =2.1, P = .04 and neocortex, P = .02).

Sample B, comparison of COV, high movement, n = 11 datasets.

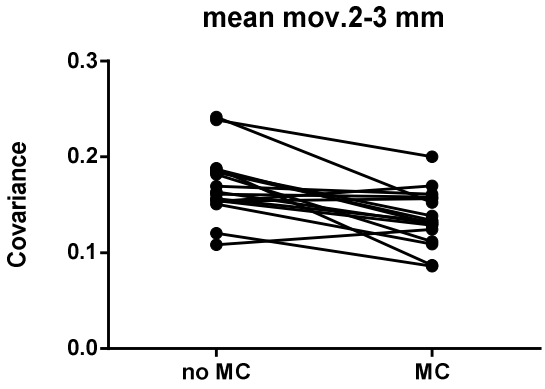

Figure 9. MC causes a highly significant decrease in COV (median COV noMC=0.17 and median COV MC=0.14, P=0.0006).

Result Summary:

MC at BP_{ND} Level

- There were no significant change of BP_{ND} values in pallidostiatum or neocortex of datasets with low movement (<1 mm), however
- There was a trend level change in neocortex for data sets with an intermediate movement (1-2 mm) (P = .07).
- MC caused a significant increase in BP_{ND} in pallidostriatum and neocortex in datasets with high movement (2-3 mm) (P =.04 and P = .02, respectively).

MC effects on BP_{ND} COV

- The BP_{ND} COV in low movement datasets (< 1mm) do not change significantly by applying MC, however a trend was detected (P = .07).
- MC decreased BP_{ND} COV in intermediate (1-2 mm) and high (2-3 mm) movement datasets significantly (P =.002 and P =.001, respectively).

Impact of Motion Correction in a test-retest setting

To investigate the effect of MC on test-retest datasets the percentage change in BP_{ND} from test to retest was calculated before and after applying MC in 17 individual ROIs

on 28 dataset pairs as described above. The covariance before and after MC from the above stated percentage change from 17 ROIs was subsequently analysed.

Table 1. The individual computed difference between test-retest BP_{ND}, BP_{ND} % was tested pair-wise between the MC and the no MC data. Significant probability values ($P \leq .05$) were Bonferroni corrected (17 teste) ($P_{corrected}$).

Region	BP_{ND} %		Percent difference of medians	*P*-value ($P_{corrected}$)
	No MC	MC		
HighBinding	8%	5%	2%	.06
Ant.cin.gy.	9%	9%	0%	.46
Thalamus	7%	5%	2%	.07
Caudatus	9%	6%	3%	.05 (.85)
Putamen	8%	6%	2%	.02 (.34)
Sup.temp.gy.	9%	9%	0%	.34
Par.cor.	12%	11%	1%	.34
Med.inf.temp.gy	7%	8%	0%	.58
Supr.temp.gy	13%	11%	3%	.05 (.85)
Occipital cor.	7%	7%	0%	.90
Sen.mot.cor	11%	8%	3%	.01 (.17)
Post.cin.gy	9%	8%	1%	.03 (.51)
Amygdala	10%	9%	1%	.85
Pallidostriatum	8%	6%	2%	.03 (.51)
Neocortex	8%	7%	1%	.10
Frontal Cortex	10%	8%	2%	.04 (.68)
Midbrain	6%	7%	-1%	.54

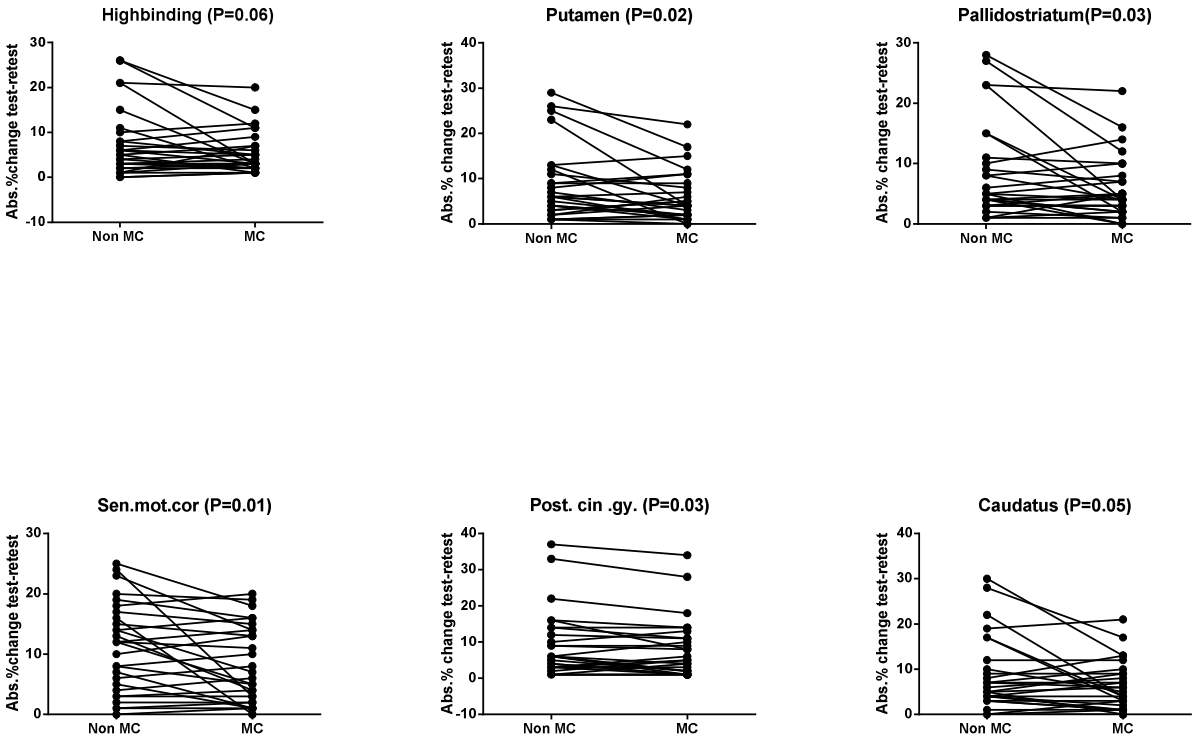

Figure 10-15. In six out of the 17 investigated regions applying MC had a significant impact on the test-retest change (uncorrected for multiple comparisons), in all cases the difference form test to retest was lower in the MC dataset.

The covariance of BP_{ND} % was compared before and after MC

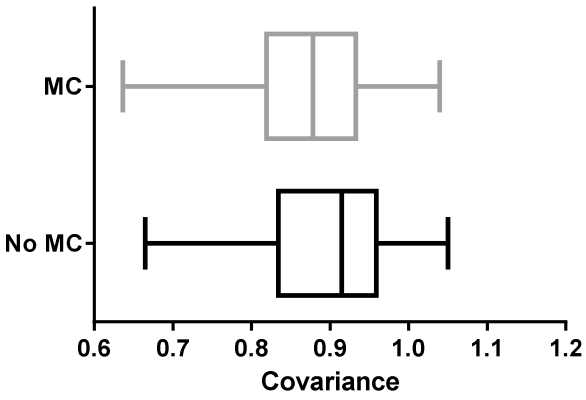

Figure 16. MC did not cause a significant difference in BP_{ND} % COV (P = .34)

Result summary, test-retest data:

- The test-retest difference decreased when MC was applied, this decrease (demonstrated as a

A lower relative percentage test-retest change) was significant in 6 out of 17 investigated regions (uncorrected for multiple comparisons).

- The covariance of the test-retest difference did not change significantly after MC (P = .34)

Conclusion:

The use of MC does not bias BP_{ND} values when the median voxel movement is less than 2 mm. However, MC decreased BP_{ND} values in pallidostriatum and neocortex in images with a median voxel movement of 2-3 mm. The BP_{ND} covariance in datasets with a median voxel movement less than 1 mm do not change significantly by applying MC, but in datasets with a median voxel movement higer than 1 mm MC causes a significant decrease in the covariance. However, it should be noted that even though the change in covariance was significant the median covariance did not differ dramatically in either group (1-2 mm: from 0.17 to 0.16 and 2-3 mm: from 0.17 to 0.14).

In accordance with this, we also saw a reduction in test-retest differences upon MC in 6 out of 17 investigated regions. We did not observe a significant change in the covariance of the test-retest data. In conclusion, MC generally moves data into a more narrow distribution, biased towards a lower median. Notably, a low median voxel movement (less than 1 mm) was not associated with any significant change after MC neither on a BP_{ND} level nor in the covariance of data points. Theses results suggests that MC can be generally applied in the pre-processing of data, as it does not induced unacceptable noise to the datasets that does not need it.

The effect of injected mass of DASB on SERT binding potential

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Background and aim

The positron emission topography (PET) radiotracer 11Carbon-labeled 3-amino-4-(2-dimethylamino-methyl-phenyl-sulfanyl)benzonitrile (^{11}C-DASB) is a highly selective radiotracer that can visualise the serotonin transporter (SERT) in vivo with high accuracy (Wilson, Ginovart, Hussey, Meyer, & Houle, 2002). However, the ratio of hot compound (radiolabelled) and cold compound (unlabelled) will vary across radiotracer productions. As labelling does presumably not affect either affinity or the ability to pass the blood brain barrier, the hot and cold compound will compete for target sites upon entering the brain. As follows, a high mass injected cold mass can potentially violate tracer assumptions by affecting the systems under investigation leading to a bias towards an underestimation of SERT non-displaceable binding potential (BP_{ND}). Such mass-dose effects has been described for other radiotracers i.e. ^{11}C-SB207145, where BP_{ND} corrections are recommend if injected mass is high (Madsen, Marner, Haahr, Gillings, & Knudsen, 2011). The aim of this report is to investigate if the injected mass DASB or the injected mass DASB adjusted to body weight (injected mass/kg) affects the binding potential.

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Material and Methods

A total of 108 ^{11}C-DASB PET HRRT scans conducted on healthy subjects below the age of 35 years and with a BMI less that 30 (n=108, 78 females) entered the analysis. The correlation of injected mass DASB and BP_{ND} values from 3 regions of interest (a pooled subcortical highbinding region (highbinding), amygdala and a pooled cortical region (neocortex)) where investigated with simple linier regression to test if injected DASB predicted BP_{ND}. In addition, a sub-analysis was performed in 19 datasets where the amount of injected DASB exceeded 5 μg (10 females).

Results

Neither total inj.mass DASB nor inj.mass DASB/kg correlated with BP_{ND} values irrespective of region.

Results can be appreciated in table 1 and 2 (Figure 1).

Table 1. Effects of injected mass DASB on SERT BP_{ND}

Sample	**Total sample** **n = 108**			**Sub-sample > 5 µg,** **n = 19**		
mean±SD (range)	1.94±2.35 µg (0.39 – 13.84 µg)			7.72±2.28 µg (5.10 – 13.84 µg)		
parameter	r^2	estimate	*P* – value	r^2	estimate	*P*-value
highbinding	0.01	- 0.009	.48	0.02	-0.004	.55
amygdala	0.01	-0.010	.41	0.03	-0.09	.48
neocortex	0.02	-0.004	.11	0.02	-0.004	.55

Table 2. Effects of injected mass DASB per kg bodyweight on SERT BP_{ND}

Sample	**Total sample** **n = 108**			**Sub-sample > 5 µg,** **n = 19**		
mean±SD (range)	0.028±0.035 µg/kg (0.005– 0.25 µg/kg)			0.0032±0.044 µg/kg (0.0045 – 0.25 µg/kg)		
	r^2	estimate	*P*- value	r^2	estimate	*P*-value
highbinding	< 0.01	-0.34	.59	0.01	-0.48	.64
amygdala	0.01	-0.71	.39	0.03	-1.15	.46
neocortex	0.02	-0.28	.12	0.01	-0.12	.68

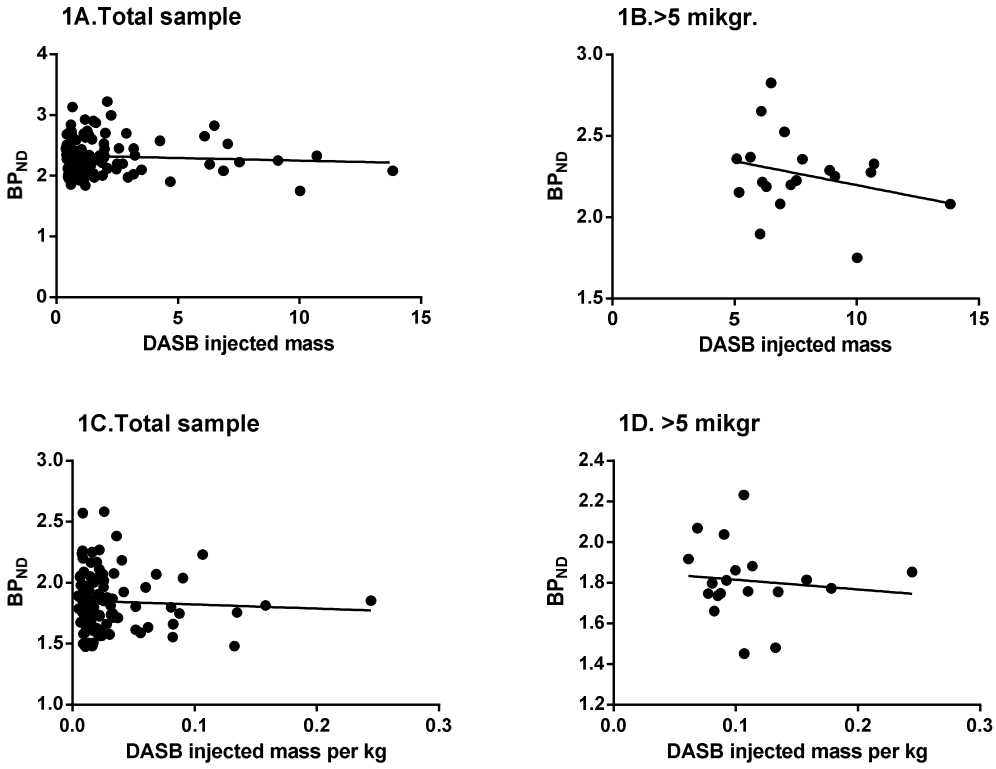

Figure 1: Correlations of injected mass DASB (μg) and SERT BP_{ND} in a pooled highbinding region. **1A and 1B:** Total amount injected DASB; **1C and 1D:** injected DASB per kg.

Conclusion

Neither in the total sample nor in the subsample of high-mass datasets did the injected mass DASB bias the outcome parameter SERT BP_{ND}. Based on this, a limit of 10 μg DASB could safely be administered. Moreover, studies with in these ranges of injected DASB do not need to control for injected does in statistical models. However, we can not exclude that mass-dose effects will occur at higher doses, to address this dose-response experiments are warranted.

References

Madsen, K., Marner, L., Haahr, M., Gillings, N., & Knudsen, G. M. (2011). Mass dose effects and in vivo affinity in brain PET receptor studies--a study of cerebral 5-HT4 receptor binding with [11C]SB207145. ***Nucl Med Biol, 38*****(8), 1085-1091. doi: 10.1016/j.nucmedbio.2011.04.006**

Wilson, A. A., Ginovart, N., Hussey, D., Meyer, J., & Houle, S. (2002). In vitro and in vivo characterisation of [11C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. ***Nucl Med Biol, 29*****(5), 509-515.**