PET Analysis of the Effect of Season, Seasonal Affective Disorder and Light Therapy on Serotonin Transporter Binding

By

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Seasonal affective disorder (SAD) is characterized by recurrent major depressive episodes during fall-winter with remission in spring-summer. SAD has an annual prevalence of 1-6% with higher rates at more extreme latitudes. However, there is limited understanding of its neuropathophysiology as no brain biomarkers have been identified that are associated with SAD. Interestingly, serotonin transporter binding (5-HTT BP_{ND}), an index of 5-HTT levels, varies seasonally in health; a finding replicated by four independent research groups. The 5-HTT has a key role in affect-regulation, given its involvement in serotonin reuptake. Yet, there have been no longitudinal studies of seasonal fluctuation in 5-HTT BP_{ND} in SAD. Accordingly, we investigated seasonal change in 5-HTT BP_{ND} in the anterior cingulate and prefrontal cortices (ACC and PFC, respectively), using positron emission tomography (PET) in SAD and health, scanning across summer and winter. Greater seasonal change in 5-HTT BP_{ND} was observed in SAD relative to health.
Additionally, there has been no investigation of deliberate light exposure upon 5-HTT BP_{ND} in the human brain despite evidence from previous PET studies, in health, of an inverse correlation between sunshine duration and 5-HTT BP_{ND}. Thus, in our second and third studies, we examined the effect of light therapy during fall-winter on 5-HTT BP_{ND} in the ACC and PFC, in health and SAD, respectively, PET scanning before and after treatment. In study two, we observed a decrease in 5-HTT BP_{ND} in the ACC following light therapy in health. In study three, light therapy reduced 5-HTT BP_{ND} across all examined brain regions in SAD.

The results of these studies have two important implications. First, the seasonal change in 5-HTT BP_{ND} in study one was comparable to the reduction in 5-HTT BP_{ND} following light therapy in study three. Hence, changes in light exposure may represent a sufficient environmental condition to account for seasonal variation in 5-HTT BP_{ND} in SAD. Second, the results of studies two and three demonstrate that light therapy reaches a therapeutic target relevant to prevention and treatment of SAD. Overall, we have identified a biomarker affected by season, associated with SAD pathophysiology that is involved in treatment response to light.
DEDICATION

In memory of Laura Kaitlin Peters whose life and struggle inspired this work.
ACKNOWLEDGMENTS

Firstly, I would like to extend my most heart-felt thanks and gratitude to my primary Ph.D. supervisor Dr. Jeffrey Meyer whose mentorship has been invaluable to both to my academic and personal growth. I met Dr. Meyer in early fall 2011 whilst completing an honours specialization in physiology and psychology at the University of Western Ontario. It had been my long-term goal to pursue translational research in the field of mood/anxiety disorders, with particular focus on receptor-ligand neuroimaging and I was excited to have the opportunity to participate in a project with the potential to have real world impact. Although I had been trained in pre-clinical research as an undergraduate, Dr. Meyer was always patient and willing to teach me the basics of how to run a clinical study, of the mathematical modelling underlying brain imaging techniques and to share his knowledge of how to best prepare findings for publications in high-impact journals and at conferences. Furthermore, although I did not arrive in his laboratory as the most confident trainee, he understood my commitment to high quality research and my drive for success – to this end he encouraged me to be confident in my own results, better my public speaking skills and reminded me to always be optimistic and solution-oriented in the face of challenge. He believed that I could be successful even when I doubted my own abilities. I have been lucky to have such a Ph.D. mentor and I owe my skills, expertise and current position to both his dedication and the excellent training environment in his lab.

I would also like to thank Drs. Robert Levitan and Jose Nobrega. Dr. Levitan’s expertise in the field of seasonal affective disorder was invaluable in informing the direction for this series of studies, guiding me toward relevant literature and
encouraging me to present at conferences geared toward to seasonal research. Dr. Nobrega’s input regarding interpretation of my PET findings, suggestions for statistical analyses and possible extensions of this research to preclinical animal models were always welcome and allowed me to challenge any preconceived notions I may have held regarding my work.

I would like to thank everyone at the CAMH Research Imaging Centre for making this series of work possible. Peter Bloomfield and Dr. Pablo Rusjan were kindly willing to lend their time explaining PET physics or the fundamentals of kinetic modelling. Laura Nguyen, Alvina Ng, Hillary Bruce and Anusha Ravichandran helped me to book PET/MRI scan slots when I had participants urgently in need of seasonal or post-treatment scanning, while Dr. Cynthia Xu and Laura Miler kindly stepped in to accompany a participant to a scan when I had double-booked myself. I would also like to thank Dr. Alan Kahn for his willingness to provide medical coverage for my PET scans, for his advice regarding medical school post-Ph.D. and for allowing me to shadow him during patient assessments for the last 2 years of my Ph.D.

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LIST OF ABBREVIATIONS

5-HIAA 5-Hydroxyindoleacetic Acid
5-HT 5-hydroxytryptamine; Serotonin
5-HTP 5-Hydroxytryptophan
5-HTT Serotonin Transporter
5-HTT BP_{ND} Serotonin Transporter Binding (non-displaceable)

[^123]ADAM (123)I-labeled 2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine
[^123]CIT 2β-carbomethoxy-3β-(4-iodophenyl)tropane

[^11]C\text{DASB} 11C-labeled-3-amino-4-[2-dimethylaminomethylphenylsulfanyl]benzonitrile

[^11]C\text{HOMADAM} C-11-labeled N,N-dimethyl-2-(2'-amino-4'-hydroxymethylphenylthio)benzylamine


Aβ β-Amyloid

ACC Anterior Cingulate Cortex

AADC Aromatic Amino Acid Decarboxylase
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ATD</td>
<td>Acute Tryptophan Depletion</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BN</td>
<td>Bulimia Nervosa</td>
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<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
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<tr>
<td>Cl⁻</td>
<td>Chloride Ion</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>DAS</td>
<td>Dysfunctional Attitudes Scale</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine Transporter</td>
</tr>
<tr>
<td>DBS</td>
<td>Deep Brain Stimulation</td>
</tr>
<tr>
<td>DMH</td>
<td>Dorsal Medial Nucleus of the Hypothalamus</td>
</tr>
<tr>
<td>DRD4</td>
<td>Dopamine Receptor D₄ Gene</td>
</tr>
<tr>
<td>DRN</td>
<td>Dorsal Raphe Nucleus</td>
</tr>
<tr>
<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders Version IV – Text Revision</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography–Mass Spectrometry</td>
</tr>
<tr>
<td>HRRT</td>
<td>High-Resolution Research Tomograph</td>
</tr>
<tr>
<td>IGL</td>
<td>Intergenticulate Leaflet</td>
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<tr>
<td>K⁺</td>
<td>Potassium Ion</td>
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<tr>
<td>MAO-A</td>
<td>Monoamine Oxidase A</td>
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<tr>
<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>m-CPP</td>
<td><em>meta</em>-Chlorophenylpiperazine</td>
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<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
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<tr>
<td>MDE</td>
<td>Major Depressive Episode</td>
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<tr>
<td>MIP</td>
<td>Mood Induction Procedure</td>
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<tr>
<td>MPA</td>
<td>Medial Preoptic Area</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MRN</td>
<td>Median Raphe Nucleus</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium Ion</td>
</tr>
<tr>
<td>Na⁺/K⁺ ATPase</td>
<td>Sodium-Potassium Pump</td>
</tr>
<tr>
<td>NET</td>
<td>Norepinephrine Transporter</td>
</tr>
<tr>
<td>NPAS</td>
<td>Neuronal PAS Domain-Containing Protein Gene</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal Anti-Inflammatory Drug</td>
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<tr>
<td>NSS</td>
<td>Neurotransmitter Sodium Symporter</td>
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<tr>
<td>p38MAPK</td>
<td>p38 Mitogen-Activated Protein Kinase</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PET-CT</td>
<td>Positron Emission Tomography - Computed Tomography</td>
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<td>PER3</td>
<td>Period Circadian Protein Homolog 3 Gene</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
</tr>
<tr>
<td>RGC</td>
<td>Retinal Ganglion Cell</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SAD</td>
<td>Seasonal Affective Disorder</td>
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<tr>
<td>SCID-I/II</td>
<td>Structured Clinical Interview for Axis I/II Disorders</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic Nucleus</td>
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<tr>
<td>SIGH-ADS</td>
<td>Structured Interview Guide for the Hamilton Depression Rating Scale with Atypical Depression Supplement</td>
</tr>
<tr>
<td>SIGH-SAD</td>
<td>Structured Interview Guide for the Hamilton Depression Rating Scale with Seasonal Affective Disorder Supplement</td>
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<tr>
<td>SLC6A4</td>
<td>Solute Carrier Family 6 Member 4 Gene</td>
</tr>
<tr>
<td>SNRI</td>
<td>Serotonin–Norepinephrine Reuptake Inhibitor</td>
</tr>
<tr>
<td>SPAQ</td>
<td>Seasonal Pattern Assessment Questionnaire</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-Photon Emission Computed Tomography</td>
</tr>
<tr>
<td>SPVZ</td>
<td>Sub-Paraventricular Zone</td>
</tr>
<tr>
<td>SRTM2</td>
<td>Simplified Reference Tissue Method 2</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>TPH2</td>
<td>Tryptophan Hydroxylase 2</td>
</tr>
<tr>
<td>TSPO</td>
<td>Translocator Protein</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>VNTR</td>
<td>Variable Number Tandem Repeat</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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1 INTRODUCTION

1.1 Statement of Problem

In 2008 the World Health Organization (WHO) identified Major Depressive Disorder (MDD) as the leading cause of death and disability in moderate to high income nations indicating that new treatment and prevention methods are needed (Mathers et al., 2008). Seasonal affective disorder (SAD) is a subtype of MDD characterized by recurrent major depressive episodes (MDEs) which occur fall-winter with full remission in the spring-summer (Rosenthal et al., 1984). The annual prevalence rate of SAD is estimated at 1 to 6 percent, with higher rates occurring at more extreme latitudes (Magnusson, 2000). For example: Haggarty et al., reported a 7 percent rate of SAD and 22 percent rate of MDE in a Northern Canadian sample, while, in Alaska, Booker et al., found that 9 percent of individuals surveyed met criteria for SAD (Booker et al., 1992; Haggarty et al., 2002). Furthermore, SAD poses a heavy burden on both sufferers and the healthcare system as, 40 percent of cases progress to spontaneous non-seasonal MDD and amongst the subtypes of MDD, SAD has the highest frequency of MDEs being almost yearly; for instance, it is not atypical for individuals with SAD to experience greater than 9 seasonal MDEs by the third to fourth decade of life (Faedda et al., 1993; Lam et al., 2006; Modell et al., 2005; Schwartz et al., 1996). Those with SAD also tend to be heavy users of an already burdened healthcare system, as they typically present with somatic symptoms such as fatigue, lethargy, bingeing, weight gain and hypersomnia that require costly investigation (Eagles et al., 2002). As such, in primary care settings, SAD is frequently misdiagnosed and only fifty percent of specialist referrals are psychiatric in nature.
(Eagles et al., 2002). There is additional reason to study the seasonal impact upon mood since 25 percent of healthy individuals experience seasonally related changes in mood, energy, appetite and sleep that affect daily functioning (i.e. subsyndromal SAD) (Chotai et al., 2004; Kasper et al., 1989; Okawa et al., 1996; Perry et al., 2001; Rosen et al., 1990). Given the high prevalence of SAD, its role in predisposing to MDD and the common problem of impaired function from seasonal variation in mood there is an urgent need for research to identify biological mechanisms relevant to understanding illness pathology so as to better develop strategies for illness prevention and treatment.

Light therapy is an evidence-based first-line treatment for SAD with a similar efficacy to that of antidepressant treatment (Lam et al., 1995; Lam et al., 2006; Moscovitch et al., 2004). There have been several advances in the technique of light therapy, insofar as it is well accepted that a light intensity of 10,000 lux is superior to 3000 lux, and that treatment in the early morning has greater efficacy as compared to evening administration (Lewy et al., 1998; Terman et al., 1990). However, at present, there is no consensus as to the mechanism by which light therapy exerts its antidepressant effects and 45 percent of SAD cases do not adequately remit after this treatment, indicating a need for improvement (Lam et al., 2006). Some theories as to the mechanism for light exposure in regards to ameliorating seasonal depression during winter include circadian phase advance, suppression of melatonin and enhancing resilience against tryptophan depletion (Lam et al., 1996b; Lewy et al., 2006; Lewy et al., 1987; Neumeister et al., 1997). However, it is generally agreed that these mechanisms are still under investigation and the means by which light therapy exerts its antidepressant effects is an on-going area of research.
Advances in mental health research are needed to improve mental health care and research utilizing neuroimaging techniques is integral to this forward progress. Receptor-ligand neuroimaging studies (i.e. positron emission tomography, PET; single photon emission tomography, SPECT) provide a means to visualize and quantify the neuropathophysiology of mental illness in vivo in the living human brain. In combination with information about illness phenomenology, such findings can be used to better understand illness pathophysiology and to determine novel biological targets for treatment or prevention. A significant body of research has focused upon investigation of monoaminergic systems (i.e. serotonin, dopamine, norepinephrine) to identify biomarkers associated with neuropsychiatric illness so as to better understand the etiology and pathophysiology of such disorders. In regards to SAD, there is most evidence of seasonal rhythmicity within the serotonin (5-HT) system, and thus it is a promising candidate for study in regards to understanding the neuropathophysiology of this disorder and to explore possible avenues by which to improve existing strategies for prevention and treatment of SAD (Levitan, 2007).

The serotonin transporter protein (5-HTT), a component of the serotonin system, is involved in clearance of extracellular 5-HT from the synaptic cleft and regulates magnitude, duration and termination of serotonergic neurotransmission (Murphy et al., 2004). Serotonin transporter binding potential (non-displaceable) (5-HTT BP\textsubscript{ND}) equals the ratio of specifically bound to free and non-specifically bound radioligand in tissue at equilibrium, an index of serotonin transporter protein (5-HTT) levels, that is commonly used in neuroimaging studies (Meyer, 2007). Interestingly, there is particularly strong evidence from neuroimaging studies of seasonal change in levels of this brain protein in health, with higher 5-HTT BP\textsubscript{ND} observed in winter
relative to summer; a finding that has been replicated by four independent research groups. (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). Yet, there are key components missing from current evidence connecting seasonal changes in mood and behaviour to seasonal variation in 5-HTT levels and environmental factors such as light exposure.

Previous neuroimaging studies of seasonal variation in 5-HTT BP_{ND} utilized retrospective data from healthy participants and were cross-sectional in design. As such, there have been no longitudinal neuroimaging studies investigating the effect of season on 5-HTT BP_{ND} in a clinical sample of individuals, such as those with SAD, a clinical population that displays marked seasonal changes mood and behaviour, relative to healthy volunteers. This is a key issue because there have been no brain biomarkers identified that are associated with SAD and thus, there is limited understanding of the neuropathophysiology of this illness. Furthermore, the efficacy of light therapy of SAD, is only 55 percent and the mechanism by which light therapy exerts its therapeutic effects remains unclear. Notably, two of the aforementioned studies using high quality imaging methods and large samples found an inverse relationship between duration of daily sunlight and 5-HTT BP_{ND}, indicating this biomarker is sensitive to light exposure (Kalbitzer et al., 2010; Lam et al., 2006; Praschak-Rieder et al., 2008). However, there have been no neuroimaging studies evaluating the effect deliberate light exposure upon 5-HTT BP_{ND} in human brain in either health or in SAD. Therefore, an investigation of the effect of light therapy upon 5-HTT BP_{ND} would be valuable in order to determine the feasibility of developing a brain biomarker for light therapy so as to improve response to this treatment.
1.2 Statement of Purpose of the Study and Objective

There is ample research in support of seasonal rhythmicity of the serotonin system, 5-HT itself has a key role in modulation of behaviours and affective states that change seasonally and there is evidence of aberrant function of the serotonin system in SAD. The 5-HTT is particularly important for further study in SAD given that this brain protein has a key role in clearance of extracellular 5-HT and 5-HT depletion is associated with depressive symptoms (Bel et al., 1992, 1993; Delgado et al., 1990; Jennings et al., 2006; Leyton et al., 2000; Mathews et al., 2004; Shen et al., 2004; Young et al., 1985). In addition, there is consistent evidence from neuroimaging studies, using both PET and SPECT technologies, of seasonal fluctuation in 5-HTT BP$_{ND}$, an index of 5-HTT levels, in health, with marked elevation in fall-winter relative to spring-summer (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). To elaborate, in regards to seasonal variation of 5-HTT BP$_{ND}$, neuroimaging studies of reasonably large sample size consistently reported this finding in a high proportion of brain regions sampled: a [$^{11}$C]DASB PET study of 88 subjects in Toronto, Canada, found seasonal variation in [$^{11}$C]DASB 5-HTT BP$_{ND}$ across all examined brain regions including the prefrontal cortex, anterior cingulate cortex (PFC and ACC, respectively) and hippocampus (Praschak-Rieder et al., 2008). Similarly, an independent [$^{11}$C]DASB PET study of 57 participants in Copenhagen, Denmark found evidence of seasonal fluctuation in 5-HTT BP$_{ND}$ in three of four brain regions with significant change in the caudate and putamen, a trend in the thalamus and no effect in the midbrain (Kalbitzer et al., 2010). Two additional studies in Amsterdam, Netherlands and Hamberg Germany, using lesser quality technology, applying [$^{123}$I]β-CIT SPECT and [$^{11}$C]McN5652 PET, respectively, investigated the
thalamus and midbrain and both found seasonal variation in 5-HTT BP_{ND} in the midbrain (the Amsterdam study included both healthy and non-SAD MDD patients) (Buchert et al., 2006; Ruhe et al., 2009). However, as previously stated, there has been no longitudinal study investigating the effect of season on 5-HTT BP_{ND} in SAD as compared to health and thus, the magnitude by which this brain protein fluctuates across seasons in this clinical population relative to health is unknown. Accordingly, the objective of study one was to use [^{11}C]DASB PET to determine and compare the magnitude of change in 5-HTT BP_{ND} in healthy individuals and those with SAD using a within-subject design across winter and summer seasons.

Light therapy is an evidence-based, front-line therapeutic for seasonal depression and there have been several advances in regards to optimizing this technique since its initial application for treatment of SAD by Rosenthal et al., in 1984 (Lam et al., 1999; Rosenthal et al., 1984). It is now well accepted that a light intensity of 10,000 lux is superior to 3000 lux, and that treatment in the early morning has greater therapeutic efficacy as compared to evening administration (Lewy et al., 1998; Terman et al., 1990). However, 45 percent of SAD cases do not remit following light therapy indicating need for improvement and there is no consensus as to the means by which this treatment ameliorates seasonal depressive symptoms, further complicating its development (Lam et al., 2006). Interestingly, both preclinical rodent studies and PET investigations report a reduction in 5-HTT binding concomitant with an increase in light exposure, and blockade of the 5-HTT has been shown to be important for antidepressant response (Kalbitzer et al., 2010; Meyer et al., 2001; Meyer et al., 2004b; Praschak-Rieder et al., 2008; Rovescalli et al., 1989). Taken together, these results suggest that there may be a relationship between light
exposure, 5-HTT binding and mood. As such, the objectives of studies two and three were to evaluate the effect of light therapy on 5-HTT $B_{ND}$ in health and in SAD, respectively, using [$^{11}$C]DASB PET.

1.3 Rationale and Statement of Research Hypotheses

1.3.1 Study 1: The Effect of Season on 5-HTT $B_{ND}$

Although information about SAD is accumulating, a critical gap is the lack of direct brain investigations of this illness, thus, there have been no brain biomarkers identified for this neuropsychiatric illness. Biological abnormalities of SAD include, in winter, increased duration and/or delay of nocturnal melatonin secretion, blunted norepinephrine, cortisol and prolactin response to challenge with the non-selective serotonin receptor agonist meta-Chlorophenylpiperazine (m-CPP), and decreased rod sensitivity to light as measured by flash electroretinography (Lavoie et al., 2009; Levitan et al., 1998; Schwartz et al., 1997; Wehr et al., 2001). Vulnerability markers include altered polymorphism frequencies of clock genes NPAS (neuronal PAS domain-containing protein) and PER3 (period circadian protein homolog 3), as well as a variable number tandem repeat on exon 3 of the DRD4 (dopamine receptor D4) gene (Johansson et al., 2003; Levitan et al., 2006). However, given the substantial burden of SAD, its high prevalence, and the lack of knowledge regarding the brain abnormalities associated with this disorder, there is a clear need to identify neurochemical and neuropathological markers associated with SAD.

One strategy for selecting a target to investigate in SAD is to choose a functionally relevant brain marker that is sensitive to seasonal effects in healthy humans. Once a biomarker has been chosen, the magnitude of seasonal change in
the target of interest can be examined in SAD relative to health to determine if seasonal rhythmicity differs between populations, indicating a possible biological abnormality associated with illness pathology. Brain markers influenced by season in health include greater striatal L-Dopa uptake in the fall and winter, decreased 5-HT_{1A} receptor binding in limbic regions in winter, altered whole brain 5-HT turnover and greater 5-HTT BP_{ND} in the fall-winter as compared to spring-summer (Buchert et al., 2006; Eisenberg et al., 2010; Kalbitzer et al., 2010; Lambert et al., 2002; Praschak-Rieder et al., 2008; Ruhe et al., 2009; Spindelegger et al., 2012). However, as previously stated, evidence is most consistent in regards to seasonal variation of 5-HTT BP_{ND} in health, as such findings have been reported by four independent research groups with reasonably large sample sizes (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). Notably, the 5-HTT is also an important target for controlling affect given that polymorphisms in the 5-HTT promotor region are associated with risk of developing MDD, medications that influence the 5-HTT influence both cognitive recall of emotionally valent material as well as negative cognitive interpretations of life events; and that overexpression of 5-HTT in regions controlling affect are associated with depressive behaviors in rodents (Caspi et al., 2010; Harmer et al., 2004; Line et al., 2014; Meyer et al., 2003; Mouri et al., 2012).

Given the consistency of seasonal change in 5-HTT BP_{ND} in health and the importance of the role of 5-HTT in affect regulation, we first hypothesized that the magnitude of seasonal variation in 5-HTT BP_{ND} in the PFC and ACC would be greater in SAD compared to health. We prioritized the PFC and ACC as these regions had considerable seasonal variation in previous study, contain structures with key roles in
mood regulation and cognitive processing of emotion, and are the regions for which 5-HTT overexpression is associated with depressive behaviors (Line et al., 2014; Mouri et al., 2012; Praschak-Rieder et al., 2008; Ressler et al., 2007). The second hypothesis was that seasonal variation in 5-HTT BP\textsubscript{ND} in the PFC and ACC would be associated with severity of SAD symptoms. The rationale for the second hypothesis is that SAD is well known to be a dimensional illness with a continuous distribution within health (such that 25% of healthy individuals experience mild seasonal symptoms) through to SAD of moderate to high severity (Bartko et al., 1989; Kasper et al., 1989; Rohan et al., 2011; Terman, 1988); and in MDD the magnitude of brain biomarker abnormalities is often correlated with severity, reflecting that MDD is a complex neuropsychiatric illnesses for which any individual pathology is more likely to present when MDD is more severe (Chiuccariello et al., 2014; Deschwanden et al., 2011; Fujita et al., 2012; Meyer, 2012; Sanacora et al., 2004; Setiawan et al., 2015). We also examined other structures such as the hippocampus, ventral striatum, thalamus, dorsal putamen, dorsal caudate and midbrain as 5-HTT density is high in these regions (Backstrom et al., 1989; Cortes et al., 1988; Laruelle et al., 1988).

1.3.2 Study 2: The Effect of Light Therapy on 5-HTT BP\textsubscript{ND} in Health

There is reason to study seasonal impact on mood in healthy individuals who have not yet developed SAD as 25 percent of healthy individuals experience seasonally related changes in mood, energy, appetite and sleep that adversely affect daily functioning during the winter months (Kasper et al., 1989). Interestingly, two previous studies in healthy volunteers, including one from our laboratory, detected an inverse correlation between duration of daily sunshine and 5-HTT BP\textsubscript{ND} (Praschak-
Rieder et al., 2008; Kalbitzer et al., 2010). Thus, it was our intent to examine effect of light therapy, a standard intervention for SAD, on 5-HTT BP_{ND} during fall-winter in health to investigate the potential of 5-HTT BP_{ND} as a biomarker for light exposure and to develop prevention strategies for new-onset SAD. We elected to first study healthy volunteers (i.e. without the confound of SAD pathophysiology) with the intent to further examine this effect in full-syndrome SAD in study three.

However, in regards to prophylactic interventions for new-onset SAD, direct investigation of prevention strategies through clinical trials are both difficult and labor-intensive since only a subset of individuals develop SAD. Similarly, direct investigation of treatment strategies with large scale clinical trials is also challenging as these trials must be oriented to the winter months. Hence a valuable intermediate approach is to develop biomarkers to assess the effect of intervention strategies. The magnitude of effect of the intervention strategy on the biomarker can then be applied to identify therapeutics that should be carried forward in development. One candidate biomarker for this approach is the serotonin transporter binding potential (non-displaceable) (5-HTT BP_{ND}), as measured using PET with the radioligand [^{11}\text{C}]DASB. Interestingly, in four independent studies, 5-HTT BP_{ND} has been found to be elevated across multiple brain regions in the fall-winter months compared to spring-summer in healthy volunteers, suggestive of the sensitivity of this biomarker to seasonal effects (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). In addition, in two of these previous studies of seasonal variation in 5-HTT BP_{ND}, using [^{11}\text{C}]DASB PET, an inverse correlation between duration of daily sunlight and 5-HTT BP_{ND} was found, suggesting that this might be a promising biomarker for light therapy (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008).
The 5-HTT also has an important role in affect-regulation given that in humans, lowering 5-HT via acute tryptophan depletion associated with low mood, particularly in those vulnerable to developing MDEs such as those with family histories of MDE, or past histories of MDE (Acta Psychiatrica et al., 2013; Delgado et al., 1990; Leyton et al., 2000; Young et al., 1985). 5-HT itself and/or its neuromodulatory role is also strongly implicated in mood disorders, given the many selective serotonin re-uptake inhibitors (SSRIs) that raise extracellular serotonin are associated with amelioration of depressive symptoms in individuals suffering from major depressive disorder (Owens et al., 1994, 1998). Modulators of extracellular 5-HT are important because it is well established that 5-HT plays a role in physiology and behaviours reported to change with season including mood, sleep, appetite and energy (Canli et al., 2007). Collectively, these findings illustrate that the importance of the 5-HTT, in terms of affect-regulation, is this protein’s influence on extracellular 5-HT levels.

As such, it was our intent evaluate the potential of 5-HTT BP
ND as a biomarker for light therapy in healthy volunteers who had not yet developed SAD in order to develop prevention strategies for new-onset SAD (Boyce et al., 2013). We hypothesized that administration of light therapy during the fall and winter months would reduce 5-HTT BP
ND in the ACC and PFC in healthy volunteers. The ACC and PFC (and/or subregions of these structures) are often activated in mood induction studies (reflecting processes that generate sad mood and they also participate in cognitive functions such as those leading to pessimism that create a sad mood (Liotti et al., 2001; Liotti et al., 2002; Mayberg et al., 1999; Sharot et al., 2007; Tom et al., 2007). Other regions included the thalamus, basal ganglia, hippocampus and midbrain
were also examined because 5-HTT density is high in these regions (Backstrom et al., 1989; Cortes et al., 1988; Laruelle et al., 1988).

1.3.3 Study 3: The Effect of Light Therapy on 5-HTT BP\textsubscript{ND} in SAD

Light therapy is an evidence-based first-line treatment for SAD, a highly impactful disease, consisting of numerous, reoccurring, major depressive episodes spanning the fall and winter with full remission in the spring and summer (Faedda et al., 1993; Rosenthal et al., 1984). There have been several advances in the technique of light therapy, insofar as light intensity of 10,000 lux is superior to 3000 lux, and treatment in the early morning has greater efficacy relative to evening administration (Lewy et al., 1998; Terman et al., 1990). However, only 55 percent of individuals with SAD experience remission of seasonal depressive symptoms following light therapy and therefore it is important to develop strategies to further improve this treatment so as to better ameliorate winter depressive symptoms (Lam et al., 2006).

It is generally recognized that use of PET with specific radioligands is an effective tool for developing therapeutics. For example, $[^{11}\text{C}]$DASB PET is often applied to predict optimal dosing of antidepressants with high affinity for the serotonin transporter while $[^{11}\text{C}]$raclopride PET is often utilized to determine suitable dosing of antipsychotics with high affinity for the D\textsubscript{2} receptor (Kapur et al., 2000; Meyer et al., 2001; Meyer et al., 2004b). More recently, a newer direction regarding the role of PET receptor-ligand neuroimaging is its application quantifying markers of disease pathology, such as amyloid burden in Alzheimer's disease or microglial activation in major depressive episodes, so as to determine if these markers may be successfully targeted by therapeutics (Rinne et al., 2010; Setiawan et al., 2015).
The intent of the present study was to develop a PET-based measure of SAD pathophysiology as a biomarker for light therapy. 5-HTT BP<sub>ND</sub> had previously been found to be inversely correlated with duration of daily sunshine in health (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008). As a priori regions, the ACC and PFC were prioritized because these regions have a key role in mood regulation and cognitive processing of emotion (Liotti et al., 2002; Mayberg et al., 1999; Ressler et al., 2007; Sharot et al., 2007; Tom et al., 2007). The ACC participates in production of sad emotions, where regional activation has been observed during provocation of sad mood in MDD patients and decreased activity follows recovery from depression (Liotti et al., 2002; Mayberg et al., 1999; Ressler et al., 2007). The PFC is involved in the cognitive emotional processing; regional reductions in activity have been found to be correlated with increased sensitivity to potential loss of reward (i.e. “loss aversion”) whereas enhanced activity accompanies recall of positive life events (Sharot et al., 2007; Tom et al., 2007). As such, our primary hypothesis was that, during fall-winter, 5-HTT BP<sub>ND</sub> would be reduced in the ACC and PFC following light therapy. Other brain regions with high 5-HTT BP<sub>ND</sub> and/or roles in affect regulation were also examined, including the hippocampus, thalamus, dorsal putamen, ventral striatum, and midbrain, in order to assess the extent to which the effects of light therapy would be more global or region-specific as in study two (Backstrom et al., 1989; Cortes et al., 1988; Laruelle et al., 1988).
1.4 Overview of the Serotonin System

1.4.1 The Raphe Nuclei

In the mammalian brain, the raphe nuclei are found in the reticular formation within the medial portion of the brain stem. These nuclei are divided into (i) the raphe nuclei of the medulla oblongata (*nucleus raphe obscurus, nucleus raphe magnus and nucleus pallidus*), raphe nuclei of the pontine reticular formation (*nucleus raphe pontis, nucleus centralis inferior*) and (iii) raphe nuclei of the midbrain reticular formation (*nucleus raphe dorsalis; dorsal raphe nucleus and nucleus centralis superior; median raphe nucleus*) (Lowry et al., 2008). The majority of neurons with the raphe nuclei are serotonergic, but of these nuclei, it is the dorsal and median raphe nuclei (DRN and MRN, respectively) of the midbrain that project to ascending brain areas, including cortical and subcortical regions, in addition to the brainstem (Vertes et al., 2008). Serotonergic innervation within the central nervous system (CNS) is extensive and the 5-HT neurotransmitter itself has a key role in modulation of affect, cognitive function and behaviours such as sleep, appetite and energy (Vertes et al., 2008). This latter topic regarding the influence of 5-HT on mood and behaviour will be covered extensively in subsequent sections.

There is also evidence that serotonergic neurons in the DRN and MRN play an important role in regulation of circadian behaviours such as diurnal variation in temperature and hormone levels, alertness and the sleep-wake cycle. However, the exact mechanism by which 5-HT modulates circadian rhythmicity in regards to functional and structural connections within the brain is complex and will require further research (Deurveilher et al., 2008). Interestingly, there is evidence of a direct
retino-raphe projection between a small population of light-sensitive, non-photopic retinal ganglion cells in the eye and serotonergic neurons within the DRN and there are also pronounced serotonergic reciprocal connections between the DRN and MRN (Ren et al., 2013; Vertes et al., 2008). The MRN provides the majority of serotonergic input to the suprachiasmatic nucleus (SCN) of the hypothalamus, the “circadian pacemaker” of the brain and also receives input from this region, indirectly, via the dorsomedial nucleus of the hypothalamus (Deurveilher et al., 2008; Vertes et al., 2008). The DRN projects indirectly to the SCN via the intergeniculate leaflet and receives input from the SCN via the medial preoptic area (Deurveilher et al., 2008; Vertes et al., 2008). As previously stated, the DRN and MRN project to both cortical and subcortical regions within the brain and also areas of the brainstem (Vertes et al., 2008). Thus, this complex signaling pathway between the retina, serotonergic neurons within raphe nuclei of the midbrain, SCN and efferent projection regions may be one means by which photoperiodic information from the environmental is transduced into a biological signal so that circadian rhythmicity in the CNS and periphery is entrained to the external light-dark cycle (Figure 1-1).

Figure 1-1: A schematic summary of the reciprocal connections between the suprachiasmatic nucleus (SCN) and the midbrain dorsal raphe nucleus (DRN) and median raphe nucleus (MRN). The SCN sends efferent projections (solid lines) to the DRN and MRN via putative relays in the medial preoptic area (MPA) and dorsal medial hypothalamus (DMH), respectively; the sub-paraventricular zone (SPVZ) may also serve as an intermediary via its projections to the DMH. In turn, the SCN receives afferent projections (broken lines) directly from serotonergic neurons in the MRN, and indirectly from serotonergic neurons in the DRN via the thalamic intergeniculate leaflet (IGL). The DRN and MRN are also reciprocally connected (solid arrows). [Modified from Deurveilher et al., 2008]
1.4.2 Serotonin Biosynthesis, Neurotransmission, Recycling and Degradation

5-HT is an indolamine monoamine neurotransmitter synthesized, in the CNS, within presynaptic serotonergic neurons with the superior raphe nuclei from its amino acid precursor L-tryptophan in a three step biosynthesis pathway (Mohammad-Zadeh et al., 2008). As a first step in this pathway, L-tryptophan is oxidized by tryptophan hydroxylase 2 (TPH2) to form 5-hydroxy-L-tryptophan (5-HTP) and this is the rate limiting step of 5-HT biosynthesis (Mohammad-Zadeh et al., 2008). Subsequently, 5-HTP is decarboxylated by aromatic amino acid decarboxylase (AADC) to form 5-hydroxytryptamine (serotonin; 5-HT) (Figure 1-2). 5-HT is then packaged into presynaptic vesicles for eventual release from synaptic terminals and also at somatodendritic sites (Dankoski et al., 2013).

Figure 1-2: The 5-HT Biosynthesis Pathway
Serotonin (5-HT) is synthesized from its amino acid precursor, L-tryptophan in a three step biosynthesis pathway. (i) L-Tryptophan is first oxidized by tryptophan hydroxylase 2 (TPH2) into its metabolic intermediate 5-hydroxy-L-tryptophan (5-HTP). (ii) 5-HTP is then decarboxylated by aromatic amino acid decarboxylase (AADC) to form 5-hydroxytryptamine (5-HT)
[Modified from Druce M et al., 2009]
After release into the synaptic cleft, this neurotransmitter interacts with both presynaptic and postsynaptic 5-HT receptors to initiate downstream effects. There are seven classes and fourteen subtypes of 5-HT receptors, all of which are G-protein coupled receptors, excepting the 5-HT₃R which is a cation-selective ion-gated ligand channel (Hannon et al., 2008). 5-HT binding to a G-protein coupled 5-HT receptor activates second messenger cascades that produce either excitatory or inhibitory effects within the CNS (Hannon et al., 2008). Some 5-HT receptors, such as the 5-HT₁D receptor, are located predominantly on presynaptic neurons and act as autoreceptors regulating serotonergic neurotransmission via negative feedback (Hannon et al., 2008). Other 5-HT receptors such as the 5-HT₂A receptor and 5-HT₂C receptor mediate the postsynaptic downstream effects of 5-HT such as regulation of mood, sleep, appetite, locomotion and cognitive function (Hannon et al., 2008). Lastly, 5-HT receptors, such as the 5-HT₁A receptor or 5-HT₁B receptor, can act as either autoreceptors on presynaptic neurons or postsynaptic receptors depending upon their regional location with the brain (Hannon et al., 2008). Regional distribution of 5-HT receptor subtypes also differs within the brain. For instance, the 5-HT₁B receptor and 5-HT₄ receptor are most abundant within subcortical regions, such as the striatum, thalamus and brainstem, whereas density of the 5-HT₁A receptor and 5-HT₂A receptor are more heavily concentrated within cortical and limbic regions such as the hippocampus, amygdala and isocortex (Varnäs et al., 2004).

Serotonergic neurotransmission is terminated via re-uptake of 5-HT by the serotonin transporter (5-HTT) into the presynaptic neuron upon which is either broken down by monoamine oxidase A (MAO-A) to produce 5-hydroxyindoleacetic acid (5-HIAA) or recycled via repackaging into presynaptic vesicles (Mohammad-Zadeh et al., 2008; Youdim et al., 2004). Thus, there is an inverse relationship between available 5-
HTT and clearance of extracellular 5-HT. For example: selective serotonin reuptake inhibitors (SSRIs), which block the 5-HTT, elevate levels of extracellular 5-HT, 5-HTT knockout mice have greater extracellular 5-HT levels and mice overexpressing 5-HTT have low extracellular 5-HT levels (Bel et al., 1992, 1993; Jennings et al., 2006; Mathews et al., 2004; Shen et al., 2004).

1.4.3 The Serotonin Transporter (5-HTT)

The human 5-HTT is encoded by the SLC6A4 (solute carrier protein family 6 membrane 4) gene which spans approximately 40 kilobases of DNA, is localized on chromosome 17, centered at 17q11.2 and comprised of 14 exons (Murphy et al., 2008) (Figure 1-3). The membrane-bound 5-HTT protein is comprised of approximately 630 amino acids with 12 transmembrane spanning helices, an extracellular region comprised of three extracellular loops (extracellular loops 2, 4 and 6) and intracellular amino and carboxy-terminal tails (Coleman et al., 2016; Murphy et al., 2008) (Figures 1-4 and 1-5).

Figure 1-3: Organization of the human serotonin transporter gene (SLC6A4). The human SLC6A4 maps to chromosome 17q11.2 and is composed of 14 exons that span ~40 kb. Functional variants that modulate transcriptional activity include variation in the length of the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR), single nucleotide polymorphisms, rs25531 and rs25532, located upstream of the transcriptional start site and variable number of tandem repeats (VNTR) at intron 2. [Modified from Murphy et al., 2008]
The 5-HTT is a monoamine transporter protein and a member of the neurotransmitter sodium symporter (NSS) family, which also includes the dopamine and norepinephrine transporters (DAT and NET, respectively) (Coleman et al., 2016). The major physiological role of 5-HTT is the transport of 5-HT from the synaptic cleft into the presynaptic neuron and thus, the 5-HTT has a key role in regulating magnitude, duration and termination of serotonergic neurotransmission (Mohammad-Zadeh et al., 2008). Reuptake of 5-HT by the 5-HTT is coupled with co-transport of sodium (Na\(^+\)), chloride (Cl\(^-\)) and potassium (K\(^+\)) ions into the cell. Na\(^+\) and Cl\(^-\) concentrations are higher outside the cell, than intracellularly, whereas the concentration of K\(^+\) is greater inside relative to outside the cell (Murphy et al., 2004). This ion concentration gradient is generated by the membrane-bound sodium-potassium pump (Na\(^+\)/K\(^+\) ATPase) and this ATPase is necessary for transporter-mediated monoamine reuptake (Murphy et al., 2004).

Figure 1-4: A schematic of the membrane-bound 5-HTT protein. The 5-HTT is comprised of 630 amino acids with 12 transmembrane spanning helices, an extracellular region comprised of three extracellular loops (extracellular loops 2, 4 and 6) and intracellular amino and carboxy-terminal tails [Modified from Murphy et al., 2008]
In regards to the mechanism of 5-HT reuptake, a single multifunctional binding site on the extracellular surface of the 5-HTT allows for movement of Na\(^+\), Cl\(^-\), K\(^+\) ions and 5-HT across the cell membrane (Murphy et al., 2004). Initially, a 5-HT molecule, a Na\(^+\) and Cl\(^-\) ion bind simultaneously to this exterior binding site. Subsequent conformational change impedes access of other molecules/ions to this binding site and transports both 5-HT, Na\(^+\) and Cl\(^-\) ions to the cytoplasmic side of the membrane (Murphy et al., 2004). 5-HT, Na\(^+\) and Cl\(^-\) then disassociate from the 5-HTT and one intracellular K\(^+\) ion is transported to the extracellular side of the membrane to restore the 5-HTT to its active conformation (Murphy et al., 2004). Na\(^+\)/K\(^+\) ATPases maintain this ion gradient to allow for continued function of the 5-HTT (Murphy et al., 2004).

**Figure 1-5: Architecture of Human 5-HTT as visualized by X-ray crystallography.** The protein is viewed parallel to the membrane. The (S)-citalopram molecules denoting central and allosteric site are shown as sticks in dark green and cyan, respectively. Sodium ions are shown as spheres in salmon. [Modified from Coleman et al., 2016]
Most 5-HTT are located at outer cell membranes, primarily perisynaptically and along axons (axolemma) (Zhou et al., 1998) (Figures 1-6 and 1-7). Diffusion of 5-HT away from serotonergic presynaptic terminals to distances of up to 20μm have been reported (Zhou et al., 1998). Accordingly, perisynaptic 5-HTT may modulate reuptake of 5-HT near synapses, whereas axonal 5-HTT may facilitate termination of serotonergic neurotransmission at more distal locations (Zhou et al., 1998). In the human brain, 5-HTT density varies by region and is highest in the raphe nuclei, elevated in subcortical regions (i.e. thalamus and basal ganglia) and lower in density in limbic and cortical regions (i.e. hippocampus, ACC, PFC). The cerebellar cortex (excepting the vermis), is nearly devoid of 5-HTT thus, in regards to quantification of the 5-HTT via PET imaging, this brain area is an excellent reference region (Backstrom et al., 1989; Cortes et al., 1988; Kish et al., 2005; Laruelle et al., 1988).

Figure 1-6: A scanning electron micrograph immunostained for the 5-HTT. A thin section of an asymmetric synapse can be seen along a 5-HTT-positive axon. Small arrows indicate the post-synaptic density and the portion of presynaptic membrane opposed to the synaptic cleft is devoid of 5-HTT. The perisynaptic area adjacent to the presynaptic membrane is distinctly stained (circled in red), indicating the presence of 5-HTT immunograin [Modified from Zhou et al., 1998]
Figure 1-7: A scanning electron micrograph immunostained for the 5-HTT of proximal axon bundles near the raphe nucleus. As can be seen, 5-HTT immunograins are located on the along the axolemma (i.e. along the length of the axon) (circled in red) and not within in the axoplasm. [Modified from Zhou et al., 1998]

1.5. Seasonality and the Serotonin System

Of the monoamine neurotransmitter systems (i.e. serotonin, dopamine, norepinephrine), there is most evidence of seasonal rhythmicity within the serotonin system. In regards to preclinical investigation, in rodents, a shortened photoperiod (i.e. to mimic shortened “winter-like” day length) has been found to decrease 5-HT release and increase both 5-HTT density and clearance of 5-HT in the hypothalamus and SCN of the hypothalamus (Blier et al., 1989; Rovescalli et al., 1989). In post-mortem study, Carlsson and colleagues, reported seasonal variation in 5-HT levels in the human hypothalamus with lower levels in late winter and higher levels in late summer (Carlsson et al., 1980). Lambert et al. also observed seasonal variation in whole brain 5-HT turnover in a sample of one hundred and one healthy males using jugular vein catheterization and found that the rate of 5-HT production was correlated with duration of daily sunshine (Lambert et al., 2002). Using this technique, it is possible to quantify overflow of 5-HT and its metabolites from the brain into jugular venous effluent, thus providing an indirect measure of 5-HT turnover in the brain (Lambert et al., 2002). More recently, in a neuroimaging study involving thirty-six healthy volunteers,
Spindelegger et al., used [carbonyl-$^{11}$C]WAY-100635 PET to examine the relationship between 5-HT$_{1A}$ receptor binding, an index of 5-HT$_{1A}$ receptor levels, season and light exposure in both cortical and subcortical limbic regions. A positive correlation was observed between post-synaptic 5-HT$_{1A}$ receptor binding and duration of daily sunlight, with greater binding observed in spring-summer relative to fall-winter (Spindelegger et al., 2012).

As the 5-HTT has a key role in regulating magnitude, duration, and termination of serotonergic neurotransmission, the majority of neuroimaging studies investigating seasonal fluctuation in the serotonin system have focused on this transporter protein. To date, there have been four cross-sectional neuroimaging studies of high to moderate quality to support evidence of seasonal variation in 5-HTT BP$_{ND}$ (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). In a study of eighty-eight healthy volunteers in Toronto, Canada, [$^{11}$C]DASB PET was used to assess seasonal change in 5-HTT BP$_{ND}$ and a marked elevation in 5-HTT BP$_{ND}$ was observed in winter relative to summer in a number of affect-modulating brain regions including the ACC and PFC, in addition to the basal ganglia, thalamus and midbrain (Praschak-Rieder et al., 2008) (Figure 1-8).
Kalbitzer et al., subsequently replicated this finding in Copenhagen, Denmark, also applying $[^{11}\text{C}]$DASB PET in a study of fifty-four subjects, reporting seasonal variation in 5-HTT BP$_{ND}$ in the caudate and putamen (Kalbitzer et al., 2010). Interestingly, in both studies, an inverse relationship was found between duration of daily sunshine and 5-HTT BP$_{ND}$, suggesting that light exposure may be one external environmental factor that may facilitate this seasonal change in 5-HTT BP$_{ND}$ (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008) (Table 1-1). Other studies applying different and lesser quality techniques and radioligands have also shown similar results (i.e. 5-HTT BP$_{ND}$ using $[^{123}\text{I}]{\beta}$-CIT SPECT is quantifiable only in the midbrain whereas 5-HTT BP$_{ND}$ cannot be measured in cortical regions using $[^{11}\text{C}]$McN5652 PET) (Brücke et al., 1993; Ikoma et al., 2002; Parsey et al., 2000). Nonetheless, in Amsterdam, Netherlands, Ruhé et al., applied $[^{123}\text{I}]{\beta}$-CIT SPECT to examine the relationship between season and 5-HTT BP$_{ND}$ in forty-nine healthy and forty-nine depressed subjects and observed elevated 5-HTT BP$_{ND}$ in winter relative to summer in the
midbrain (Ruhe et al., 2009). In Hamburg, Germany, Buchert et al., used $[^{11}\text{C}]$McN5652 PET in thirty-nine healthy participants and also reported the season-related finding in the midbrain, but not in the thalamus (Buchert et al., 2006). Collectively, these findings from four independent research groups in four different countries indicate that 5-HTT BP$_{ND}$ is higher across multiple brain regions in winter relative to summer (Table 1-2).

![Table 1-1: Negative correlations between average sunshine duration and day length in Toronto, Ontario, Canada and brain 5-HTT BP$_{ND}$ in 88 healthy study participants. [Modified from Praschak-Rieder et al., 2008]](attachment:table1-1)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Duration of Sunshine $^a$</th>
<th>Day Length $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anteromedial prefrontal cortex</td>
<td>$-0.21$</td>
<td>$-0.20$</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>$-0.21$</td>
<td>$-0.21$</td>
</tr>
<tr>
<td>Caudate</td>
<td>$-0.26$</td>
<td>$-0.29$</td>
</tr>
<tr>
<td>Putamen</td>
<td>$-0.23$</td>
<td>$-0.26$</td>
</tr>
<tr>
<td>Thalamus</td>
<td>$-0.21$</td>
<td>$-0.21$</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>$-0.39$</td>
<td>$-0.38$</td>
</tr>
</tbody>
</table>

$^a$No significant correlation was found between age and duration of sunlight ($r = 0.06, P = .60$) or between age and day length ($r = 0.01, P = .91$). $^b$Spearman rank sum correlation coefficient.

Table 1-2: Seasonal fluctuation in 5-HTT binding as replicated by four independent research groups; $^a$applicable to only to midbrain regions; $^b$5-HTT binding not measurable in cortex

In conclusion, there is evidence from both preclinical, post-mortem, clinical and neuroimaging studies in support of seasonal variation within the serotonin system. However, the majority of these studies focused upon investigation of this
monoaminergic system in health. In order to examine the effect of seasonal fluctuation of the serotonin system in relation to seasonal changes in affect and behaviour, it is necessary to study a clinical population of individuals who experience marked seasonal variation in mood, such as those with SAD.

1.6 Seasonal Affective Disorder

1.6.1 Definition

SAD is a subtype of MDD, characterized by a pattern of MDEs that occur seasonally as part of an existing mood disorder (American Psychiatric Association, 2013). A key criterion is a consistent pattern of onset and remission at characteristic times of the year, which is not better explained by seasonally linked psychosocial stressors (American Psychiatric Association, 2013). In addition, this seasonal pattern of MDE recurrence and remission must persist for a minimum of two consecutive years, during which there is no occurrence of non-seasonal MDEs (American Psychiatric Association, 2013). Lastly, the lifetime number of seasonal MDEs must be greater than the number of non-seasonal MDEs (American Psychiatric Association, 2013). This seasonal pattern of MDEs commonly occurs in fall-winter, with full remission in spring-summer (i.e. “winter SAD”) (Levitan, 2007; Rosenthal et al., 1984). Winter MDEs must include low mood or anhedonia, but are also often accompanied by atypical depressive symptoms such as decreased energy, hypersomnia and increased appetite or weight gain (Rosenthal et al., 1984).
1.6.2 Neuropathophysiology of SAD

There have been several lines of investigation in regards to identifying biological markers associated with the SAD phenotype. Lavoie and colleagues examined SAD patients using flash electroretinography and found decreased rod sensitivity to light in winter relative to summer (Lavoie et al., 2009). Seasonal variation in nocturnal melatonin secretion has also been reported such that, in SAD as compared to health, duration the secretion is longer in winter than in summer, indicating that circadian rhythm dysregulation may play a role in the pathology of this disorder (Wehr et al., 2001). There is also evidence of circadian rhythm delay in seasonal depression, such that intrinsic melatonin and temperature rhythms are misaligned with external light-dark cycle (Lewy et al., 1988). Genetic markers identified in SAD include altered polymorphic frequencies in the clock genes NPAS and PER3 and a variable number tandem repeat on exon 3 of the DRD4 gene (Johansson et al., 2003; Levitan et al., 2006). However, to date, a major body of work has focused upon the role of monoamine neurotransmitter systems (i.e. serotonin, dopamine, and norepinephrine) in SAD pathophysiology (Levitan, 2007). Of note, there has been particular emphasis on the serotonin system given evidence of its seasonal rhythmicity and the importance of 5-HT in regulating behaviours and affective states that change with season such as mood, sleep and appetite.

1.6.2.1 Role of Serotonin Physiology in Seasonal Behaviour

It is well established that 5-HT plays a role in physiology and behaviours related to mood, sleep and appetite. As such, this section will provide an overview of
research regarding the importance of 5-HT in regards to the modulation of affective and behavioural states that change seasonally in SAD.

Results of clinical studies indicate that pharmacological manipulations that affect function of the serotonin system and/or extracellular 5-HT levels have a profound impact on mood and also in SAD. Depletion of tryptophan, the amino acid precursor of 5-HT, has been estimated to reduce 5-HT levels by approximately 80 percent and this decrease in 5-HT is associated with low mood (Young et al., 1985). Accordingly, reduction of extracellular 5-HT via acute tryptophan depletion (ATD) is accompanied by the return of depressive symptoms in remitted MDD patients following antidepressant treatment and relapse into MDE in SAD patients both after light therapy in winter and during summer remission (Delgado et al., 1990; Neumeister et al., 1997; Neumeister et al., 1998). Conversely, elevation of extracellular 5-HT levels via administration of intravenous $d$-fenfluramine, a medication that causes 5-HT release, is followed by happier mood and increased energy in healthy individuals, and a reversal of depressive symptoms in SAD (Meyer et al., 1996; O'Rourke et al., 1989). Administration of the non-selective 5-HT receptor agonist, m-CPP, to individuals with SAD during winter, has been associated with reports of increased energy and euphoria (“activation-euphoria”), and also blunted norepinephrine, cortisol and prolactin response, suggestive of either a season-dependent downregulation or a dysfunction of postsynaptic 5-HT receptors (Levitan et al., 1998; Schwartz et al., 1997). Lastly, both tryptophan supplementation and use of SSRIs, treatments which raise levels of extracellular 5-HT, are effective in ameliorating symptoms of seasonal depression during winter (Lam et al., 1997; Lam et al., 2006). Thus, there is
substantial evidence to support hypofunction of serotonin system both in regards to depressed mood and in SAD.

Sleep disturbances are a common in SAD with approximately 80 percent of patients reporting symptoms of hypersomnia during winter MDEs (Kaplan et al., 2009). As such, antidepressants that inhibit 5-HT reuptake and increase levels of extracellular 5-HT can help to alleviate excessive sleepiness during winter in SAD and these medications are also sometimes associated with the side-effect of transient insomnia (Aszalos, 2006; Wilson et al., 2005). In addition, there is strong support from preclinical animal studies of a relationship between the activity of 5-HT releasing neurons in the DRN and the state of the sleep-wake cycle (Jacobs et al., 1999). To elaborate, relative to the awake state, firing of 5-HT releasing neurons decreases during slow wave sleep and is virtually absent during rapid eye movement sleep (Jacobs et al., 1999; McGinty et al., 1976). Accordingly, during slow wave and rapid eye movement sleep, respectively, extracellular 5-HT levels have been observed to decrease by 50 percent and 38 percent, respectively, in the DRN of cats and by 58 percent and 37 percent, respectively, in the frontal cortex of rats (Portas et al., 1998; Portas et al., 1994). In summary, hypersomnia is a hallmark of the symptomatic phase of SAD, medications that elevate 5-HT are helpful in alleviating excessive sleepiness, and extracellular 5-HT levels in both the DRN and efferent projection regions are altered in a consistent manner during sleep relative to waking, suggestive of a neuromodulatory role for 5-HT in the sleep-wake cycle.

Hyperphagia and carbohydrate cravings are core features of SAD (Rosenthal et al., 1984). Appetite control is mediated through serotonergic neuromodulation at the paraventricular nucleus of the medial hypothalamus (Blundell, 1984; Fletcher et al.,
1989) and it has been found that ingestion of high carbohydrate meals increases uptake of tryptophan from the blood into the brain thereby elevating brain 5-HT levels (Fernstrom, 1986). Interestingly, individuals with SAD experience seasonal alterations in the ability to detect “sweet taste” such that this taste sensitivity is blunted in winter and normalizes during the summer months, a variation that is not observed in healthy volunteers (Andersen et al., 2014; Arbisi et al., 1996). As such, it has been postulated that, in SAD, seasonal depressive symptoms of increased appetite and carbohydrate cravings are attributable to hypofunction of the serotonin system during winter and that such behaviours may reflect a compensatory mechanism to elevate low levels of 5-HT via excessive carbohydrates (i.e. sugary and starchy foods) (Andersen et al., 2014; Arbisi et al., 1996).

It is also important to note that there strong evidence of an anorexiogenic role for 5-HT in regards to appetite regulation; thus, low levels of extracellular 5-HT would be expected to induce symptoms of hyperphagia and carbohydrate craving, as commonly seen in the symptomatic phase of SAD, whereas pharmacological manipulations that elevate extracellular 5-HT would likely reduce appetite (Fernstrom, 1985). Much research regarding 5-HT and appetite stems from both preclinical and clinical studies of appetite regulation with a primary focus on treatment of obesity, but as individuals with SAD commonly suffer from bingeing and significant weight gain during the winter, symptoms which profoundly impact individual health and well-being, the relationship between 5-HT and food intake is an important topic for discussion (Rintamaki et al., 2008; Wurtman, 1993). Accordingly, in rodents, injection of 5-HT into the paraventricular nucleus of the medial hypothalamus has been found to decrease food intake and administration of \(d\)-fenfluramine has been shown to reduce both body
weight and food intake (Blundell, 1984; Fletcher et al., 1989). This latter finding regarding d-fenfluramine has been corroborated clinically in two double-blind studies of obese volunteers in which chronic use of this medication was significantly reduced food intake and body weight relative to placebo (Drent et al., 1995; Guy-Grand et al., 1989). Other supplements and medications that affect the serotonin system or raise levels of extracellular 5-HT have also been found to suppress appetite (Halford et al., 2007). For instance, in a double-blind placebo-controlled trial, twenty obese participants treated for six weeks with 5-HTP experienced a reduction in body weight, carbohydrate craving and earlier satiety as compared to volunteers receiving placebo treatment (Cangiano et al., 1992). There also some evidence that treatment with m-CPP, a medication that causes “activation-euphoria” in SAD during winter, reduces both appetite and body weight in obese patients and in those with SAD (Levitan et al., 1998; Sargent et al., 1997; Schwartz et al., 1997). As m-CPP has relatively high affinity for post-synaptic 5-HT2C receptors that are primarily involved in regulation of appetite and food intake, these findings suggest that agonism of this receptor subtype may induce hypophagia and weight loss (Kennett et al., 1997; Sargent et al., 1997). Lastly, appetite can be reduced by SSRIs, such as fluoxetine, that are commonly used to treat winter MDEs in SAD and raise levels of extracellular 5-HT (Goldstein et al., 1995; Lam et al., 1995; Pijl et al., 1991). To conclude, symptoms of hyperphagia, carbohydrate craving and weight gain, commonly observed in patients with SAD during winter, may be compensatory behaviours to offset hypofunction of the 5-HT system and elevate low levels of 5-HT. Accordingly, medications that raise levels of extracellular 5-HT or mimic the actions of this neurotransmitter are effective in
reducing appetite and inducing weight loss in individuals experiencing such symptoms.

Taken together, these findings suggest that processes that influence extracellular 5-HT levels across seasons are highly promising mechanisms to explain seasonal variation in symptoms commonly observed in SAD, such as low mood, hypersomnia, hyperphagia, carbohydrate craving and weight gain.

1.7 Light Therapy

In 1984, Rosenthal and colleagues published a seminal paper describing SAD as a syndrome and reporting the efficacy of the light therapy, a chronotherapeutic treatment, in mitigating the symptoms of seasonal depression (Rosenthal et al., 1984). Seasonal variation in day-length (i.e. photoperiod) had been observed at latitudes where SAD was particularly prevalent, and it had been hypothesized that the symptoms of seasonal depression might be an outcome of shortened photoperiod in winter (Levitan, 2007; Rosen et al., 1990). Accordingly, it was postulated that extension of day-length during the winter months might ameliorate seasonal depressive symptoms and that exposure to daily bright light might be a means by which to lengthen shortened photoperiod during this season (Levitan, 2007). In a double-blind, placebo-controlled, cross-over design, Rosenthal et al., randomized patients with SAD into either bright light (2,500 lux) or dim light conditions (100 lux), during which participants were asked to sit in front of a fluorescent lamp daily, for three hours upon waking and before sleeping, for a period of two weeks (Rosenthal et al., 1984). A robust antidepressant effect of bright light was observed after two weeks.
of treatment whereas little or no therapeutic effect was found following exposure to the alternative dim light condition (Rosenthal et al., 1984) (Figure 1-9).

![Graph showing effect of light on mood in seasonal depression. Bright white light had significant antidepressant effects (t=8.45, p<.001, two-tailed paired t-test, Bonferroni intervals). There was no significant effect with dim light.](Image)

**Figure 1-9:** Effect of light on mood in seasonal depression. Bright white light had significant antidepressant effects (t=8.45, p<.001, two-tailed paired t-test, Bonferroni intervals). There was no significant effect with dim light. [Modified from Rosenthal et al., 1984]

However, the lengthy duration of such a daily treatment regime required to achieve therapeutic response necessitated investigating the feasibility of a briefer more intense period of light exposure as compared to this initial treatment protocol. As such, Terman and colleagues investigated the efficacy of thirty minutes of light therapy at an intensity 10,000 lux and found its antidepressant effects to be comparable to two hours of light therapy at an intensity of 2,500 lux (Terman et al., 1990). Moreover, light therapy in the early morning was observed to be superior to
evening administration, with reported remission rates of 54.3 percent relative to 33.3 percent respectively (Terman et al., 1990; Terman et al., 1998).

In further support of its therapeutic effects, randomized controlled trials comparing light therapy to antidepressant treatment found that its efficacy was comparable to that of SSRIs with overall remission rates of approximately 55 percent (Lam et al., 2006; Ruhrmann et al., 1998). Interestingly, the positive effects of light therapy occurred within one to two weeks whereas antidepressant response typically required two to four weeks of treatment (Lam et al., 2006; Ruhrmann et al., 1998).

At present, light therapy is regarded as a first-line treatment for SAD and clinical consensus guidelines for treatment of seasonal depression recommend daily
use of a fluorescent lamp emitting full-spectrum white light fluorescent white light at a “dose” of 10,000 lux for thirty minutes each morning (Lam et al., 1999). It is also recommended that the height of the lamp be adjusted such that individual's eyes are level with the centre of the screen and angle of the lamp is tilted approximately 15° downward. This lamp angle and level of light exposure are considered optimal for amelioration of seasonal depressive symptoms as clinical trials using this protocol have reported positive results (Lam et al., 2006; Levitan, 2005; Terman et al., 1998). However, as 45 percent of SAD cases fail to achieve remission following a course of light therapy, it is important to develop strategies to further improve this treatment (Lam et al., 2006).

Despite evidence of the seasonal rhythmicity of the serotonin system, the importance of 5-HT in regulation of mood and behavioural states that change with season and evidence of aberrant function of the serotonin system in SAD, there have been few studies evaluating the effect of deliberate light exposure on the serotonin system. Some theories as to the mechanism by which light therapy exerts its antidepressant effects include circadian phase advance, phase shift of endogenous melatonin rhythm and enhancing resilience against tryptophan depletion (Lam et al., 1996b; Lewy et al., 1987; Neumeister et al., 1997; Terman et al., 2001). There is some evidence, albeit from small clinical studies, suggestive of the involvement of serotonin system in therapeutic response to light in SAD. For example, tryptophan supplementation has been reported to augment the antidepressant effects of light therapy, and therapeutic response following combination treatment with light therapy and citalopram, an SSRI, has been found to be superior to light therapy alone (Lam et al., 1997; Thorell et al., 1999). Interestingly, in rodents, an inverse relationship
between light exposure and 5-HTT density has been observed in the hypothalamus and SCN of the hypothalamus (Rovescalli et al., 1989). Furthermore, in PET studies of healthy volunteers, an inverse relationship between duration of daily sunlight and 5-HTT BP_{ND} has also been observed, suggesting this transporter protein may be sensitive to light exposure and thus a potential brain biomarker by which to assess the effects of light therapy (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008).

1.8 Summary of Hypotheses

**Study 1: The Effect of Season on 5-HTT BP_{ND}**

**Objective:** To determine whether magnitude of seasonal variation in 5-HTT BP_{ND}, an index of 5-HTT levels, in the PFC and ACC is greater in SAD as compared to health.

Hyp [1] The magnitude of seasonal fluctuation in 5-HTT BP_{ND} in the PFC and ACC will be significantly greater in SAD relative to health.

Hyp [2] Seasonal variation in 5-HTT BP_{ND} in the PFC and ACC will be associated with degree of seasonal change in mood and behaviour

**Study 2: The Effect of Light Therapy on 5-HTT BP_{ND} in Health**

**Objective:** To evaluate the effect of light therapy on 5-HTT BP_{ND}, an index of 5-HTT levels, in the ACC and PFC of healthy individuals during fall and winter.

Hyp [3] In fall and winter, 5-HTT BP_{ND} will be significantly reduced in the ACC and PFC after light therapy as compared to placebo treatment in healthy volunteers.

**Study 3: Effect of Light Therapy on 5-HTT BP_{ND} in SAD**
**Objective:** To evaluate the effects of light therapy on 5-HTT BP_{ND}, an index of 5-HTT levels, in the PFC and ACC of SAD subjects during the winter.

**Hyp [4]** In SAD, light therapy will be associated with reduced 5-HTT BP_{ND} in the ACC and PFC in winter as compared to baseline (i.e. prior to start of treatment).

**Note:** In study 3, as we chose to examine the effect of light therapy on 5-HTT BP_{ND} in a sample SAD of patients with fairly severe seasonal depressive symptoms, a study design including a placebo control group, as in study 2, was not feasible. In such a clinical situation, in which an evidence based first-line therapeutic is available, the ethical standard at CAMH is that a placebo should not be given.

In regards to all three studies, the ACC and PFC were prioritized since structures within these regions have a key role in mood regulation and cognitive processing of emotion (Liotti et al., 2001; Liotti et al., 2002; Mayberg et al., 1999; Sharot et al., 2007; Tom et al., 2007). *Our secondary hypotheses are that similar changes in 5-HTT BP_{ND} will occur in other brain regions assayed, including the hippocampus, ventral striatum, dorsal putamen, dorsal caudate, thalamus and midbrain.*
MATERIALS AND METHODS

2.1 Rationale For Use Of [$^{11}$C]DASB Positron Emission Tomography

At present, [$^{11}$C]DASB (11C-labeled 3-amino-4-[2-dimethylaminomethyl-phenylsulfanyl]benzonitrile) is the preferred radioligand for neuroimaging of the 5-HTT. It is a high quality radioligand with several useful properties including selectivity for the 5-HTT, excellent brain uptake, reversible kinetics, negligible sensitivity to endogenous 5-HT, a high ratio of specific to free/non-specific binding allowing for its quantification in multiple brain regions and its reliability is well established (Ginovart et al., 2001; Houle et al., 2000; Wilson et al., 2002; Wilson et al., 2000). In addition, binding of DASB to the 5-HTT, in vitro, has been observed to be saturable only in environments mimicking extracellular conditions and not intracellularly (Quelch et al., 2012). Accordingly, this result suggests that changes in 5-HTT BP$_{ND}$ observed in vivo using [$^{11}$C]DASB PET may largely represent fluctuations in levels of 5-HTT protein on the cell surface (i.e. extracellular environment) and not within the cell. Furthermore, the major role of the 5-HTT is its ability to transport extracellular 5-HT from the synaptic cleft into the presynaptic neuron and this functionality is dependent upon its embedding in the cell membrane. As such, it would be expected that fluctuations in 5-HTT BP$_{ND}$ observed in vivo using [$^{11}$C]DASB PET would correlate to alterations in extracellular 5-HT reuptake.

There are also a number of other radioligands which have been used for 5-HTT neuroimaging including β[$^{123}$I]CIT, [$^{11}$C]McN5652, [$^{123}$I]ADAM, [$^{11}$C]MADAM and [$^{11}$C]HOMADAM. These radioligands have been applied for several years, and have modeling, displacement studies, and reliability data in humans. Their properties will be
summarized briefly and are outlined in further detail in the table below (Table 2-1) (Meyer, 2014).

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>Nonselective—near 1:1 affinity for 5-HTT to DAT (Lamelle et al., 1994; Carroll et al., 1995)</td>
<td>Likely selective 10:1 to 100:1 affinity for 5-HTT over NET (Shank et al., 1988; King et al., 1998)</td>
<td>Highly selective 1000:1 affinity for 5-HTT over NET or DAT; negligible affinity for many other targets (Wilson et al., 2000, 2002)</td>
<td>Highly selective 1000:1 affinity for 5-HTT over NET or DAT (Choi et al., 2000; Oya et al., 2000)</td>
<td>Highly selective (Hallidin et al., 2005; Chalon et al., 2003)</td>
<td>Highly selective (Jarkas et al., 2005)</td>
</tr>
<tr>
<td>Displaceability of specific binding</td>
<td>Incomplete (Pirker et al., 1995; Tanscher et al., 1999)</td>
<td>In most reports (Kent et al., 2002; Parsey et al., 2000; Suhara et al., 2003)</td>
<td>Highly displaceable (Meyer et al., 2001, 2004a, b; Wilson et al., 2000, 2002)</td>
<td>Highly displaceable (Choi et al., 2000; Oya et al., 2000; Erlandsson et al., 2005)</td>
<td>Highly displaceable (Hallidin et al., 2005; Chalon et al., 2003; Lundberg et al., 2007)</td>
</tr>
<tr>
<td>Reversibility (in human imaging)</td>
<td>Good (Kuikka et al., 1993; Brücke et al., 1993)</td>
<td>Not adequate to adequate, depending upon region (Back et al., 2000; Parsey et al., 2000; Ikoma et al., 2002)</td>
<td>Adequate in midbrain, good to very good in other regions (Ginovart et al., 2001; Ichise et al., 2003; Houle et al., 2000)</td>
<td>Adequate in midbrain, good to very good in other regions (Erlandsson et al., 2005; Catafau et al., 2005)</td>
<td>Adequate in midbrain, good to very good in other regions</td>
</tr>
<tr>
<td>Brain uptake</td>
<td>Adequate (Kuikka et al., 1993; Brücke et al., 1993)</td>
<td>Good (Parsey et al., 2000; Ikoma et al., 2002)</td>
<td>Very good (Ginovart et al., 2001; Ichise et al., 2003; Houle et al., 2000)</td>
<td>Adequate (Erlandsson et al., 2005; Catafau et al., 2005)</td>
<td>Good (Lundberg et al., 2006)</td>
</tr>
<tr>
<td>Sensitivity to endogenous serotonin</td>
<td>Not known</td>
<td>Not known</td>
<td>Negligible (Talbot et al., 2005; Praschak-Rieder et al., 2005)</td>
<td>Not known</td>
<td>Not known</td>
</tr>
<tr>
<td>Specific binding to free and nonspecific binding ratio</td>
<td>Good</td>
<td>Not adequate in most regions; adequate in thalamus (Ikoma et al., 2002; Frankle et al., 2005)</td>
<td>Adequate to very good, depending upon region (Ginovart et al., 2001; Ichise et al., 2003)</td>
<td>Not adequate in most regions; adequate in midbrain (Erlandsson et al., 2005; Catafau et al., 2005)</td>
<td>Adequate to very good (Lundberg et al., 2005, 2006)</td>
</tr>
<tr>
<td>Reliability of 5-HTT BP</td>
<td>Not measured</td>
<td>Modest (Kent et al., 2002)</td>
<td>Very good to excellent (Praschak-Rieder et al., 2005; Meyer et al., 2001)</td>
<td>Most regions reasonable (Catafau et al., 2005)</td>
<td>Good to very good (Lundberg et al., 2006)</td>
</tr>
<tr>
<td>5-HTT BP measurable in multiple regions?</td>
<td>Brain stem only (Kuikka et al., 1993; Brücke et al., 1993)</td>
<td>Measurable in thalamus (Ikoma et al., 2002), not measurable in cortex (Parsey et al., 2000)</td>
<td>Yes (Ginovart et al., 2001; Ichise et al., 2003)</td>
<td>Measurable in midbrain; unclear for other regions (Erlandsson et al., 2005; Catafau et al., 2005)</td>
<td>Yes (Lundberg et al., 2006)</td>
</tr>
</tbody>
</table>

**Table 2-1: Comparison of PET and SPECT Radiotracers for the 5-HTT**

[Modified from Meyer et al., 2014]

The first 5-HTT radioligands were β[123I]CIT and [11C]McN5652, applied using SPECT and PET imaging, respectively, although reliable 5-HTT quantification using both techniques was difficult due to significant methodological constraints (Brücke et al., 1993; Szabo et al., 1995). Measurement of the 5-HTT using β[123I]CIT SPECT is feasible only in the midbrain, a region in which density of both the 5-HTT and the DAT
are high (Brücke et al., 1993). In addition, the β-CIT ligand has near equal affinity for both monoamine transporters and this further complicated quantification of the 5-HTT in the midbrain (Laruelle et al., 1994). In regards to [¹¹C]McN5652, this radioligand has modest reversibility and a low ratio of specific relative to free and non-specific binding, both characteristics which prohibited reliable quantification of 5-HTT in cortical regions (Buck et al., 2000; Ikoma et al., 2002; Parsey et al., 2000).

Later generation radioligands include [¹¹C]DASB, [¹²³I]ADAM, [¹¹C]MADAM and [¹¹C]HOMADAM (Meyer, 2014). The properties of [¹¹C]DASB were described in the first paragraph of this section and are further outlined in the table below for comparison purposes. [¹²³I]ADAM, a SPECT radioligand, has some advantage over β[¹²³I]CIT in regards to its high affinity for the 5-HTT relative to the DAT and NET, but its low specific to free and non-specific binding ratio limits its use to midbrain regions (Catafau et al., 2005; Choi et al., 2000; Erlandsson et al., 2005; Oya et al., 2000). The [¹¹C]MADAM PET radioligand approaches the quality of [¹¹C]DASB, although [¹¹C]DASB ligand has been observed to have better brain uptake and test-retest reliability in comparison to [¹¹C]MADAM (Lundberg et al., 2006). Finally, although there is evidence that the PET radioligand [¹¹C]HOMADAM displays better reversibility and a greater specific to free and non-specific binding ratio relative to [¹¹C]DASB, in vivo investigations of its test-retest reliability and sensitivity to endogenous 5-HT have not been conducted and such studies are necessary for further development of this tracer (Ginovart et al., 2001; Nye et al., 2008). Thus, [¹¹C]DASB was applied to measure 5-HTT BP_{ND} in the following series of studies.

Information regarding PET scan radiation dose for [¹¹C]DASB was included in study consent forms, which also detailed scanning procedure and associated risks.
This information was explained to study participants in accordance with CAMH standard operating procedures, both within the consent forms and in the initial interview by investigators AET or SJH. Dose of $[^{11}C]$DASB administered to each subject was less than 2mSv/scan, well within Health Canada guidelines for a PET study and less than the amount of radiation received from natural sources over one year (3mSv). Delay between scans ranged from 2 weeks (study three) to 6 months (study one). Risk to participants was described as minimal, as such doses and associated scanning intervals have never been associated with adverse effects. Additional questions regarding the scanning procedure were addressed by principal investigator JHM.

2.2 Study 1: The Effect of Season on 5-HTT $BP_{ND}$

2.2.1 Participants

Twenty SAD participants (14 women and 6 men; mean [SD] age: 31.3 [4.8] years; age range: 24-39) and twenty healthy volunteers (13 women and 7 men; mean [SD] age: 30.5 [4.2] years; age range: 24-39) were recruited from the Greater Toronto Area between June 2012 and July 2015. Healthy subjects were age-matched to SAD subjects within 3 years. Demographics are listed in the table below (Table 2-2)

<table>
<thead>
<tr>
<th></th>
<th>SAD (n = 20)</th>
<th>Healthy (n = 20)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>31.3 (4.8)</td>
<td>30.5 (4.2)</td>
<td>t_{28}=0.56 (p=0.58)</td>
</tr>
<tr>
<td>Female to male ratio</td>
<td>14 : 6</td>
<td>13 : 7</td>
<td>χ²(1)=0.11 (p=0.74)</td>
</tr>
<tr>
<td>Body mass index (SD)</td>
<td>24.6 (3.5)</td>
<td>23.2 (2.2)</td>
<td>t_{28}=1.37 (p=0.18)</td>
</tr>
<tr>
<td>Years in climatic area</td>
<td>22.3 (12.2)</td>
<td>22.2 (11.4)</td>
<td>t_{28}=0.013 (p=0.99)</td>
</tr>
<tr>
<td>Age of SAD onset</td>
<td>21.9 (5.4)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>No. of seasonal MDE</td>
<td>9.2 (2.2)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Winter SGIH-SAD (HDSR-29)</td>
<td>26.0 (9.9)</td>
<td>1.56 (1.9)</td>
<td>t_{28}=0.79 (p&lt;0.0000)</td>
</tr>
<tr>
<td>Summer SGIH-SAD (HDSR-29)</td>
<td>2.7 (3.05)</td>
<td>1.3 (1.8)</td>
<td>t_{28}=1.78 (p&lt;0.0000)</td>
</tr>
<tr>
<td>SPAQ (Global Seasonality Score)</td>
<td>16.8 (3.2)</td>
<td>2.1 (1.7)</td>
<td>t_{28}=18.13 (p&lt;0.0000)</td>
</tr>
</tbody>
</table>

*Chi-Square test for association. †No. of seasonal MDEs consistent with reports from the literature (Lam et al. 2006; Modell et al. 2005). ‡Welch's independent samples t-test for unequal variances.

Table 2-2: Demographic Characteristics
Criteria for all included being between the ages of 18 to 40, non-smoking and in good physical health, no history of alcohol or substance abuse, no antidepressant use within the past 6 months and no use of prescription medications or herbal supplements within the past 2 months. In addition, as light exposure has been found to reduce $[^{11}\text{C}]\text{DASB} \ 5\text{-HTT BP}_{\text{ND}}$ during the winter months and $[^{11}\text{C}]\text{DASB} \ 5\text{-HTT BP}_{\text{ND}}$ has been shown to be inversely correlated with duration of daily sunshine, both use of light therapy within the past 3 months and travel to more southern latitudes during the study period were exclusionary (Harrison et al., 2015; Praschak-Rieder et al., 2008). Exclusion criteria for female subjects included use of oral contraceptives, current pregnancy, postpartum or recent abortion (within one year), and in perimenopause or menopause. No female subject tested positive for pregnancy at the time of scanning as indicated by urine dip stick testing for human chorionic gonadotropin (hCG).

Subjects were asked not to take over the counter medications one week prior to scanning, to avoid alcohol 4 days prior to scanning and not to consume caffeinated beverages within 2 days of the PET scan. In addition, lifetime history of Axis I or Axis II disorders was exclusionary for healthy subjects and comorbid Axis I or Axis II disorders were exclusionary for SAD subjects. Screening instruments included the Structured Clinical Interview for DSM-IV-TR (SCID-I/II). Accordingly, all subjects received both SCID-I/II assessments and a consultation with a study psychiatrist to verify either (i) the presence of recurrent MDD with a seasonal pattern specifier (SAD group) or (ii) the absence of lifetime psychiatric illness (healthy volunteers). Urine drug screening was performed at initial assessment and on each PET scanning day to rule out recent drug and medication use. Urine toxicology was performed using gas
Participants also completed the Seasonal Pattern Assessment Questionnaire (SPAQ) from which a summed global seasonality score was calculated to determine degree of seasonality (i.e. seasonal change in sleep, mood, energy, appetite, weight and social activity) (Rosenthal et al., 1987). The SPAQ was administered in accordance with the season in which subjects were scanned. All participants were scanned first in the season during which they were assessed (i.e. winter or summer) and the distribution of subjects scanned initially in summer or winter, did not differ between groups (summer: 10 healthy, 8 SAD; winter: 10 healthy, 12 SAD; \( \chi^2_{(1)}=0.40, p=0.53 \)). Subjects were defined categorically as healthy (with no seasonality) for SPAQ scores below 12, moderate SAD for scores between 12 and 16, and severe SAD for scores equal to or greater than 16 (Bartko et al., 1989; Terman, 1988). All healthy participants had SPAQ scores of less than 7 (mean [SD]: 2.1 [1.7], range 0-6, Table 2.1). On each scan day the Structured Interview Guide for the Hamilton Depression Rating Scale with Seasonal Affective Disorder Supplement (SIGH-SAD) was also administered. For each participant, written informed consent was obtained after the procedures were fully explained. The study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health (CAMH), University of Toronto.
2.2.2 Image Acquisition and Analysis

All scans occurred at the CAMH Research Imaging Centre. All participants underwent two $[^{11}C]$DASB PET and MRI scans: one in spring-summer and the other in fall-winter, in randomized order, to measure the seasonal percent change in 5-HTT $BP_{ND}$. Scan dates of healthy controls were matched to SAD participants within two to four weeks. To minimize any potential effects of circadian rhythm all scans were scheduled in the morning and took place at either 9:30am or 11:30am. All participants were non-smoking and on each PET scan day underwent laboratory tests (plasma sampling for cotinine, calcium and thyroid hormones, complete blood cell count) to verify non-smoking status and ensure physical health.

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

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

2.2.3 Statistical Analysis

Seasonal percent change in 5-HTT BP\textsubscript{ND} (% Δ 5-HTT BP\textsubscript{ND}) was calculated in each region for each subject [(winter 5-HTT BP\textsubscript{ND} - summer 5-HTT BP\textsubscript{ND})/summer 5-HTT BP\textsubscript{ND}]. As one value pertaining to a particularly severe SAD case was outside of the normal distribution, non-parametric tests were used for all statistics. The % Δ 5-HTT BP\textsubscript{ND} in the PFC and ACC was compared between SAD and healthy groups using the Mann-Whitney U test. To assess the relationship of % Δ 5-HTT BP\textsubscript{ND} to severity, the primary method was to categorize SAD into two groups, moderate and severe, applying a cut-off of greater than 16 on the SPAQ as previously described (Bartko et al., 1989; Terman, 1988). To determine whether a difference was present amongst healthy, moderate SAD and severe SAD groups, the Kruskal–Wallis H test was applied to assess % Δ 5-HTT BP\textsubscript{ND} in the PFC and ACC, and then the Mann-Whitney U test was used to compare healthy with severe SAD. As a secondary approach, all analyses were applied in the other regions of interest including the dorsal putamen, thalamus, dorsal caudate, midbrain, ventral striatum and hippocampus.
As an additional analysis, the effect of severity of SAD upon seasonal change in 5-HTT BP<sub>ND</sub> was investigated. Seasonal change in 5-HTT BP<sub>ND</sub> (Δ 5-HTT BP<sub>ND</sub>) was calculated for each participant (winter 5-HTT BP<sub>ND</sub> - summer 5-HTT BP<sub>ND</sub>). To determine whether a difference was present amongst healthy, moderate SAD and severe SAD groups, the Kruskal–Wallis H test was applied to assess Δ 5-HTT BP<sub>ND</sub> in the PFC and ACC and other examined regions, followed by the Mann-Whitney U to compare healthy with severe and moderate SAD groups. The Kruskal–Wallis H test was also used to determine if 5-HTT BP<sub>ND</sub> values differed across groups in winter and in summer.

Further assessments of the relationship to severity of symptoms, were to determine the Spearman correlation coefficients between % Δ 5-HTT BP<sub>ND</sub> in the PFC and ACC and seasonal depressive symptoms, as measured by the SPAQ. These correlations were also determined for other regions of interest. Lastly, as an exploratory analysis, Spearman correlation coefficients were used to assess the relationship between Δ 5-HTT BP<sub>ND</sub> and seasonal depressive symptoms in all brain regions assayed.

2.3. **Study 2: The Effect of Light Therapy on 5-HTT BP<sub>ND</sub> in Health**

2.3.1 **Participants**

Twenty-one healthy volunteers (11 women and 10 men; mean [SD] age 25.8 [5.8] years; age range 19-39) were recruited through fliers posted in community locations within the Toronto area. We elected to study healthy volunteers, rather than SAD patients because we were interested in investigating the potential of 5-HTT BP<sub>ND</sub> as a biomarker for light exposure, so as to develop prevention strategies for those
who had not yet developed SAD. This was plausible because previous observations of
correlations between 5-HTT BP_{ND} and season were in healthy samples (Kalbitzer et
al., 2010; Praschak-Rieder et al., 2008). For each participant, written informed
consent was obtained after the procedures were fully explained. The study and
recruitment procedures were approved by the Research Ethics Board for Human
Subjects at the Centre for Addiction and Mental Health, University of Toronto.
Participants were recruited as non-smoking healthy volunteers, with no history of
major medical or psychiatric illness, no history of alcohol or substance abuse and no
recent use of prescription or over the counter medications, including herbal
supplements. Female subjects were free from oral contraceptives. Subjects were
screened using the Structured Clinical Interview for DSM-IV–Non-Patient-Edition to
rule out psychiatric disorders (current or in remission), current suicidal ideation, history
of self-harm, anger dyscontrol or impulsive behavior. Urinalysis and urine drug
screening were also performed to rule out recent herbal, drug or medication use (i.e. within 5 half-lives).

Two subjects did not complete the study. One subject withdrew due to
discomfort in the scanner. A second subject had a positive urine drug screen for a
recreational drug and was subsequently withdrawn. Thus, 19 participants (10 women
and 9 men) completed the study.

2.3.2 Study Design

Volunteers participated in a single-blind, placebo-controlled, within-subject,
counterbalanced, crossover design. All subjects completed two experimental
conditions separated by a four week washout period. In Condition 1, participants
received five sessions of daily light therapy and in Condition 2, participants received five sessions of placebo treatment. In each treatment condition, sessions were 30 minutes in length and took place between 7:00am and 7:30am for five consecutive mornings. Subjects were randomly assigned in regards to the order in which they received each condition and assignment of conditions was balanced in both subject number and order.

**Condition 1 (Light Therapy):** Subjects were seated approximately 30 cm in front of a Day-Light Classic light box (Uplift Technologies Inc., Dartmouth, NS, Canada), which emitted broad-spectrum fluorescent white light at 10,000 lux for 30 minutes; the standard treatment dose for SAD (Terman et al., 1990; Terman et al., 2005). The height of the box was adjusted so that the subject’s eyes were level with the centre of the screen and the box was tilted at an angle of approximately 15° downward. During the sessions, subjects were asked to read during so that gaze was cast downward and head position and posture were monitored by a study investigator.

**Condition 2 (Placebo):** In a room separate from that in which subjects had undergone the light therapy condition, subjects were tethered to an inactive negative ionization system (Sphere One Inc., Chattanooga, TN) with a Velcro wrist strap and seated approximately 30 cm in front of the ionizer. Posture and position were monitored by a study investigator. As the negative ionizer was turned off with only the fan running to simulate activity, this was not expected to produce an effect on either mood or 5-HTT BPND. In addition, to control for expectancy bias, this intervention was described as another active condition. Subjects were instructed to read for the duration of the 30 minute session.
Mood-related symptoms were assessed at screening and on day five of each condition using the Structured Interview Guide for the Hamilton Depression Rating Scale with Atypical Depression Supplement (SIGH-ADS), Beck Depression Inventory (BDI) and the 10cm Visual Analogue Scale (VAS) for mood (i.e. happy–depressed), energy (i.e. most–least), and anxiety (i.e. relaxed–tense). Participants also wore an Actiwatch (Philips Electronics, Murrysville, PA), a waterproof light-weight wrist-watch, for 24 hours/day during both light therapy and placebo treatment to collect information about how such variables might differ across conditions. At the end of each condition subjects also underwent a $[^{11}\text{C}]$DASB PET scan to measure 5-HTT $\text{BP}_{\text{ND}}$.

**2.3.3 Image Acquisition and Analysis**

Participants underwent a $[^{11}\text{C}]$DASB PET scan at the end of each treatment condition, spaced a minimum of four weeks apart. All scans were scheduled for the morning and took place at either 9:30am or 11:30am. Lighting conditions in the scan room were kept constant during scanning and participants were instructed to keep their eyes closed for the scan duration. Participants were required not to consume any caffeine or alcohol 48 hours prior to scanning. On the day of scanning, subjects also underwent laboratory tests (complete blood cell count, plasma sampling for calcium, cotinine and thyroid hormones) to ensure physical health and non-smoking status, and a urine drug screen to rule out recent herbal, drug or medication use (within 5 half-lives). All subjects were medication-free prior to scanning, save for three participants who tested positive for over the counter medications, none of which seemed likely to influence the serotonin transporter (ibuprofen, aspirin, pseudoephedrine). In addition,
removal of these subjects from analysis did not affect the significance of the results and therefore, these data were included in the final analyses.

Synthesis and measurement of 5-HTT BP\(_{\text{ND}}\) with \([^{11}\text{C}]\text{DASB}\) reported in this study are the same as those used in previous studies (Ginovart et al., 2001; Houle et al., 2000; Meyer et al., 2001; Meyer et al., 2004b; Praschak-Rieder et al., 2008; Praschak-Rieder et al., 2005). Briefly, PET images were acquired using a High Resolution Research Tomograph (HRRT) PET camera (in-plane resolution; full-width half-maximum, 3.1mm; 207 axial sections of 1.2mm; Siemens Molecular Imaging, Knoxville, TN, USA). Prior to each scan, an intravenous bolus of 10 mCi (370 MBq) of \([^{11}\text{C}]\text{DASB}\) was injected. The \([^{11}\text{C}]\text{DASB}\) was of high radiochemical purity (99.35% ± 0.57%) and high specific activity (84.78 ± 31.14 GBq/μmol) at the time of injection. The emission scan was reconstructed in 15 frames of 1 minute, followed by 15 frames of 5 minutes, totaling to a scan duration of 90 minutes in length (Ginovart et al., 2001; Ichise et al., 2003). The images were corrected for attenuation using a cesium 137–labeled transmission scan and reconstructed by filtered back-projection (Hann filter) at Nyquist cut-off frequency. Additional details regarding the scanning acquisition have been previously published (Meyer et al., 2009). Each participant also underwent magnetic resonance imaging (GE Signa 1.5-T scanner, spin-echo sequence proton density–weighted image, x, y, z voxel dimensions; 0.78, 0.78, and 3mm, GE Medical Systems, Milwaukee, WI USA) for the region of interest (ROI) delineation. ROIs were delineated on these magnetic resonance images using a semi-automated method based on linear and nonlinear transformations of an ROI template in standard space to each individual magnetic resonance image (MRI), followed by a refinement process based upon the gray matter probability (Rusjan et al., 2006). The MRI was co-
registered to the summated $[^{11}\text{C}]$DASB PET image using a mutual information algorithm and the resulting transformation was applied to sample the ROIs from the PET image (Studholme et al., 1999). The location of the ROI was verified by visual assessment on the summated $[^{11}\text{C}]$DASB PET image. The posterior half of the cerebellar cortex under exclusion of vermis and cerebellar white matter served as the reference region. The borders of the reference tissue were at least 1 full width at half maximum (5.5 mm) from the venous sinuses and occipital cortex. At a distance of 1 full width at half maximum, spillover from the occipital cortex (which has specific binding) or the venous sinuses is negligible.

5-HTT $\text{BP}_{\text{ND}}$ values were determined using the non-invasive Logan method (PMOD Technologies Ltd, Zurich, Switzerland) (Logan et al., 1996). This method provides valid and reproducible $[^{11}\text{C}]$DASB PET measurements of 5-HTT $\text{BP}_{\text{ND}}$ values with low between-subject variance in 5-HTT $\text{BP}_{\text{ND}}$ for most brain regions (Meyer et al., 2004a; Meyer et al., 2004b; Praschak-Rieder et al., 2007; Praschak-Rieder et al., 2005). A secondary analysis was conducted using SRTM2 (Wu et al., 2002).

### 2.3.4 Statistical Analysis

Participants were divided into fall (September 22 to December 20) and winter (December 21 to March 19) groups corresponding to the season in which they were scanned. The mean scan date [SD] of the fall group was, November 17, 2009 [18.6 days] and the mean scan date of the winter group was February 13, 2010 [16.1 days].

For each region, within each group, the Shapiro-Wilk test was applied to assess the normality of the distribution of the difference in 5-HTT $\text{BP}_{\text{ND}}$ between light
and placebo conditions. PET data was also plotted as a histogram for each group and visually inspected to ensure normality and concordance with the Shapiro-Wilk test. In the fall group, the difference in 5-HTT BP$_{ND}$ between light and placebo was normally distributed across all brain regions assayed. In the winter group, PET data was normally distributed across all brain regions with the exception of the thalamus (Shapiro-Wilk test, W=0.82, $p=0.03$).

The primary analyses were repeated-measures multivariate analyses of variance (MANOVA) applied to each group to assess the effect of treatment (light therapy versus placebo) on 5-HTT BP$_{ND}$ (the repeated dependent variable) in the ACC and PFC within each season. Regional univariate repeated-measures ANOVAs were applied only if there was a significant omnibus effect in the repeated-measures MANOVA. To correct for 4 multiple comparisons (2 seasons, fall and winter; 2 a priori brain regions, ACC and PFC), significance for each ANOVA was set at a $p$-value of 0.0125. As a secondary analysis, for each group, a repeated-measures MANOVA was applied to assess for a global effect of treatment (light therapy versus placebo) on 5-HTT BP$_{ND}$ in all brain regions assayed, with the exclusion of data from the thalamus pertaining to the winter group, which was not normally distributed.

In addition, for each group, exploratory two-tailed paired t-tests were also performed to examine the effects of treatment in all brain regions, with the exception of winter data from the thalamus, for which the non-parametric Wilcoxon Signed Ranks test was applied. For these exploratory analyses, to correct for 14 multiple comparisons (2 seasons, fall and winter; 7 brain regions), significance was set at a $p$-value of 0.0036. For each group (with the exclusion of winter data from the thalamus), effect size (Cohen’s $d$) was calculated for all regions, defined as mean difference
across conditions (light versus placebo) divided by the standard deviation of the difference across conditions. For winter data from the thalamus, effect size was calculated by dividing the test statistic from the non-parametric Wilcoxon Signed Ranks Test by the square root of the number of observations.

As an additional exploratory measure, Pearson correlation coefficients were also applied to determine whether there was a relationship between reduction in 5-HTT BP_{ND} in the ACC, a region heavily involved in mood regulation, following light therapy relative to placebo, and improvement on scales of mood-related symptoms (BDI, VAS Mood, Anxiety, Energy and SIGH-ADS) (Ressler et al., 2007). All analyses were performed using the Statistical Package for Social Sciences version 20 (IBM SPSS Statistics, Chicago, IL).

2.4 Study 3: Effect of Light Therapy on 5-HTT BP_{ND} in SAD

2.4.1 Participants

For each participant, written informed consent was obtained after the procedures were fully explained. The study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto. Eleven SAD participants (9 women and 2 men; mean [SD] age: 32.8 [2.6] years; age range: 26-39) were recruited from the Greater Toronto Area between December 2013 and December 2015. Participant demographics are included in Table 2-3.

Criteria included being age 18 to 40, non-smoking and in good physical health with no history of alcohol or substance abuse, no antidepressant use within the past 6
months and no use of prescription medications or herbal supplements within the past 2 months. Comorbid Axis I or Axis II disorders were exclusionary. In addition, both use of light therapy within the past 3 months and travel to more southern latitudes during the study period were also exclusionary as potentially confounding variables (Harrison et al., 2015; Praschak-Rieder et al., 2008). Exclusion criteria for female subjects included use of oral contraceptives, current pregnancy, postpartum or recent abortion (within one year), and in perimenopause or menopause. Seven of eleven participants had previous trials of antidepressant medications (SSRIs, SNRIs or bupropion) for treatment of winter MDEs, although no participant had used psychotropic medication in the past six months. One participant had previously bought a light box but had never used it for a consistent period and had not used it within the past six months. Screening instruments included the Structured Clinical Interview for DSM-IV-TR (SCID-I/II) and Seasonal Pattern Assessment Questionnaire (SPAQ) from which a summed global seasonality score was calculated to determine degree of seasonality (i.e. seasonal change in sleep, mood, energy, appetite, weight and social activity (Rosenthal et al., 1987).

Table 2-3: Demographic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>2.6</td>
</tr>
<tr>
<td>Female:Male Ratio</td>
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</tr>
<tr>
<td>Body Mass Index</td>
<td>24.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Years in Climatic Area</td>
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</tr>
<tr>
<td>Age of SAD Onset</td>
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<td>4.9</td>
</tr>
<tr>
<td>No. Seasonal MDE(^a)</td>
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</tr>
<tr>
<td>SIGH-SAD (Baseline)</td>
<td>30.3</td>
<td>4.8</td>
</tr>
<tr>
<td>SIGH-SAD (Week 2)</td>
<td>11.3</td>
<td>7.1</td>
</tr>
<tr>
<td>SIGH-SAD (Week 6)</td>
<td>6.5</td>
<td>5.5</td>
</tr>
<tr>
<td>SPAQ(^b) (Global Seasonality Score)</td>
<td>17.8</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\(^a\)No. Seasonal MDEs consistent with reports from literature
\(^b\)SPAQ: Seasonal Pattern Assessment Questionnaire
All SAD subjects received a consultation with a study psychiatrist to verify diagnosis and attended clinical follow-up appointments two weeks after beginning light therapy and at the end of treatment (week six). Initial consultations and follow-up appointments included assessment for emergence of manic symptoms (which did not occur in this sample), but a scale based measurement specifically for manic symptoms (i.e. Young Mania Rating Scale) was not applied (Sit et al., 2007). Urine drug screening was performed at initial assessment and on each PET scanning day to rule out recent drug and medication use.

### 2.4.2 Treatment Protocol

Volunteers participated in an open-label, within-subject investigation during which they received a total of six weeks of daily morning light therapy. Enrollment began in late fall, starting from November 1 and all participants completed the light therapy protocol by February 15, so as reduce the possibility of spontaneous spring remission (Lam et al., 2006). The study was conducted over three consecutive winters (2013/2014-2015/2016). Participants were asked to use a Day-Light Classic light box (Uplift Technologies Inc., Dartmouth, NS, Canada) each morning for a 30 minute period between 7:00am and 8:00am. All subjects underwent two [$^{11}$C]DASB PET and MRI scans: one set of scans before beginning light therapy and another after two weeks of treatment to measure the effect of light therapy on 5-HTT BP$_{ND}$. This duration between scans was chosen because usually, most of the clinical response of seasonal MDEs to light therapy occurs within two weeks and it was our intent to evaluate neurochemical changes concomitant with this dramatic reduction in
depressive symptoms (Lam et al., 2006; Ruhrmann et al., 1998). At the end of treatment, subjects also came in for a final clinical assessment.

Participants were trained in how to use the light box and provided with an instruction sheet detailing its use. Training and instructions were consistent with clinical consensus guidelines for the use of light therapy for treatment of SAD (Lam et al., 1999). Briefly, subjects were asked to sit approximately 30cm in front the light box which emitted broad-spectrum fluorescent white light at 10,000 lux for 30 minutes; the standard treatment dose for SAD (Terman et al., 1990; Terman et al., 2005). The height of the box was adjusted so that the subject’s eyes were level with the centre of the screen and the box was tilted at an angle of approximately 15° downward. During the sessions, participants were instructed not to stare directly at the light and instead, asked to read so that gaze and head position were cast downward.

Mood-related symptoms were assessed at screening with the Structured Clinical Interview for DSM-IV, and in addition, on each PET scan day and when participants returned for their final assessment following six weeks of treatment, assessment tools included the Hamilton Depression Rating Scale with the Seasonal Affective Disorder Supplement (SIGH-SAD), Beck Depression Inventory (BDI), Dysfunctional Attitudes Scale (DAS) and the 10cm Visual Analogue Scale (VAS) for mood (i.e. happy–depressed), energy (i.e. most–least), and anxiety (i.e. relaxed–tense) (Williams J et al., 1994). Applying the SIGH-SAD, treatment response was defined as a reduction of at least 50% compared to baseline with a score of less than or equal to 14 and remission was defined as a score less than or equal to 8 (Terman et al., 1998).
2.4.3 Image Acquisition and Analysis

Participants underwent two $[^{11}\text{C}]$DASB PET and MRI scans, one set of scans before beginning light therapy and another after two weeks of treatment to measure the effect of light therapy on 5-HTT BP$_{ND}$. Participants were asked not to take over-the-counter medications one week prior to scanning, to avoid alcohol 4 days prior to scanning and not to consume caffeinated beverages within 2 days of the PET scan. In addition, to minimize any potential effects of circadian rhythm all scans were scheduled in the morning and took place at either 9:30am or 11:30am. All participants were non-smoking and on each PET scan day underwent laboratory tests (plasma sampling for cotinine, calcium and thyroid hormones, complete blood cell count) to verify non-smoking status and ensure physical health.

Synthesis of $[^{11}\text{C}]$DASB has been described previously (Ginovart et al., 2001; Wilson et al., 2000). Briefly, $[^{11}\text{C}]$-CH$_3$I was trapped in a high-performance liquid chromatography sample loop coated with a solution of the N-normethyl precursor (1 mg) in dimethylformamide (80 µl). After 5 minutes at ambient temperature, the contents of the sample loop were injected onto a reverse-phase high-performance liquid chromatography column, and the fraction containing the product was collected, evaporated to dryness, formulated in saline, and filtered through a 0.2-µ filter. Prior to each scan, an intravenous bolus of 10 mCi (370 MBq) of $[^{11}\text{C}]$DASB was injected. The $[^{11}\text{C}]$DASB was of high radiochemical purity (98.60% ± 0.61%) and high specific activity (58.10 ± 33.17 GBq/µmol) at the time of injection. PET images were obtained using a high-resolution PET/CT Siemens-Biograph HiRez XVI scanner (81 axial sections of 2mm; coverage from medulla through to superior frontal cortex; Siemens
Molecular Imaging, Knoxville, TN, USA). The emission scan was reconstructed in 15 frames of 1 minute, followed by 15 frames of 5 minutes, totaling to a scan duration of 90 minutes in length. The images were corrected for attenuation using a germanium 68–labeled transmission scan and reconstructed using 2D filtered back projection algorithms with a ramp filter. Subsequent to the initial PET scan, each participant also underwent a magnetic resonance imaging scan (GE 3.0-T scanner, fast spin echo – XL sequence, proton density–weighted image, x, y, z voxel dimensions; 0.37, 0.37, and 0.90 mm, GE Medical Systems, Milwaukee, WI, USA) for the region of interest (ROI) delineation.

To minimize head movement during the PET scan, a custom fitted thermoplastic mask was made for each subject and used with a head fixation system. After reconstruction denoised dynamical images were visually inspected for potential head movement. Motion was determined by defining the outline of the cortex in the transverse and coronal planes of the integral PET image and then by transposing this outline onto sequential frames which were visually inspected for shift using Analyze version 9.0 (AnalyzeDirect, Inc., KS, USA). When motion was visible, it was corrected using frame realignment with respect a reference frame characterized by a high signal-to-noise ratio (Mawlawi et al., 2001). Roto-translation transformations were calculated on denoised frames images using the Automatic Image Registration algorithm (Woods et al., 1992). When motion correction was applied the reference frame was the first 5 minute frame.

ROIs were delineated on the magnetic resonance images using a semi-automated method in which regions of a template MRI are transformed onto the individual MRI based on a series of transformations and deformations that matched
the template image to the individual co-registered MRI, as well as segmentation of the individual MRI to select gray matter voxels as previously described (Meyer et al., 2009; Rusjan et al., 2006). ROIs on the MRI were subsequently located on the PET image using the rigid body transformations from co-registration of the MRI to PET image via a mutual information algorithm. All ROIs were bilateral and included the anterior cingulate cortex, prefrontal cortex, ventral striatum, dorsal putamen, thalamus, hippocampus, midbrain and cerebellar cortex. The location of the ROI was verified by visual assessment on the summated $[^{11}\text{C}]$DASB PET images. The posterior half of the cerebellar cortex excluding the vermis and cerebellar white matter served as the reference region.

Reference tissue methods have been validated for $[^{11}\text{C}]$DASB to calculate 5-HTT BP$_{ND}$ (Ginovart et al., 2001; Ichise et al., 2003). We applied the non-invasive Logan method, which has a modest underestimate but the advantage of having the lowest coefficient of variation (i.e. standard deviation/mean) of calculated 5-HTT BP$_{ND}$ values (Logan et al., 1996). An additional analysis was conducted using the simplified reference tissue method 2 (SRTM2) which has a negligible underestimate but a higher coefficient of variation (Wu et al., 2002).

2.4.4 Statistical Analysis

The primary analysis for the region of interest method was a repeated-measures multivariate analysis of variance (rm-MANOVA) to assess the effect of light therapy on 5-HTT BP$_{ND}$ (the repeated dependent variable) in the ACC and PFC. As a secondary important analysis, a rm-MANOVA was also applied to evaluate the effect of light therapy across all examined brain regions. Regional univariate repeated-
measures ANOVAs were applied only if there was a significant omnibus effect in the rm-MANOVA. Effect size (Cohen’s $d$), was calculated for all regions as mean difference across conditions (light vs. baseline) divided by the standard deviation of the difference across conditions. Percentage change in 5-HTT BP$_{ND}$ was calculated for each region as: $[(\text{Light}-\text{Baseline})/\text{Baseline}]$.

As exploratory measure, Pearson correlation coefficients were calculated to determine whether there was a relationship between reduction in 5-HTT BP$_{ND}$, following light therapy, in the ACC and PFC, regions heavily involved in mood regulation (26), and improvement on scales of mood-related symptoms (BDI, VAS Mood, Anxiety, Energy and SIGH-SAD), as well as SPAQ scores.
3 RESULTS

3.1 Study 1: The Effect of Season on 5-HTT BPND

3.1.1 Rationale

Although information regarding the neuropathophysiology of SAD is accumulating, a critical gap is the lack of direct brain investigations of this illness. As such, there is a clear need to identify brain biomarkers associated with SAD to better understand its underlying neurobiology and develop strategies for prevention and treatment. There is strong evidence that 5-HTT BPND displays seasonal fluctuation with greater binding in the winter than summer in healthy participants (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009), but there have been no longitudinal studies examining seasonal change in 5-HTT BPND in SAD or in health. Thus, we scanned healthy participants and individuals with SAD, using [11C]DASB PET, in summer and in winter, to measure seasonal variation in 5-HTT BPND in the PFC and ACC and compare the magnitude of seasonal change in this biomarker in these brain regions between groups. Furthermore, as SAD is a dimensional illness with a continuous distribution within health (such that 25% of healthy individuals experience mild seasonal symptoms) through to SAD of moderate to high severity, we also assessed the relationship between degree of seasonal change in mood and behaviour and magnitude of seasonal fluctuation in 5-HTT BPND (Bartko et al., 1989; Kasper et al., 1989; Rohan et al., 2011; Terman, 1988).
### 3.1.2 Effect of SAD and Severity Category on % Δ 5-HTT BP<sub>ND</sub>

Seasonal % Δ 5-HTT BP<sub>ND</sub> was greater in SAD as compared to health in the PFC and ACC (Mann-Whitney U, U=126.5 and 114.0, p=0.046 and 0.02, respectively, Table 3-1). However, the strongest finding was a main effect of group (healthy, moderate SAD, and severe SAD) upon seasonal fluctuation in 5-HTT BP<sub>ND</sub> in the PFC and ACC (Kruskal–Wallis H test, χ<sup>2</sup>(2)=8.82, p=0.01 and χ<sup>2</sup>(2)=9.62, p=0.008, respectively, Figure 3-1 and Table 3-2). Similar findings were present across other regions of interest (χ<sup>2</sup>(2)=7.01-10.45, p=0.005-0.03, Figure 3-1 and Table 3-2), excepting the hippocampus in which a trend-level effect was observed (χ<sup>2</sup>(2)=5.26, p=0.07; Figure 3-1 and Table 3-2).

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Mann-Whitney U-test</th>
<th>SAD vs Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p-Value</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>126.5</td>
<td>0.046</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>114.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Dorsal putamen</td>
<td>140.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Thalamus</td>
<td>147.0</td>
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</tr>
<tr>
<td>Dorsal caudate</td>
<td>154.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>154.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Midbrain</td>
<td>96.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>153.0</td>
<td>0.20</td>
</tr>
</tbody>
</table>

5-HTT BP<sub>ND</sub>: serotonin transporter binding potential (non-displaceable); Mann-Whitney U, non-parametric equivalent of an independent samples t-test.

<sup>a</sup>Mann-Whitney U-test statistic.

**Table 3-1: Group differences in seasonal percent change in 5-HTT BP<sub>ND</sub>**

These findings were primarily explained by greater seasonal % Δ 5-HTT BP<sub>ND</sub> in the PFC and ACC of severe SAD cases relative to healthy volunteers, (Mann-
Whitney $U$, $U= 42.5$ and $37.0$, $p=0.005$ and 0.003, respectively, Figure 3-1 and Table 3-2), an effect also observed in other regions of interest ($U=40.0-62.0$, $p=0.004-0.048$; Figure 3-1 and Table 3-2), excepting the midbrain for which seasonal $\% \Delta$ 5-HTT BP$_{ND}$ was significantly greater across all SAD participants relative to healthy volunteers ($U=96.0$, $p=0.005$; Figure 3-1 and Table 3-1). In contrast, seasonal $\% \Delta$ 5-HTT BP$_{ND}$ in moderate SAD subjects was similar to healthy individuals with no difference in any region of interest (Mann-Whitney $U$, $U=51.0-106.0$, $p=0.07-0.96$; Figure 3-1 and Table 3-2). Seasonal $\% \Delta$ 5-HTT BP$_{ND}$ was consistent within individuals across brain regions (Cronbach's alpha, $\alpha=0.89$)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Kruskal–Wallis $H$-test</th>
<th>Mann–Whitney $U$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2(2)$</td>
<td>$p$-Value</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>8.82</td>
<td>0.01</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>9.62</td>
<td>0.008</td>
</tr>
<tr>
<td>Dorsal putamen</td>
<td>10.09</td>
<td>0.006</td>
</tr>
<tr>
<td>Thalamus</td>
<td>10.45</td>
<td>0.005</td>
</tr>
<tr>
<td>Dorsal caudate</td>
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</tr>
<tr>
<td>Ventral striatum</td>
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</tr>
<tr>
<td>Midbrain</td>
<td>8.71</td>
<td>0.013</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5.26</td>
<td>0.07</td>
</tr>
</tbody>
</table>

5-HTT BP$_{ND}$, serotonin transporter binding potential (non-displaceable); Kruskal–Wallis $H$, non-parametric test; Mann–Whitney $U$, non-parametric equivalent of an independent samples $t$-test.

$a_n=9$ (Moderate SAD) vs $n=20$ (Healthy).

$b_n=11$ (Severe SAD) vs $n=20$ (Healthy).

$c$Kruskal–Wallis test statistic.

$q$Mann–Whitney $U$-test statistic.

Table 3-2: Group differences in seasonal percent change in 5-HTT BP$_{ND}$ in participants undergoing [$^{11}$C]DASB PET in spring-summer and fall-winter.
Seasonal % Δ 5-HTT BP<sub>ND</sub> measured applying the SRTM2 was similar to that of the non-invasive Logan method (Pearson correlation coefficient, r=0.93-0.98, p<0.0001, across regions) and yielded similarly consistent main results.

![Figure 3-1: Seasonal percent change in serotonin transporter binding potential (% Δ 5-HTT BP<sub>ND</sub>) as measured in 8 brain regions of interest (ROIs). Open (healthy, n=20) and red (moderate SAD, n=9) and blue (severe SAD, n=11) triangles represent individual subject % Δ 5-HTT BP<sub>ND</sub>. Black bars represent mean % Δ 5-HTT BP<sub>ND</sub> for each group. % Δ 5-HTT BP<sub>ND</sub> was significantly greater in the prefrontal and anterior cingulate cortices (severe SAD vs health; Mann-Whitney U, U<sub>1</sub>= 42.5 and 37.0, p=0.005 and 0.003, respectively; greater magnitude in severe SAD of 35.10% and 14.23%, respectively) with similar findings observed in other regions (U<sub>i</sub> = 40.0 to 62.0, p=0.004 to 0.048; greater magnitude in severe SAD of 13.16% to 17.49%). To compare groups, the Kruskal-Wallis H test was also applied at each ROI. <sup>a</sup>p-value ≤ 0.005; <sup>b</sup>p-value ≤ 0.01; <sup>c</sup>p-value ≤ 0.05. Seasonal % Δ 5-HTT BP<sub>ND</sub> was consistent within individuals across brain regions (Cronbach's alpha, α=0.89).

3.1.3 Effect of SAD and Severity Category on Δ 5-HTT BP<sub>ND</sub>

The effects of SAD and severity on Δ 5-HTT BP<sub>ND</sub> were consistent with those on % Δ 5-HTT BP<sub>ND</sub> (figure 3-2). A main effect of group was observed on Δ 5-HTT BP<sub>ND</sub> in the PFC and ACC (Kruskal–Wallis H test, χ<sup>2</sup>(2)=8.32, p=0.016 and χ<sup>2</sup>(2)=9.00, p=0.01, respectively) and across other regions of interest (χ<sup>2</sup>(2)= 6.45-10.56, p=0.005-0.04, Figure 3-2). These findings were similarly driven by increased Δ 5-HTT BP<sub>ND</sub> of
severe SAD cases relative to healthy volunteers (Mann-Whitney $U$, $U=38.5-52.5$, $p=0.003-0.018$) with no difference in $\Delta$ 5-HTT BP\textsubscript{ND} values upon comparison of moderate SAD and healthy groups ($U=60.0-108.0$, $p=0.16-0.91$, Figure 3-2).

As compared to healthy and moderate SAD groups, mean regional 5-HTT BP\textsubscript{ND} values of severe SAD cases were greater in fall-winter by 0-7.89% and lower in spring-summer by 11.47-20.83%, however, these differences were not significant (Kruskal-Wallis H test, fall-winter: $\chi^2(2)=0.01-1.59$, $p=0.45-0.99$; spring-summer: $\chi^2(2)=1.61-5.11$, $p=0.08-0.45$, Table 3-3).
Relationship of Symptoms to % Δ $[^{11}\text{C}]$DASB 5-HTT $\text{BP}_{\text{ND}}$

In SAD participants, a positive correlation was also observed between magnitude of seasonal % Δ 5-HTT $\text{BP}_{\text{ND}}$ and SAD severity, as measured by the SPAQ GSS, which was significant in the PFC (Spearman’s rank correlation coefficient, $p=0.52$, $p=0.018$) and trend-level in the ACC (Spearman’s rank correlation coefficient, $p=0.42$, $p=0.07$, Table 3-4). Similar relationships were observed in other examined brain regions ($p=0.44$-$0.55$, $p=0.012$-$0.055$), with the exception of the midbrain, in which no significant correlation was observed ($p=0.25$, $p=0.29$; Table 3-4). However, in healthy participants, for which the range in SPAQ GSS scores was narrow, no significant correlations were observed between seasonal % Δ 5-HTT $\text{BP}_{\text{ND}}$ and SPAQ GSS in any brain region ($p=0.18$-$0.36$, $p=0.12$-$0.92$; Table 3-4).

Table 3-4: Correlations between seasonal percent change in 5-HTT $\text{BP}_{\text{ND}}$ and seasonal pattern assessment questionnaire global seasonality score
Correlations between SPAQ scores and $\Delta$ 5-HTT BP$_{ND}$ in SAD and healthy groups were comparable to those of seasonal % $\Delta$ 5-HTT BP$_{ND}$ in all examined brain regions (Figure 3-3, Table 3-5).

**Figure 3-3:** In SAD participants, a positive correlation was observed between magnitude of seasonal $\Delta$ 5-HTT BP$_{ND}$ and SAD severity, as measured by the SPAQ GSS which was significant in the PFC (Spearman’s rank correlation coefficient, $\rho=0.52$, $p=0.02$) and trend-level in the ACC (Spearman’s rank correlation coefficient, $\rho=0.41$, $p=0.07$). Correlations between SPAQ scores and $\Delta$ [${}^{11}$C]DASB 5-HTT BP$_{ND}$ were comparable to those of seasonal % $\Delta$ [${}^{11}$C]DASB 5-HTT BP$_{ND}$.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>SAD (n=20)</th>
<th>Health (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p^{ab}$</td>
<td>$p$-value</td>
</tr>
<tr>
<td>Prefrontal Cortex</td>
<td>0.52</td>
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</tr>
<tr>
<td>Hippocampus</td>
<td>0.50</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Table 3-5: Correlations between seasonal change in 5-HTT BP$_{ND}$ value and Seasonal Pattern Assessment Questionnaire Global Seasonality Score
In SAD, *post-hoc* exploratory analyses comparing a breakdown of the GSS by mood, energy and appetite clusters found that the strongest correlations between seasonal change in mood symptoms and seasonal % Δ 5-HTT BP<sub>ND</sub> occurred in the PFC (ρ=0.53, p=0.015), dorsal putamen (ρ=0.66, p=0.002), dorsal caudate (ρ=0.62, p=0.004), ventral striatum (ρ=0.63, p=0.003) and thalamus (ρ=0.59, p=0.006). As for seasonal changes in energy levels, the strongest correlations were also observed in the PFC (ρ=0.52, p=0.019), dorsal putamen (ρ=0.70, p=0.001), dorsal caudate (ρ=0.68, p=0.001), ventral striatum (ρ=0.71, p<0.0001) and thalamus (ρ=0.66, p=0.001). Similar relationships were found between changes in appetitive behaviors and seasonal % Δ 5-HTT BP<sub>ND</sub> with the strongest correlations observed in the PFC (ρ=0.50, p=0.024), thalamus (ρ=0.54, p=0.014), dorsal putamen (ρ=0.48, p=0.03), hippocampus (ρ=0.48, p=0.033) and ventral striatum (ρ=0.42, p=0.069). Results are presented in Table 3-6.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>SAD</th>
<th>Health</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mood</td>
<td>Energy</td>
</tr>
<tr>
<td></td>
<td>ρ</td>
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</tr>
<tr>
<td>Hippocampus</td>
<td>0.28</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Spearman’s Rank Correlation Coefficient

Table 3-6: Correlations between seasonal percent change in 5-HTT BP<sub>ND</sub> and domains of the seasonal pattern assessment questionnaire
3.2 Study 2: The Effect of Light Therapy on 5-HTT BP\textsubscript{ND} in Health

3.2.1. Rationale

Seasonal change in mood and behavior is a significant problem as approximately twenty-five percent of healthy individuals report impaired functioning in winter due to low mood, increased appetite and decreased energy (Chotai et al., 2004; Kasper et al., 1989; Okawa et al., 1996; Perry et al., 2001; Rosen et al., 1990). In addition, only 55% of individual suffering from seasonal depressive symptoms fully remit after light therapy, a first-line treatment for SAD (Lam et al., 2006). As such, there is an urgent need for research to optimize this treatment for prevention of SAD. In two previous studies of seasonal variation in 5-HTT BP\textsubscript{ND} using [\textsuperscript{11}C]DASB PET, an inverse correlation between duration of daily sunlight and 5-HTT BP\textsubscript{ND} was observed in health, suggesting that this might be a promising biomarker for light therapy (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008). However, there has been no investigation of the effects of deliberate bright light exposure upon 5-HTT BP\textsubscript{ND} in human brain. Thus, we used [\textsuperscript{11}C]DASB PET to examine the effects of light therapy on 5-HTT BP\textsubscript{ND} in the ACC and PFC during the fall and winter, in healthy volunteers, in order to investigate the potential of 5-HTT BP\textsubscript{ND} as a biomarker for light exposure to develop prevention strategies for new-onset SAD.

3.2.2 5-HTT BP\textsubscript{ND} Decreases in the ACC Following Light Therapy in Winter

In winter group, there was a main effect of treatment on 5-HTT BP\textsubscript{ND} in the ACC and PFC (repeated-measures MANOVA, $F_{(2,8)}=19.54, p=0.001$). Subsequent univariate pairwise ANOVAs showed this treatment effect to be significant only in the
ACC ($F_{(1,9)}=18.04$, $p=0.002$) after correction for multiple comparisons where a decrease in 5-HTT BP$_{ND}$ of 12% was observed following light therapy relative to placebo, but not in the PFC (magnitude -1.1%, $F_{(1,9)}=0.23$, $p=0.64$). As a secondary analysis, the repeated-measures MANOVA was re-run with all ROIs, excluding the thalamus, and an effect of treatment was observed ($F_{(6,4)}=14.53$, $p=0.01$); however, since this was an exploratory analysis among a number of such analyses, greater than five, this was viewed as a non-significant finding. Subsequent exploratory t-tests revealed some level of reduction in 5-HTT BP$_{ND}$ in the ventral striatum after light therapy compared to placebo (magnitude -9.9%, paired t test, $t_9=2.85$, $p=0.02$) and hippocampus (magnitude -10%, paired t test, $t_9=1.73$, $p=0.12$), but the effect in the ventral striatum did not remain significant after correction for multiple comparisons (Figure 3-4 and Table 3-7). In addition, in the winter group, upon application of the two-tailed non-parametric Wilcoxon Signed Ranks Test, we did not observed a significant effect of treatment (light therapy versus placebo) on 5-HTT BP$_{ND}$ in the thalamus ($Z=-1.79$, $p=0.08$). In the fall group, there was no significant effect of treatment on 5-HTT BP$_{ND}$ observed in the ACC and PFC (repeated-measures MANOVA, $F_{(2,7)}=0.93$, $p=0.44$) or, upon additional comparison, in any other examined region (Table 3-7).
Table 3-7: Group differences in brain 5-HTT BP_{ND} values in healthy subjects undergoing [^{11}C]DASB PET in winter (n=10) and fall (n=9) following light therapy and placebo conditions (inactive negative ionization)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Winter Mean % change*</th>
<th>Winter Mean difference† (SD)</th>
<th>Winter Effect size</th>
<th>Winter P-value</th>
<th>Fall Mean % change*</th>
<th>Fall Mean difference† (SD)</th>
<th>Fall Effect size</th>
<th>Fall P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate cortex</td>
<td>−12.0%</td>
<td>−0.064 (0.048)</td>
<td>1.34</td>
<td>0.002</td>
<td>1.2%</td>
<td>0.013 (0.019)</td>
<td>0.04</td>
<td>0.88</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>−1.1%</td>
<td>−0.006 (0.053)</td>
<td>0.15</td>
<td>0.84</td>
<td>6.1%</td>
<td>0.017 (0.056)</td>
<td>0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>−9.9%</td>
<td>−0.205 (0.227)</td>
<td>0.90</td>
<td>0.02</td>
<td>−2.4%</td>
<td>−0.096 (0.246)</td>
<td>0.35</td>
<td>0.33</td>
</tr>
<tr>
<td>Dorsal putamen</td>
<td>1.6%</td>
<td>0.035 (0.136)</td>
<td>0.03</td>
<td>0.93</td>
<td>5.4%</td>
<td>0.108 (0.251)</td>
<td>0.43</td>
<td>0.23</td>
</tr>
<tr>
<td>Thalamus</td>
<td>4.0%</td>
<td>0.095 (0.275)</td>
<td>0.40**</td>
<td>0.019†</td>
<td>2.6%</td>
<td>0.082 (0.257)</td>
<td>0.32</td>
<td>0.06</td>
</tr>
<tr>
<td>Midbrain</td>
<td>−3.6%</td>
<td>−0.115 (0.202)</td>
<td>0.44</td>
<td>1.39</td>
<td>0.20</td>
<td>−0.032 (0.452)</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>−10.0%</td>
<td>−0.103 (0.189)</td>
<td>0.55</td>
<td>1.73</td>
<td>0.12</td>
<td>−3.4%</td>
<td>−0.036 (0.208)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

5-HTT BP_{ND}, serotonin transporter binding potential (non-displaceable); dPFC, dorsolateral prefrontal cortex; vPFC, ventrolateral prefrontal cortex; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex.
*Mean % change calculated as: (Light − Placebo)/Light.
†Mean difference calculated as: Light − Placebo.
‡Cohen’s d.
*Paired, two-tailed t-test, uncorrected P-value shown.
**Similar results observed in sub-regions of the PFC (dPFC, vPFC, mPFC and OFC).
***Calculated as: Wilcoxon signed-ranks test statistic/Number of observations.
††Non-parametric Wilcoxon signed-ranks test.

Figure 3-4: Serotonin transporter binding potential (5-HTT BP_{ND}) measured across conditions in 6 brain regions of interest (ROIs) during the winter (n=10). Open (placebo) and closed (light therapy) triangles represent individual subject 5-HTT BP_{ND} values for each condition and red bars represent the group mean. ACC refers to anterior cingulate cortex. 5-HTT BP_{ND} was significantly decreased in the ACC of healthy individuals following light therapy relative to placebo. Trend-level reductions in 5-HTT BP_{ND} were also observed in the ventral striatum and hippocampus. Error bars were omitted for clarity.
Similar results were obtained when analyzing 5-HTT BP\textsubscript{ND} values obtained from applying SRTM2. 5-HTT BP\textsubscript{ND} values were highly correlated across the two methods in each region, within both light therapy and placebo conditions (Pearson correlation coefficient, $r=0.90-0.99$, $p=<0.001$ and $r=0.92-0.99$, $p=<0.001$, respectively).

An exploratory analysis was also conducted to determine whether there was a relationship between reduction in 5-HTT BP\textsubscript{ND} in the ACC following light therapy relative to placebo and improvement of mood-related symptoms. As small changes in mood-related symptoms would be expected of healthy subjects prior to and after light therapy, behavioural data was pooled across fall and winter groups (n=19) to increase statistical power. Accordingly, a modest positive, trend-level correlation was observed between reduction in 5-HTT BP\textsubscript{ND} in the ACC following light therapy relative to placebo and improvement of scores on the BDI (Pearson correlation coefficient, $r=0.45$, $p=0.06$). However, no significant or trend level correlations were observed between the other scales of mood symptoms (VAS Mood, Anxiety, Energy, or SIGH-ADS) and change in 5-HTT BPND in the ACC or PFC.

We also collected sleep data via actigraphy (Actiwatch Spectrum, Philips Respironics, Pennsylvania, USA) because we were interested in investigating the relationship between changes in sleep measures and in 5-HTT BP\textsubscript{ND} following light therapy. However, we did not observe a significant correlation between change in ACC 5-HTT BP\textsubscript{ND} and any sleep measure (time of awakening, bed-time, number of awakenings, sleep duration, sleep efficacy, sleep latency, $r=0.20-0.04$, $p=0.40-0.87$) across conditions and upon further analysis, did not find group differences in changes
in these parameters (Table 3-8). Most likely the reason for this was that the sample had fairly normative levels of these measures.

<table>
<thead>
<tr>
<th>Sleep parameter</th>
<th>Winter, Mean (SD)</th>
<th>Fall, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Placebo</td>
</tr>
<tr>
<td>Bed time‡</td>
<td>23:42:00 (1:36:00)</td>
<td>23:31:00 (1:17:00)</td>
</tr>
<tr>
<td>Wake-up time‡</td>
<td>05:55:20 (0:54:00)</td>
<td>05:46:05 (0:48:00)</td>
</tr>
<tr>
<td>Sleep latency (mm:ss)</td>
<td>5:11 (3:34)</td>
<td>6:33 (7:37)</td>
</tr>
<tr>
<td>Sleep duration (h:mm:ss)</td>
<td>5:18:12 (0:52:00)</td>
<td>5:25:00 (0:42:00)</td>
</tr>
<tr>
<td>Awakening (a)</td>
<td>11:15 (3:49)</td>
<td>12:29 (4:33)</td>
</tr>
<tr>
<td>Sleep efficacy (%)</td>
<td>86.44 (5.01)</td>
<td>87.00 (3.45)</td>
</tr>
</tbody>
</table>

*Paired, two-tailed t-test, uncorrected P-value shown.
†24-hour time notation.

Table 3-8: Group differences in sleep parameters in healthy subjects undergoing [11C]DASB PET in winter (n=10) and fall (n=9) following light therapy and placebo conditions (inactive negative ionization)

3.3 Study 3: The Effect of Light Therapy on 5-HTT BP<sub>ND</sub> in SAD

3.3.1 Rationale

The efficacy of light therapy, a first-line treatment for SAD, is comparable that of antidepressant use (Lam et al., 1995; Lam et al., 2006; Moscovitch et al., 2004). In addition, the positive effects of light therapy tend to occur within one to two weeks whereas response to antidepressants typically begins after two to four weeks. This treatment is also associated with a lower frequency of adverse effects relative to antidepressant use (Lam et al., 2006; Ruhrmann et al., 1998). However, only 55% of individual suffering from SAD fully remit following light therapy, indicating a need to improve this treatment (Lam et al., 2006). In the first study, we observed a seasonal fluctuation in 5-HTT BP<sub>ND</sub> across examined regions, including the PFC and ACC in SAD, predominantly in severe SAD cases, with an elevation in winter relative to
summer. In the second study, we observed a region-specific decrease in 5-HTT BPND in the ACC of healthy individuals, a brain region involved in affect-regulation following light therapy (Ressler et al., 2007). In this study, we scanned individuals with severe SAD, in which we had previously observed increased magnitude of seasonal change in 5-HTT BPND before and after light therapy to determine (i) if light therapy could reduce the winter elevation in 5-HTT BPND to ameliorate seasonal depressive symptoms and (ii) whether the findings of study two of, in health, were generalizable to individuals with full-syndrome severe SAD.

3.3.2 Global Reduction in 5-HTT BPND Following Light Therapy in SAD

The primary finding was a main effect of treatment on 5-HTT BPND in the ACC and PFC (repeated-measures MANOVA, F(2,9)=6.82, p=0.016). Subsequent univariate pairwise ANOVAs showed this effect to be significant in both the ACC (F(1,10)=15.11, p=0.003) and PFC (F(1,10)=8.33, p=0.016), with decreases in 5-HTT BPND of 11.94% and 9.13%, respectively, following light therapy (Figure 3-5, Table 3-9). Similarly, a multivariate effect of treatment was also observed when the repeated-measures MANOVA included 5-HTT BPND as the dependent variable from all examined brain regions (repeated-measures MANOVA, F(4,7)=8.54, p=0.028). Significant reductions in 5-HTT BPND were found in the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain (F(1,10)= 36.94 to 8.02, p<0.0001 to 0.018; magnitude -16.74 to -8.83; Figure 3-5, Table 3-9). Effect sizes in the ACC and PFC were 1.17 and 1.03, respectively, with similar values observed in the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain (Cohen’s d: 0.85-1.83, Table 3.9). The change in 5-HTT BPND following light therapy measured applying the SRTM2 was similar to that of
the non-invasive Logan method (Pearson correlation coefficient, \( r=0.93-0.98, p<0.0001 \), across regions) and yielded similarly consistent main results.

![Table 3-9: Change in 5-HTT BP\(_{ND}\) values in SAD participants before and after light therapy](image)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>5-HTT BP(_{ND}), Mean (SD)</th>
<th>Effect Size(^a)</th>
<th>Mean % Change (SD)(^b)</th>
<th>F(_{1,12})(^c)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Cingulate Cortex</td>
<td>0.42 (0.071)</td>
<td>1.71</td>
<td>-11.94 (10.45)</td>
<td>15.11</td>
<td>0.003</td>
</tr>
<tr>
<td>Prefrontal Cortex</td>
<td>0.24 (0.054)</td>
<td>1.03</td>
<td>-9.13 (11.09)</td>
<td>8.33</td>
<td>0.016</td>
</tr>
<tr>
<td>Ventral Striatum</td>
<td>1.22 (0.12)</td>
<td>1.11</td>
<td>-9.17 (6.60)</td>
<td>13.65</td>
<td>0.004</td>
</tr>
<tr>
<td>Dorsal Putamen</td>
<td>1.26 (0.15)</td>
<td>1.16</td>
<td>-8.93 (7.72)</td>
<td>14.89</td>
<td>0.003</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.63 (0.19)</td>
<td>1.29</td>
<td>-12.44 (9.02)</td>
<td>19.45</td>
<td>0.002</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1.11 (0.16)</td>
<td>0.65</td>
<td>-13.47 (16.18)</td>
<td>8.02</td>
<td>0.018</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.57 (0.11)</td>
<td>1.63</td>
<td>-16.74 (9.16)</td>
<td>35.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\)Cohen's \( d \); \(^b\)Mean % Change calculated as: ([Light-Baseline]/Baseline); \(^c\)Repeated-measures ANOVA

![Figure 3-5: Serotonin transporter binding potential (5-HTT BP\(_{ND}\)) measured across conditions in 7 brain regions of interest (ROIs) in SAD participants before and after two weeks of daily morning light therapy. Closed (baseline) and open (light therapy) triangles represent individual subject 5-HTT BP\(_{ND}\) values for each condition with the connecting lines depicting respective change in 5-HTT BP\(_{ND}\) for each subject. Red bars represent the group mean. ACC refers to anterior cingulate cortex. A significant global reduction in 5-HTT BP\(_{ND}\) was observed following light therapy (repeated-measures MANOVA, \( F_{(4,7)}=8.54, p=0.028 \). Region-specific significant reductions were present in the ACC (\( F_{(1,10)}=15.11, p=0.003 \), magnitude of decrease, 11.94%), PFC (\( F_{(1,10)}=8.33, p=0.016 \), magnitude of decrease, 9.13%), and also in the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain (\( F_{(1,10)}=8.02 \) to 36.94, \( p<0.0001 \) to 0.018; magnitude of decrease, 8.83% to 16.74%). Effect sizes in the ACC and PFC were 1.17 and 1.03, respectively, with similar values observed in the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain (Cohen's \( d \): 0.85-1.83). Error bars were omitted for clarity.](image)
There was some tendency for a relationship between degree of seasonal variation in mood and behaviour as measured by the SPAQ and reduction in 5-HTT BP\textsubscript{ND} in the ACC and PFC following treatment (Pearson correlation coefficient, r=-0.49, \( p=0.13 \) and r=-0.52, \( p=0.10 \), respectively). After two weeks of light therapy, clinical response and remission were observed in 54.5\% and 27.3\% of SAD cases, respectively, and after six weeks of light therapy, clinical response and remission occurred in 81.8\% and 54.5\% of SAD cases, respectively. During clinical assessment, there was no evidence of manic symptoms in any participant, consistent with mood and energy ratings on the SIGH-SAD and VAS scales. There was no significant relationship between reduction in 5-HTT BP\textsubscript{ND} in the ACC and PFC following light therapy and improvement in scores on scales of mood symptoms (SIGH-SAD, VAS Mood and Energy or BDI).
4 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 General Discussion of Results

4.1.1 Study 1: The Effect of Season on 5-HTT BP_{ND}

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

Greater seasonal fluctuation of 5-HTT BP_{ND} across all examined brain regions, including the PFC and ACC, has important implications for SAD pathophysiology, particularly in regards to severe SAD. [{^{11}}C]DASB has a strong preferential binding to 5-HTT on the cell surface where functional 5-HTT are located and the binding of [{^{11}}C]DASB is insensitive to competition by endogenous serotonin as demonstrated by the lack of effect of physiologically tolerable serotonergic manipulations in humans, such as acute tryptophan depletion (Praschak-Rieder et al., 2005; Quelch et al., 2012; Talbot et al., 2005). As such, the changes in 5-HTT BP_{ND} observed in vivo using [{^{11}}C]DASB PET are best interpreted to reflect greater availability of the 5-HTT to clear 5-HT from extracellular space in the winter, thereby lowering levels of extracellular 5-HT. This is a key issue, given that overexpression of 5-HTT in the PFC is associated
with decreased stimulation induced release of 5-HT from serotonergic neurons and differential expression of the 5-HTT is associated with magnitude of response to anxiogenic stimuli (Jennings et al., 2010; Lesch et al., 1996; Mouri et al., 2012). In addition, while greater seasonal variation in 5-HTT BP_{ND} was observed in severe SAD relative to health, 5-HTT BP_{ND} values did not differ across groups in summer and winter seasons, suggesting that, in SAD, change across seasons is more relevant than the 5-HTT BP_{ND} levels, themselves. Taken together, these findings suggest that across the shift from summer to winter, seasonal change in 5-HTT levels and/or affinity may alter the dynamics of extracellular 5-HT release within the PFC, ACC and subcortical structures thereby dysregulating systems adversely affected in severe SAD, such as mood, energy and appetite.

Identifying a new brain biomarker in SAD is critical for therapeutic advances because brain biomarkers are an essential guide for developing treatments of complex neuropsychiatric illnesses with multiple underlying biological phenotypes. While it is well accepted that novel therapeutics require target engagement, it is a newer direction in therapeutic development to assess the effects of treatment on the target biomarker itself. Knowledge that the biomarker has been engaged then allows for assessment of whether an adequate number of phenotypes have been targeted in clinical trials with symptom burden as the primary outcome. In the present investigation, the biomarker identified provides opportunities to create novel prevention methods for SAD: it is clear that the environmental combination of seasonal variables including light, temperature, and humidity, influence 5-HTT BP_{ND}, especially in those with severe SAD. Future studies could identify combinations of specific environmental factors and their exposure thresholds that induce seasonal
change in 5-HTT BP\textsubscript{ND} so that by staying below such thresholds or adding other preventative interventions, such as light therapy, the winter elevation in 5-HTT BP\textsubscript{ND} could be avoided. Avoidance of environmental qualities and exposure thresholds that induce seasonal fluctuations in 5-HTT BP\textsubscript{ND} could then be incorporated into larger scale clinical studies as prevention strategies in high risk communities, such as those at more Northern latitudes where the prevalence of SAD exceeds six percent (Magnusson, 2000).

The present study found no seasonal differences in 5-HTT BP\textsubscript{ND} in healthy volunteers with minimal seasonality, as measured by the SPAQ, or in SAD participants with mild-moderate symptom severity, whereas several previous studies found seasonal changes in 5-HTT BP\textsubscript{ND} in healthy subjects who were not selected on the basis of seasonal fluctuation in mood and, presumably, had a wide range of seasonal variation in symptoms (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). It is important to note that the sample populations of the present study are different from those of earlier studies and likely account for this discrepancy. For example, it may be seasonal variation in 5-HTT BP\textsubscript{ND} would be observed in healthy individuals with a greater degree of change in seasonal symptoms. In regards to why 5-HTT BP\textsubscript{ND} did not change with season in mild-moderate SAD, it is plausible that multiple pathophysiological mechanisms contribute to SAD and that, of these, a single individual pathology, such as seasonal fluctuation in 5-HTT BP\textsubscript{ND}, is more likely to be present and of greater magnitude in those suffering from a more severe form of the illness.

There are some limitations of this study typical of SAD investigations and human brain studies of neuropsychiatric disease. First, it was not always possible to
scan SAD participants when their winter MDE was at its most severe. As such, completion of winter scanning prior to the symptomatic nadir may have underestimated the strength of the relationship between severity of SAD and seasonal % Δ 5-HTT BPND. Second, as our approach was to determine if the severity of SAD was related to seasonal change in 5-HTT BPND, we deliberately chose healthy volunteers with negligible severity of seasonal symptoms for comparison to SAD subjects with more extreme symptoms, and this may have reduced our ability to detect seasonal differences in healthy subjects. Since 25 percent of healthy people experience seasonal variation in mood symptoms, an additional interesting future direction would be to assess % Δ 5-HTT BPND in a group of healthy subjects with substantial seasonality to further characterize this brain phenotype (Kasper et al., 1989). Third, exposure to different seasonal environmental influences (i.e. light, temperature, humidity) are highly inter-correlated and this does not allow for differentiation of their individual effects in regards to the contribution of each factor to seasonal change in 5-HTT BPND. Lastly, while has strongly preferential binding to 5-HTT on outer cell membranes, 5-HTT BPND, is a measure of both 5-HTT density and its affinity for [11C]DASB (Quelch et al., 2012). Thus, it is not possible to differentiate between changes in 5-HTT density and affinity, although it would be expected that when the affinity of the serotonin transporter is altered there is a similar functional effect on the dynamics of extracellular 5-HT concentrations.

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

4.1.2 Study 2: The Effect of Light Therapy on 5-HTT BP\textsubscript{ND} in Health

This is the first investigation to compare the effect of light therapy to placebo upon 5-HTT BP\textsubscript{ND} in the human brain. The primary finding is that, during the winter, administration of light therapy significantly decreased 5-HTT BP\textsubscript{ND} in the ACC of healthy humans and this reduction remained significant after correction for multiple comparisons. In the fall group, no significant change was observed in any examined brain region. These results have important implications as they suggest a novel mechanism by which light exposure may exert an antidepressant effect and also provide a basis upon which to better develop light therapy for prevention of SAD.

At present, there is no consensus by which light therapy facilitates amelioration of depressive symptoms such as low mood. However, the finding that light therapy, a first-line treatment for SAD, affects 5-HTT BP\textsubscript{ND} in the ACC is in accordance with literature supporting the role of this brain region in regulation of mood and antidepressant response. The ACC participates in production of sad emotions, where regional activations occur during transient sadness and a reduction in activity follows
recovery from depression (Mayberg et al., 1999). In addition, activity in the ACC has
been observed to decrease in response to antidepressant drug treatment, cognitive
behavioural therapy, transcranial magnetic stimulation, and deep brain stimulation
(DBS) (Kreuzer et al., 2015; Mayberg et al., 1999; Mayberg et al., 2005; Ressler et al.,
2007; Siegle et al., 2006). Interestingly, 5-HT function in the ACC may be important for
treatment response since, in rodent models of depressive behaviour, 5-HT depletion
abolishes the antidepressant effects of DBS in the ACC (Hamani et al., 2010; Hamani
et al., 2012).

One explanation for reduced 5-HTT BP\textsubscript{ND} in the ACC following light therapy is
that, during the winter, light exposure may increase signaling between the retina,
dorsal raphe nucleus (DRN) of the midbrain and ACC to influence 5-HTT BP\textsubscript{ND} in the
ACC. To elaborate upon this putative mechanism, it has been reported that retinal
sensitivity, as measured by electroretinography, changes after light therapy in
individuals with SAD and also varies seasonally in both subsyndromal and full-
syndrome SAD (Hebert et al., 2002; Lavoie et al., 2009). In addition, in a recent
preclinical study, it was shown that, following light exposure, a distinct population of
non-visual DRN-projecting retinal ganglion cells (RGCs) were able to modulate
affective behavior via increased input to 5-HT neurons in the DRN (Ren et al., 2013).
Furthermore, in rodents, sleep deprivation, an anti-depressant treatment that
increases neuronal firing in the DRN, has been found to down-regulate levels of
monoamine transporters such as the 5-HTT and NET in efferent limbic structures
(Hipolide et al., 2005; Mogilnicka et al., 1980; Ursin, 2002). The DRN has projections
to the ACC and 5-HTT BP\textsubscript{ND} in the ACC varies inversely with duration of daily
sunshine (Porrino et al., 1982; Praschak-Rieder et al., 2008). In future study,
preclinical investigation would be helpful in regards to identifying specific cellular mechanisms underlying this light-induced change in 5-HTT BP_{ND}.

As approximately twenty-five percent of healthy individuals experience seasonal changes in mood and energy that impair functioning and only 55 percent of individuals suffering from seasonal depressive symptoms fully remit after light therapy, there is an urgent need for research to optimize this treatment for prevention of SAD (Chotai et al., 2004; Kasper et al., 1989; Lam et al., 2006; Okawa et al., 1996; Rosen et al., 1990). [^{11}\text{C}]DASB PET is currently applied to guide new antidepressant drug development for medications that bind to the 5-HTT (Meyer, 2012; Meyer et al., 2004a; Meyer et al., 2001; Meyer et al., 2004b). Our finding of a decrease in 5-HTT BP_{ND} in the ACC of healthy individuals and an associated increase in mood after light therapy, represents the first central biomarker associated with therapeutic response to light exposure. This reduction in 5-HTT BP_{ND} in the ACC, a brain region heavily involved in mood regulation, following light therapy, may provide a means to improve the efficacy of this treatment (Ressler et al., 2007). For example: [^{11}\text{C}]DASB PET could be similarly applied to determine what aspects (i.e. light colour, duration, intensity, time of day, etc.) of light exposure best reduce 5-HTT BP_{ND} in the ACC to optimize this treatment.

One limitation of the present study is that it may have been underpowered to detect changes in 5-HTT BP_{ND} in regions other than the ACC, such as the ventral striatum and hippocampus which had similar magnitudes of change but greater variability of such change. Second, while our cut-off between winter and fall was based on standardized definitions of season, it is possible that the true cut-off at which 5-HTT BP_{ND} in the ACC decreases after light therapy might be earlier, as a cut-off of
November 27 would have yielded similar results (magnitude -12.81%, repeated-measures ANOVA, $F_{(1,11)}=7.34$, $p=0.02$). Third, we chose to examine the effect of light therapy in healthy volunteers, thus, the extent to which these findings are generalizable to a clinical population of individuals with SAD is unclear. Another challenge in studying healthy volunteers is that they would be expected to score low on measures of depressed mood and energy both prior to and after light therapy. This floor effect may have limited our ability to detect significant correlations between 5-HTT BP_{ND} and symptom-related scales.

We studied healthy individuals in the fall and winter since the primary aim of this study was to evaluate the potential of 5-HTT BP_{ND} as a biomarker for light exposure to develop prevention strategies for new-onset SAD. Nevertheless, these results suggest several future directions: one future direction should examine the effect of light therapy on 5-HTT BP_{ND} in individuals with sub-syndromal or full-syndrome SAD to determine whether there would be a greater level of response. Another interesting future direction would be to assess the effect of light therapy on 5-HTT BP_{ND} in the spring and summer to further characterize the seasonal variation in response.

In summary, this is the first study to examine the effects of light therapy upon 5-HTT BP_{ND} in the living human brain. The main finding is that 5-HTT BP_{ND} in the ACC was significantly reduced after light therapy relative to placebo treatment in healthy individuals during the winter months. These results provide evidence of a relationship between 5-HTT binding in the ACC and light therapy, and identify, for the first time, a central biomarker associated with the therapeutic intervention of light therapy. As such, this central biomarker represents a new approach by which, in the future,
different candidate light therapy strategies could be screened with the best performing method upon reducing 5-HTT $\text{BP}_{\text{ND}}$ in the ACC being advanced to clinical trial for preventing SAD.

4.1.3 **Study 3: The Effect of Light Therapy on 5-HTT $\text{BP}_{\text{ND}}$ in SAD**

This is the first study of the effects of light therapy on 5-HTT $\text{BP}_{\text{ND}}$ in SAD. The primary finding was a main effect of treatment on 5-HTT $\text{BP}_{\text{ND}}$ across all brain regions, including a significant reduction in 5-HTT $\text{BP}_{\text{ND}}$ in the ACC and PFC. Similarly, significant decreases in 5-HTT $\text{BP}_{\text{ND}}$ were also observed in other brain regions assayed including the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain. These findings provide important direction for discerning the most salient environmental factor in the etiology of the seasonal change in 5-HTT $\text{BP}_{\text{ND}}$ in SAD. These results also demonstrate that light therapy reaches an key therapeutic target in the treatment of SAD and provide a basis for further development of this treatment through application of $[^{11}\text{C}]\text{DASB}$ PET.

The findings of the present study have implications for understanding the etiology of the seasonal change in 5-HTT $\text{BP}_{\text{ND}}$ across summer and winter seasons. We previously found that SAD subjects, particularly severe SAD cases with SPAQ global seasonality scores of greater than 16, had significantly greater seasonal variation in 5-HTT $\text{BP}_{\text{ND}}$ relative to healthy controls across all brain regions assayed (Tyrer et al., 2016). In the present study, all participants had SPAQ global seasonality scores of 16 or greater (Tyrer et al., 2016). However, in the previous study of seasonal variation, identification of the environmental cause inducing this seasonal change in 5-HTT $\text{BP}_{\text{ND}}$ was not resolvable since the main environmental factors that vary with
season, such as light, temperature and humidity, are highly inter-correlated and cannot be differentiated with respect to their relationship to seasonal fluctuation in this biomarker. In the present study, both the magnitude of the effect on 5-HTT BP_{ND} following two weeks of light therapy and the global reduction in this brain biomarker was in close accordance with the seasonal change in 5-HTT BP_{ND} across winter to summer seasons previously observed in SAD (Mc Mahon et al., 2016; Tyrer et al., 2016). As such, our findings suggest that changes in light exposure represent a sufficient environmental condition to account for the seasonal variation in 5-HTT BP_{ND} in SAD.

Our results of significant reductions in 5-HTT BP_{ND} in the ACC and PFC following light therapy, a first-line treatment for SAD, are in accordance with literature supporting the role of these regions in affect control (Liotti et al., 2002; Mayberg et al., 1999; Ressler et al., 2007; Sharot et al., 2007; Tom et al., 2007). However, it is notable that, following treatment, decreases in 5-HTT BP_{ND} were observed across all other examined regions including the hippocampus, ventral striatum, dorsal putamen, thalamus and midbrain, suggesting that the effects of light therapy on this biomarker may be more global than region-specific. Interestingly, dysregulated functioning, neurochemistry and/or structure has been implicated in some of these other regions in MDD. For instance, in the hippocampus, increased CA1 pyramidal cell neuron density and reduced astrocyte activation have been found to be associated with illness duration in recurrent/chronic MDD (Cobb et al., 2016; Cobb et al., 2013). The ventral striatum plays a role in both reward-processing and in the symptom of anhedonia, and this region has been a target of DBS for treatment of MDD with positive results in some open trials (Grubert et al., 2011). Lastly, the superior raphe nuclei within the
midbrain have a strong influence on circadian rhythm, including the sleep wake cycle (Vertes et al., 2008). Hence it is plausible that changes in 5-HTT BP\textsubscript{ND} in these other regions could also contribute to antidepressant response following light therapy. Hence it is plausible that changes in 5-HTT BP\textsubscript{ND} in these other regions could also contribute to effects of light therapy.

Determination of the mechanism underlying the substantial global reduction in 5-HTT BP\textsubscript{ND} following light therapy will require further study. A possible explanation for the global reduction in brain 5-HTT BP\textsubscript{ND} following light therapy is that light induces signalling directly between the retina and the DRN and also, indirectly, through the suprachiasmatic nucleus and then the dorsomedial nucleus of the hypothalamus to the median raphe nuclei MRN (Ren et al., 2013; Shen et al., 1994; Vertes et al., 2008). There are also strong reciprocal connections between the DRN and MRN (Vertes et al., 2008). This signalling to the raphe nuclei of midbrain may modulate firing of efferent serotonergic neurons within these structures to decrease 5-HTT levels in subcortical and cortical brain regions. A direct link between alterations in firing of the DRN and MRN and reduction of 5-HTT levels in efferent regions has not been fully established, but in rodents, sleep deprivation, a chronotherapeutic treatment, has been shown to increase neuronal firing in the DRN and down-regulate 5-HTT levels in efferent limbic structures (Hipolide et al., 2005; Mogilnicka et al., 1980; Ursin, 2002). Identification of specific cellular mechanisms underlying this light-induced change in 5-HTT BP\textsubscript{ND} would be a valuable direction for future study.

While results of phase 3 studies are the overriding determinant of therapeutic usefulness, to address the phenotypic heterogeneity of neuropsychiatric illnesses and verify target engagement, a new direction for advancing personalized medicine in
clinical trials is to apply PET receptor-ligand neuroimaging in phase 0 studies. This is intended as a solution to the concern that the reason clinical trials fail at a high frequency for treatment of neuropsychiatric illnesses is due to a mismatch between the therapeutic and the target as a result of illness heterogeneity or because the treatments themselves do not affect the target. Other insightful information can be obtained from such trials in similar circumstances. For example, the novel therapeutic Bapineuzumab, a humanized monoclonal antibody against the β-amyloid (Aβ) N-terminus, has been found to reduce accumulation of amyloid more prominently in mild-moderate relative to severe Alzheimer's disease, suggesting that the therapeutic might be best developed for an earlier phase of the illness than initially intended (Liu et al., 2015). Although it is the clinical response which ultimately matters, similar strategies of pure target engagement have a longstanding history of use in MDD; for instance, threshold occupancies of 80% for the 5-HTT and greater than 14% for the DAT are considered optimal in new antidepressant development (Meyer et al., 2002; Meyer et al., 2001; Meyer et al., 2004b). Accordingly, the present investigation was designed as a phase 0 study to develop a PET-based biomarker of light therapy. As such, in future study, the magnitude of reduction in 5-HTT BP_{ND}, as measured by [^{11}C]DASB PET, could be used to identify aspects of light therapy that could be modified so as to improve this biological response. It is anticipated that treatments with a greater biological response (i.e. decrease in 5-HTT BP_{ND}) would achieve a greater level of clinical response in large scale clinical trials.

As we chose to examine the effect of light therapy on 5-HTT BP_{ND} in a sample SAD patients with fairly severe seasonal depressive symptoms, a study design including a placebo control group was not feasible. In this clinical situation, in which an
evidence based first-line therapeutic is available the ethical standard at our site is that a placebo should not be given. As such, it is difficult to differentiate the effects of light therapy on 5-HTT BP<sub>ND</sub> from putative effects of placebo on this biomarker. However, it should be noted that therapeutic interventions for treatment for MDD, such as mirtazapine, a tricyclic antidepressant with an efficacy equal to that of SSRIs, which do not selectively target the 5-HTT, but presumably include placebo effects, do not induce change in 5-HTT BP<sub>ND</sub> as observed in the present study (Cipriani et al., 2009; Lundberg et al., 2012). In addition, there is reason to believe 5-HTT BP<sub>ND</sub> is reasonably resilient to effects of social interaction since simple administration of a protocol in a laboratory environment, such as ATD, does not affect 5-HTT BP<sub>ND</sub>. (Praschak-Rieder et al., 2005; Talbot et al., 2005). Lastly, although we did not include a control group of healthy volunteers, we have previously published two sets of reliability data using this technique demonstrating no systematic change in 5-HTT BP<sub>ND</sub> in health, thus it is unlikely that our results can be attributed to repeated-measurement alone (Meyer et al., 2001; Praschak-Rieder et al., 2005). As such, we favour the interpretation that the decrease in 5-HTT BP<sub>ND</sub> is best attributed to effect of the light therapy.

There are a few limitations of this study. First, we acknowledge it would have been desirable to conduct this study with a larger sample. However, it is notable that decreases in 5-HTT BP<sub>ND</sub> following light therapy were observed in all 11 subjects in the ACC and in 10 of 11 subjects in the PFC, with similar reductions in other regions, so it is highly unlikely that these decreases could be attributable to chance alone. Second, given our sample size, we may have been underpowered to detect significant correlations between therapeutic response to light therapy and reduction in 5-HTT
BPND. However, this investigation was designed as a "proof of concept" phase 0 study to develop a PET-based biomarker for light therapy and we note that sample sizes of over one hundred patients are typically required to distinguish dose effects of therapeutics, such as SSRIs in MDD (Fabre et al., 1995; Feighner et al., 1999). Third, the present study demonstrates the influence of light therapy on one therapeutic target, but other targets may also be important in regards to predicting treatment response. For example, Kohno et al., using PET, recently found increased uptake of [18F]-fluorodeoxyglucose in the right olfactory bulb of healthy humans after light therapy (Kohno et al., 2016). Fourth, while [11C]DASB has strong preferential binding to 5-HTT on outer cell membranes, 5-HTT BPND, is a measure of both 5-HTT density and its affinity for [11C]DASB (Quelch et al., 2012). Thus, it is not possible to differentiate between changes in these measures; however, it would be anticipated that alterations in both parameters would have similar effects upon extracellular 5-HT concentrations. Lastly, as we chose to investigate the effects of light therapy on 5-HTT BPND in SAD, it is unclear as to whether the effects of this treatment are specific to SAD, generalizable to other psychiatric populations or may also occur in healthy subjects. As such, a key direction of future study would be to examine the effect of this treatment on 5-HTT BPND in psychiatric illnesses for which light therapy has been shown to have efficacy, such as in bipolar disorder or in unipolar MDD, and to compare the effects in healthy non-seasonal healthy controls (Lam et al., 2016; Tseng et al., 2016).

In summary, this is the first investigation of the effects of light therapy on 5-HTT BPND during winter in SAD. The primary findings were significant reductions in 5-HTT BPND following light therapy in the ACC, PFC, as well as the hippocampus, ventral
striatum, dorsal putamen, thalamus and midbrain. Since \(^{11}C\)DASB preferentially binds to functional 5-HTT on the cell surface, this implies that one mechanism by which light therapy may exert antidepressant effects is via an elevation in extracellular serotonin levels (Quelch et al., 2012). These results also identify, for the first time, a central brain marker that has potential for use as a predictor to identify optimal modifications of light therapy in SAD, via use of \(^{11}C\)DASB PET as an imaging-calibration technique, which could then be brought forward for assessment in clinical trials. Given that the magnitude of the effect of light therapy on 5-HTT \(B_{ND}\) in SAD and the global reduction in this brain biomarker following light therapy is in close accordance with the effect of season on 5-HTT \(B_{ND}\), these results strongly suggest that seasonal change in light is the environmental factor mediating the seasonal variation in 5-HTT \(B_{ND}\) in SAD.

4.2 Summary of Findings and Conclusions

The importance of the 5-HTT, in regards to affect-regulation, is its influence on extracellular 5-HT levels. There is an inverse relationship between available 5-HTT and clearance of extracellular 5-HT. In support of this relationship, SSRIs that block the 5-HTT raise extracellular 5-HT, 5-HTT knockout mice have greater extracellular 5-HT and mice with overexpression of 5-HTT have low extracellular 5-HT (Bel et al., 1992, 1993; Jennings et al., 2006; Mathews et al., 2004). In addition, many SSRIs that raise extracellular 5-HT are associated with amelioration of depressive symptoms in individuals suffering from MDD and such modulators of extracellular 5-HT are important because it is well established that 5-HT plays a role in physiology and behaviours reported to change with season including mood, energy and appetite (Owens et al., 1994, 1998). There is also strong support from neuroimaging research
of seasonal variation in 5-HTT BP\textsubscript{ND}, an index of 5-HTT levels, in healthy volunteers and 5-HTT BP\textsubscript{ND} has been found to be inversely correlated with exposure to light (Buchert et al., 2006; Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Ruhe et al., 2009). However, until this series of studies, there had been no longitudinal investigation of the effect of season on 5-HTT BP\textsubscript{ND} in SAD as compared to health. In addition, there had been no studies evaluating the effect of deliberate bright light exposure upon 5-HTT BP\textsubscript{ND} in human brain, although light therapy is a front-line treatment for SAD and there is limited knowledge of the mechanism by which it exerts its antidepressant effects. Thus, the primary aim of this thesis was to examine the effects of both season and light exposure, upon 5-HTT BP\textsubscript{ND} in SAD and health.

The purpose of study one was to determine whether the magnitude of seasonal variation in 5-HTT BP\textsubscript{ND} was greater in SAD as compared to health in the PFC and ACC, brain regions involved in affect-regulation (Ressler et al., 2007). SAD participants and healthy volunteers underwent \([^{11}\text{C}]\text{DASB}\) positron emission tomography scans in summer and winter to measure seasonal change in 5-HTT BP\textsubscript{ND}. In accordance with our hypotheses, seasonal variation in 5-HTT BP\textsubscript{ND} was greater in SAD relative to health, across all examined brain regions, including in the PFC and ACC, and this fluctuation was primarily due to differences between severe SAD cases and healthy volunteers (Tyrer et al., 2016). We also observed significant and trend-level positive correlations between seasonal fluctuation in regional 5-HTT BP\textsubscript{ND} and degree of seasonality in our SAD participants (Tyrer et al., 2016). Our interpretation of these findings is that the seasonal change in 5-HTT BP\textsubscript{ND} reflects greater availability of the 5-HTT to clear 5-HT from extracellular space in winter, thereby lowering levels of extracellular 5-HT to dysregulate systems adversely affected in SAD, such as
mood, energy and appetite, and predispose such individuals to experiencing recurrent seasonal MDEs.

There are a number of highly inter-correlated environmental factors that vary with season, such as light, temperature, and humidity, which might promote seasonal change in 5-HTT BP$_{ND}$. If such variables and their exposure thresholds inducing this seasonal change were to be identified, it might be possible for individuals predisposed to developing recurrent winter MDEs via avoidance of the winter elevation in 5-HTT BP$_{ND}$ by staying below such thresholds or adding other preventative interventions, such as light therapy. In both previous studies of seasonal variation in 5-HTT BP$_{ND}$ using [$^{11}$C]DASB PET, including one from our laboratory, an inverse correlation was observed between 5-HTT BP$_{ND}$ and duration of daily sunshine in health, suggesting that changes in exposure to light might affect 5-HTT BP$_{ND}$ levels (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008). Accordingly, the objective of study two was to investigate the effect light therapy, a standard intervention for SAD treatment, on 5-HTT BP$_{ND}$ during fall and winter in health. We elected to first study healthy volunteers, rather than SAD patients, because we were interested in investigating the potential of 5-HTT BP$_{ND}$ as a biomarker for light exposure to develop prevention strategies for new-onset SAD, with the intent to further examine this effect in full-syndrome SAD in study three. Our hypothesis was that, in fall and winter, 5-HTT BP$_{ND}$ would be significantly reduced in the ACC and PFC after light therapy. The primary finding was a region-specific decrease in 5-HTT BP$_{ND}$ in the ACC of healthy volunteers following light therapy in winter, with no significant change observed in any examined brain region in individuals scanned during the fall (Harrison et al., 2015).
The results of study two suggest that, in healthy participants who have not yet developed SAD, the response to light therapy may be confined to the ACC, a brain region heavily involved in regulation of affect and antidepressant response (Ressler et al., 2007). The ACC participates in production of sad emotions, where regional activations have been observed during transient sadness in healthy volunteers and decreases in activity follow recovery from depression (Mayberg et al., 1999). Activity in this region has also been observed to decrease following various antidepressant treatments (i.e. use of SSRIs, cognitive behavioural therapy, transcranial magnetic stimulation, and DBS) (Ressler et al., 2007). Furthermore, appropriate 5-HT function in the ACC may be important for treatment response, as in rodent models of depressive behaviour, 5-HT depleting lesions abolish the antidepressant effects of DBS (Hamani et al., 2010; Hamani et al., 2012). Finally, twenty-five percent of healthy individuals experience daily impairment during the winter due to marked seasonal changes in mood, energy and appetite (Kasper et al., 1989). Thus, our observation of a region-specific decrease in 5-HTT BP$_{ND}$ following light therapy in winter suggests that the effects of light exposure on this biomarker are most pronounced during a period in which even healthy individuals experience some level of seasonal depressive symptoms.

In study one, we observed a greater magnitude seasonal variation in 5-HTT BP$_{ND}$ in SAD, primarily in severe SAD cases, as compared to health across multiple brain regions suggesting that it might be a biomarker associated with SAD pathology (Tyrer et al., 2016). In study two, we found a region-specific decrease in 5-HTT BP$_{ND}$ in the ACC following light therapy during winter in healthy volunteers, suggesting that 5-HTT BP$_{ND}$ might have potential as a biomarker in regards to evaluating the effects of
light therapy (Harrison et al., 2015). As a final step, it was our intent to examine light therapy’s effect on 5-HTT BP\textsubscript{ND} in severe SAD, in order to determine whether this PET-based measure of SAD pathophysiology could be useful in assessing treatment response to light in this clinical population and ameliorate seasonal change in 5-HTT BP\textsubscript{ND}. As such, we scanned SAD participants before and after light therapy during the winter, with the hypothesis that 5-HTT BP\textsubscript{ND} would be reduced across brain regions, including the ACC and PFC, following this treatment. Interestingly, we discovered a marked and consistent decrease in 5-HTT BP\textsubscript{ND} across all examined regions, including the ACC and PFC, following light therapy, with a greater spatial distribution of effect and more robust statistical significance relative to study two. 5-HTT BP\textsubscript{ND} has been previously observed to be elevated in winter relative to summer across multiple brain regions in SAD (McMahon et al., 2016; Tyrer et al., 2016). Although we did not observe an association between magnitude of decrease in 5-HTT BP\textsubscript{ND} and clinical remission or response following treatment, our conceptualization of light therapy is that this treatment may facilitate its therapeutic effects via interaction with multiple biological targets and it is our view that 5-HTT is one such target. As such, the lack of relationship between treatment response and reduction in this biomarker would be expected if light therapy affects multiple biological targets each of which, collectively, contribute to its antidepressant effects. Accordingly, our findings suggest that one means by which light therapy may exert its antidepressant effects during winter in SAD is to ameliorate the seasonal change in 5-HTT BP\textsubscript{ND}, thereby decreasing the availability of the 5-HTT to clear 5-HT from synapse and increasing levels of extracellular 5-HT.
In summary, we have identified a biomarker that is affected by season and associated with SAD pathology, which is also involved in treatment response to light. As such, the results of this series of studies have two important implications. First, the findings of studies one and three provide a basis for understanding the etiology of the seasonal variation in 5-HTT BP_{ND} in SAD. To elaborate, in SAD, the magnitude of seasonal change in 5-HTT BP_{ND} observed across regions moving from winter to summer in study one was comparable to the global reduction in 5-HTT BP_{ND} found following light therapy during winter in study three. Taken together, these results suggest that seasonal change in light exposure may be a sufficient environmental factor to mediate the seasonal variation in 5-HTT BP_{ND} in SAD, an explanation supported by findings from both preclinical and clinical neuroimaging studies, in which 5-HTT binding has been observed to be inversely correlated with exposure to light (Kalbitzer et al., 2010; Praschak-Rieder et al., 2008; Rovescalli et al., 1989). Second, the results of studies two and three demonstrate that light therapy reaches an important therapeutic target relevant to both prevention and treatment of SAD and these findings provide an opportunity to further develop this therapeutic through application of [11C]DASB PET. Twenty-five percent of healthy individuals experience marked seasonal changes in mood, energy and appetite which impair function and full-syndrome SAD is a common problem with an annual prevalence rate of 1 to 6 percent. However, only 55 percent of individual suffering from seasonal depressive symptoms fully remit after light therapy, indicating a need for improvement. Our finding of a decrease in 5-HTT BP_{ND} in both in healthy individuals who have not yet developed SAD and in those with full-syndrome SAD, represents the first central biomarker associated with treatment response to light therapy. Accordingly, in future
study, the magnitude of reduction in 5-HTT BP<sub>ND</sub>, as measured by [<sup>11</sup>C]DASB PET, could be used as a predictor to identify aspects of light therapy (i.e. light colour, duration, intensity, time of day, etc.) that could be modified so as to optimize SAD prevention and treatment.

4.3 Recommendations for Future Studies

4.3.1 Differentiating the Effects of Mood vs. Seasonality on 5-HTT BP<sub>ND</sub> in SAD

There is evidence that the SAD represents a complex phenotype influenced by multiple interacting, but independent, vulnerability factors including both the presence seasonality (i.e. seasonal change in mood and behaviour) and a predisposition to development of MDEs, among other factors (Levitan, 2007). Although there is strong relationship between individuals who report a high degree seasonality, as measured by the SPAQ GSS and incidence of SAD, the former measure is generally regarded as a dimensional construct (Bartko et al., 1989; Terman, 1988). As such, seasonality describes seasonal changes in mood and behaviour that may or may not cause clinical impairment, as is necessary for diagnosis of SAD and there is some overlap in degree of seasonality between those suffering from subsyndromal SAD and full-syndrome SAD (American Psychiatric Association, 2013; Terman, 1988).

In study one, we investigated the seasonal change in 5-HTT BP<sub>ND</sub> in SAD by scanning participants in winter during their symptomatic phase and in summer following remission from their seasonal MDEs. As such, all SAD subjects reported significantly greater seasonality, relative to asymptomatic healthy controls and all met criteria for moderate to severe MDEs during the winter months (Tyrer et al., 2016). In this first study we found greater seasonal variation 5-HTT BP<sub>ND</sub> in SAD relative to
health, primarily due to increased magnitude of seasonal change in 5-HTT BP_{ND} in severe SAD relative to moderate SAD cases (as measured by the SPAQ GSS) (Tyrer et al., 2016). The third study included SAD participants with a high degree of seasonality (SPAQ GSS ≥ 16), and moderate to severe MDEs, subjects who had previously displayed an increased magnitude of seasonal change in 5-HTT BP_{ND} in study one. In this subsequent study, we found a significant decrease 5-HTT BP_{ND} across all brain examined brain regions after light therapy during winter. As both studies included SAD participants with a high degree of seasonality and moderate to severe MDEs, it was not possible to conclusively establish whether the effects of season and light therapy on 5-HTT BP_{ND} in SAD were primarily attributable to degree of seasonality or to scanning an MDE and again while in remission. Determination of key components modulating the season and light-induced change in 5-HTT BP_{ND} is critical in order to better identify individuals with SAD in which this biological abnormality is present and whom may achieve greater benefit from interventions that preferentially target the 5-HTT.

As a first step, separation of the components of the mood and seasonality in SAD as contributing factors to change in 5-HTT BP_{ND}, as observed in studies one and three would require individual assessment the impact of each factor (i.e. seasonality or mood) on 5-HTT BP_{ND}, while keeping the other variable constant, as not to confound results. There are two means by which to do this; the first would involve using [{\textsuperscript{11}}C]DASB PET to scan participants with SAD before and after acute manipulation of mood during summer remission (i.e. lower mood during a characteristic period of remission, without the confound of change of season) to assess change in 5-HTT BP_{ND}. A second way by which to this would require scanning
participants with unipolar MDEs that report low seasonality but are similar in severity and degree of atypical depressive symptoms to those with SAD, within the same summer and winter scanning windows as those previously used in study one (i.e. alter season without the confound of change in mood, as individuals would, presumably, be depressed at during both time-points) (Tyrer et al., 2016). The following two subsections will review the methods by which the contributions of the effects of seasonality and mood could be assessed, followed by brief outline of study design

4.3.1.1 Acute Manipulation of Mood in SAD during Summer Remission

In regards to the first point, common procedures to facilitate acute and transient lowering of mood include mood induction paradigms and acute tryptophan depletion. There are numerous mood induction procedures (MIPs), all of which engender sad mood in individuals by different means. Interestingly, to the best of our knowledge, manipulation of mood via MIPs has never been examined in SAD. Common MIPs include Velten mood induction (reading aloud self-referential statements progressing from neutral to negative mood), music mood induction (participants listen to sad or neutral musical pieces so as to invoke the mood expressed by the music) and film-clip induction (subjects are presented a short film and explicitly asked to imagine themselves in the situation in order to generate the desired mood) (Gilet, 2008; Velten, 1968). Of these, the Velten MIP is used most widely by researchers studying changes in affective states, given its efficacy an altering subjective emotional states and it is often applied combination with other approaches such as music mood induction in order to increase efficacy (Dowlati et al., 2014; Frost et al., 1982; Gilet, 2008; Velten, 1968). Thus, a combination MIP, preferentially using the Velten and
music MIPs simultaneously, could be used to induce neutral (i.e. control condition) and sad mood, in SAD participants during summer remission in a randomized order with $[^{11}\text{C}]\text{DASB}$ PET scanning before and after each MIP (Dowlati et al., 2014; Gilet, 2008). If the effects of season and light on 5-HTT $\text{BP}_{\text{ND}}$ were primarily attributable to changes in mood as opposed to seasonality, it would be expected that an increase in 5-HTT $\text{BP}_{\text{ND}}$ would be observed following induction of sad mood in summer, with no change after induction of neutral mood. A change in 5-HTT $\text{BP}_{\text{ND}}$ would not be expected if seasonality was the primary factor mediating change in this biomarker.

In regards to acute tryptophan depletion (ATD), Neumeister and colleagues examined the effects of this procedure during characteristic summer remission in individuals with SAD (Neumeister et al., 1998). In comparison with sham depletion, ATD caused transient relapse into MDE in 73 percent of the remitted SAD sample, suggestive of a “trait-like” vulnerability toward depressive relapse following perturbation of the serotonergic function in SAD (Neumeister et al., 1998). In a follow-up study Neumeister et al., found that all but one of the SAD participants who had relapsed after ATD in summer, experienced a recurrent seasonal depressive episode during the subsequent winter (Neumeister et al., 1999). Furthermore, ATD has been found to facilitate relapse in remitted SAD patients following light therapy (Lam et al., 1996). These results suggest that individuals with SAD, sensitive to acute serotonergic manipulations, may be at high risk for development of recurrent winter MDEs. It is notable that there is evidence of a similar “trait-like” vulnerability toward serotonergic function in unipolar MDD since ATD has also been shown to facilitate depressive relapse in unmedicated remitted MDD participants (Neumeister et al., 2004). Taken together, these results suggest that vulnerability to serotonergic
dysfunction, as unmasked by ATD, may be an underlying trait that is present in those predisposed to development of MDEs.

There are only two neuroimaging studies that have applied receptor-ligand neuroimaging techniques to investigate the effect of ATD relative to sham depletion on 5-HTT BP_{ND} (Praschak-Rieder et al., 2008; Talbot et al., 2005). Both investigations used [^{11}C]DASB PET, were conducted using healthy volunteers and found negligible differences in 5-HTT BP_{ND} across brain regions between conditions. Thus, there have been no PET or SPECT investigations studying the effects of ATD on mood and 5-HTT BP_{ND} in SAD. As such, to differentiate the contributions of the effects of seasonality and mood on 5-HTT BP_{ND}, an alternative approach to an MIP would be to use [^{11}C]DASB to scan individuals with SAD, during summer, before and after sham and ATD. [^{11}C]DASB is insensitive to displacement by endogenous 5-HT, use of an ATD procedure (i.e. a serotonergic manipulation) would not be a confounding factor in regards to quantification of 5-HTT BP_{ND}. For example, if 5-HTT BP_{ND} was found to be unaffected by ATD, yet mood was altered, this would suggest that the effect of season and light on this biomarker do not result from alterations in mood as induced by ATD. Interestingly, Lam et al. have demonstrated that tryptophan depletion affects mood in SAD responders to light therapy, but measurement of 5-HTT BP_{ND} using [^{11}C]DASB PET was not a component of this study (Lam et al., 1996). The results of such as study would also be important, as this would be the first investigation of the effect of ATD on 5-HTT BP_{ND} in unmedicated SAD subjects in remission from depression.
4.3.1.2 Assessment of 5-HTT BP$_{ND}$ in Non-Seasonal MDD during Winter and Summer

Acute manipulations of mood, as proposed in the experiments outlined, above are useful as they allow for assessment of the effects of alterations in mood in the population of interest (i.e. SAD), during the summer season, which is a period of characteristic remission and can be completed in a relatively short time period. However, mood induction paradigms are acute and transient in nature, spanning minutes (i.e. MIPs), to days (i.e. ATD), and the season and light-induced changes in 5-HTT BP$_{ND}$ upon mood as observed in studies one and three occurred over months and weeks, respectively. As such, it is possible that a brief period of depressed mood invoked by mood induction, as outlined above, might not be sufficient in duration to elevate 5-HTT BP$_{ND}$ in those with SAD.

An alternative method by which to differentiate the components of the mood and seasonality in SAD as contributing factors to change in 5-HTT BP$_{ND}$ would be to apply [$^{11}$C]DASB PET to scan individuals with unipolar MDD, low in seasonality, but of similar severity and degree of atypical symptoms to those of the severe SAD group, longitudinally, during summer and winter (participants would be experiencing MDEs at both time-points). In this population, changes in 5-HTT BP$_{ND}$ across summer and winter seasons could then be compared to that of healthy and SAD subjects (Tyrer et al., 2016). If 5-HTT BP$_{ND}$ was found to be elevated during both seasons relative to non-seasonal healthy controls and levels of 5-HTT BP$_{ND}$ were roughly equivalent to those observed in SAD during winter, this would be evidence of depressed mood as a primary factor mediating the season and light-induced changes observed in SAD. Furthermore, as light therapy has shown efficacy as a therapeutic for non-seasonal
unipolar MDD and has been found to reduce 5-HTT BP\textsubscript{ND} in SAD, as observed in study three, an additional promising future direction would be to extend the investigation regarding the effects of light therapy on 5-HTT BP\textsubscript{ND} to those with non-seasonal unipolar depression (Lam et al., 2016).

4.3.2 Generalizability of Findings to “Seasonal Bulimia Nervosa”

It has become clear that some biological abnormalities are not specific to a single disorder, but present across a range of neuropsychiatric illnesses that share common behavioural features (i.e. dysphoric mood, anhedonia, etc.). For example, levels of MAO-A, an enzyme that degrades dopamine, norepinephrine, and 5-HT, as measured by $[^{11}\text{C}]$haranine PET, have been found to be elevated in MDD, postpartum depression, alcoholism, perimenopause and borderline personality disorder (Kolla et al., 2016; Matthews et al., 2014; Meyer et al., 2009; Rekkas et al., 2014; Sacher et al., 2015). Similarly, the density of translocator protein (TSPO), a marker of neuroinflammation, as measured by $[^{18}\text{F}]$EPPA PET has been observed to be elevated both in both MDD and Alzheimer’s disease. (Setiawan et al., 2015; Suridjan et al., 2015). As such, it has become increasingly common to study behavioural phenotypes and/or biomarkers that may be shared across a neuropsychiatric disorders to better understand the underlying neurochemistry of such illnesses, clarify overlap and boundaries between disorders and identify optimal therapeutic targets to improve treatment response (Morris et al., 2012).

Seasonal patterns of behaviour are present in psychiatric disorders other than SAD such as bulimia nervosa (BN) (Lam et al., 1996a; Levitan et al., 1994; Yamatsuji et al., 2003). BN is an eating disorder commonly characterized by dysfunctional eating
behaviours such as periods of extreme overeating (i.e. bingeing) followed by compensatory behaviours such as self-induced vomiting, purging (i.e. laxative and/or diuretic use), over-exercise or fasting (American Psychiatric Association, 2013). It is notable that there is a high rate of psychiatric comorbidity between SAD and this eating disorder, as it has been reported that 21 to 32 percent of patients with BN also meet criteria for SAD, with a concomitant decrease in mood and marked increase in carbohydrate craving, hyperphagia and compensatory purging behaviours during winter relative to summer (Lam et al., 1996a; Lam et al., 1991). Accordingly, the prevalence of SAD in BN is 4 to 5 times greater than the 1 to 6 percent prevalence rate reported in the general population at similar latitudes. In addition, many individuals with BN who do not meet full criteria for SAD still display seasonal patterns in dysfunctional eating behaviours and mood of greater magnitude than of healthy controls (Gruber et al., 1996; Lam et al., 1996a; Lam et al., 1991). Interestingly, in one study evaluating “seasonal BN” patients, 31 percent had SPAQ global seasonality scores equal to or greater than 16, equivalent to those of our severe SAD patients in which we observed seasonal change in 5-HTT BP_{ND} in study one and decrease in 5-HTT BP_{ND} following light therapy in study three (Lam et al., 1991; Tyrer et al., 2016). Furthermore, a correlation has also been observed between frequency of bingeing and purging behaviours in BN in photoperiod, with a peak in dysfunctional eating behaviours during winter, and a symptomatic nadir in summer (Blouin et al., 1992).

Similar to SAD, BN is often a chronic illness with poor outcome following treatment as only 45 percent of individuals attain full recovery, 27 percent achieve partial remission, but remain symptomatic while 23 percent experience a protracted course of illness spanning many years (Steinhausen et al., 2009). The efficacy of
fluoxetine, the only approved pharmacotherapy for BN, in reducing the behavioural symptoms of this disorder is comparable to its antidepressant efficacy in SAD, as only 56 and 67 percent of BN patients experience significant reductions in vomiting and binge-eating, respectively, after 8 weeks of treatment, while only 55 percent of SAD patients remit from winter depression following 8 weeks of treatment with this SSRI (Fluoxetine Bulimia Nervosa Collaborative Study Group., 1992). Interestingly, light therapy also been found to ameliorate both low mood and bingeing and purging behaviours in patients with seasonal BN. For example: in an open-label 4 week trial of light therapy of bulimic patients with a comorbid diagnosis of moderate to severe SAD, clinical response was observed in 56 percent of participants as indicated by a 50 percent reduction in SIGH-SAD scores and a reduction in bingeing and purging behaviour of 50 percent was observed in 55 and 45 percent of patients, respectively (Lam et al., 2001). Similar studies of effects of light therapy on reducing dysfunctional eating behaviours in “seasonal” BN patients have shown similar results (Lam et al., 1994).

Just as there is evidence of hypofunction of the serotonin system in SAD, so too is there similarly strong evidence of serotonergic dysfunction in BN. Significantly greater levels of depression, sadness and desire to binge have been found in women with BN relative to healthy volunteers following ATD, suggesting increased vulnerability to serotonergic manipulations and altered modulation of the serotonin system (Kaye et al., 2000). 5-HIAA levels, a metabolite of 5-HT, have been found to be reduced in the cerebral spinal fluid of symptomatic BN patients and this reduction is inversely correlated with frequency of binge-eating episodes (Jimerson et al., 1992). Brewerton and colleagues administered oral m-CPP, a non-selective 5-HT receptor
agonist, to BN patients and found seasonal variation in prolactin response in BN with no difference in healthy volunteers and also lower peak cortisol and prolactin responses in BN compared to health (Brewerton et al., 1992). Seasonal variation in prolactin levels was also seen following administration of $L$-tryptophan in BN and notably, blunted prolactin and cortisol responses were observed patients with both BN and MDD, relative to health (Brewerton et al., 1992). In a double-blind, randomized, placebo-controlled study, Levitan et al., replicated this finding of blunted cortisol and prolactin response following intravenous m-CPP challenge in bulimic patients and also observed subjective responses on mood ratings such as decreased anxiety, increased feeling of calmness and altered self-awareness, similar to mood-altering effects reported using the same paradigm in symptomatic individuals with SAD (Levitan et al., 1998; Levitan et al., 1997). Hormonal response following infusion of intravenous 5-HTP, a metabolic intermediate of 5-HT biosynthesis, has also been investigated with blunted prolactin levels observed in BN patients relative to healthy volunteers, further indicating hypofunction of the serotonin system (Goldbloom et al., 1996). In support of this observation, administration of $d$-fenfluramine, a 5-HT releasing agent, has been associated with similarly blunted prolactin response in this clinical group, whereas this neuroendocrine response is not observed in those who have recovered from BN (Jimerson et al., 1997; Monteleone et al., 1998; Wolfe et al., 2000). Collectively, these findings provide strong support of hypofunction of the serotonin system in BN.

However, despite evidence of a seasonal dimension to BN, the large body of research supporting hypofunction of the serotonin system in this disorder, a high rate of comorbidity between SAD and BN, and poor treatment response to SSRIs, a first-line evidence-based treatment for this disorder, there have been relatively few PET
studies of investigating 5-HTT BP_{ND} in BN and no study investigating seasonal change in 5-HTT BP_{ND} in "seasonal BN" or in BN following light therapy. To date, most studies have included relatively small samples of recovered, or symptomatic non-seasonal BN patients (i.e. ≤10 participants), applying inferior neuroimaging techniques and importantly, have not controlled for seasonal effects on 5-HTT BP_{ND}, thus findings regarding 5-HTT BP_{ND} in BN have been inconclusive. For example: Taucher and colleagues used \([^{123}]\beta\)-CIT SPECT and found reduced 5-HTT BP_{ND} in the thalamus and hypothalamus of 10 BN patients relative to 10 healthy controls, although the authors acknowledged potential confounds such as the near equal affinity of the tracer for the 5-HTT and DAT, the small sample size and history of anorexia nervosa (AN) in 20 percent of the BN group (Tauscher et al., 2001). Bailer et al., used [\(^{11}\)C]McN5652 PET to assess 5-HTT BP_{ND} in 9 women recovered from BN and found higher binding in the antero-ventral striatum relative to 7 women who had recovered from bulimia-type AN, but also noted the small sample size and limitations of the neuroimaging technique which precluded measurement of 5-HTT BP_{ND} in the neocortex (Bailer et al., 2007). The only study to apply [\(^{11}\)C]DASB PET found higher 5-HTT BP_{ND} in the anterior cingulate cortex and superior temporal gyrus in recovered BN patients relative to healthy volunteers, but this investigation was also confounded by small sample size and an unequal division of scan dates by season, thus it was not possible to assess the effect of season on 5-HTT BP_{ND} (Pichika et al., 2012).

Given evidence of a seasonality present in this eating disorder subtype, the high rate of comorbidity between SAD and BN, findings of serotonergic dysfunction in this disorder and the efficacy of both light therapy and SSRI treatment in ameliorating binge-purge behaviour in BN, it would be valuable to conduct a [\(^{11}\)C]DASB PET study
with an identical design to that of study one, to determine whether individuals with BN that are high in seasonality (i.e. as measured by the SPAQ with a GSS score ≥ 16, without comorbid SAD), also display seasonal variation in 5-HTT BP\textsubscript{ND}. The magnitude of this seasonal fluctuation in “seasonal BN” patients could then be compared to that previously observed in SAD and health (Mc Mahon et al., 2016; Tyrer et al., 2016). Such a study would provide insight into the neurobiology underlying the symptomatic peak in dysphoric mood and dysfunctional eating behaviours that individuals with “seasonal BN” report during the winter months. In addition, as both individuals with SAD and “seasonal” BN report low mood and dysregulating eating during winter, this would suggest the presence of a common neurobiological abnormality associated with seasonal fluctuations in these symptoms that are shared across separate neuropsychiatric disorders. As a logical next step, the effects of light therapy on 5-HTT BP\textsubscript{ND} could also be investigated during winter in a small sample of "seasonal BN" patients, to determine if a similar reduction symptoms and in this biomarker would be observed, as was found in SAD in study three.

4.3.3 Neurobiology Underlying the Effect of Season and Light on 5-HTT BP\textsubscript{ND}

In this series of studies we observed greater magnitude of seasonal variation in 5-HTT BP\textsubscript{ND}, an index of 5-HTT levels, in SAD relative to health with an elevation in winter relative to summer. We also found that, during winter, light therapy could ameliorate this seasonal change in 5-HTT BP\textsubscript{ND} in region-specific manner in health, with a significant reduction in this biomarker in the ACC and more globally in SAD, with significant decreases observed across all examined brain regions. However, preclinical investigation of the neurobiology underlying the effect of season and light
on the 5-HTT is beyond the scope of this thesis. This is a key issue for future study because at present there is no consensus as to a putative serotonergic mechanism underlying SAD development and maintenance, and there is limited understanding of the means by which light therapy exerts its antidepressant effects; such an investigation is needed to better understand the interplay between season, light and seasonal changes in mood and behaviour, and their effects on the 5-HTT on a cellular level. Possible mechanisms underlying the effect of season and light on the 5-HTT include alterations in gene expression of the 5-HTT or changes in trafficking (i.e. upregulation or downregulation) of the 5-HTT protein to the cell surface. As [\textsuperscript{11}C]DASB binds preferentially to the 5-HTT on the cell surface, we favour the view that changes in 5-HTT BP\textsubscript{ND} may reflect an alterations in regulation of the 5-HTT to the plasma membrane and that the study of molecular and cellular mechanisms by which this may occur is a promising direction for future study.

Endogenous regulation of 5-HTT expression and activity is complex and occurs at multiple levels (i.e. transcriptional, translational, post-translational) (Blakely et al., 1998). Nonetheless, in order to explain the effect of season and light on the 5-HTT, an environmental stressor must be identified that, when present, is able to activate cellular machinery in serotonergic neurons within the brain to alter 5-HTT levels. Interestingly, there is evidence that regulation of stress-activated p38 mitogen-activated protein kinase (p38MAPK) is under photoneural control, such that activity of this kinase peaks during darkness and is inhibited by exposure to bright light (Chik et al., 2004). In addition, serotonergic neurons in the DRN express p38MAPK which, when phosphorylated, increases both trafficking of the 5-HTT to the cell surface and 5-HT clearance. Accordingly, this kinase has a critical role in mediating 5-HTT density...
at the plasma membrane of serotonergic neurons and also in regulation of extracellular 5-HT levels (Samuvel et al., 2005; Zhu et al., 2005).

Importantly, there is also evidence that activity of p38MAPK is critical for appropriate behavioural response to stress (Bruchas et al., 2011). It is notable that, in rodent models of depressive-like behaviour, genetic deletion or pharmacological inhibition of this kinase in serotonergic neurons of the DRN reduces social avoidance following a social defeat paradigm, stress-induced reward seeking behaviour and immobility time during the forced swim test, relative to animals with intact p38MAPK (Bruchas et al., 2011). On a cellular level, exposure to these stressors has been found activate this kinase, increase 5-HTT density on the plasma membrane of serotonergic neurons and decrease extracellular 5-HT levels, a result that is not observed upon genetic knock-out or pharmacological inhibition of p38MAPK (Bruchas et al., 2011). Collectively, these findings indicate that, in animal models, exposure to stress initiates a cascade of cellular events in which activation of p38aMAPK induces a hyposerotonergic state leading to depressive-like behaviours in rodents.

It is notable that, DASB has been shown to bind preferentially to 5-HTT on outer cell membranes suggesting that the season and light-induced changes in 5-HTT BP\textsubscript{ND} observed in this series of [\textsuperscript{11}C]DASB PET studies reflect altered 5-HTT density at the plasma membrane. Consequently, alterations in activity of p38MAPK may be critical in mediating these changes in cell surface 5-HTT levels. To elaborate, individuals with SAD are sensitive to changes in photoperiod and as such, in winter, shortened photoperiod and/or lack of light exposure may act as environmental stressors facilitating activation of stress-induced p38MAPK, subsequent upregulation in trafficking of the 5-HTT to the cell surface, extracellular 5-HT loss and development
of winter MDEs (Wehr et al., 2001). In the absence of such a stressor, for instance, during the summer months or following bright light exposure in winter (i.e. light therapy) this increase in trafficking of the 5-HTT to the cell surface and concomitant lowering of mood would not be expected to occur.

In future study, in order investigate the possible role of p38MAPK in mediating the effects of season and light on the 5-HTT and in SAD, the selection of an appropriate animal model is critical. To date, such studies have been challenging, as most rodent species used in preclinical research are nocturnal and thus have opposite rest-activity cycles to humans and other diurnal species (Barak et al., 2013). In addition, most tests of depressive-like behaviour in animal models have been designed for commonly used nocturnal laboratory rodents (i.e. rats and mice), and therefore modification of these paradigms is often necessary (Barak et al., 2013). However, there has been significant progress in regards to establishing both face, construct and predictive validity of certain diurnal rodent species.

Specifically, the diurnal Mongolian gerbil has been investigated as a highly promising animal model of SAD. Notably, in these animals, afferents have been found to project directly from a select population of light-sensitive non-visual retinal ganglion cells (RGCs) to serotonergic neurons in the DRN and light-deprivation has been shown to induce depressive-like behavior (Fite et al., 1999; Lau et al., 2011; Luan et al., 2011). Ren and colleagues found that this distinct population of non-visual DRN-projecting RGCs was critical in modulating affective behavior in response to light via increased input to serotonergic neurons in the DRN and also that the level of neural activity in these RGCs was highly correlated with both 5-HT levels in the DRN and reduction in depressive-like behaviour (Ren et al., 2013). Serotonergic neurons within
the DRN projects to both subcortical and cortical areas and increased neuronal firing in this region has been found to down-regulate levels of monoamine transporters, including the 5-HTT (Hipolide et al., 2005; Mogilnicka et al., 1980; Porrino et al., 1982). Thus, it is possible that light exposure may exert its antidepressant effects via increased signaling along this retina-raphe pathway, thereby regulating 5-HTT levels in efferent brain regions.

As such, a possible direction of future study would be to expose groups of Mongolian gerbils to different photoperiodic conditions (i.e. 12:12 LD, 16:8 LD 8:16 LD, constant light, constant darkness). Subsequent to exposure to these photoperiodic conditions, western blotting would be useful in regards to quantifying levels of phosphorylated (i.e. active) p38MAPK in different brain regions. To measure levels of 5-HTT on the cell-surface, a membrane impenetrant biotinylation procedure could be used followed by affinity chromatography to isolate this protein and western blotting for 5-HTT quantification. In terms of analysis of these results, one could then examine the relationship between phosphorylated p38MAPK and both total and cell surface 5-HTT across photoperiodic conditions. If exposure to darkness facilitates trafficking of the 5-HTT to the cell surface via interaction with p38MAPK, it would be expected that levels of this kinase and cell-surface 5-HTT would elevated in conditions of shortened photoperiod and constant darkness, whereas p38MAPK and cell-surface 5-HTT levels would be reduced following exposure to constant bright light or lengthened photoperiod. This could be extended into studies evaluating the dependence of this process on the presence or absence of p38MAPK to further establish causality.
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LIST OF PUBLICATIONS

