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THE LONGITUDINAL BIOLOGY OF DEPRESSION: PET STUDIES OF THE DOPAMINE AND SEROTONIN SYSTEMS

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Cover: [^{11}C]MADAM PET data from a healthy human subject, overlaid on MR image. Sagital view of 3D visualization generated using 3DSlicer (www.slicer.org).

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The Longitudinal Biology of Depression: PET Studies of the Dopamine and Serotonin Systems

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To Frans and Vera

"The information I was able to find on the internet about the effects of hormones on the workings of the psyche left an impression of confusion and incoherence . . . serotonin was linked to self-esteem, to the sense of recognition obtained from the group. But in any case, it was essentially produced in the intestine, and it had been found to exist in a great variety of living creatures, including amoebas. What feeling of self-esteem could exist among amoebas? What sense of recognition from the group? I gradually reached the conclusion that medical art remained confused and imprecise about these matters, and that antidepressants were among the many medications that work (or don't) without anyone knowing exactly why."

Excerpt from *Serotonin* (2019). By Michel Houellebecq.

Translated by Shaun Whiteside. London: William Heinemann

This too shall pass

-Sufi proverb

ABSTRACT

The involvement of monoamine neurotransmission in the pathophysiology of depression was established already in the 1960s. However despite an abundance of research, the exact role of the serotonin (5-HT) and dopamine systems in depression have remained elusive.

Positron emission tomography (PET) is a nuclear imaging technique that makes the study of the living human brain possible at a molecular level. In this thesis, PET methods were validated (study I) and applied to study aspects of the serotonin and dopamine systems in depression. This was done through patient and control comparisons (study II and III), through examining correlations between key 5-HT proteins (study IV), and through a longitudinal approach testing patients before and after treatments with known efficacy (study II and III).

In study I, binding characteristics of the D2 dopamine receptor (D_2R) radioligand [^{11}C]raclopride was evaluated in brain regions where D_2R density is low (i.e., outside striatum). In most extrastriatal brain regions, little or no decrease in binding was observed after administration of a pharmacological competitor, lending no support for valid quantification. Further, extrastriatal test-retest repeatability was poor. The results indicate that [^{11}C]raclopride PET is not suitable for D_2R quantification in extrastriatal brain regions.

The aim of study II was to investigate D_2R availability in patients with severe depression, compared with healthy controls at baseline and before and after electroconvulsive treatment. Nine patients hospitalized for depression were examined using [^{11}C]raclopride PET before and after a series of electroconvulsive therapy treatments, and nine healthy, matched controls were examined twice. Lower striatal D_2R availability was observed in patients compared with controls. No significant change in [^{11}C]raclopride binding was observed in the patient group following treatment. The results suggest that low D_2R is associated with severe depression.

In Study III, [^{11}C]MADAM PET was used to quantify the 5-HT transporter (5-HTT) in 17 patients with depression before and after treatment with cognitive behavioral therapy. Matched healthy controls were examined once with [^{11}C]MADAM PET. Depression severity decreased and 5-HTT availability increased significantly in patients following the treatment. No significant difference in [^{11}C]MADAM binding was observed between controls and patients at baseline. The results indicate that previous findings of 5-HT dysregulation in patients with depression reflect a temporary state rather than a permanent trait.

The aim of study IV was to evaluate the correlation between 5-HTT availability and 5-HT 1B receptor (5-HT1B) availability in the human brain. [^{11}C]MADAM and [^{11}C]AZ10419369 PET was used to quantify 5-HTT and 5-HT1B respectively. 17 healthy individuals were examined with both radioligands. Strong correlations were observed in cortical regions while very weak correlations were observed in most subcortical regions. The results could be indicative of a strong transsynaptic regulation of the 5-HT system in cortical regions. However, the analysis was exploratory and [^{11}C]MADAM signal to noise ratio is poor in cortex so the results should be interpreted with caution.

LIST OF SCIENTIFIC PAPERS INCLUDED IN THESIS

The following four papers are included after the summary chapter and are referred to by their roman numerals in the text.

I. **Svensson JE**, Schain M, Plavén-Sigray P, et al. Validity and reliability of extrastriatal [11C]raclopride binding quantification in the living human brain. Neuroimage 2019; 202: 116143.

II. Tiger M, **Svensson J**, Liberg B, et al. [11C]raclopride positron emission tomography study of dopamine-D2/3 receptor binding in patients with severe major depressive episodes before and after electroconvulsive therapy and compared to control subjects. Psychiatry Clin Neurosci 2020; 263–269.

III. **Svensson JE**, Svanborg C, Plavén-Sigray P, Kaldo V, Halldin C, Schain M, Lundberg J. Serotonin transporter availability increases in patients recovering from a depressive episode. *Submitted.*

IV. **Svensson JE**, Plavén-Sigray P, Halldin C, Schain M, Tiger M, Lundberg J. In vivo correlation of serotonin transporter and 1B receptor availability in the human brain – a PET study. *Manuscript.*

LIST OF NON-THESIS SCIENTIFIC PAPERS

I. Almskog, LM, Wikman, A, **Svensson, J**, Wanecek, M, Bottai, M, van der Linden, J, & Ågren, A (2020). Rotational thromboelastometry results are associated with care level in COVID-19. Journal of Thrombosis and Thrombolysis.

II. **Svensson J**, Schain M, Knudsen GM, Ogden T, Plaven-Sigray P (2020): Early stopping in clinical PET studies: how to reduce expense and exposure. medRxiv 2020.09.13.20192856.

III. Freiburghaus, T, **Svensson, JE**, Matheson, GJ, Plavén-Sigray, P, Lundberg, J, Farde, L, & Cervenka, S (2020). Low convergent validity of [11C]raclopride binding in extrastriatal brain regions: a PET study of within-subject correlations with [11C]FLB 457. NeuroImage, 117523.

IV. Almskog, LM, Hammar, U, Wikman, A, Östlund, A, **Svensson, J**, Wanecek, M, Ågren, A, 2020. A retrospective register study comparing fibrinogen treated trauma patients with an injury severity score matched control group. Scand. J. Trauma. Resusc. Emerg. Med. 28, 5.

V. **Svensson, JE**, Schain, M, Plavén-Sigray, P, Cervenka, S, Tiger, M, Nord, M, Halldin, C, Farde, L, Lundberg, J, 2019. In response to the letter "[11C]raclopride and extrastriatal binding to D2/3 receptors." Neuroimage 116371.

VI. Arakawa, R, Stenkrona, P, Takano, A, **Svensson, J**, Andersson, M, Nag, S, Asami, Y, Hirano, Y, Halldin, C, Lundberg, J, 2019. Venlafaxine ER Blocks the Norepinephrine Transporter in the Brain of Patients with Major Depressive Disorder: a PET Study Using [18F]FMeNER-D2. Int. J. Neuropsychopharmacol. 22, 278–285.

CONTENTS

LIST OF ABBREVIATIONS

5-HT	5-Hydroxytryptamine (serotonin)
5-HTT	5-Hydroxytryptamine Transporter
^{11}C	Carbon-11
^{18}F	Flour-18
ACC	Anterior Cingulate Cortex
BP_{ND}	Binding Potential Non-Displaceable
CBT	Cognitive Behavioral Therapy
C_{ND}	Non-displaceable Radioactivity Concentration
C_S	Specifically Bound Radioactivity Concentration
C_T	Total Radioactivity Concentration
CSF	Cerebrospinal Fluid
D2R	Dopamine 2 Receptor
DLPFC	Dorsolateral Prefrontal Cortex
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECT	Electroconvulsive Therapy
HRRT	High Resolution Research Tomograph
HPA	Hypothalamic–Pituitary–Adrenal
ICC	Intraclass Correlation Coefficient
ICBT	Internet-delivered Cognitive Behavioral Therapy
IR	Immediate Release
MADRS	Montgomery Asberg Depression Rating Scale
MDD	Major Depressive Disorder
MR	Magnetic Resonance
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
ROI	Region Of Interest
SD	Standard Deviation
SPM	Statistical Parametric Mapping
SRTM	Simplified Reference Tissue Model
SSRI	Selective Serotonin Reuptake Inhibitor

SUV	Standardized Uptake Value
TC	Temporal cortex
TAC	Time-activity curve
VAR	Absolute Variability
V_T	Total Volume of Distribution
WAPI	Wavelet Aided Parametric Imaging
XR	Extended Release

1 INTRODUCTION

1.1 RATIONALE FOR THIS THESIS

The history of psychopharmacology is to a large part a history of serendipity findings. In a somewhat circular argumentation, these chance findings have then laid the foundation on which the dominant biological explanations for many psychiatric diseases rest. For depression, the monoamine hypothesis followed the discovery that pharmacological agents increasing cerebral monoamine levels alleviate depressive symptoms[1,2]. Since this theory gained popularity in the 1960s, much research has been directed towards the monoamines, mainly serotonin, but also dopamine. However, despite solidifying the fact that monoamine neurotransmission is important in the pathophysiology of depression, the exact role of these neurotransmitters remains elusive. The original idea of low monoamine levels as the root cause of depressive symptoms is now considered too simplistic.[3] Among the evidence indicating a more complex biology is the fact that more than 30% of patients with depression fail to respond to existing pharmacological treatments inhibiting monoamine reuptake[4]. As such, there is a great need for new methods and improved study designs to shed light on the role of monoamines in depression, with the end goal of finding better treatment options.

The nuclear imaging methodology positron emission tomography (PET) allows the study of biology at a molecular level in the living human brain. PET brain research is a living field in which methods continuously are being developed creating ways to study biological systems previously unavailable for in vivo research.

Longitudinal studies – where patients are examined when depressed and after treatment – offer a way to differentiate between "state" and "trait", where the depressive episode is considered "state" and susceptibility to depression "trait"[5]. A pre-post design also makes it possible to study treatments with known clinical efficacy where the direct or downstream mechanism of action is unknown.

The general aim of this thesis was to validate and apply PET methodology to study the serotonin and dopamine systems, their role in the pathophysiology of depression, and in the effects of established treatments of the disorder.

1.2 MAJOR DEPRESSIVE DISORDER

Major depressive disorder (MDD) is a common and devastating brain disorder, often chronic or recurring and with deleterious consequences in social and professional functioning as well as in overall health for afflicted individuals. MDD has a lifetime prevalence of about 20% and is twice as prevalent in women as in men[6]. It is the leading cause of disability in the world[7], a fact that is explained by both the number of years lived with disease and years of life lost, where the latter to a substantial degree is driven by suicide. Around one million

individuals commit suicide every year globally; of these more than half is estimated to meet the criteria for current depressive disorder[8].

1.2.1 Definition

There exist two commonly used classification systems used for psychiatric diagnosis: the International Classification of Diseases, maintained by the World Health Organization, and the Diagnostic and Statistical Manual of Mental Disorders (DSM), maintained by the American Psychiatric Association. Although not identical, application of these two systems is considered to identify approximately the same cohort of subjects. In all studies contained in this thesis, the DSM criteria were applied.

Central to the MDD diagnosis is a depressed mood and/or loss of pleasure in most activities. The severity of the disorder is determined by the number and severity of symptoms, and the degree of functional impairment. In the current version of the DSM (DSM-5[5]), five or more of the symptoms presented in Box 1 must be present during the same 2-week period to make the diagnosis of an episode of MDD. At least one of the symptoms must be either (1) depressed mood or (2) loss of interest or pleasure. Additionally, the symptoms must "cause clinically significant distress or impairment in important areas of functioning" [5].

Box 1. DSM-5 criteria for Major depressive episode

1. Depressed mood most of the day, nearly every day.
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day.
3. Significant weight loss when not dieting or weight gain, or decrease or increase in appetite nearly every day.
4. Insomnia or hyperomnia nearly every day
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nearly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt nearly every day.
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day.
9. Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

In the studies included in this thesis, the previous version of DSM (DSM-IV [9]) was used. The one significant change between DSM-IV and DSM-5 was the removal of the bereavement exclusion criterion, stating that the diagnosis should not be used within two months of the death of a loved one.

1.2.2 Pathophysiology

The biological underpinnings of MDD are to a large degree still unknown. Several mutually non-exclusive theories exist (see Ferrari 2017 for a review[10]). Briefly, non-exhaustively, and in no specific order:

1.2.2.1 Neuroplasticity

MDD patients show smaller volumes in many brain regions, including hippocampus, when compared with healthy controls, and an increase in hippocampal volume has been observed in MDD following treatment with antidepressants [11]. One PET study shows lower synaptic density in MDD patients with more severe symptoms [12].

1.2.2.2 Immune system abnormalities

The sickness behavior accompanying an infection (e.g., influenza) share many similarities with depression (e.g., low mood, lethargy, sleep disturbances, anxiety, loss of appetite) lending support to the role of the immune system in MDD[13]. Plasma levels of proinflammatory cytokines have been shown to be increased in patients with MDD[14]. Recent PET-data have shown support of cerebral immune activation in MDD[15].

1.2.2.3 The stress system

Environmental stress is a well described risk factor of depression[16]. The hypothalamic–pituitary–adrenal (HPA) system is a main regulator of stress response[16]. Dysregulation of the HPA system has been shown to be associated with MDD[16,17] and treatment with antidepressants has been shown to normalize an impairment in the glucocorticoid-mediated negative feedback of the HPA system associated with depression[18].

1.2.2.4 The monoamine hypothesis

In the 1960s, the discovery that drugs increasing monoamine levels in the brain were effective in treating depression led to the theory that depression was caused by low levels of monoamines[19]. Several subsequent empirical findings support the notion that monoamine neurotransmission is involved in MDD pathophysiology[20–22]. However, the view that low levels of e.g., serotonin is the sole cause of the disorder have since been criticized as too simplistic[3,10].

1.2.3 Treatment

1.2.3.1 Cognitive behavioral therapy (CBT)

During the 1980s and 1990s, cognitive and behavioral psychotherapeutic techniques were merged under the umbrella of cognitive behavioral therapy. This new approach quickly gained popularity and was shown to be effective in the treatment of most psychiatric disorders[23]. A defining feature of CBT is the idea that symptoms and dysfunctional behaviors often are cognitively mediated and, following from this, that various psychiatric conditions can be treated by modifying dysfunctional thinking and beliefs[24].

Specifically in the treatment of MDD, several CBT techniques have proven effective, e.g., behavioral activation and cognitive restructuring. An integral part of the treatment is the use of homework and a lot of the progress takes place between therapy sessions. CBT targeting MDD has comparable effectiveness as pharmacological treatment[25].

1.2.3.2 Electroconvulsive therapy (ECT)

ECT has been used since the 1940s to treat both unipolar and bipolar depression. With remission rates above 50%[26], it is the most effective treatment of MDD known today[27]. Using electricity, a seizure is induced in the brain of the patient. The procedure is performed under muscle relaxation and general anesthesia. Typically, 2-3 treatments are administered per week and usually a treatment cycle consists of 6-12 treatments.[28] Due to the small, but non-trivial, risks involved in general anesthesia, ECT is typically reserved for severely depressed patients[29].

The molecular mechanism of action of ECT is still largely unknown. Modulation of the dopamine system has been proposed as one possible mediator of the mood altering effect of ECT[30].

1.2.3.3 Pharmacotherapy

The first line pharmacological treatment of MDD are selective serotonin reuptake inhibitors (SSRIs)[31] which act through blocking the serotonin transporter (5-HTT)[32], increasing synaptic serotonin levels. Though there exist pharmacologic treatment options without any known serotonergic effect (e.g., bupropion), most common drugs targeting MDD have some effect on the serotonin system. It is not known how SSRIs alleviate depressive symptoms downstream the inhibition of the 5-HTT and though about 2/3 of patients with MDD respond to treatment only about 1/3 reach remission when treated with an SSRI[4]. In the studies included in this thesis, no pharmacological treatment was used.

1.2.4 Criticism of the diagnosis, implications for PET research

The MDD diagnosis has received criticism for being too heterogeneous; e.g., there are in theory 16,400 combinations of the criteria outlined in Box 1 that will allow a MDD diagnose to be made[33]. This has also been shown empirically; in one analysis over 1,000 unique depression-symptom profiles was observed in 3,700 patients diagnosed with MDD[33]. Further, the criteria for MDD do not include information regarding the length of the current episode or the psychosocial situation of the patient. DSM-5 has the diagnosis of "adjustment disorder" for excessive psychological symptoms in response to a stressor, and "persistent depressive disorder" for depressions lasting more than two years. However, an individual under a large degree of environmental stressors and with more than one year of continuous depressive symptoms could qualify for a MDD diagnosis as could an individual under no stress and with a sudden onset of symptoms two weeks prior to the diagnosis.

Following from this it can seem unrealistic to believe that anything meaningful can be detected in studies with about 20 participants, a typical sample size for a clinical PET study.

A counterargument to this is that even if both the cause and the symptoms of the disorder differ between individuals, it could well be that there exists a common biological underpinning somewhere in the causal path for a large majority of patients with MDD.

In the two clinical studies in this thesis, we have tried to mitigate the problem with heterogeneity; in study II by including only severely ill patients requiring hospitalization, and in study III by only including patients with recurrent disease, i.e., with at least one prior episode of MDD.

1.3 POSITRON EMISSION TOMOGRAPHY

In contrast to X-ray radiography, where electromagnetic radiation is produced and projected from the scanner and detected on the other side of the body, PET functions through injecting a radiolabeled medical compound (radioligand), usually a drug that binds to a specific protein. The scanner then passively detects the radiation emitted from the body. Clinically PET, and its close cousin, single photon emission tomography, is mainly used to locate increased metabolism (e.g., malignancies, inflammatory disease)[34], but more recently also in the diagnosis of Parkinson's disease and Lewy body dementia[35]. In brain research, PET is among other things used to study the molecular underpinnings of neurological and psychiatric diseases and in the development of new drugs[36].

Hitherto, radioligands have been developed targeting around 50 different proteins with varying function (e.g., transporters, enzymes, receptors) in the human brain[37]. In theory, a radioligand could be developed to study almost any biological process in the body. The next radioligand could putatively hold the key to unlock schizophrenia, or migraine, or any other disorder. This virtually endless well of possible discoveries stands in sharp and important contrast to most other brain imaging modalities.

1.3.1 The PET examination

In a typical PET experiment, the radioligand is intravenously injected in tracer dose (i.e., a very low dose, to avoid significant occupancy and pharmacological effects[38]) into the research subject. After passage over the blood brain barrier, the radioligand binds to its target molecule. The two most used isotopes in PET radioligands are Flour-18 (^{18}F) and Carbon-11 (^{11}C), both with short half-lives (about 110 min and 20 min, respectively). When the isotope decays, it emits a positron that travels a short distance (typically <1mm) before it meets its antiparticle, an electron, causing the annihilation of both the electron and the positron and producing two high energy (511 keV) gamma photons moving in approximately opposite directions.

In the PET scanner, scintillation crystals detect gamma photons. Several rings of detector units surround the head of the participant. When two crystals simultaneously (i.e., within a few nanoseconds) detects a photon it is called a coincidence. When this happens, the system records a line of response (figure 1). A coincidence can either be caused by a "true", "random" or "scatter" event. A random event is when, by chance, one photon from each of

two different decaying positrons hit detectors simultaneously; scatter is when a photon changes direction on the way to the detector ring due to interaction with other particles. Both random and scatter events add noise to the data; this is addressed through automated statistical processing in the reconstruction step, where the registered data is converted to a 3D image.

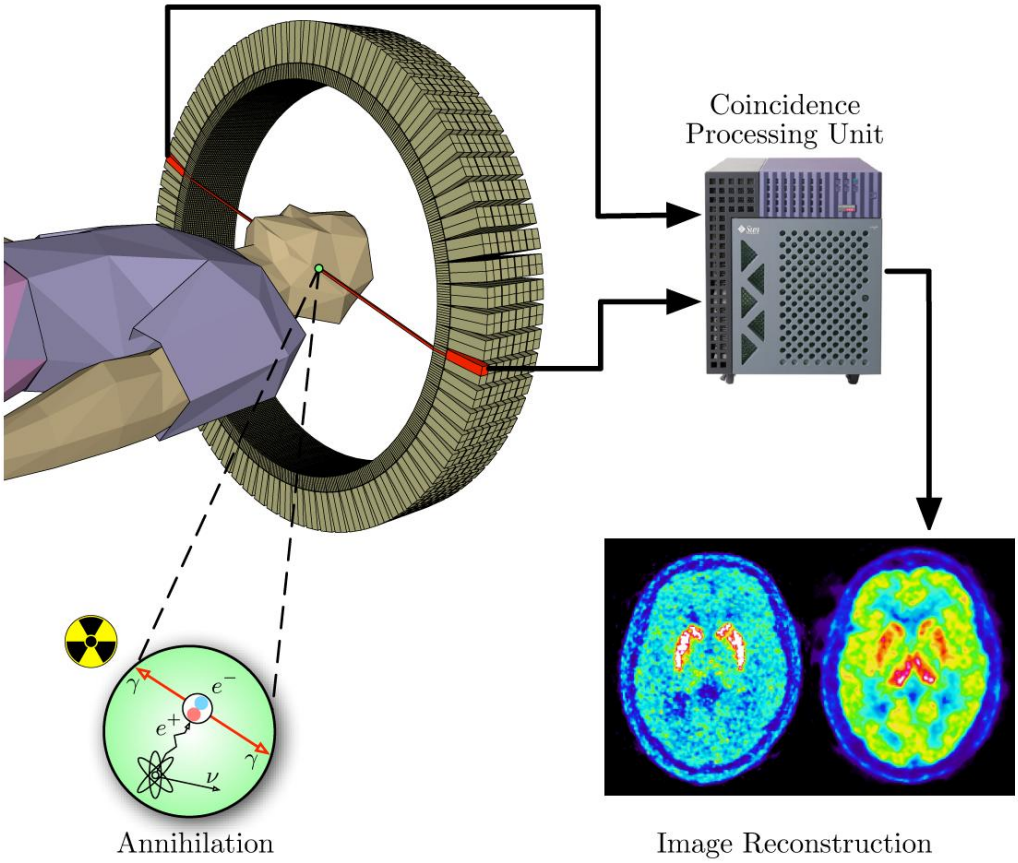

Figure 1. *The positron meets an electron and is annihilated, resulting in two gamma photons traveling in opposite direction. The photons interact with scintillation crystals in the detector ring causing a coincidence and the PET-system registers a line of response (the red line passing through the head in the image). The stored data is reconstructed to a 3D image. Modified image from the public domain (created by Jens Maus).*

1.3.2 Quantification of PET-data

When nuclear imaging is used in the clinic (e.g., to look for cancer metastasis), quantification of the radioligand binding is not always necessary. In brain research, however, commonly groups of individuals are to be compared, making binding quantification a necessity.

The information contained in PET data for a given examination in a given brain region is the total radioactivity concentration (denoted C_T, commonly expressed as kBq/mL) over time. This value will vary depending on several factors aside from the density of the protein that is to be quantified (e.g., injected dose, body mass, rate of radioligand metabolism, etc). To be able to make comparisons between examinations effectively, the data therefore needs to be

normalized. An easy, albeit error-prone, way to do this is through calculating a Standardized Uptake Value (SUV) where C_T is divided by injected dose and body mass. A more reliable method is to calculate the ratio of C_T to some reference concentration. If arterial blood is collected during the examination, the volume of distribution (V_T) can be calculated. Conceptually, V_T can be thought of as the amount of plasma units needed (at equilibrium) to reach the same radioactivity as one unit of tissue. For example, a V_T of 5 in a brain region of 1 cm^3 means that a volume of 5 cm^3 plasma would be needed to reach the same amount of radioactivity as what was recorded in the brain tissue[39].

Quantification methods using arterial blood as an input function require relatively few assumptions and are commonly considered gold standard in PET quantification; however the process of gathering this data is cumbersome, uncomfortable for the research subject, and adds to the sources of potential measurement error. It is therefore common to apply reference tissue quantification whenever possible. A valid reference region should be devoid of the protein that is quantified (i.e., no specific binding of the radioligand) and the non-specific uptake should be the equal to the target region. The typical outcome measure when applying reference tissue quantification is BP_{ND}, the binding potential with respect to non-displaceable uptake. BP_{ND} is the ratio of the concentration of specifically bound (C_S) to non-displaceable radioligand (C_{ND}) at equilibrium:

$$BP_{ND} = \frac{C_S}{C_{ND}} \quad (1)$$

C_{ND} is defined as the combined uptake from free and non-specifically bound radioligand. The term non-displaceable is derived from the fact that, in contrast to specifically bound radioligand, free and non-specifically bound radioligand is not "displaced" by a drug that competes at the binding site. Just based on the PET measurement from the target region (i.e., C_T), there is no reliable way to disentangle what part of the registered radioactivity is due to C_{ND} and what is due to C_S. However, using a reference region as a surrogate for the C_{ND}, BP_{ND} can be calculated according to (at equilibrium):

$$BP_{ND} = \frac{C_T^{target} - C_T^{reference}}{C_T^{reference}} \quad (2)$$

BP_{ND} calculated using reference tissue methods can thus be described as "how many percent more radioactivity is measured in the target region compared to the reference region". A reference tissue generated BP_{ND} of 0.5 means that there is 50% more radioligand present in the target region compared with the reference region, and, if all assumptions hold, that there is twice as much non-displaceable as specifically bound radioligand in the target region. BP_{ND} is unitless and the value holds no interesting biological information in itself, but derives meaning from the fact that it is proportional to the density of the protein that is to be quantified.[39]

1.3.3 A short note on consequences of violations of the assumptions of reference tissue methods

To appreciate the results of study I, it is helpful to understand two points on how the BP_{ND} estimate is affected when assumptions underlying reference tissue quantification are violated: (1) If the reference tissue uptake is lower than the true non-displaceable uptake in the target region, then the BP_{ND} estimate will be biased upwards, while the opposite effect (false low BP_{ND}) will be the result if the reference uptake is higher than the true non-displaceable uptake. (2) If the ratio of specific binding to non-displaceable uptake is low in the target region (i.e., a low "true" BP_{ND}), then small violations of the reference tissue assumptions will have a big effect on the BP_{ND} estimate (Box 2 for an example). Consequently, it becomes even more important to check the validity of the BP_{ND} estimates in low binding regions before interpreting results from these regions. See Salinas[40], for an in-depth discussion of violations of reference tissue models.

Box 2. To illustrate the difference in consequences of violation of reference tissue assumptions between low and high binding regions, consider 10% lower tissue radioactivity concentration in the reference region (C_T^{ref}=0.9) compared with non-displaceable uptake in the target region (C_{ND}^{target}=1), in two cases: 1) specific binding (C_S^{target}), is 0.1, and 2) where C_S^{target} is 5.

Case1:

$$BP_{ND}^{true} = \frac{C_S^{target}}{C_{ND}^{target}} = \frac{0.1}{1} = 0.1$$

$$BP_{ND}^{observed} = \frac{C_T^{target} - C_T^{ref}}{C_T^{ref}} = \frac{1.1 - 0.9}{0.9} = 0.22$$

Case 2:

$$BP_{ND}^{true} = \frac{C_S^{target}}{C_{ND}^{target}} = \frac{5}{1} = 5$$

$$BP_{ND}^{observed} = \frac{C_T^{target} - C_T^{ref}}{C_T^{ref}} = \frac{6 - 0.9}{0.9} = 5.7$$

In case 1, the observed BP_{ND} is 120% higher than the true value, while, in case 2, the observed BP_{ND} is 14% higher. The same absolute difference in non-displaceable uptake between target and reference will have larger consequences in low binding target regions.

1.3.4 Validity and reliability of radioligand binding estimates

As part of the development of a new radioligand validity and reliability of the binding quantification must be assessed. A visual representation of the classical interpretation of validity and reliability can be seen in figure 2. In short, a measuring method is valid if it measures what it purports to measure (i.e., it has a low degree of bias)[41]; it is reliable if a result can be reproduced when the measurement is repeated under the same conditions.[42]

In the field of PET, validity is usually defined as whether, and to what extent, a radioligand binds to the protein of interest. A common approach to test the validity of a radioligand is through a pharmacological challenge, in which PET experiments are performed before and after pretreatment with a competitor, i.e., a drug binding to the same target as the radioligand, but from a different chemical class. Reduced binding after pretreatment is a sign of validity. Reliability is usually interpreted as repeatability, i.e. to what degree do we achieve similar results when the same phenomenon is measured more than once. In this context, the center of the arrowboards in figure 2 can be viewed as the true underlying quantity and the blue crosses as repeated measures. This is commonly evaluated using a test-retest design in which a group of individuals are examined twice with the same radioligand. The variability of the measurement, both between and within individuals, is evaluated[42].

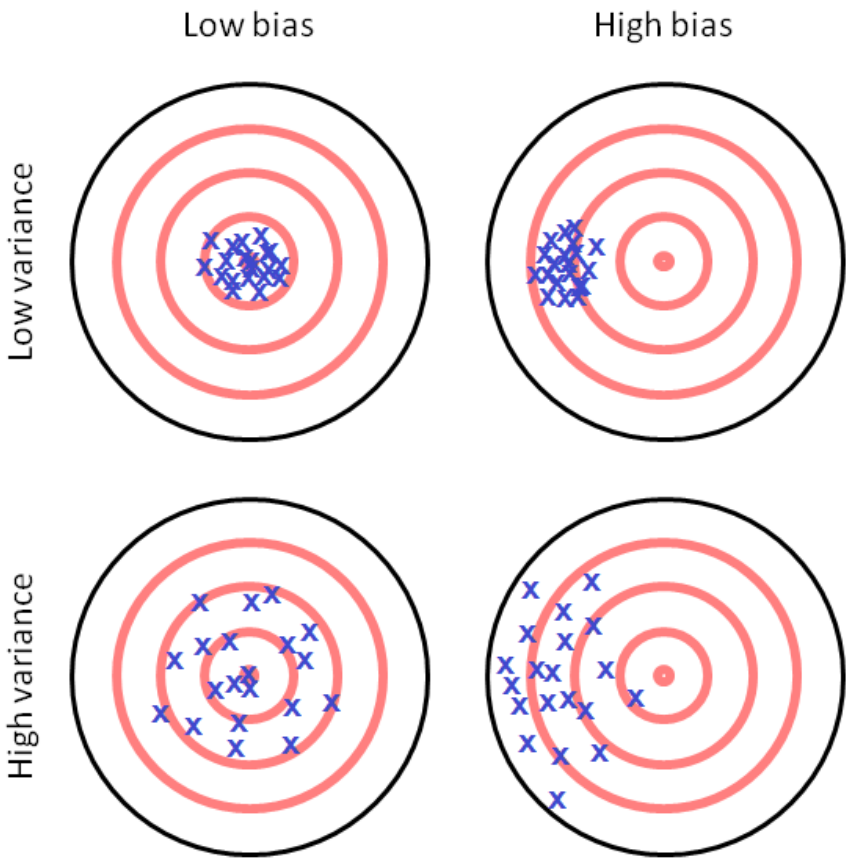

Figure 2. *A measure can deviate from the "true" score in two ways. Either through a systematic deviation (i.e., bias) from the true score (left panels vs. right panels), or through a high degree of variance (upper panels vs. lower panels).*

1.4 BRAIN NEUROTRANSMISSION

The cells that make up the human brain can be sorted in two categories: glial cells and neurons[43]. Glial cells produce no electrical impulses, and their main function is to support the neurons[44]. Neurons are structurally and functionally diverse and complex cells that transmit electrical signals (action potentials) and communicate with other cells through converting the action potential to a chemical signal[45]. The point of communication is called a synapse, and there are many synapses in the brain. It has been estimated that the about16 billion neurons[43] in the human cerebral cortex each has 7000 synapses[46], totaling 0.15 quadrillion synapses, or 10^{12} (one million times one million) per cm^3 [47]. In the synapse, the presynaptic neuron releases a molecule (neurotransmitter) that interacts with receptors on the postsynaptic neuron (figure 3)[48]. The chemical transmission can also occur through volume transmission, where the neurotransmitter diffuse some distance after release and interacts with extrasynaptically located receptors[49].

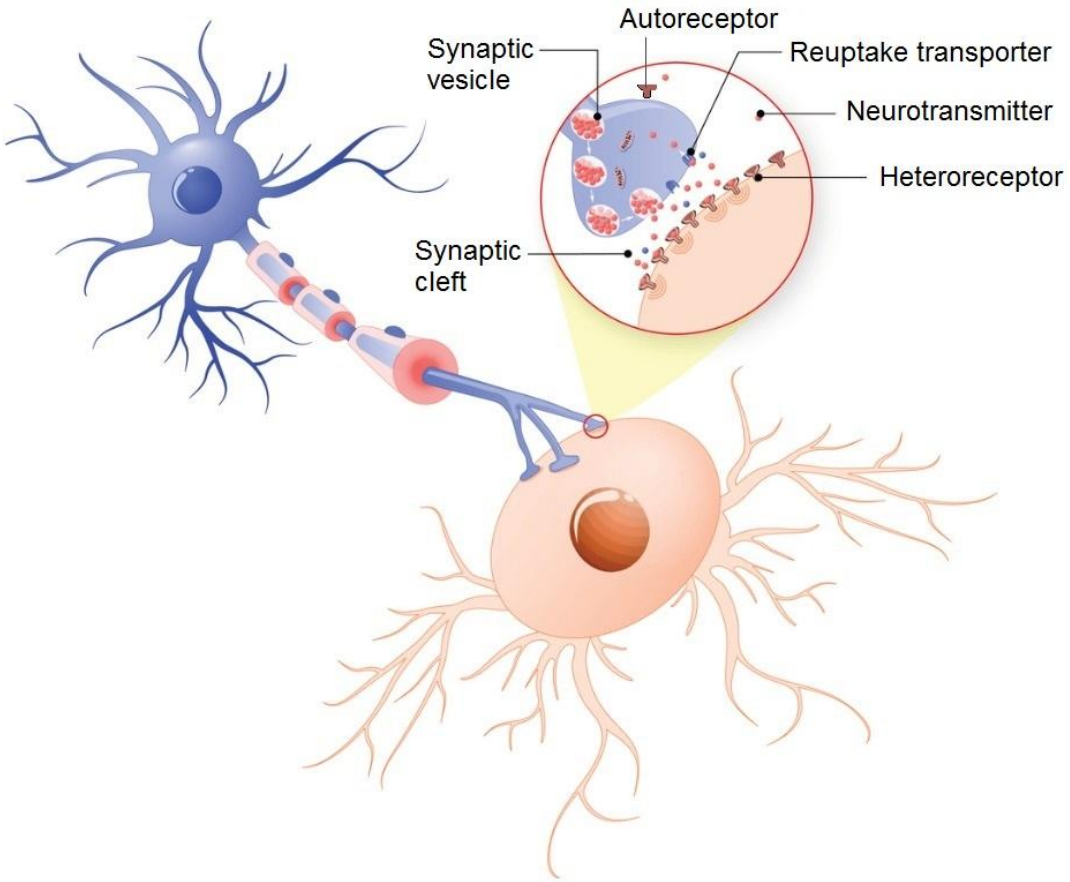

Figure 3*. Schematic image of a synapse. Neurotransmitter is released to the synaptic cleft from synaptic vesicles in the presynaptic neuron. The neurotransmitter interacts with receptors, either on the postsynaptic neuron (heteroreceptors) or on the presynaptic neuron (autoreceptors). The signal is terminated through transport back into the presynaptic neuron via a reuptake transporter. Source: Modified image purchased at istockphoto.com*

Two broad families of receptors are present in the central nervous system: metabotropic and ionotropic receptors[45,50]. Upon activation by a neurotransmitter, a ionotropic receptor opens a linked ion channel allowing the influx of ions to the cell, whereas a metabotropic receptor triggers a second messenger system (e.g., through activating a G-protein) within the neuron[45].

Neurons are commonly classified according to the (main) neurotransmitter they produces. The vast majority of neurons in the brain produce either *gamma*-Aminobutyric acid (GABA) or glutamate[48]. The effect of GABA neurotransmission is usually inhibitory (decreasing the chance of an action potential occurring) and for glutamate excitatory (increasing the chance of an action potential occurring). However, the effect of any neurotransmitter depends on the type of receptors on the post-synaptic neuron.

1.4.1 Monoamine neurotransmitters

The monoamines constitute a functionally important group of neurotransmitters. With regard to psychiatric and neurological disorders, the three monoamines dopamine, serotonin and norepinephrine are most relevant[48]. In this thesis, the serotonin and dopamine systems are studied.

Following synthesis (for dopamine from the amino acid tyrosine and serotonin from tryptophan), the neurotransmitters are loaded into synaptic vesicles through the vesicular monoamine transporter 2. After depolarization of the presynaptic neuron, the vesicle is released to the synapse[48]. For both dopamine and serotonin, a large part of the chemical transmission has been suggested to occur through volume transmission[49]. The primary mechanism of termination of the signal is through active transport back into the presynaptic neuron; for dopamine through the dopamine transporter, and for serotonin through the 5-HTT. Both neurotransmitters are catabolized to inactive metabolites by enzymes, monoamine oxidase A and B, and for dopamine also by catechol-O-methyltransferase[48].

1.4.2 Dopamine

The dopamine system in man consists of around 400.000 neurons with the cell bodies mainly situated in substantia nigra and the ventral tegmental area in the midbrain, with projections to cortex, limbic structures and basal ganglia.[51] The relatively small number of dopamine neurons (less than 0.005 ‰ of the total amount of neurons[43]) does not stand in relation to their functional importance. Dopaminergic neurotransmission is, among other things, central to functions like movement and reward[52,53] and is implicated in the pathophysiology of both neurological (e.g., Parkinson´s disease[54]) and psychiatric disorders (e.g., mood disorders, schizophrenia[55] and substance abuse[56]).

1.4.2.1 Dopamine receptors

There are five subtypes of dopamine receptors described, all metabotropic and G-protein coupled[48]. They are typically divided into two groups, dopamine 1-like receptors and dopamine 2-like receptors (D_2R)[57]. Of the two, D_2R is the variant most implicated in the pathophysiology of psychiatric disorders and is the most studied receptor system with PET[36].

1.4.2.2 Dopamine and depression

Postmortem studies of patients who suffered from depression have shown reduced levels of dopamine metabolites in cerebrospinal fluid (CSF) and in brain striatal brain regions compared with healthy controls.[58] Postmortem receptor data are more contradictory with some studies showing higher density of dopamine receptors in striatum and amygdala[59–61]. Studies applying in vivo PET have mainly focused on striatum where patients have had either decreased binding[62], increased binding[63], or no significant difference, compared with controls[64]. A possible limitation of these studies is that only out-patients were included, a group that usually displays less severe symptoms compared to hospitalized patients. Psychomotor retardation and parkinsonism – symptoms well known to be associated with dopamine transmission – are more pronounced in patients with more severe depression[65].

1.4.3 Serotonin

Serotonin is a neurotransmitter found in most living things, including plants and algae[66,67]. In man, it is most abundantly found in the gut where it regulates digestion[68]. Similar to the dopamine system, the amount of serotonin neurons located in the central nervous system is vanishingly small relative to the total number of neurons[67], but, as is the case with dopamine, this does not mirror their functional importance. Serotonergic neurotransmission has been shown to affect body temperature, mood, sleep, eating behavior, sexual behavior, cognition and pain[67,68], and has been implicated in a large number of brain disorders, e.g., migraine, depression, schizophrenia and autism[67]. The cell bodies of serotonin neurons are mainly located in the raphe nuclei in the brainstem from where they project to a broad array of cortical and subcortical regions[67].

1.4.3.1 Serotonin receptors and transporter

Presently, 14 variants of serotonergic receptors are described; they are classified into seven families (5-HT1–7) based on their molecular and biological characteristics[67,69]. Except for the ionotropic 5-HT3 receptor, all serotonin receptors are metabotropic and G-protein coupled[70]. The receptors that have most often been implicated in psychiatric disorders are the inhibitory serotonin 1A and 1B and excitatory 2A receptors (5-HT1A; 5-HT1B and 5-HT2A)[45,71,72]. 5-HT1A and B function both as heteroreceptors and as autoreceptors regulating neurotransmission[48,73] (figure 3). Upon binding to a 5-HT1A or B autoreceptor, serotonin inhibits formation of the second messenger cyclic adenosine, thereby decreasing release of serotonin to the synapse[71]. The serotonin transporter, 5-HTT, is the main route of clearing serotonin from the synapse[48]. In the human brain, 5-HTT is only expressed in serotoninergic neurons[67].

1.4.3.2 Serotonin and depression

A number of findings support the role of serotonin in MDD: serotonin metabolite levels in CSF of MDD patients has been shown to be decreased compared to controls[20]; further, depletion of tryptophan, the precursor to serotonin, has been shown to induce lower mood in

MDD patients in remission[74]; and, importantly, the symptoms of depression are alleviated when patients suffering from MDD are treated with pharmacological agents known to increase cerebral serotonin levels[4,19]. In the study of depression using in vivo PET, 5-HTT it the protein that has been most extensively researched. Though individual results differ, meta-analyses indicate reduced 5-HTT availability in patients with MDD[75,76]. For 5-HT1B only two small (both n=10) patient-control PET studies have been published, both showing low 5-HT1B availability in the brains of patients compared to controls[77,78].

2 AIMS

In this thesis, PET methods were validated (study I) and applied to study aspects of the serotonin and dopamine systems in major depressive disorder. This was done through patient and control comparisons (study II and III), by examining correlations between key serotonin proteins (study IV), and through a longitudinal approach testing patients before and after treatments with known efficacy (study II and III).

The specific aims were as follows:

In study I, the validity and reliability of quantification of the radioligand [^{11}C]raclopride outside of striatum was evaluated.

In study II, dopamine D_2 receptor density was compared between healthy controls and patients with severe depression, and patients before and after electroconvulsive treatment.

In study III, brain serotonin transporter density was compared in healthy controls and patients with depression, and in patients before and after cognitive behavioral therapy.

In study IV, the correlation between serotonin transporter availability and serotonin 1B receptor availability was evaluated in the brains of healthy individuals.

3 MATERIAL AND METHODS

All studies were approved by the Ethics Committee of the Stockholm Region, and the Radiation Safety Committee of the Karolinska University Hospital. All subjects gave their written and verbal informed consent before initiation of study procedures.

3.1 STUDY SUBJECTS

All subjects were healthy according to: clinical interview, magnetic resonance (MR) imaging of the brain, physical examination, routine blood tests, and urine drug screen both at inclusion and at the day of PET examinations. Aside from a diagnosis of MDD for patients in study II and III, the subjects had no history of diseases involving the central nervous system, or any history of alcohol use disorder or drug addiction.

Subjects in study I consisted of two cohorts, the healthy controls from study II (see below), and eleven healthy male subjects (25±2.5 years, mean±SD) that participated in a previously published study[79]. Patients in study II (n=9; 3 males; 48±11 years) were recruited at psychiatric wards in Stockholm, Sweden. The controls (n=9; 3 males; 51±12 years), matched for age and sex, were recruited via advertisement in local newspapers. In study III, patients (n=17; 4 males; 47±13 years) and healthy control subjects (n=17; 4 males; 47±14 years), matched for age, sex and intellectual ability were recruited by advertisements in local newspapers. The healthy controls from study III also participated in study IV.

3.2 STUDY INTERVENTIONS

3.2.1 Quetiapine

Quetiapine is a multimodal drug with D_2R antagonist properties[80]. The drug is used in psychiatric care to treat various conditions, including psychotic disorders, bipolar disorder and as an adjunctive treatment to MDD[81]. In study I, PET-data from a previously published occupancy study of quetiapine was reanalyzed to evaluate D_2R competition of [^{11}C]raclopride binding in extrastriatal brain regions.

3.2.2 ECT

In study II, hospitalized patients with severe depression were treated with ECT. See section 1.2.3.2 for an overview of ECT.

3.2.3 Internet-delivered cognitive behavioral therapy (ICBT)

In study III, patients with MDD were treated with ICBT. The main component of the ICBT treatment program is 10 text modules covering specific themes such as psychoeducation and behavioral activation. The modules end with a home assignment. All participants are assigned an online psychologist with thorough training in CBT. The psychologist supervised the progress and provided individual feedback on home assignments. The standard duration of

treatment is 12 weeks. ICBT has been found to be as effective as face-to-face CBT, and is now a standard treatment option[82]. The ICBT treatment protocol applied in study III has been tested in randomized trials[83], and has been shown to be effective in clinical practice [84].

3.3 PET EXAMINATIONS

The PET examinations in all studies were performed using a High Resolution Research Tomograph (HRRT; Siemens Molecular Imaging, USA) with a maximum spatial resolution of about 2mm full-width-half-maximum[85].

Before the PET examination, a plaster helmet was made for each subject and used with a head fixation device in order to minimize head movement in the scanner [86]. Transmission scans were performed prior to each PET examination in order to correct for signal attenuation. In each PET experiment, a saline solution containing the radioligand was injected into an antecubital vein as a bolus (<10 seconds). The cannula was thereafter immediately flushed with 10 mL saline. Brain radioactivity was recorded continuously for 51–93 minutes depending on the radioligand applied. The resulting data was reconstructed in 3D and binned into consecutive time frames.

3.3.1 Applied Radioligands

3.3.1.1 [^{11}C]raclopride

The dopamine D_2R binding radioligand [^{11}C]raclopride was developed in the 1980s[87] and is one of the most frequently used PET radioligands in brain research to date. Due to its relatively low affinity for D_2R (Kd = 1.3 nM), [^{11}C]raclopride has primarily been used to study receptor availability in striatal regions. The validity and reliability for quantification of [^{11}C]raclopride binding in striatum is well established[88–90]. Extrastriatally, however, where the concentration of D_2R ranges from 1–10% of that in striatum[91], the data is sparse. [^{11}C]raclopride was used in study I and II and was prepared as described previously[92].

3.3.1.2 [^{11}C]MADAM

[^{11}C]MADAM was developed at Karolinska Institutet's PET group in the early 2000s. It is a radiolabeled selective 5-HTT inhibitor, N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine (MADAM), that binds selectively and reversibly to 5-HTT[93]. [^{11}C]MADAM is similar both in chemistry and pharmacokinetic properties to [^{11}C]DASB, a more widely used 5-HTT radioligand. [^{11}C]MADAM was prepared by methylation, using [^{11}C]methyl triflate, as previously described[93], and used for quantification of 5-HTT in studies III and IV.

3.3.1.3 [^{11}C]AZ10419369

The 5-HT1B receptor radioligand [^{11}C]AZ10419369 was developed as a joint venture between AstraZeneca and Karolinska Institutet's PET group[94]. [^{11}C]AZ10419369 binds selectively to the 5-HT1B receptor and has shown good test-retest reliability[95,96].

[^{11}C]AZ10419369 was prepared by methylation, using [^{11}C]methyl triflate, as previously described[97] and was used for quantification of 5-HT1B receptors in study IV.

3.3.2 Image processing

T1-weighted MR images were acquired using a 1.5 Tesla (part of studies I and II), or a 3 Tesla (studies III and IV and part of studies I and II) GE Signa system (GE Medical Systems, USA).

3.3.2.1 Image preprocessing

The dynamic PET images were corrected for head motion using a between-frame-correction algorithm implemented in Statistical Parametric Mapping (SPM, Wellcome Department of Cognitive Neurology, University College, London, UK). Using SPM the T1-weighted MR images were then co-registered to a summated PET image. To derive regional time-activity curves (TACs), the resulting co-registration matrix was used to project regions of interest (ROIs) on the realigned dynamic PET-image.

3.3.2.2 Regions of interest

For all studies, FreeSurfer (version 6.0, http://surfer.nmr.mgh.harvard.edu/)[98] was applied to the MR images to generate the reference tissue ROI. In studies I, III and IV, the FreeSurfer segmentation was also used to define the ROIs included in the analysis.

In study II, ROIs were defined using an atlas of functional, connectivity-based, subdivision of striatum[99]. The atlas was projected to the dynamic PET data in two steps: first via transformation to each subject's MR image using the FLIRT and FNIRT functions in FSL5 (FMRIB Software Library v5.0), and then via co-registration to the summated PET image, using the MR-to-PET co-registration matrix.

3.3.2.3 Quantification

Quantification using reference tissue methods were applied in all studies. Cerebellar gray matter was used as reference since negligible amounts of specific binding have been observed here for all three radioligands[91,97,100,101]. In all studies, BP_{ND} was used as the parameter of interest.

In studies I and II, the simplified reference tissue model (SRTM)[102] was applied. SRTM was originally developed to improve reference tissue quantification of [^{11}C]raclopride binding and has been shown to often produce good fits with stable parameter estimation of kinetic PET data[103]. In addition to the assumptions underlying all reference tissue models (see section1.3.2), the SRTM assumes that radioligand kinetics can be represented by a one-tissue model in both reference and target tissue[40].

The Logan graphical analysis is a linear method for quantification of PET data[104] requiring no assumptions of compartment kinetics. In studies III and IV, a multilinear version of the non-invasive Logan model was used to calculate BP_{ND}[105]. The model requires the time at which

the linear phase starts (t*) as input. For [^{11}C]MADAM, t* was set to 45 minutes, corresponding to 8 frames; for [^{11}C]AZ10419369 t* was set to 33 minutes, corresponding to 10 frames. The reference region efflux rate constant (k2') is also needed as input, either in the form of a population average, or from each individual. In studies III and IV the k2' was derived using the SRTM in representative regions for each individual.

Parametric images were generated using the 3D stationary wavelet-aided parametric imaging (WAPI) procedure, where the non-invasive Logan plot, fitted with multilinear regression, is applied on TACs from individual voxels[106].

3.4 STATISTICS

In study I, a paired one sided *t*-test was used to evaluate differences in regional BP_{ND} values obtained at baseline and after quetiapine administration. Absolute variability (VAR) and intraclass correlation coefficient (ICC) was used to evaluate test-retest reliability (see below).

In study II, a repeated measures, between-group analysis of variance was used to test the difference between groups over time (i.e., if ECT treatment in patients with MDD changed [^{11}C]raclopride binding when compared with controls measured twice). Paired two sided *t*-tests were used to test for baseline differences between patients and controls. Pearson´s correlation coefficients were calculated to assess associations between variables. Effect sizes were quantified using Cohen's d_z, i.e., a paired version of the classical Cohen's *d* where the standard deviation of the difference score is used as denominator[107].

In study III, paired t-test was used to assess differences in [^{11}C]MADAM BP_{ND} within patients before and after CBT (two sided), as well as between patients at baseline and their matched control subjects (one sided). A multilevel model for repeated measures was used to assess change in the score of self rated Montgomery Asberg Depression Rating Scale (MADRS-S)[108,109], used as a marker for depression symptom load. To test for an association between change in MADRS-S and [^{11}C]MADAM BP_{ND}, individual slopes from the MADRS-S multilevel model were entered as an independent variable into a regression model, predicting BP_{ND} from PET2 while controlling for BP_{ND} from PET1.

In study IV, the partial Pearson correlation coefficient was calculated to assess the correlation between [^{11}C]MADAM and [^{11}C]AZ10419369 BP_{ND} in relevant brain regions, while controlling for age. The reason for controlling for age is that the expression of both 5-HTT and 5-HT1B have been shown to decrease with age[110,111].

All statistical analyses and data visualization were performed using R[112].

3.4.1 Multiple comparisons

In study I and IV, no corrections for multiple comparisons were performed due to an exploratory analytical approach in these studies. In study II, Bonferroni correction was applied. In study III, the analysis protocol (both image and statistical analysis) was preregistered and the analyses were clearly marked as "confirmatory" or "exploratory". Two

confirmatory comparisons were made for each of the two research questions tested. In the longitudinal analysis, no correction was made since there was a strong correlation between the compared variables. In the cross-sectional analysis, a Bonferroni correction was applied.

3.4.2 Test-retest metrics

3.4.2.1 Absolute variability (VAR)

$$VAR = \frac{|BP_{ND}^{PET1} - BP_{ND}^{PET2}|}{\frac{1}{2}(BP_{ND}^{PET1} + BP_{ND}^{PET2})} \times 100 \quad (3)$$

VAR is a measure of the absolute reliability of a measurement expressed as a percentage of the average BP_{ND} value. PET1 refers to the first PET measurement, and PET2 refers to the second PET measurement. The reported value is the average VAR for all subjects.

3.4.2.2 Intraclass correlation coefficient (ICC)

$$ICC = \frac{MS_B - MS_W}{MS_B + MS_W} \quad (4)$$

where MS_B denote the between-subjects mean sum of squared variance and MS_W the within-subject mean sum of squared variance. ICC normalizes the measurement error to the between-subject variance and will give information on how well a test can distinguish between individuals. The score can vary between 1 and -1, values closer to 1 indicate that most of the variance is due to between-subject rather than within-subject variation [113].

4 RESULTS AND COMMENTS

4.1 STUDY I: VALIDITY AND RELIABILITY OF EXTRASTRIATAL [^{11}C]RACLOPRIDE BINDING QUANTIFICATION IN THE LIVING HUMAN BRAIN

Very little is published on the validity of [^{11}C]raclopride binding quantification outside of the striatum. Despite this, binding data for regions with low density of D_2R, e.g., the neocortex, is frequently reported in the scientific literature. The primary aim of study I was to assess the validity and reliability of extrastriatal [^{11}C]raclopride binding quantification. Validity was assessed through reanalyzing an occupancy dataset where eleven healthy subjects were examined with [^{11}C]raclopride PET before and after dosing with an extended (XR) and immediate release (IR) formulation of 300 mg quetiapine, a D_2R antagonist. Reliability was assessed using a test-retest design where a separate sample of nine healthy controls were examined twice with [^{11}C]raclopride PET.

Significant decrease in [^{11}C]raclopride BP_{ND} following pretreatment with quetiapine was observed in all striatal regions, the temporal cortex (TC) and the thalamus, but not in frontal regions (Figure 4 and 5).

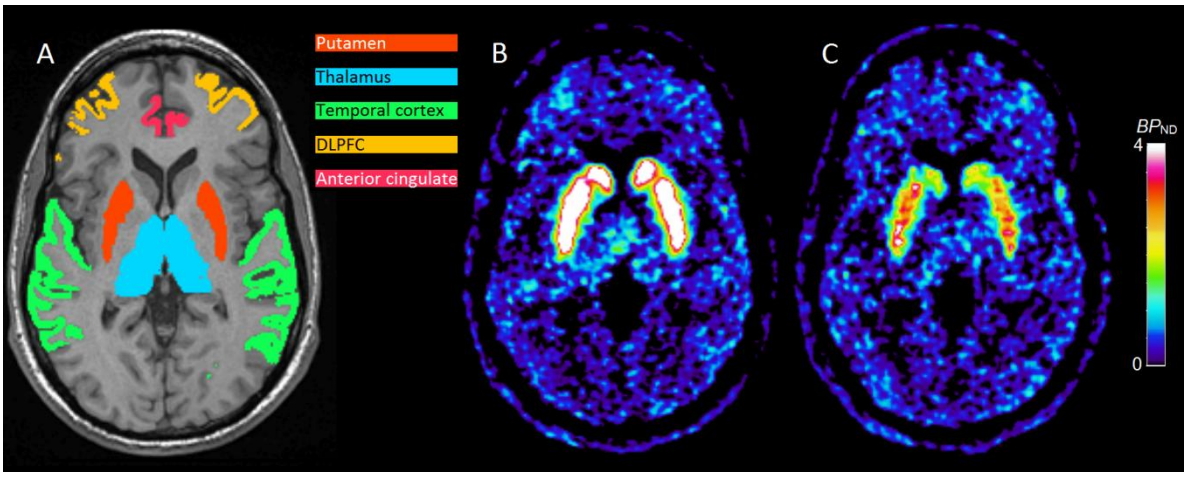

Figure 4. *MRI and parametric PET images for one subject. A) MRI with regions of interest overlaid B) [^{11}C]raclopride binding at baseline C) [^{11}C]raclopride binding after pretreatment with quetiapine. DLPFC, dorsolateral prefrontal cortex.*

In putamen, the percentage decrease (i.e., the occupancy value for quetiapine) of [^{11}C]raclopride BP_{ND} in the IR examination was 50.6±4.1% (mean±SD), in thalamus 19.5±16.6% and in TC 17.8±16.8%. The occupancy in TC and thalamus was significantly lower compared to the occupancy in putamen. The analysis of the test-retest data using equation 3 for calculation of VAR and 4 for calculation of ICC showed a VAR of 3.7% and ICC 0.88 in putamen; in thalamus 16.6% and 0.27, (VAR and ICC respectively); in TC 13.8% and 0.7; in dorsolateral prefrontal cortex 26% and 0.81.

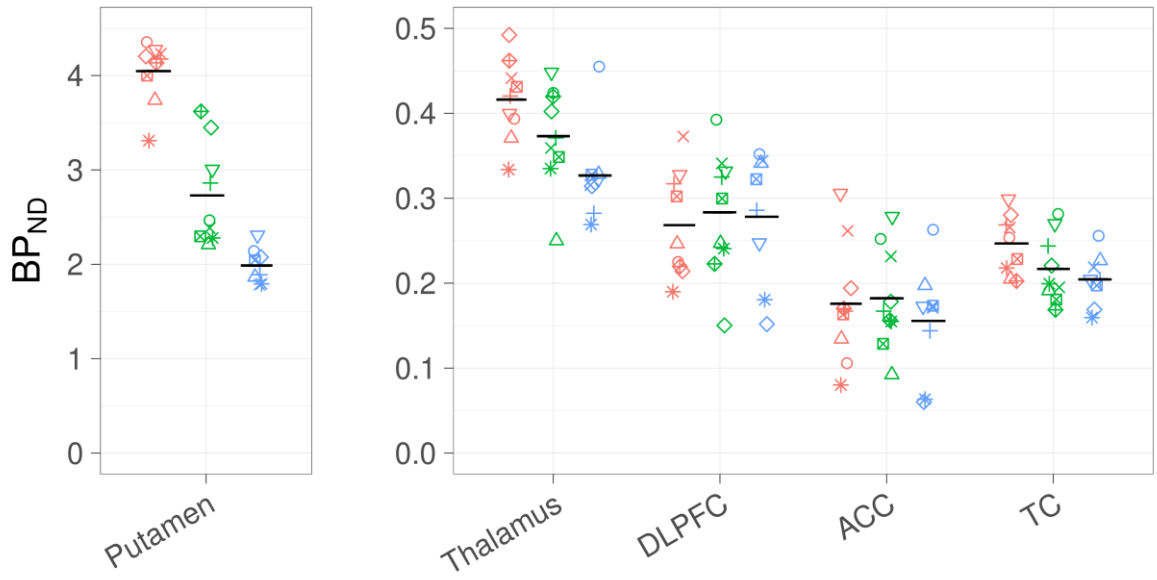

Figure 5. *BP_{ND} data. For each ROI three PET examinations were analyzed. From left to right: Baseline (red); after Quetiapine XR pretreatment (green); after Quetiapine IR pretreatment (blue). Horizontal bars represent mean BP_{ND}. Each shape represents one subject. DLPFC, dorsolateral prefrontal cortex; ACC, Anterior cingulate cortex; TC, Temporal cortex*

The observation of lower occupancy in thalamus and TC compared with striatum, and no significant decrease of [^{11}C]raclopride binding in frontal regions, indicates that some degree of the BP_{ND} estimates in these regions reflect something else than specific binding. Using the arrowboard analogy presented in figure 2, the lower right panel (high bias and high variance) best describes the results we observe in most extrastriatal regions.

4.2 STUDY II: [^{11}C]RACLOPRIDE POSITRON EMISSION TOMOGRAPHY STUDY OF DOPAMINE-$D_{2/3}$ RECEPTOR BINDING IN PATIENTS WITH SEVERE MAJOR DEPRESSIVE EPISODES BEFORE AND AFTER ELECTROCONVULSIVE THERAPY AND COMPARED TO CONTROL SUBJECTS

In study II, [^{11}C]raclopride PET was used to study D_2R in severely depressed patients before and after ECT and in a matched cohort of healthy controls measured twice and receiving no intervention. The main hypothesis tested in this study was that D_2R binding would be different in MDD patients compared to controls. The secondary hypothesis was that ECT would be associated with a change in D_2R binding towards that of the control group.

For two patients, the post-treatment PET examination had to be excluded from analysis for technical reasons. The patient-control comparison was thus performed in nine patients and nine controls, while the longitudinal analysis (before and after treatment) was performed in seven patients and seven matched controls. MDD symptoms were quantified at the time of PET using MADRS.

MADRS at baseline was 35±7 and after treatment 12±4 ($p<0.001$). The analysis of the baseline [^{11}C]raclopride BP_{ND} between patients and controls showed a significantly lower D_2R availability in patients in all three examined regions ($p<0.05$ corrected for multiple comparisons) (figure 6, panel A). The average [^{11}C]raclopride BP_{ND} increased 10–17% in the three regions after ECT, corresponding to Cohens *dz* of 0.37–0.65; however the repeated measures between-group analysis comparing the pre and post PET measurements of patients with controls was not significant (figure 6, panel B).

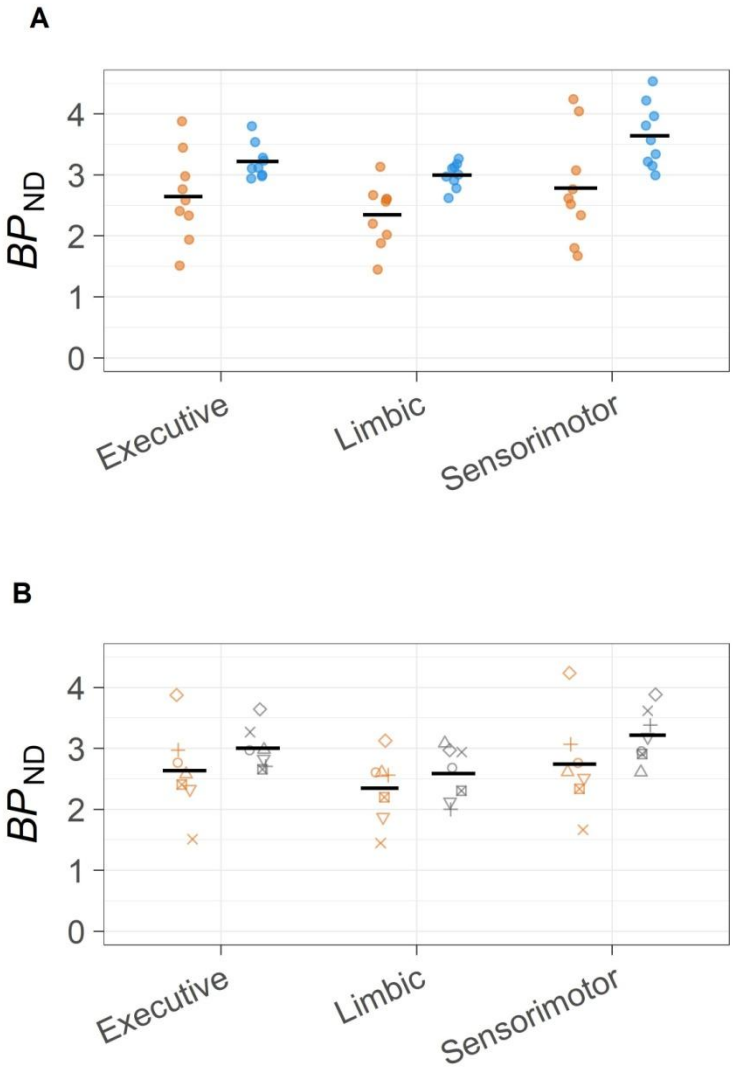

Figure 6. *[^{11}C]raclopride binding potential (BP_{ND}) in the executive, limbic, and sensorimotor striatum in (A) patients with major depressive disorder (MDD) pre electroconvulsive therapy (ECT; red) and control subjects (blue; n = 9) and (B) MDD patients pre- (red) and post-ECT (gray; n = 7; subject identity is indicated by the shape of the marker). Horizontal line indicates mean value.*

The results support the notion that severe MDD is associated with lower D_2R binding in striatum. We noted a non-significant, numerical increase in D_2R binding in the patient group after response ECT.

4.3 STUDY III: SEROTONIN TRANSPORTER AVAILABILITY INCREASES IN PATIENTS RECOVERING FROM A DEPRESSIVE EPISODE

The primary aim of study III was to compare serotonin transporter availability between healthy controls and patients with depression, as well as between pateitents before and after psychotherapy. The longitudinal design, in combination with a treatment that has no known direct effect on the serotonin system, makes it possible to study 5-HTT during the natural course of a depressive episode. Previous molecular imaging studies have shown low cerebral concentration of 5-HTT in MDD patients[75]. Whether or not this also is present before disease onset and after remission (i.e. a trait), or only at the time of the depressive episode (i.e. a state) is a research question possible to address with this design.

Seventeen patients with an episode of MDD were examined twice with [^{11}C]MADAM PET, before and after treatment with ICBT. 17 matched healthy control subjects were examined once with [^{11}C]MADAM PET. Confirmatory analysis of the longitudinal and cross-sectional data were performed in 1) a composite region consisting of eight pre-specified cerebral regions and 2) in the median raphe nuclei. The treatment effect was evaluated using MADRS-S.

The MADRS-S score at start of the CBT treatment was 28±4 and at completion of treatment 15±9, the decrease was significant ($p<0.001$). In the longitudinal PET analysis, [^{11}C]MADAM binding increased on average by 10% in the composite region following CBT ($p = 0.01$). No change was observed in the median raphe. In the cross-sectional (patient-control) comparison, no evident difference in [^{11}C]MADAM binding was observed in the composite region or in median raphe (figure 7).

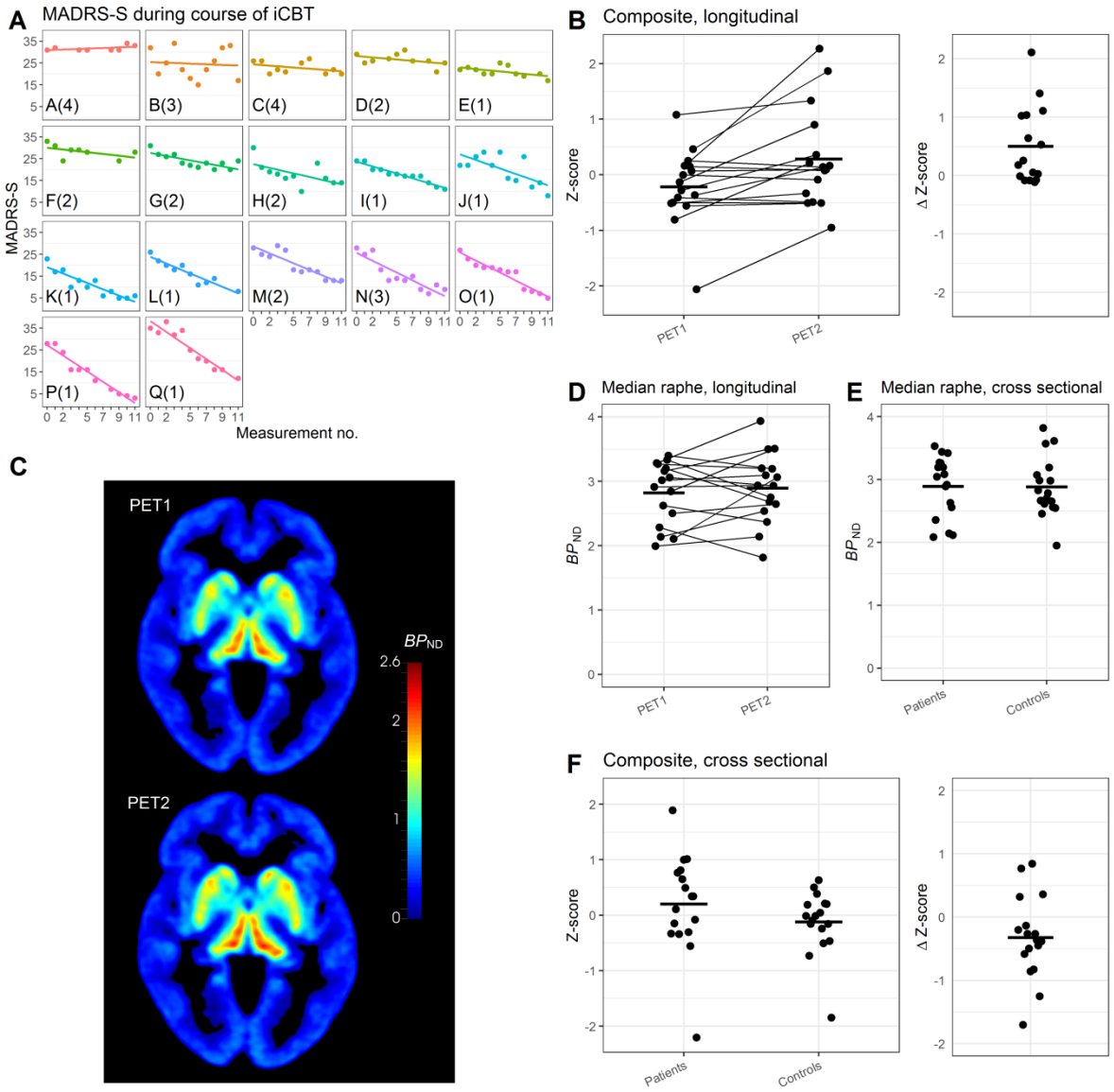

Figure 7. *In (A) MADRS-S measurements during the course of CBT. Each cell represents one patient. In (B) longitudinal [^{11}C]MADAM data, PET1 (baseline) and PET2 (after treatment). Right panel shows difference scores between scans. In (C) mean parametric image of patients at baseline (upper image) and after treatment (lower image). In (D) longitudinal data, median raphe, BP_{ND} at PET1 (baseline, left) and PET2 (after treatment, right). In (E) cross-sectional data, median raphe, BP_{ND} for patients with MDD at baseline (left) and healthy controls (right). In (F) cross-sectional [^{11}C]MADAM data, comparing patients with MDD at baseline to matched healthy controls. Right panel shows difference scores between matched pairs.*

The fact that the control group only performed one PET examination is a limitation of the study design, adding to the list of assumptions needed in order to draw any causal inference from the results. Under the null hypothesis that 5-HTT have no association to either depression or CBT treatment, we would expect no change in [^{11}C]MADAM binding between

PET1 and PET2. Lacking a second measurement in the controls, we can still make the (reasonable) assumption that there should be no systematical change in [^{11}C]MADAM when a healthy control is examined twice with a few weeks apart. This would imply that the observed increase in cerebral [^{11}C]MADAM binding as MDD patients improve in their symptoms is caused either as a direct effect by the CBT treatment or by the alleviation of depressive symptoms.

4.4 STUDY IV: IN VIVO CORRELATION OF SEROTONIN TRANSPORTER AND 1B RECEPTOR AVAILABILITY IN THE HUMAN BRAIN – A PET STUDY

Both the 5-HT1B receptor and 5-HTT are important in regulating cerebral serotonergic transmission, and both proteins have been implicated in the pathophysiology of MDD. In study IV, the primary aim was to investigate whether a correlation was present between 5-HTT and 5-HT1B expression in the brain of healthy individuals.

Seventeen healthy individuals were examined with PET twice the same day using the radioligands (in a random order) [^{11}C]AZ10419369, binding to 5-HT1B, and [^{11}C]MADAM, binding to 5-HTT. BP_{ND} was calculated for a set of ROIs, including median and dorsal raphe, and the correlation between the binding estimates of the two radioligands was calculated using partial Pearson correlation coefficient, controlling for age.

The point estimate of the [^{11}C]AZ10419369 and [^{11}C]MADAM BP_{ND} correlation was positive in all examined brain regions. In most cortical regions, the correlation was strong, e.g., in the temporal cortex, $r(15) = 0.6$, $p = 0.01$ and in the parietal cortex, $r(15) = 0.79$, $p < 0.01$, while in most subcortical regions, the correlation estimate was very weak (figure 8). No clear pattern was discernible in the raphe data.

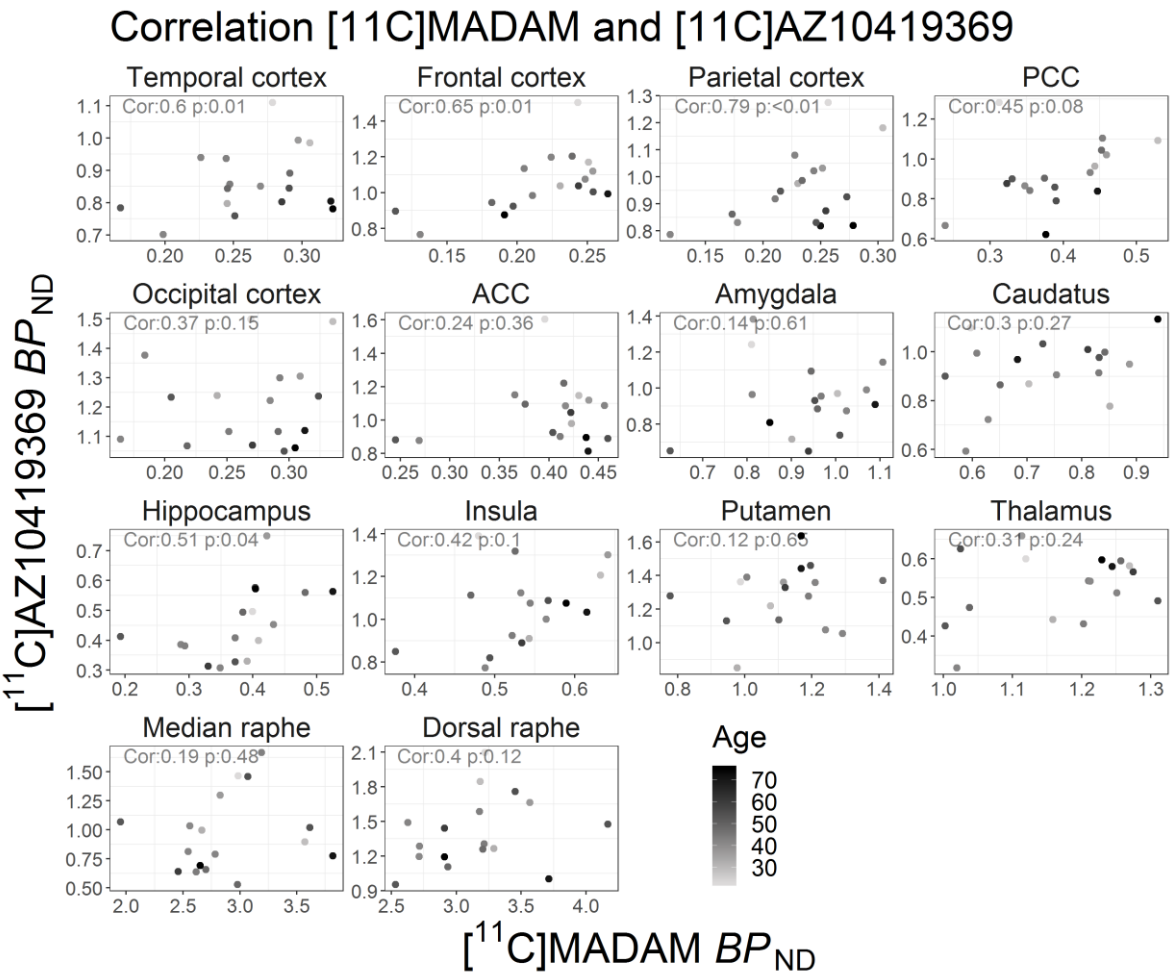

Figure 8. *Correlation between [^{11}C]AZ10419369 and [^{11}C]MADAM BP_{ND} in different brain regions. The reported correlations are controlled for age. To visualize the effect of age lighter grayscale is used for younger subjects and darker for older subjects in the scatter plots. ACC, Anterior cingulate cortex; PCC, Posterior cingulate cortex*

The strong correlation observed in cortical regions must be interpreted with caution. [^{11}C]MADAM BP_{ND} is low in most of the cerebral cortex, with specific binding several times lower compared with the non-displaceable uptake. This means that random variation of non-interesting phenomena (e.g., differences in anatomy of the reference region, small discrepancies in the delineation ROIs) may have a substantial impact on the quantification, and, importantly, could affect the BP_{ND} estimate of both radioligands in the same direction. In support of this interpretation a high correlation (r=0.66, p=0.006) was observed in the centrum semiovale, a brain region only containing white matter. In brain regions with good signal-to-noise for both radioligands, no obvious correlations between the proteins were observed.

5 CONCLUSIONS

Study I: In most brain regions outside striatum, little or no decrease in BP_{ND} was observed after pretreatment with a competitor, lending no support for valid [^{11}C]raclopride binding quantification here. We suggest that a violation of reference tissue assumptions (lower C_{ND} in the reference tissue compared with the target regions), inflating the BP_{ND} estimate in extrastriatal regions, is the most likely cause for our results. Extrastriatal test-retest repeatability was relatively poor in most regions. Our findings indicate that [^{11}C]raclopride PET is not suitable for quantification of D_2R in most regions outside of striatum. At a minimum, our results cannot establish satisfactory validity in extrastriatal regions, and any researcher who wishes to publish studies using [^{11}C]raclopride data in these regions should perform an independent validation study. The results from study I were used when deciding on the analysis plan for study II.

Beyond the implications for [^{11}C]raclopride research, the results in study I raises questions regarding the interpretation of test-retest metrics in PET research. ICC normalizes the measurement error to the between-individual variance. Higher between-individual variance will increase the ICC (indicating better reliability), other things being equal. Our results indicate that extrastriatal [^{11}C]raclopride BP_{ND} is biased due to a violation of reference tissue assumptions. If the size of the bias is not identical between individuals, the between-individual variance will increase. Assuming the bias is stable between measurements, we would expect ICC to be overestimated. The VAR is sensitive to the magnitude of the signal. Given a (stable) bias inflation of BP_{ND}, the VAR will be underestimated (indicating better reliability). There is thus a risk that reliability metrics can indicate a false high reliability due to a lack of validity in the outcome measure. This can create a veneer of credibility for a measurement where little or no connection to the target it purportedly measures exists. Before interpreting reliability metrics of a radioligand, it is therefore important to establish validity. This is especially pertinent in low-binding regions where violations of assumptions in the quantification method will have a disproportionally large effect.

Study II: The role of dopamine transmission in affective disorders is unclear, as is the molecular mechanism of action for ECT, the most effective MDD treatment known. In study II, we used [^{11}C]raclopride PET to quantify striatal D_2R binding before and after a treatment series of ECT in patients hospitalized due to MDD. We also examined a group of matched healthy controls twice. The results showed an association between severe MDD and lower dopamine D_2R binding in striatum, compared with controls. The [^{11}C]raclopride BP_{ND} increased (i.e., in the direction of the controls) after response to ECT. However due to the small sample size, we were powered to detect only large effects, and though the average change in BP_{ND} was substantial (an absolute increase of 10-17%) it was not statistically significant.

Study III: An increase in 5-HTT availability was observed as depressive symptoms were alleviated in patients suffering from a MDD episode. These results suggest a degree of plasticity in the serotonin system with regard to depressive episodes, indicating that previously reported cross-sectional findings of lower 5-HTT binding in patients with MDD compared with controls most likely reflect the depressive state rather than a trait.

A speculative interpretation of the results in study III is that the observed increase in 5-HTT binding could constitute the normalization of a system attempting to compensate for the stress associated with a depressed state. Under this model, environmental stress decreases cerebral 5-HTT availability as (part of) an adaptive response. Some individuals will still develop MDD, after which, hypothetically, either continual environmental stress or the stress inherent to the depressive state will keep 5-HTT levels low. As the patient recovers, either due to spontaneous remission or treatment, e.g., CBT, 5-HTT levels could be expected to increase towards premorbid levels.

Study IV: The analysis plan in study IV was not preregistered and the correlation between 5-HTT and 5-HT1B BP_{ND} was tested in a large number of brain regions in an exploratory approach. We observed strong correlations mainly in cortical regions where 5-HTT density is known to be low. A similarly strong correlation was observed in centrum semiovale. The explanation that these results are driven by an artifact must therefore be considered and the results interpreted with caution. If the results are due to a true underlying correlation between the expression of 5-HTT and 5-HT1B in cortex, this opens up for interesting interpretations. The strictly pre-synaptic 5-HTT and, in cortex, mainly post-synaptic 5-HT1B are synthesized by different neurons. A strong correlation between the expression of the two proteins could therefore be indicative of a robust transsynaptic regulation of the serotonin system in cortical regions.

6 ETHICAL CONSIDERATIONS

From an ethical perspective, the characteristic that sharply distinguish PET studies from other psychiatric research is the injection of radioactivity into the research subjects. Each PET examination can roughly be translated to the radiation dose that a person would get from the background radiation for 18 months living in Sweden. Although the risk of causing any disease is considered minimal, it is impossible to escape the fact that the radiation causes some measure of harm. However, currently PET is the only effective way to quantify a given protein in the brain in vivo. I can see no viable option to answer the research questions we are addressing in the present studies. Given the limited knowledge of MDD, the suffering the disorder imposes on the affected individuals along with the economic burden to society, my belief is that the risks are acceptable.

The PET examination itself is not entirely pleasant. The research subjects must lay still for approximately 100 minutes with the head inside a narrow tunnel. The plaster helmet we use to minimize head movement in the camera can sometimes cause discomfort but is generally well tolerated. Some pain arises from catheterization of a peripheral vein used for injection of radioligand. The full procedure is described in detail to the research subjects prior to them signing the informed consent.

Patients and healthy controls receive a cash payment of SEK 1000–3000 for participation. Other potential benefits include undergoing an MR examination that is reviewed by a neuroradiologist. This, however, is a double-edged sword. Though it is possible to detect disease at an early stage, it is not uncommon to find abnormalities that are clinically unimportant but can cause psychological distress for the subject and possibly lead to unnecessary treatments. During the recruitment for study III, we had a case where signs of previous inflammation in the brain were observed. It may have been caused by some nonspecific disease many years ago that the patient did not know of and that would never have caused any problems, but it may also have been multiple sclerosis where the patient's prognosis can be improved by discovering the disease early.

In study III, we preregistered the analysis plan. Preregistration reduces the researcher’s degrees of freedom which can lower the risk of false positive findings, which is an inherent ethical good.

Looking at the potential impact the studies might have in society, I cannot see any major ethical pitfalls. Politically, the field we investigate is rather uncontroversial (in my opinion). If we find that the 5-HTT, or D_2R, play a role in depression, it is unlikely to directly change any treatment standards or put the concept of depression in a drastically different light. Rather it will be another building block in the effort to close in on an understanding of what goes on in the brain during depression.

7 FUTURE PERSPECTIVES

Regarding study I, the fact that in many cortical regions no decrease was observed in BP_{ND} after administration of a competitor is interesting and warrants further investigation. The explanation we propose for this phenomenon is that most of the BP_{ND} in cortex reflect a bias caused by lower C_{ND} in the reference tissue compared with the target regions. If this is the case then it could be specific to [^{11}C]raclopride, but it is also possible that it is connected to a fundamental difference in the anatomy of cerebellum compared with cerebral regions. E.g., the resolution of today's PET systems is at best about 2mm, whereas the average thickness of the cerebellar cortex is about 1mm[114]. It is thus unavoidable that when cerebellar cortex is used as a reference region, the voxels will contain some amount of white matter and some CSF. The cerebral cortex is about 2.5mm and profoundly less convoluted[114]. Even if C_{ND} is exactly the same in cerebellar and cerebral cortical tissue, it is unlikely that the C_{ND} contribution of radioactivity measured with PET in e.g., dorsolateral prefrontal cortex, will be exactly the same as that in a cerebellar reference region. This is trivially true for any given individual, but it is likely true also on group level. If this is the cause of the results observed in study 1, it is possible that a similar effect is present for other radioligands where cerebellum is used as a reference tissue. In high binding regions, a small discrepancy between C_{ND} in target and reference region has little consequence, but it is not uncommon that radioligands are validated in high binding regions, but later low binding regions are reported in the literature. One way to test if a general effect exists is to examine occupancy datasets applying other radioligands, comparing low and high binding regions for a pattern like what we observe in study I.

Out of the four studies contained in this thesis I think study III is the most important to continue to build on. The observed increase in 5-HTT availability in MDD patients following CBT can be interpreted in many ways and could have important implications. It has been suggested that the main role of the cerebral serotonin system is to enhance adaptive responses to adverse conditions, mainly through improving an individual's resilience to stress[3,72]. When applied to psychiatric disorders, serotonin transmission could then be hypothesized to be part of an innate coping system in the brain, reducing the risk of long-lasting stress induced symptoms, and, by extension, psychiatric conditions. Under this theory, the findings in study III could be explained as that 5-HTT was relatively low in response to the stress underlying the depression. As the depressive symptoms were alleviated following therapy, the coping system was no longer needed and 5-HTT levels increased towards premorbid levels. If this theory of serotonin mediated coping is verified, it could shift how the serotonin system is regarded in relation to MDD with the potential to open for new lines of research and, by extension, new treatments.

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