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Imaging serotonin and dopamine transporters in social anxiety disorder

Characterization, treatment and expectancy effects

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ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020

ISSN 1652-9030
ISBN 978-91-513-0895-1
urn:nbn:se:uu:diva-406312

Dissertation presented at Uppsala University to be publicly examined in Sal XI, Universitetshuset, Biskopsgatan 3, Uppsala, Friday, 24 April 2020 at 10:15 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Professor Eduard Maron (Imperial College London).

Abstract
Hjorth, O. 2020. Imaging serotonin and dopamine transporters in social anxiety disorder. Characterization, treatment and expectancy effects. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Social Sciences* 176. 85 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0895-1.

The monoamines serotonin and dopamine are likely to be involved in the pathophysiology of social anxiety and other affective disorders, but their respective contributions and putative interactions in the causes and cures of these disorders are still not well understood. It is also largely unknown if and how expectations of treatment success affect brain neurochemistry and neural activations, and if expectations interact with antidepressants like selective serotonin reuptake inhibitors (SSRIs). In this thesis some of these issues were addressed by use of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Using the highly selective radiotracers [^{11}C]DASB and [^{11}C]PE2I to characterize the availability of serotonin (SERT) and dopamine (DAT) transporter proteins, study I compared non-displaceable binding potentials (BP_{ND}), probing the transporters, between patients with social anxiety disorder and healthy controls. Increased SERT binding was observed in the reward related region nucleus accumbens (NAcc), in the social anxiety group. Moreover, increased DAT binding was associated with severity of the disorder and social anxiety was also associated with higher SERT-DAT co-expression in fear- and reward-related areas, including the amygdala and NAcc. Study II showed that verbal instructions regarding expected treatment efficacy strongly affected the clinical outcome of SSRI-treatment. Overt treatment, when patients with social anxiety disorder were correctly informed, was vastly superior to covert SSRI treatment, when patients expected an ineffective placebo. Groups were also differentiated on objective brain activity measures. Study III further demonstrated different SERT and DAT binding changes in limbic and striatal areas with overt as compared to covert SSRI-treatment. Decreased DAT BP_{ND} in the striatum, as assessed with PET, correlated with improvement in the overt group, suggesting increased dopaminergic signalling. Study IV compared treatment-induced changes in SERT and DAT binding after cognitive-behavior therapy (CBT) combined with an SSRI or placebo in patients with social anxiety disorder. Both groups showed initial co-expression similar to study I. The SSRI+CBT and placebo+CBT combinations yielded dissimilar transporter change patterns. Higher SERT occupancy in the NAcc correlated with reduced symptoms and this relationship was moderated by the change in DAT BP_{ND}. The results of this thesis support that functional interactions between serotonin and dopamine modulate social anxiety symptomatology and are important brain targets for successful treatment. Further it demonstrates that the treatment success of SSRIs in social anxiety disorder depends on how the treatment is presented. These results can be informative for the practice of clinicians, but also highlights an ethical dilemma because a large portion of the total treatment effects is elicited by processes within the patient itself.

Keywords: PET, serotonin, dopamine, placebo, SSRI, CBT, MRI

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ISSN 1652-9030
ISBN 978-91-513-0895-1
urn:nbn:se:uu:diva-406312 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-406312)

To Sahar, Love and Nora

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Hjorth, O. R., Frick, A., Gingnell, M., Hoppe, M. J., Faria, V., Hultberg, S., Alaie, I., Månsson, K. N. T., Wahlstedt, K., Jonasson, M., Lubberink, M., Antoni, G., Fredrikson, M., Furmark, T. (2019). Expression and co-expression of serotonin and dopamine transporters in social anxiety disorder. A multitracer positron emission tomography study. *Molecular Psychiatry*.

II Faria, V., Gingnell, M., Hoppe, J. M., Hjorth, O. R., Alaie, I., Frick, A., Hultberg, S., Wahlstedt, K., Engman, J., Månsson, K. N. T., Carlbring, P., Andersson, G., Reis, M., Larsson, E. M., Fredrikson, M., Furmark, T. (2017). Do you believe it? Verbal suggestions influence the clinical and neural effects of escitalopram in social anxiety disorder. A randomzied trial. *EBioMedicine*, 24: 179-188.

III Hjorth, O. R., Frick, A., Gingnell, M., Hoppe, M. J., Faria, V., Hultberg, S., Alaie, I., Månsson, K. N. T., Rosén, J., Reis, M., Wahlstedt, K., Jonasson, M., Lubberink, M., Antoni, G., Fredrikson, M., Furmark, T. (2020). Response expectancies shape the effect of SSRI treatment on serotonin and dopamine transporters in patients with social anxiety disorder. *Manuscript in preparation.*

IV Hjorth O. R*., Frick, A*., Gingnell, M., Engman, J., Björkstrand, J., Faria, V., Alaie, I., Carlbring, P., Andersson, G., Jonasson, M., Lubberink, M., Antoni, G., Larsson, E. M., Reis, M., Wahlstedt, K., Fredrikson, M., Furmark, T. (2020) Changes in serotonin and dopamine transporter availability after combined treatment with escitalopram and cognitive-behavioral therapy in patients with social anxiety disorder. *Manuscript in preparation.*

Contents

Abbreviations

5-HT	Serotonin
AADC	Amino acid decarboxylase
ACC	Anterior cingulate cortex
BAI	Becks anxiety inventory
BLA	Basolateral amygdala
BOLD	Blood oxygen level dependent
BP	Binding potential
BP_{ND}	Non-displaceable binding potential
cAMP	Cyclic adenosine monophosphate
CGI	Clinical global impression
CI	Confidence interval
CNS	Central nervous system
D1-R	D1 dopamine receptor
DAG	Diaglycerol
DASB	3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile
DAT	Dopamine transporter
DSM	Diagnostic and statistical manual of mental disorders
DVR	Distribution volume ratio
FWE	Family-wise error
G-protein	Guanin nucleotide-binding protein
HC	Healthy controls
IP_3	Inositol triphosphate
L-Dopa	L-3,4-dihydroxyphenylalanine
LSAS	Liebowitz Social Anxiety Scale (self-report LSAS-SR)
LSD	Lysergic acid dimethylamide
MADRS	Montgomery Åsberg Depression Rating Scale (self-report MADRS-S)
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MINI	Mini International Neuropsychiatric Interview
MNI	Montreal Neurological Institute
mPFC	Medial pre-frontal cortex
NAcc	Nucleus accumbens
NET	Norepinephrine transporter protein
NK-1	Neurokinin-1
PCPA	Para-clorophenylalanine

PE2I	N-(3-iodoprop-2E-enyl)-2b-carbomethoxy-3b-(4-methyl-phenyl) -nortropane
PET	Positron emission tomography
PFC	Pre-frontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PPI	Psychophysiological Interaction
QOLI	Quality of life index
rCBF	Regional cerebral blood-flow
RNG	Random-number generator
RPM	Receptor parametric mapping
ROI	Region of interest
SAD	Social anxiety disorder
SCID	Structured Clinical Interview for DSM
SE	Standard error
SERT	Serotonin transporter
SIAS	Social Interaction Anxiety Scale
SMD	Standardized mean difference
SNAP	Soluble NSF attachment protein
SNARE	Soluble N-ethylene-maleimide-sensitive factor-attachment protein receptors.
SNRI	Selective serotonin and norepinephrine reuptake inhibitor
SPECT	Single photon emission computerized tomography
SPM	Statistical Parametric Mapping
SPSQ	Social Phobia Screening Questionnaire
SSRI	Selective serotonin reuptake inhibitor
STAI-S	State-trait anxiety inventory - state
VMAT	Vesicular monoamine transporter protein
Vol	Volume of cluster of voxels in square millimeters
VTA	Ventral tegmental area

Introduction

As will become clear while reading this thesis, a lot of work has been performed to delineate how common social anxiety disorder (SAD) is (i.e. very common), the negative social consequences of SAD, how socially anxious individuals function cognitively, what treatments work and for how many etcetera. However, we only have a vague idea of how SAD pathophysiology is manifested in the central nervous system, and how different treatments exert their therapeutic effects on a neuronal level. Even less is known about how neurotransmission is affected by expectancies – i.e., how effective patients expect a treatment to be. This thesis will answer neither of those questions completely, but give a brief overview of systems likely to be involved and further delineate methods that can be used to unveil some answers to these important questions. As you will see, all those suffering from SAD are not helped by currently available treatments, but a better understanding of the neural underpinnings of SAD could possibly yield new insights in the process of building comprehensible pathophysiological models, which ultimately could lead to the development of more effective treatments. But let's not get ahead of ourselves.

Social anxiety disorder

Social anxiety disorder (SAD) is a life-long, highly debilitating psychiatric disorder with potentially large impact on all areas of an individual's social life (Alonso, Angermeyer, & Lepine, 2004; M. B. Stein & Kean, 2000; Wittchen et al., 2011). It is characterized by a persistent fear of social situations where one will be, or might become the focus of attention, and especially if one's behavior or performance is under scrutiny of others. Typical situations are: meeting people one does not already know, giving a presentation in school or at work, giving a speech, being observed while eating or writing etcetera (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*, 2000). Which situations that provoke the fear or anxiety varies individually, but the situations almost always provoke symptoms. The individual is afraid of acting in a way, or showing symptoms of anxiety, which might lead to humiliation or embarrassment, and people suffering from SAD typically avoid the feared social situations if possible, or otherwise endure them with intense anxiety or distress.

As mentioned, SAD is very common psychiatric disorder with 12 month and life-time prevalence estimates of around 7-8% and 13% respectively in the United States population (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012; Wang et al., 2005). Estimates of 1.9-15.6% point prevalence depending on severity has also been found in a Swedish survey (Furmark et al., 1999). As it is a common and disabling disorder, the economic societal costs are high (Acarturk et al., 2009). Furthermore, SAD is characterized by an often early onset with a debut before the age of 12 for 50% of those who receive a SAD diagnosis (M. B. Stein & Stein, 2008) and, as for many other psychiatric disorders, individuals with earlier onset often exhibit more persistent symptoms (Beesdo et al., 2007). Also, similar to other anxiety disorders, about 2/3 of those who suffer from SAD are women (M. B. Stein & Stein, 2008). Stereotypically, SAD is linked with extensive shyness, and research on personality measures and anxiety disorders has confirmed that SAD symptoms is negatively correlated with the big-5 personality trait neuroticism and extraversion (Bienvenu et al., 2001; Kotov, Watson, Robles, & Schmidt, 2007). The latter indicates that people with SAD tend to be less outgoing and more reserved and reflecting. However, there are also assays where only 50% of those who had received a SAD diagnosis reported being shy as children (Cox, MacPherson, & Enns, 2005). This makes the relationship less salient and suggests that SAD is a heterogeneous condition, also reflected by the division of SAD into the generalized and (by exclusion) non-generalized subtypes in the DSM-IV (*DSM-IV-TR*, 2000). DSM-IV criteria were used to assess SAD symptoms in the studies included in this thesis, and I therefore use the DSM-IV definitions. In the current version of DSM, i.e. DSM-5, a performance subtype is possible to specify and the reader should be aware that a new version (DSM-V) has slighty different definitions.

Treatments of social anxiety disorder

As I will cover in the coming sections, there are effective treatments for SAD with response rates between 50-65% (Leichsenring & Leweke, 2017). However, to be treated you first need to enter treatment, which means initiating contact with care givers and, given the nature of SAD, this is not an easy task. Depending on estimates, only 35-50% seek treatment for their symptoms and many of those who seek help, have suffered from their symptoms, and the social consequences of those symptoms for most of their lives (Ruscio et al., 2008; Wang et al., 2005). Therefore, new treatments should entail high availability to be able to reach those in need of psychiatric care.

Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs) selectively block the serotonin transporter (SERT) affecting serotonin signaling (see the serotonin section on page 21 in this thesis) and this class of drugs is a very common, and publicly recommended treatment of SAD (National Collaborating Centre for Mental Health (UK), 2013; Socialstyrelsen, 2017). The Swedish Nobel prize winner Arvid Carlsson and members from his group found interest in some tricyclic compounds with high SERT affinity and they developed the first SSRI, zimelidine, in collaboration with the Swiss biochemist Hans Corrodi in 1971 (Patent No. 781;105, 1972). The first zimelidine clinical trial on patients suffering from depression was performed in 1976 (Siwers, Ringberger, Tuck, & Sjoqvist, 1977) and it eventually became available on the Swedish market in 1982, only to be withdrawn again in 1983 (much to Carlsson's dismay) due to serious side effects in a few cases (Carlsson, 1999). Some years later, fluoxetine (Prozac) was developed and gained widespread commercial use.

All SSRIs possess high affinity to the SERT, but differ in pharmacokinetics (Hiemke & Härtter, 2000) and selectivity. For example, paroxetine and sertraline, two common SSRIs, possess some affinity also for the norepinephrine transporter (NET) and the dopamine transporter (DAT), which has been shown to increase extracellular levels of dopamine in nucleus accumbens (NAcc) and dorsal striatum with acute administration (Kitaichi et al., 2010), but reduced dopaminergic signaling in other regions has been hypothesized (Zhou et al., 2005). However, other SSRIs, e.g escitalopram, have a very high selectivity to SERT (Owens, Knight, & Nemeroff, 2001). Therapeutic doses vary between different compounds, but an average of 80% occupancy of the SERT is considered to be needed to elicit clinical improvement (Meyer et al., 2004; Voineskos et al., 2007). Therapeutic effects of SSRIs are not instant, but is in many cases achieved after 3 to 4 weeks. It has however been shown that many of those that do not respond to treatment after 8 weeks, do so after 12 weeks (Stein, Stein, Pitts, Kumar, & Hunter, 2002). In the treatment of SAD, the standardized mean difference (SMD) when SSRIs are compared to

wait-list groups has been estimated to -0.91 (95% CI -1.23- to -0.6), and specifically for escitalopram; -0.88 (95% CI -1.20 to -0.56) (Mayo-Wilson et al., 2014). The mean difference on the Liebowitz Social Anxiety Scale (LSAS, see methods) compared to placebo after 12-weeks of 20 mg daily escitalopram treatment was -10.1(95% CI -13.7 to -6.5) in another meta study (Baldwin et al., 2016). When trying to separate the true clinical response of SSRI treatment from psychological expectancy effects, treatment design considerations are of utmost importance. Hence, this will be covered in the next chapter.

A cognitive model of social anxiety disorder and cognitive behavioral therapy (CBT)

CBT is a commonly used framework for psychotherapy which is considered first line treatment for SAD (National Collaborating Centre for Mental Health (UK), 2013; Socialstyrelsen, 2017). There are different models for how SAD emerges and, more importantly for treatment, how it is maintained. There are multiple cognitive models of SAD that are applied in CBT (Clark & Wells, 1995; Hope, Heimberg, & Turk, 2010). The models are useful, not only for the care giver, but providing the patient with so called psychoeducation is a deliberate approach to increase the patients understanding of their symptoms and cognitions surrounding social situations they perceive as threatening. Main SAD features addressed are the exaggerated self-focus, and the safety-behaviors that socially anxious individuals use to try to hide their symptoms from others. Educating the patients is meant to lead to a cognitive reconstruction of how they perceive their symptoms and since the descriptions of the models often fit very well with thoughts and feelings the patient has identified earlier, it can also lead to a stronger alliance between therapist and patient in working towards improvement. As with almost all CBT treatment, the work consists of stepwise exposure to situations that are perceived as socially threatening. To put it simple, the patient should try to stay in the social situation, and practice to keep outward focus. Exposure can also be set up to test specific negative thoughts and afterwards the patient and therapist evaluate the outcome together.

The efficacy of CBT has been demonstrated in numerous randomized trials with one meta-analysis showing SMDs between individual CBT and waiting-list controls of -1.19 (95% CI -1.56 to -0.81) (Mayo-Wilson et al., 2014) and -0.77 (95% CI -0.94 to -0.6) in an earlier meta-analysis (Acarturk, Cuijpers, van Straten, & de Graaf, 2009). CBT has also been proven to be effective for treatment of SAD in children and adolescents (Hedge's g = -0.71 95% CI -0.98 to -0.45) (Scaini, Belotti, Ogliari, & Battaglia, 2016).

As mentioned earlier, it is difficult for individuals suffering from SAD to enter treatment because of the compromised social approach behaviors inherent in the condition. Different approaches have been tried to increase the availability of CBT and evidence indicate that social anxiety can be successfully

treated with therapy administrated online using different protocols with varying degree of patient independence (Andrews, Davies, & Titov, 2011; Berger, Hohl, & Caspar, 2009; Furmark et al., 2009; Gingnell et al., 2016; Hedman et al., 2011; Williams, O'Moore, Mason, & Andrews, 2014). SMD for internet based CBT compared to waiting-list controls has been estimated to -0.88 (95% CI -1.04 to -0.71) and another meta-analysis showed no difference between internet based guided self-help interventions and face-to-face CBT (Carlbring, Andersson, Cuijpers, Riper, & Hedman-Lagerlöf, 2018) suggesting that internet based CBT could be a feasible way to reach socially anxious individuals that otherwise find it difficult to seek effective treatment for their problems.

Combining pharmacologic and psychologic treatment

There are other treatments that should be mentioned. One of the papers in this thesis is partially based on combined treatment with the SSRI escitalopram and internet based cognitive behavioral therapy. In the report of the full sample clinical effects there were no benefits of combined SSRI + CBT as compared to CBT monotherapy after 9 weeks of treatment, however combined treatment had a comparably stronger effect at 15 month follow-up (Gingnell et al., 2016). There are a few more studies investigating combined pharmacological and psychotherapy interventions, and while the effects are not iatrogenic, no statistically significant benefit as compared to monotreatment has been identified so far (Mayo-Wilson et al., 2014).

Other treatments

The serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine , which in addition to inhibiting the SERT, also inhibits the NET has been shown to be effective in the treatment of SAD (SMD: -0.96 (95% CI -1.25 to -0.67) but might also elicit more discontinuation symptoms than some of the SSRIs (Sir et al., 2005)There are also other pharmacological treatments that provide effective symptom relief but that are typically avoided because of the potential for severe side effects such as benzodiazepines, that can induce drug dependence, a need to constantly increase the dose and withdrawal symptoms (Leichsenring & Leweke, 2017). Another pharmacological treatment that is not commonly prescribed are the monoamine oxidase inhibitors (MAOIs) that alters the activity of monoamines such as dopamine, serotonin and norepinephrine by non-selectively inhibiting the enzyme monoamine oxidase. Positive effects have been reported for patients with SAD (Blanco et al., 2010; Heimberg et al., 1998; Liebowitz et al., 1992; Versiani et al., 1992) but MAOIs have traditionally been avoided due to reports of health risks if patients consume too much dietary tyramine, and potential dangerous interactions with SSRIs. However, the potential risks could possibly be exaggerated, with some reports claiming the amount of tyramine needed to be dangerous are not consumed with a normal diet (see, Finberg & Rabey, 2016), and irre-

versible MAOIs have been administered with other non-SSRI pharmaceuticals without severe side effects (Adli et al., 2008). Considering the positive effects, irreversible MAOIs could be a good alternative if other treatments do not elicit positive results, however the need for monitoring and for the patient to take extra responsibility might make MAOIs unattractive for clinicians (Garcia & Santos, 2020). Lastly, another psychological treatment that could potentially prove useful in the near future and has a high availability for SAD patients is imaginal exposure using virtual reality paradigms (Chesham, Malouff, & Schutte, 2018).

The role of expectancies and placebo in pharmacological treatment

How much of the clinical response elicited by SSRIs are attributable to the pharmacological effect of blocking the SERT, and how much depends on the patients' perceptions of how efficacious the treatment will be? A similar question was asked, rather harshly in 1998, by Irving Kirsch and Guy Sapirstein in their influential meta-analysis "Listening to Prozac, but hearing placebo" (Kirsch & Sapirstein, 1998). The authors claimed that the placebo response to SSRIs was greater than the pharmacological response. The title was a paraphrase on the title of a book by the psychiatrist Peter Kramer called "Listening to Prozac" (Kramer, 1993) which was also a controversial piece of work, where he discussed the ethical implications of changing patients' personalities with pharmacological treatment. The paper by Kirsch and Sapirstein initiated a hotheaded debate, and a seemingly never ending flood of academic word play on the theme "Listening to X, but hearing Y" (Irving Kirsch, 1998, 2014; Klein, 1998; Rehm, 1998; Rickles, 2006), which goes on even today (Oronowicz-Jaśkowiak & Bąbel, 2019). The debate partly concerned methodological issues regarding how meta-analyses should be conducted but eventually it also fostered new research designs, and revitalized already existing placebo designs, allowing a more stringent approach to investigate the separate contributions of pharmacology and expectation.

Placebo models

There is a large number of models of the placebo effect. Classical conditioning has been suggested as a means of eliciting placebo responses by, for example administering a pharmaceutical by a pill, which subsequently elicits a placebo response when the pharmacologically active ingredient is removed (Wickramasekera, 1980). However, placebo responses can be provoked without the participants having any prior experience. It has been proposed that classical conditioning, in part, can be complemented by the expectancy theory (Stewart-Williams & Podd, 2004) which postulates that it is the expectancy of

an effect which produces the effect. Expectancies can be acquired through verbal instructions, by direct or observed experience, or other cognitive or emotional factors (Stewart-Williams, 2004). Further, emotional change theory suggests that taking a placebo increases the likelihood that participants will experience a reduction in stress and anxiety, which reduces both physical and psychological symptoms. This may in turn decrease avoidance and increase approach behaviors (Lundh, 1987).

There are also reward models of the placebo effect where expectancy of an effect from a placebo, is regarded as similar to expectancy of reward. This perspective is in concordance with biophysiological data on the brain's reward system, where the striatal structure NAcc (de la Fuente-Fernandez, 2001) and other regions innervated by the mesocorticolimbic dopamine pathways, are deeply involved (Petrovic et al., 2010; Wager, 2004). Importantly, increased dopamine signaling in the NAcc has been detected during prediction of reward (de la Fuente-Fernández et al., 2002; Phillips, Stuber, Heien, Wightman, & Carelli, 2003), and furthermore, interactions between dopamine and endogenous opioids are suggested to mediate placebo responsivity (Altier & Stewart, 1999; Cowen & Lawrence, 1999; Scott et al., 2008). It should be noted that other neurochemicals like opioids, oxytocin and cholecystokinin has also been suggested as possibly important in yielding placebo responses (Benedetti, 2014).

Placebo designs

In a typical double-blind design, participants are allocated to either the drug treatment arm or sugar pill placebo treatment by means of randomization and when results are analyzed, the placebo response in both groups are treated as equal, meaning the placebo response is theoretically supposed to be added on top of the pharmacological response in the drug treatment arm. This technique is widely used but has some issues. The assumption of equal placebo response across groups can only hold if blinding to treatment is sufficient and many drugs, including SSRIs, have known side effects. If participants can perceive them, blinding is broken, consequently boosting the placebo-response in the drug arm (Kirsch, 2000; Kirsch & Weixel, 1988; Lund, Vase, Petersen, Jensen, & Finnerup, 2014). The crossover-designs were largely abandoned for similar reasons. It's difficult to avoid unblinding when providing two temporally separated treatments to the same individuals if one of the treatments have salient side-effects and the other none (Boutron et al., 2006).

An alternative design to capture placebo responders is the placebo "run-in" design where both treatments initially receive placebo. Participants who exhibit a faster and stronger response are subsequently removed from analyses. No stable personality predictor has been identified regarding who will yield a stronger placebo response, meaning someone who responds to one type of placebo might not respond to another, and might also not respond to the same

placebo in another context or with repeated testing (Tack et al., 2005; Talley et al., 2006). Furthermore, the run-in procedure has received a lot of critique, since removing patients who meet all the inclusion criteria, and hence are eligible for the study, introduces a risk for different kinds of selection bias to affect the results (Berger, Rezvani, & Makarewicz, 2003).

The design that is considered the most stringent when it comes to capturing the true drug-response is the so called "balanced placebo design" (Kirsch & Weixel, 1988). It is constituted by four groups split into a 2×2 design. Two groups will receive the drug, but only one of them will receive correct information, whereas the other group is told they will receive a placebo. If salient side-effects are expected that might disclose blinding, the placebo can be described as an "active" placebo which will not have any effect on symptoms but might elicit similar side effects as the drug under evaluation. The remaining two groups will also be split on grounds of which information they are given but both are administered a placebo. The [told-drug, given-drug]-condition is most similar to what patients will meet in a clinical setting. More similar than a double-blind design where neither care giver nor patient know what treatment arm the patients belong to and [told-drug, given-drug] usually elicits the strongest effect (Kirsch & Weixel, 1988). The difference between the [told-drug, given-drug] and [told placebo, given-drug] conditions gives a description of the placebo (expectancy) response, and in studies where this manipulation is the most relevant, or if power concerns might arise due to too few participants distributed over four treatment arms, you can use only these two conditions. Consequently, no information about the characteristics of the [told-placebo, given-placebo] (null-response) and [told-drug, given placebo] is given.

Due to the deceptive nature of the balanced placebo designs is not uncomplicated from an ethical standpoint, and serious considerations regarding risk-benefit trade-offs are needed before eventual implementation (Miller, Wendler, & Swartzman, 2005). Another aspect is the issue of withholding the full effect of a treatment even though it depends on psychological variables. However, when justified, it can be a better tool than double-blind designs to delineate drug-psychology-interactions. A personal reflection is that I wonder what the consequences would be for the validity of clinical trials, if the balanced placebo design or its derivatives were to become the new golden standard, and if this information were to become common knowledge, and participants and patients begin to expect deception.

Neural effects of expectancy

The placebo response encompasses a wide range of conditions, treatments, and responses, which the variety of neuroimaging studies in the field reflects. As mentioned earlier, increased dopamine signaling has been suggested as a key element in the placebo response under certain circumstances. In research

on Parkinson's disease using the radiotracer [^{11}C]raclopride, de la Fuente-Fernandez and coworkers (2001) found reduced D2-receptor availability, which also correlated with reduced symptoms, when placebo was administered. When reductions in the NAcc were also found (de la Fuente-Fernández et al., 2002), the authors proposed that it is the expectation of reward that elicits the dopamine response rather than receiving the reward itself.

In a PET-study on depression, administration of placebo elicited glucose metabolism responses almost identical to the SSRI fluoxetine, and treatment response, regardless of treatment arm, was associated with metabolic changes in NAcc and orbitofrontal cortex (Mayberg et al., 2002). Neuroimaging results on anxiety has shown that placebo can affect responses to emotional faces, and these results entailed fMRI-signals previously detected in placebo analgesia (Petrovic et al., 2005). Placebo response has also been shown to attenuate stress-related amygdala activity as measured by change in rCBF, using [H_2-^{15}O]-water tracer PET, but only in patients with homozygous serotonin transporter-linked polymorphic region (5-HTTLPR) alleles (Furmark et al., 2008). In another study, extending the sample from Furmark and coworkers (2008), placebo and SSRI-responders shared attenuation patterns in the amygdala, which correlated with symptom improvement, an effect that also significantly separated responders and non-responders. Interestingly, no differences in rCBF were found between responders across conditions (Faria et al., 2012). Additionally, analyses of amygdala-prefrontal couplings in a follow-up study, revealed differences between responders and non-responders, both within and between treatment modalities (SSRI and placebo), but there was also suggestive evidence for a shared amygdala-PFC connectivity pattern between responder groups (Faria et al., 2014). In conclusion, not much neuroimaging work has been performed on placebo effects and anxiety, and there are many questions left to answer regarding the neural underpinnings of the placebo-response.

Monoamines in anxiety

Serotonin

Serotonin has, since it's discovery been shown to be important in regulation of many brain functions including, but not limited to, mood, anxiety, aggression, sleep, memory and perception (Roth, 1994). 5-hydroxytryptamine was first isolated in the year 1948 by Rapport, Green, & Page (1948) and its molecular structure was correctly identified. It was discovered in human blood serum and received its name because of its effect on the tonus of blood vessels, hence sero ("serum") – tonin ("tonus"). The largest concentrations of serotonin are found in the intestines where it facilitates intestinal motility, but it was also discovered in the brain in 1953 (Twarog & Page, 1953). Serotonin is synthesized in the nerve terminals and in the raphe nuclei (Greek for "*seam*") which is located in the midbrain and provides serotonergic innervation widely throughout the brain. The importance of the raphe nuclei for serotonergic synthesis was discovered by Dahlström and Fuxe (1964, 1965) who were also deeply involved in the discovery and mapping of the brain dopamine system. Similar to the dopamine system, serotonergic neurons have a great impact on human behavior but number only in the hundreds of thousands, which can be compared to the total number of neurons in the brain which is around 100 billion (Herculano-Houzel, 2009). The raphe nuclei are divided into the caudal and rostral groups. The rostral group, which has the highest relevance for this thesis, has serotonergic projections to the forebrain, and the caudal group has efferent projections toward the brain stem and the spine. The efferent projections from the median and dorsal raphe partly overlap (see Figure 1). The median raphe projections are characterized by thick fibers with short thin branches and project to the dorsal hippocampus, septum, and hypothalamus, and then project further towards the lateral regions of the neocortex. In contrast, the dorsal raphe projection fibers are more intricately distributed with a large number of branches. They innervate the amygdala, the adjacent parts of hippocampus and the striatum and then continue towards the medial frontal cortex where they exert a much stronger influence than the median raphe projections (Azmitia & Segal, 1978; Imai, Steindler, & Kitai, 1986). As a side note, the differences in fiber structure and regions of innervation between the dorsal and median raphe projections, could mean that they are functionally different, which could make them potential targets for future directed treatments of disorders that are today treated with pharmaceuticals which affect a larger part of the serotonin system. The raphe itself is of course also innervated. The dorsal raphe receives glutamatergic input from limbic areas, lateral habenula, tegmentum and the ACC (Behzadi, Kalén, Parvopassu, & Wiklund, 1990; Peyron, Petit, Rampon, Jouvet, & Luppi, 1997), but there is also GABAergic influence (Qing-Ping, Ochiai, & Nakai, 1992) and there are clusters of dopaminergic cell bodies within the raphe itself.

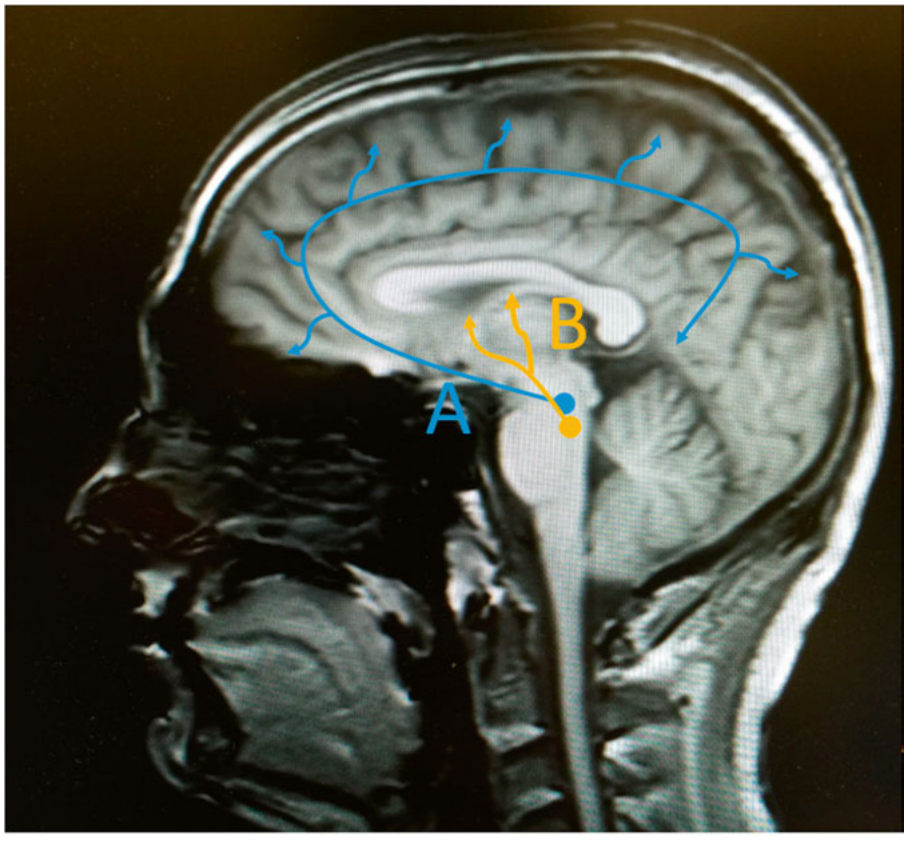

Figure 1. Serotonin pathways in the CNS. **A** is the dorsal-raphe pathway with projections towards amygdala, hippocampus, striatum and cortex; **B** is the medial raphe pathway with projections towards the dorsal hippocampus and lateral parts of the cortex.

Synthesis starts with L-tryptophan crossing the blood brain barrier and subsequently the serotonergic neurons in the raphe nuclei. L-tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase, which adds a hydroxyl group to the 6th member of the indole group of tryptophan (see Figure 2). The enzyme responsible for catalysis of 5-hydroxytryptophan to 5-hydroxytryptamine (serotonin) in the last step of synthesis is called aromatic L-amino acid decarboxylase (AADC), a PH-sensitive monoamine catalyst, which notably also catalyzes the last step of dopamine synthesis.

At the axon terminals, serotonin is transported into vesicles by the vesicular monoamine transporters VMAT1 (peripheral nervous system) and VMAT2 (central nervous system (CNS)) for which serotonin has similar affinity. Vesicles are subsequently docked by SNAP-SNARE complexes at the cell membrane adjacent to the synaptic cleft, and released into the synaptic cleft by Ca^{2+} mediated fusion between vesicle- and cell -membranes (Sanders-Bush &

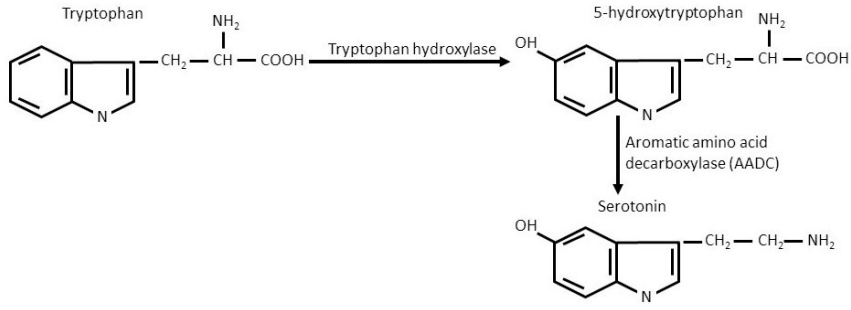

Figure 2. Simplified flow-chart of serotonin synthesis

Nichols, 2012). Serotonin receptors come in seven different classes, some of which have multiple subclasses, which amounts to at least thirteen 5-HT receptors, and likely more, in the CNS. Six of the seven main classes are coupled with different guanin nucleotide-binding proteins (G-proteins) which modulate intracellular processes. The 5-$HT_{1/5}$ receptors are coupled with the G_i and G_o-proteins respectively which inhibit the enzyme adenylyl cyclase, preventing cyclic adenosine monophosphate (cAMP) synthesis. cAMP affects protein kinases (PKA, PKC) and Ca^{2+} activity within the cell. 5-HT_1-receptors are also utilized as autoreceptors, downregulating serotonin synthesis, cell firing and release, when stimulated. Conversely, the 5-$HT_{4/6/7}$ receptors stimulate cAMP synthesis by their coupling with the G_s-protein. The 5-HT_2-receptors stimulate the $G_{q/11}$-proteins which increase the activity of phospholipase C (PLC), a promotor of diacylglycerol (DAG) and inositol triphosphate (IP_3), respectively stimulating PKC and Ca^{2+} release. Lastly, the 5-HT_3-receptor facilitates Na^+, Ca^{2+} and K^+ (among others) ion influx, depolarizing the cell (Nichols & Nichols, 2008).

The serotonin transporter (SERT) is a presynaptic low-capacity reuptake transport protein located in the cell membrane of the axon adjacent to the synaptic cleft. Its main function is to transport free serotonin from the synaptic cleft back into the presynaptic cell where it can be either reused for further signaling or catalyzed and removed. SERT is the main target for the SSRIs that bind to the SERT and prevent reuptake (See the Selective Serotonin Reuptake Inhibitor section for an overview.)

Serotonin in anxiety and social anxiety

Scientists became interested in serotonergic drugs for the treatment of psychiatric disorders when the psychiatrist Roland Kuhn noticed that many of his depressed patients, who were treated with the antihistamine imipramine for other reasons, also recovered from depression (Kuhn, 1958). Imipramine is a tricyclic which is a class of antidepressants that binds to an abundance of targets in the CNS but primarily blocks the SERTs and NETs (Baldessarini, 1989; Gillman, 2007). A few years later it was observed that imipramine also

had a positive effect on panic disorder symptoms (Klein & Fink, 1962), and when the SSRIs were introduced and found effective, the idea of serotonergic involvement in anxiety accumulated further support.

There has been a lot of debate about serotonin being anxiogenic or anxiolytic. For example, inhibiting serotonin synthesis with para-clorophenylalanine (PCPA) was found to favor approach behaviors in approach-avoidance conflicts in rats (Robichaud & Sledge, 1969) and the benzodiazepine oxazepam was found to reduce serotonin turnover (Wise, Berger, & Stein, 1972). However, the clinical effect of the tricyclics, and the SSRIs point toward an anxiolytic effect. A model of three levels of defense proposed by Blanchard & Blanchard, (1988), where different levels are activated depending on the distance from an identified threat, was utilized by Deakin & Graeff, (1991) when they proposed that different parts of the serotonin system modulated different levels of defense, which would give serotonin a dual role, enhancing anxiety and inhibiting fear expression. The theory suggests an anxiogenic effect in social anxiety disorder, and, since the theory can be tested in many ways, it has gained some support whereas other evidence is inconclusive (Graeff, Parente, Del-Ben, & Guimarães, 2003; Graeff & Zangrossi, 2010; Pinheiro, Zangrossi-Jr., Del-Ben, & Graeff, 2007).

Narrowing the scope to neurochemical studies in social anxiety, studies have found increased serotonin synthesis in SAD as compared to healthy controls, in the raphe nuclei, and limbic and striatal areas, including the amygdala (Frick et al., 2015; Furmark et al., 2016). The synthesis may also be attenuated by anxiolytic treatment (Frick et al., 2016). Further, reduced serotonin-1A receptor binding has been found, also in the amygdala, but in the ACC and the insula as well (Lanzenberger et al., 2006). Moreover, increased SERT binding potential (Frick et al., 2015; van der Wee et al., 2008) has been detected in SAD. In conclusion results indicate, in line with the dual-role hypothesis, that SAD is characterized by an overactive presynaptic serotonin system.

Dopamine

Dopamine is a slow-acting catecholamine, involved in a wide array of functions and processes in the brain ranging from voluntary movement, emotion, reward, foraging, memory, learning, habit formation etcetera (Ayano, 2016; Bressan & Crippa, 2005; Yin & Knowlton, 2006). Although 3, 4-Dihydroxyphenetylamine, dopamine's chemical name, was synthesized for the first time as early as in the year 1910 (Barger & Ewins, 1910), it was not until 1957-1959 that is became clear that it was occurrent in the mammalian brain, and it had received it's yet unfamiliar name, dopamine, just a few years earlier in 1951 (Hornykiewicz, 1986). Notably, only 4 papers were published regarding dopamine's pharmacological effects between it's discovery and the late 50's (Gurd, 1937; Hamet, 1931; Holtz, Heise, & Lüdtke, 1938; Tainter, 1930). The paper by Holtz, Heise and Lüdtke (1938) demonstrated that dopamine is synthesized by L-3,4-dihydroxyphenylalanine (L-Dopa) when they discovered

aromatic L-amino acid decarboxylase (AADC) in mammalian kidney tissue, which subsequently led to the controversial suggestion that dopamine was a precursor for noradrenaline and adrenaline, by Blaschko, (1939). A notion that later turned out to be correct.

In the late 50's Arvid Carlsson and colleagues were able to reverse reserpine akinesia using L-Dopa (Carlsson, Lindqvist, & Magnusson, 1957) and noticed that the effect was even more pronounced when monoamine oxidase inhibitors were administered, indicating that some other derivative of L-Dopa was working behind the scenes. Equipped with a new self-developed chemical fluorescent technique sensitive enough to assess even low dopamine concentrations, Carlsson's group was able to point out the basal ganglia as the main dopaminergic brain region (Bertler & Rosengren, 1959). The nigrostrial pathway from cellbodies in the midbrain structure substantia nigra, to the dorsal striatum (putamen and caudate nucleus) was identified just a few years later, and evidence of the later verified mesolimbic, mesocortical and tuberoinfundibular pathways also emerged using similar methods - see Figure 3 (Anden et al., 1964; Fuxe, 1965).

The mesocortical pathway runs from cell bodies mainly in the ventral tegmental area (VTA) of the midbrain, through infra- and pre-limbic areas, to the medial pre-frontal cortex (mPFC) where it mainly innervates deeper cortical layers with high density of D1 and D2 receptors (Vincent, Khan, & Benes, 1993), and then further extends with reduced innervation through dorsal projections through the parietal lobe (Fuxe et al., 1974; Oades & Halliday, 1987). The mesolimbic pathway's rostral projections also have it's origin in the VTA, with nerve terminals concentrated to the NAcc, amygdala, olfactory tubercle and, in part, stria terminalis. Finally, the tuberoinfundibular pathway has axons running ventrally from cell bodies in the arcuate nucleus of the hypothalamus to the median eminence (Fuxe, Hökfelt, & Ungerstedt, 1970).

Dopamine synthesis starts with the amino acid tyrosine crossing the blood-brain barrier and entering neurons in the VTA or substantia nigra through low- and high-affinity amino acid transporters. Tyrosine hydroxylase converts tyrosine to L-Dopa, which is transported from the midbrain cell soma, along the axon to the nerve terminals where L-dopa is synthesized into dopamine by dopa decarboxylase - see Figure 4 and Figure 5 (Elsworth & Roth, 1997). Solute carrier SLC18A2 vesicular protein VMAT2 transports dopamine into vesicles which are stored for later use (Erickson, Schafer, Bonner, Eiden, & Weihe, 1996).

Dopamine exocytosis into the synaptic cleft is initiated by action potentials causing calcium-ion in-flow that activates SNAP-SNARE complexes (Brunger, 2006) which subsequently fuses docked vesicles with the cell membrane, releasing dopamine into the synapse. There are 5 known receptors that are targets of dopamine, named D1-5 and they are often divided into two

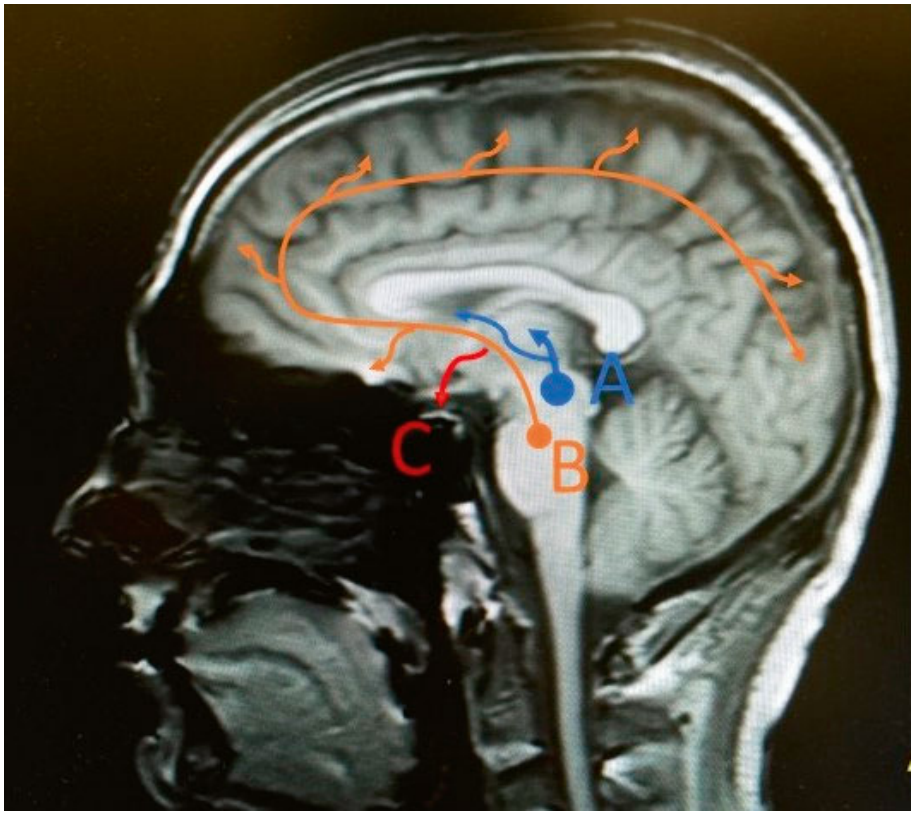

Figure 3. Dopamine pathways in the CNS. **A** is the nigro-striatal pathway with projections originating from substantia-nigra; **B** is the mesocorticolimbic pathway with projections originating from the ventral tegmental area (VTA); and **C** is the tuberofindibular pathway in the hypothalamus with projections toward olfactory cortex.

groups, the D1-like (D1 + D5) and the D2-like (D2, D3, D4) depending on similar actions within the groups (Andersen et al., 1990). They all activate intracellular G-proteins, and D1-like receptors activates the G_s-protein, which stimulates adenylyl cyclase to synthesize cAMP from adenosine triphosphate - see Figure 5. The D2-like instead inhibits adenylyl cyclase activity by affecting the same G_i-protein as 5-HT_1-receptors (Missale, Nash, Robinson, Jaber, & Caron, 1998), and similar to 5-$HT_{1/5}$-receptors, it has also been found to cause cell membrane hyperpolarization by upregulation of outward potassium-ion transport (Greif, Lin, Liu, & Freedman, 1995). Further, the activation of D2 autoreceptors downregulate the expression of tyrosine hydroxylase and the dopamine reuptake transporter protein (DAT), effectively reducing dopamine release (see Ford, 2014).

The DAT allows reuptake of extracellular dopamine by NA^+/Cl^- - coupled transport. After reuptake, dopamine is either recycled by monoamine oxidase

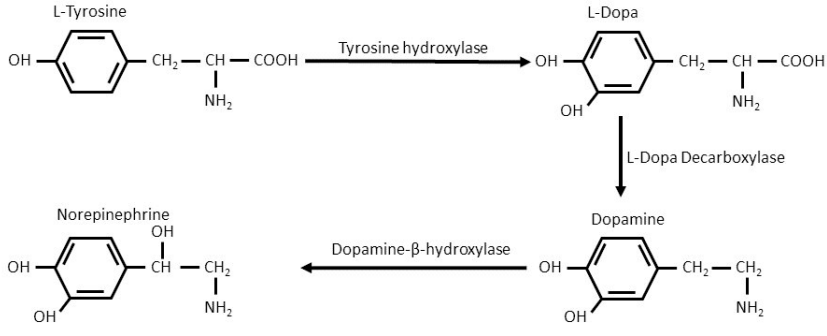

Figure 4. Simplified flow-chart of dopamine synthesis

or reused in vesicular mediated release into the synapse (Reith, Xu, & Chen, 1997).

Dopamine in anxiety

In animal studies, dopamine has been shown to influence the amygdala through VTA-mesolimbic pathway signaling, mediating anxiety responses (Marowsky, Yanagawa, Obata, & Vogt, 2005) and attenuating mPFC exerted inhibition of the baso-lateral amygdala (BLA) (Rosenkranz & Grace, 1999). Further observations have indicated that mesolimbic stimulation of the NAcc, a structure strongly implicated in hedonic reward processing and addiction, can occur due to both aversive and rewarding stimuli (de Jong et al., 2019; Lammel, Ion, Roeper, & Malenka, 2011), and meso-cortical projections has been suggested to only receive aversive information (Lammel et al., 2011). Moreover, dopamine has also been implicated as a necessary component of fear memory stabilization (Fadok, Dickerson, & Palmiter, 2009; Zweifel et al., 2011). Feedback to VTA and substantia nigra dopaminergic neurons has also been detected from structures involved in negative reward (Bernard & Veh, 2012).

The notion of dopaminergic influence on social anxiety symptoms emerged from different lines of research. Traits that are prevalent in depression, but also SAD, including anhedonia and reduced social motivation have been linked to striatal dopaminergic dysfunction (Gorwood, 2008), and studies in the latter part of the 20th century on irreversible monoamine oxidase inhibitors (MAOIs), which inhibit the breakdown of dopamine and other monoamines, demonstrated that MAOIs were more effective in treating SAD symptoms and depression with high rejection sensitivity, than the strictly serotonergic tricyclics or beta blockers (Liebowitz et al., 1985, 1992). Further, homovanillic

acid, a rest product after MAO catalysis of dopamine, was reduced in the CSF of panic patients with comorbid SAD (Johnson, Lydiard, Zealberg, Fossey, & Ballenger, 1994). The genotype of DAT has also been shown to be associated with SAD (Bergman et al., 2014; Rowe et al., 1998). Moreover, SAD has a relatively high prevalence in Parkinson's disease, a condition characterized by degeneration of neurons (Bolluk, Özel-Kizil, Akbostanci, & Atbasoglu, 2010; Gultekin, Ozdilek, & Bestepe, 2014).

There are a handful of nuclear imaging studies that has aimed to characterize dopamine signaling in SAD. One SPECT study using the radiotracer [^{123}I]iodobenzamide found that the dopamine receptor D2, which, when expressed as an autoreceptor, downregulates the DAT and dopamine synthesis, was downregulated in SAD, a pattern also detected in obsessive compulsive disorder (OCD) with comorbid SAD (Schneier et al., 2008). However, the same authors could not replicate these findings in a different cohort with generalized social anxiety using [^{11}C]raclopride (Schneier et al., 2000, 2009). A study examining D2 binding in mesocortical projections found increased olfactory- and right dorsolateral prefrontal-cortex binding in SAD (Plavén Sigray et al., 2017), and reduced D2 availability has been found to be associated with symptom improvement after CBT (Cervenka et al., 2012). Studies characterizing the DAT in SAD have yielded inconclusive results in case-control studies and decreased (Tiihonen et al., 1997), increased (van der Wee et al., 2008) as well as no difference in DAT availability have been reported (Schneier et al., 2009). Hence the contribution of DAT binding in SAD in remains unclear.

Interactions between serotonin and dopamine

Not much work has been done on serotonin-dopamine interaction in anxiety in humans but the monoaminergic systems of mammalian brains are surprisingly similar in their distribution, compared to many other systems in the CNS. This could indicate that successful translation from work performed in rodents to human conditions is more likely but there are also evidence against that view, showing differences between humans and mice in serotonin receptor gene expression in the middle temporal gyrus (Hodge et al., 2019). As may be noticed in the sections about serotonin and dopamine, both systems originate from adjacent regions in the midbrain. Serotonin from the raphe nuclei and dopamine from VTA and substantia nigra. In addition, there are clusters of dopaminergic cells in the raphe nuclei, and conversely there are serotonergic projections from raphe which innervate dopaminergic cells in VTA and substantia nigra (Hervé, Pickel, Joh, & Beaudet, 1987). Also, both systems innervate the same regions to a large extent (Fuxe, 1965a). The dorsal raphe projects to amygdala, ventral hippocampus and the striatum (Jacobs & Azmitia,

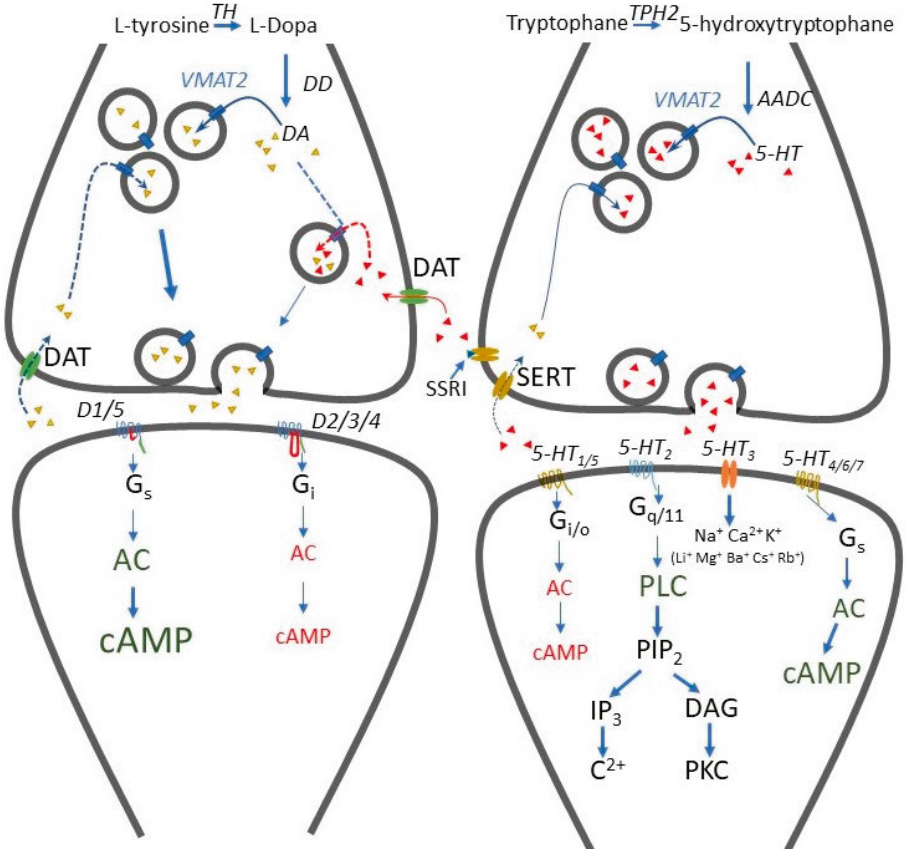

Figure 5. Simplified illustration of dopamine (left) and serotonin (right) signaling in the CNS. Abbreviations: TH, tyrosine hydroxylase; L-Dopa, L-3,4-dihydroxyphenylalanine; DD, dopa decarboxylase same as AADC, L-amino acid decarboxylase; DA, dopamine; VMAT, vesicular monoamine transporter; DAT, dopamine transporter; D1/5; dopamine "D1-like" receptors; D2/3/4, dopamine "D2-like" receptors; G_x, guanine nucleotide-binding protein; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PLC, phospholipase C; PIP_2, phosphoinositide phosphatidylinositol-4,5-biphosphate; IP_3, inositol triphosphate; DAG, Diaglycerol; TPH2, tryptophanhydroxylase; 5-HT, serotonin; SERT, serotonin transporter; 5-HT_x, serotonin receptors.

1992), and the mesolimbic dopaminergic pathway also projects to the amygdala and ventral striatum, whereas the nigro-striatal projections innervate the dorsal striatum (Anden et al., 1964; Fuxe et al., 1974; Oades & Halliday, 1987). Further there are raphe projections innervating the substantia-nigra (Dray, Gonye, Oakley, & Tanner, 1976) and multiple 5-HT-receptors are involved in regulation of dopamine release. Most of them facilitate dopamine release, but the 5-HT_{2c} mediates serotonergic inhibition of dopamine release (Di Matteo, Di Giovanni, Pierucci, & Esposito, 2008). As mentioned earlier, some SSRIs have low affinity for the DAT, but even for SSRIs with very high

affinity, changes in dopamine signaling has been detected, and there is some evidence as to why this happens. It has been suggested that the DAT can also transport serotonin - see Figure 5, and this transport increases when SERT is occupied by SSRIs (Daws, 2009). It is somewhat unclear what the consequences are of the serotonin reuptake, but it has been hypothesized that increased uptake of serotonin by the DAT leads to co-release of serotonin and dopamine which in turn should lead to decreased dopaminergic signaling due to more competition for space in the terminal vesicles (Zhou et al., 2005). Other studies have also found reductions in extracellular dopamine after SSRI administration (Dewey et al., 1995). It is largely unknown how effects of increased dopaminergic signaling due to reward expectancy would affect serotonergic transport by the DAT.

It could be hypothesized that clinical features of SAD, like approach-avoidance conflicts in daily life, involve fundamental aversive and appetitive motivational systems that depend on serotonin-dopamine interactions in the brain. For example, ventral striatum and the amygdala are involved in reward seeking but also in processing aversive stimuli. The NAcc, which is richly innervated by dopaminergic projections, also has high concentrations of serotonergic cells in parts of its shell. Further, it receives direct input from the basolateral amygdala (Shirayama & Chaki, 2006; Stuber et al., 2011), and the amygdala, which is highly innervated by serotonergic projections, also has dopaminergic inputs, which are believed to modulate emotional responses to stimuli (Kobiella et al., 2010; Okita et al., 2016). There are also evidence of PFC modulating amygdala regulation of NAcc dopamine signaling (Jackson & Moghaddam, 2001). The only neurochemical study on a SAD sample that until now has examined serotonin and dopamine transport concomitantly, found increased striatal DAT binding potential as compared to healthy controls, and increased serotonin transport in the bilateral thalamus using [^{123}I] β-CIT SPECT (van der Wee et al., 2008). In conclusion, a lot more can be learned about feasible serotonin-dopamine interactions, and their role in the pathophysiology of SAD. Correlations between serotonin and dopamine transporter binding in fear and reward-related areas has for example not been examined in SAD, and putative alterations in SAD could give more insights into the different roles of monoamines in the pathophysiology of the disorder. Furthermore, if alterations are found, it could be interesting to investigate changes in reuptake with treatment to discern if treatment normalizes values or exerts its effects through other means.

Methods

Positron emission tomography

PET is a nuclear imaging technique which can be utilized to investigate different facets of metabolism in biological tissue by pairing a radioactive isotope with a tracer, which is a neurochemical that binds to a specific target in the tissue of interest. The pairing can be performed with different techniques, but the most common radioligands used in neuroimaging are synthesized in a cyclotron, which is a particle accelerator that utilizes a radio frequency to accelerate particles contained in a static magnetic field (Wagner, 1998).

When performing a PET-scan, the subject is positioned in supine position with the head lightly fixated to avoid movement artifacts during data collection and the radiotracer is subsequently injected, typically with a venous bolus-injection. After a short waiting-period to allow the radiotracer-compound to find its target, imaging can be initiated.

The head of the participant is encircled by a detector ring equipped with gamma ray detectors, called scintillator crystals. When the crystals are struck by a gamma-ray they generate light which is subsequently detected by photomultiplier tubes that translate the light to electrical signals. As the radioligand decays a positron is emitted which travels until it encounters an electron and creates an unstable exotic atom called para-positronium. Para-positronium's mean lifetime is 0.125 nanoseconds and during decay it emits two gamma rays in an approximate 180° angle. This property makes it possible to localize the point of decay in space by measuring the time difference between gamma ray detections.

In neuroscientific studies, a wide variety of PET radiotracers are used with the ones synthesized in cyclotron having a relatively short mean half-life as compared to the radioligands of the similar imaging technique single photon emission computerized tomography (SPECT). A benefit of short half-life is that multiple biological targets can be assessed in the same patient during one day. For example, the [^{11}C]-ligand which was utilized in the PET-studies included in this thesis has a half-life of 20.3 minutes which allowed imaging of a second tracer after a waiting-period of around 60 minutes. At that time less than 1% of the activity from the first injection remained. The half-life of two other often used radiotracers, [^{18}F] and [^{15}O] are 109.7min and 2.03 min respectively. A downside of short half-life is that you need direct access to production facilities at the scanning site to ensure sufficient radioactivity at the

time of injection, but [^{18}F] allows for transport and development of small but sufficiently shielded cyclotrons increases accessibility.

When creating PET images, the most important measure is often the binding potential (BP) (Lu & Yuan, 2015), which reflects the density of available targets in a given volume. More specifically the ratio of radiotracer that binds to its target to non-displaceable (ND) radiotracer is calculated which gives the BP_{ND} (Innis et al., 2007). In earlier PET studies blood sampling was needed to determine BPs, which was distressful for certain patient populations. but contemporary techniques utilize assumption based statistical models or graphical models (Lammertsma & Hume, 1996; Logan et al., 1996). They take advantage of the fact that radioligand targets are not evenly distributed throughout the brain and therefore a region that has negligible levels of the target receptor is used as a reference region. The two methods used in this thesis were the Logan reference method and receptor parametric mapping (RPM). The Logan reference method is a graphical method which can be used when radiotracer clearance from the reference region is sufficiently fast and when target/reference-region equilibrium is sufficiently stable. The ratio of specifically bound to free and non-specifically bound tracer is denoted the distribution volume ratio (DVR) and the BP_{ND} is derived by DVR-1. Benefits are that the number of parameters are lower than in other reference-methods, which can affect noise in the BP_{ND} signal (Gallezot, Lu, Naganawa, & Carson, 2020) The second method, RPM, (Gunn, Lammertsma, Hume, & Cunningham, 1997; Lammertsma & Hume, 1996) is also a method that utilizes a region without specific binding as a reference region, but instead of a graphical approach it uses a statistical wavelet, where multiple preset basis functions are applied, and through iteration the models are simplified and linearized. Main assumptions for RPM are that the reference region is not influenced by pathology, and that unspecific binding of the radiotracer in target and reference - regions is equal. The accuracy of both methods are dependent on scanning time and the radioligands used, and both methods has shown good properties in combination with the [^{11}C]DASB and [^{11}PE2I] radiotracers (Gallezot et al., 2020; Jonasson et al., 2013; Zanderigo, Ogden, & Parsey, 2013).

General PET procedure in the studies of this thesis

In the work of this thesis, participants were scanned in a Siemens ECAT EXACT HR+ (Siemens/CTI) PET scanner. Prior to scanning participants had to fast for a minimum of 3 hours, and refrain from caffeine, nicotine and alcohol -intake for at least 12 hours before scanning. To carry out attenuation correction before radiotracer injection, a transmission scan was performed using three retractable Germanium (^{68}Ge) rotating line sources during 10 minutes. 63 contiguous planes were collected with a slice thickness of 2.46 mm which yielded an axial field of view of 155 mm. The radiotracer was administered by a venous bolus in the participant's arm. After the first scan there was a

waiting-period of approximately 60 minutes before the next scan to make sure activity from the first injection did not contaminate data from the second scan.

When preparing images, individual data were outlined on each participant's respective T1-weighted MR-image, collected on another occasion. As a side note, blood samples to estimate SSRI and metabolite blood serum concentrations were drawn during the post-treatment PET session.

[^{11}C]DASB

The tracer used to characterize the SERT, 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) (Houle, Ginovart, Hussey, Meyer, & Wilson, 2000) has high affinity for the SERT but low affinity for other monoamine transporters which makes it very useful when investigating the functions of multiple monoamine transporters in the same study (Wilson, Ginovart, Hussey, Meyer, & Houle, 2002; Wilson et al., 2000). DASB was radiolabelled with [^{11}C] and 22 images were retrieved during 60 minutes (seconds; 1×60, 4×30, 3×60, 4×120, 2×180, 8×300). BP_{ND} images were calculated using the reference Logan method.

[^{11}C]PE2I

N-(3-iodoprop-2E-enyl)-2beta-carbomethoxy-3beta-(4'-methylphenyl)-nortropane (PE2I) has a 30-fold higher affinity for the DAT as compared to other monoamine transporters (Halldin et al., 2003). An important property of PE2I when used in studies examining treatment effects of SSRIs, is that it is not affected by SSRI administration, which is the case for many other DAT tracers used in PET and SPECT imaging (Ziebell et al., 2010). PE2I was also radiolabelled with [^{11}C] and 22 images were retrieved during 80 minutes (seconds; 4×60, 2×120, 4×180, 12×300). BP_{ND} images were calculated using RPM (Gunn et al., 1997; Jonasson et al., 2013).

Magnetic resonance tomography

An MR-scanner works by first aligning hydrogen atoms in the tissue along a strong static magnetic field, with strengths often in the range between 0.5-3 tesla (T), even though even stronger magnetic fields are used in some scanners. Due to the strong magnetic field it is important to screen participants for any ferromagnetic objects or other objects that might be disrupted by strong magnetic fields, they might have outside or inside their bodies, (e.g. jewelry, pacemakers, metal shrapnel etcetera). Even if the object is not ferromagnetic it can still be conductive which might induce heating or locally block the MR-signal. Some participants might also feel uncomfortable because of the narrow space inside the MR-scanner. An external radiofrequency pulse is used to excite the aligned hydrogen atoms, which then release detectable radio signals while they again align with the static magnetic field (T1) and their spin go out

of phase (T2). Since the hydrogen atoms are affected by the binding and structure of the molecules they are bound to, it is possible to separate the signals from different tissues or fluids. In the studies included in this thesis, so called T1-weighted anatomical images were produced to be able to define the different ROIs which are difficult to discern using the more diffuse PET-images or blood oxygenation-level dependent (BOLD) fMRI images alone. T1-images were retrieved by using an echo time (TE) of 15ms, time between repetition of radio frequency pulses (TR) of 5700ms and a radio pulse inversion time of 400ms. The field of view was 230 × 230mm^2, size of voxels retrieved was 0.8 × 1.0 × 2.0mm^2, and images consisted of 60 contiguous slices. In study II, an echo planar imaging (EPI) sequence was used to perform BOLD imaging (TE = 35ms; TR = 3000ms; flip angle = 90°, acquisition matrix = 76 × 77, voxel size = 3 × 3 × 3 mm^3, gap = 1mm, 30 axial slices). BOLD-imaging measures the rate of oxygenated to deoxygenated blood, which gives an indirect signal of activity in different brain areas. All participants in all four studies were scanned in a Philips Achieva 3.0T whole body MR-scanner (Philips Medical Systems, Best, The Netherlands). In study IV, all of the participants were scanned using an 8-channel head coil, whereas in study I-III 24 controls and 5 patients were scanned with a 32-channel head coil due to a scanner upgrade. The visual stimuli in study II (emotional faces and elliptical shapes) were presented using E-prime software (Psychology Software Tools, Sharpsburg, PA, USA), and MR-compatible goggles (Visual System, NordicNeuroLab, Bergen, Norway).

Behavioral measures of anxiety

The main instrument to measure clinical outcome was the Liebowitz Social Anxiety Scale (Liebowitz, 1987) which measures social anxiety and avoidance symptoms over the past week. The 24-items describe different potentially anxiety provoking situations and the respondents estimates how anxious they felt or, if the situation did not occur during the week, how they think they would have felt had the situation occurred (0 = no anxiety, 1 = mild, 2 = moderate, 3= severe). Further the respondents estimate how often they typically avoid these situations (0 = never (0%), 1 = occasionally (1-33%), 2 = often (34-67%), 3 = usually (67-100%). Hence, the minimum score is 0 and the maximum score is = 144, higher scores reflecting more severe symptoms. There is a clinician administrated version and a self-report version (SR) of LSAS and versions are highly correlated with each other (Baker, Heinrichs, Kim, & Hofmann, 2002; Fresco et al., 2001) and the LSAS-SR has been shown to have good properties for identifying individuals with social anxiety during screening procedures (Rytwinski et al., 2009). Further, clinical global impression – improvement scale (CGI-I) was use to assess responder rates in study IV. In a double-blind setting CGI-I can be used by study clinicians to

make judgements regarding the improvement of participants after treatment. Improvement is rated from 1-7 where 1 indicates that the patient is very much improved, 4 indicates no change and 7 very much worse. Because of the overt/covert – design in study II and III, the study clinician could not be blinded, and therefore a statistical approach was applied (Jacobson & Truax, 1991), where a reliable change (>1.96) in LSAS-score, in combination with falling within 2 SDs of the normal population (LSAS-SR < 39), was rated as the patient responding to treatment.

Other measures of anxiety and depression were used during screening procedures in the studies included in this thesis. These include the Social Phobia Screening Questionnaire (SPSQ) (Furmark et al., 1999), Social Interaction Anxiety Scale (SIAS) (Mattick & Clarke, 1998), Montgomery-Åsberg Depression Rating Scale (MADRS-S) (Montgomery & Åsberg, 1979), the social anxiety section of Structured clinical interview for DSM-IV Axis I disorders (SCID-I) (First, Spitzer, Gibbon, & Williams, 1997) and the MINI international neuropsychiatric interview (Sheehan et al., 1998).

Aims

Not much is known about the influence of dopamine or serotonin/dopamine-interactions on social anxiety disorder, with regard to etiology and treatment, and how expectancies impact treatment outcome. The aim of the studies included in this thesis were to investigate possible alterations in serotonin and dopamine transporters in SAD, the effect of pharmacologic (SSRI) and non-pharmacologic (CBT) treatments on these monoamine systems and to evaluate if different response expectancies, induced by verbal instructions, would affect the outcome of SSRI-treatment as measured with self-reports and neuroimaging methods.

I

This cross-sectional case-control PET study aimed to examine differences in serotonin and dopamine transporter binding potentials between untreated SAD patients and healthy controls. Of special interest was putative group differences in the balance (co-expression) between monoamine transporters.

II

We here sought to test if the beneficial effect of escitalopram was influenced by verbal instructions by giving one group (overt) correct information while telling the other group (covert) they received an active placebo. We also aimed to examine differences on fMRI brain activity responses between the groups.

III

The aim of this PET study was to investigate if expectancy effects, induced by verbal instructions, would yield measurable effects on serotonin and dopamine transporters. In a subsample of study II, parallel changes in serotonin and dopamine transporters were evaluated by means of PET in SAD patients randomized to overt or covert SSRI-treatment.

IV

In this PET-study, we aimed to examine changes in serotonin and dopamine transporter binding in SAD after combined treatment with SSRI+CBT or placebo+CBT to investigate changes with treatment and if pharmacological and non-pharmacological treatments yield their effects through similar or different means.

Summary of studies

Study I

Background

Serotonin and dopamine have been implicated as important agents in the neuropathogenesis of social anxiety disorder (Berger, Gray, & Roth, 2009; Brühl, Delsignore, Komossa, & Weidt, 2014; Gordon & Hen, 2004; Richey et al., 2017) where serotonin alterations, so far, have been more thoroughly studied using PET imaging, with results indicating a hyperactive serotonergic system in SAD. (Frick et al., 2015; Furmark et al., 2016; Lanzenberger et al., 2006) Dopamine is known for its important role in voluntary movement, social behaviors and reward seeking (Berridge & Kringelbach, 2008, 2015; Wacker & Smillie, 2015), and this is one reason why dopamine has gained some attention in SAD research (Richey et al., 2017). However, nuclear imaging studies targeting dopamine have been inconclusive with regards to patient-control differences. This applies to studies targeting the D2-receptor (Plavén Sigray et al., 2017; Schneier et al., 2000, 2008, 2009) as well as the DAT (Schneier, Abi-Dargham, et al., 2009; Tiihonen et al., 1997; van der Wee et al., 2008). Using [^{11}C]DASB, a radioligand with high affinity for SERT and [^{11}C]PE2I to characterize DAT binding, the aim of the study was to examine SERT and DAT alterations and interactions in SAD. An additional aim was to replicate earlier findings from a previous study demonstrating increased SERT BP_{ND} in SAD (Frick et al., 2015).

Methods

A total of 27 SAD patients (17 men) and 43 healthy controls (HC) (23 men) underwent [^{11}C]DASB PET for 60 minutes, and 80 minutes of [^{11}C]PE2I PET with a 45-60 minute long waiting period in-between. Participants' PET images were co-registered to their corresponding T1-weighted MR images and BP_{ND} images were retrieved using Reference Logan (Logan et al., 1996) for [^{11}C]DASB and RPM for [^{11}C]PE2I (Gunn et al., 1997). Groups were compared on SERT and DAT BP_{ND} using parametric two-sample t-tests with family-wise error correction (FWE). Co-expression of SERT and DAT BP_{ND} was

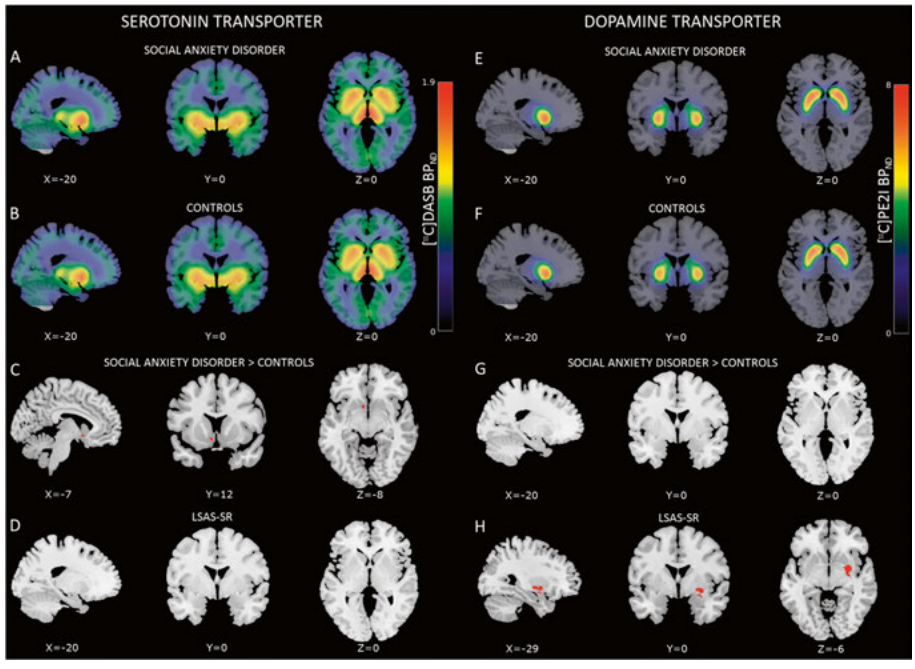

Figure 6. Mean SERT BP_{ND} for (**A**) SAD patients and (**B**) HC is displayed on the left-hand side while panel **C** shows increased NAcc SERT BP_{ND} in the SAD group. (**D**) No correlation with symptom severity was detected. **E** and **F** shows DAT BP_{ND} for SAD and HC respectively. No DAT BP_{ND} between group differences (**G**) were detected, but SAD symptoms correlated with DAT BP_{ND} (**H**) in the right amygdala, left hippocampus, right pallidum and right putamen (Hjorth et al., 2019).

assessed with voxel-wise Pearson partial correlations, and Fisher transformations were made to allow between group comparisons of correlation coefficients. Analyses were performed in MATLAB2018a (Mathworks Inc., Nantucket, MA, USA) using age and sex as covariates. Additionally, multiple regressions were used to investigate if SERT and DAT binding was associated with symptom severity (LSAS-SR score) in the SAD group.

Results

When analyzing the separate tracers, group analyses revealed a difference in SERT BP_{ND} in the NAcc, the SAD group showing increased binding as compared to HCs - see Figure 6C and Table 1. Also, several other regions exhibited effects in the same direction but at a more lenient statistical threshold (i.e. without FWE-correction). However, no linear relationship between SERT BP_{ND} and symptom severity was found. An inverse pattern was detected when examining DAT BP_{ND} differences. There were no significant between group

effects at the corrected p-level, but DAT BP_{ND} in the right amygdala, left hippocampus, right pallidum and right putamen, showed positive correlations with SAD symptom severity scores - see Figure 6H.

Co-expression analyses indicated increased co-expression in the SAD group as compared to the HCs in the amygdala, caudate nucleus, putamen, NAcc and posterior ventral thalamus- see Figure 7 and Table 2. One cluster with decreased transporter co-expression was detected in the dorsomedial thalamus.

Table 1. *Group differences in SERT and DAT BP_{ND} between a sample with SAD and HC individuals. Results from multiple regression regarding transporter binding and symptom severity are also presented.*

	X[a]	Y[a]	Z[a]	Cluster volume[b]	Z	P_{FWE}
SERT; SAD > HC						
L NAcc	-6	12	-8	512	2.65	.018
DAT; SAD vs. HC						
n.s						
SERT-LSAS						
n.s						
DAT-LSAS; positive						
R Amygdala	32	-2	-12	88	2.85	.033
L Hippocampus	-20	-38	-2	32	3.34	.026
R Putamen	30	0	-6	240	3.25	.031
R Pallidum	26	0	-6	208	3.07	.019

[a] MNI coordinates

[b] Cluster volume in mm^3

Table 2. *Group differences in SERT and DAT co-expression between a sample with SAD patients and HC individuals.*

	X[a]	Y[a]	Z[a]	HC *r*[b]	SAD *r*[b]	Diff *r*[c]	Diff *p*	Cluster volume[d]
SAD > HC								
L Amygdala	-30	0	-26	-.176	.405	0.582	.024	576
R Caudate	16	22	-8	.280	.726	0.445	.019	1344
R Putamen	34	4	4	.362	.742	0.380	.033	1088
R Nacc	10	12	-12	.071	.546	0.475	.044	384
R Thalamus								
HC > SAD								
R Thalamus	8	-20	8	.608	.120	0.488	.030	512

[a] MNI coordinates; L = Left, R = Right

[b] Pearson correlation coefficient, *r*

[c] Difference in correlation coefficients between groups

[d] Cluster volume in mm^3

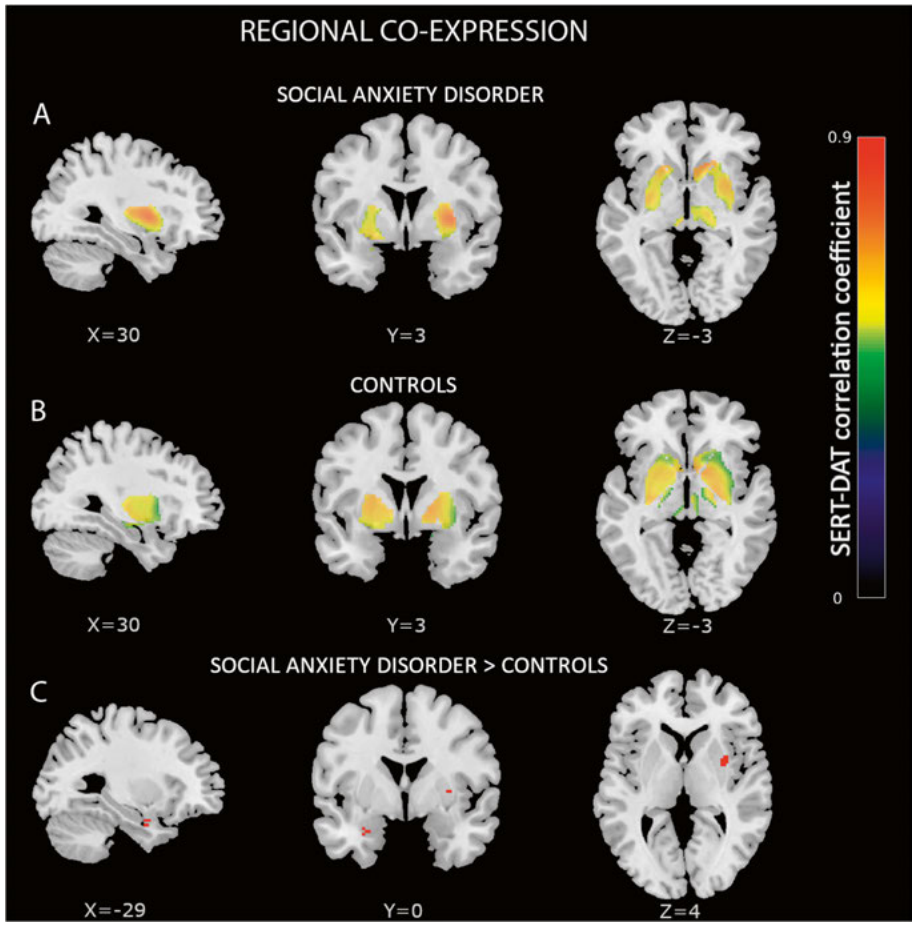

Figure 7. Regional co-expression of SERT([^{11}C]DASB) and DAT([^{11}C]PE2I) as reflected by voxel-wise partial Pearson correlation coefficients. Panels show transporter co-expression in (A) SAD patients, (B) HC, and bottom panel (C) depicts statistically significant clusters of increased co-expression in SAD (Hjorth et al., 2019).

To further investigate if SAD diagnosis could be predicted by SERT and DAT BP_{ND} interactions, logistic regressions were performed with analyses indicating significant interaction effects in the amygdala ($p = 0.032$, $Z = -2.15$), putamen ($p = 0.036$, $Z = -2.09$) and dorsomedial thalamus ($p = 0.013$, $Z = 2.49$). When examining the model, the interaction effect (SERT BP_{ND} × DAT BP_{ND}) significantly increased McFadden R^2 explained variance in the amygdala, putamen and thalamus -see Table 3. Social anxiety symptoms as a continuous variable was however not predicted by transporter interactions.

Table 3. *Changes in logistic regression model fit when adding the SERT BP_{ND} and DAT BP_{ND} interaction term.*

	R^2 main effect	R^2 interaction	Z	P
Amygdala	.18	.25	-2.15	.032
Putamen	.19	.24	-2.09	.036
Thalamus	.15	.25	2.49	.013

Discussion

This is the first PET case-control study conducted where serotonin and dopamine transporters are examined in the same sample. Results showed increased SERT binding in NAcc in SAD, while increased DAT binding in both limbic and striatal areas was significantly associated with increased social anxiety symptom severity. SAD patients also exhibited a higher SERT/DAT co-expression than HCs in these regions, deeply involved in fear and reward processing (Adhikari et al., 2015; Carlezon & Thomas, 2009; Kohls et al., 2013; Ohman, 2005; Schultz, 2016). Interregional signaling between amygdala and NAcc is important for emotion regulation and these two areas are regarded as part of a ventral emotion processing system (Cardinal, Parkinson, Hall, & Everitt, 2002; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). Also, the shell of NAcc is known to harbor dense concentrations of serotonergic neurons and in rodents, innervations projected from amygdala to NAcc has been found to reinforce D1-R mediated reward-seeking behavior (Stuber et al., 2011). Our data, when taken together with earlier studies suggests an overactive presynaptic serotonin system, but for dopamine there might be reason to believe signaling is attenuated. Rodent studies show that reduced DAT expression occurs concomitantly with increased free synaptic dopamine and when faced with standard behavior testing, anxious behavior is reduced in DAT knockout mice (Carpenter, Saborido, & Stanwood, 2012; Zhuang et al., 2001). Therefore, higher DAT BP_{ND} might be a reflection of faster dopamine clearance, resulting in less dopamine signaling which in turn might contribute to alterations in social and reward-seeking motivation.

Important limitations were low sample size, possible SAD sample heterogeneity, and lack of approach behavior measurements. Moreover, though we detected increased co-expression in SAD, the techniques used do not leave room for mechanistic inferences regarding interactions between serotonin and dopamine signaling in SAD.

Study II

Background

The true efficacy of SSRI's has been up for debate for many years both regarding depression (Bschor & Kilarski, 2016; Khan & Brown, 2015) and anxiety (Roest et al., 2015; Sugarman, Loree, Baltes, Grekin, & Kirsch, 2014) although academic correspondence regarding depression has drawn more attention. It has been suggested that the therapeutic effect of SSRI is only partly pharmacological and that the differences found between placebo and SSRI treatment groups are enlarged by perceived SSRI side effects that reveal for the participant which treatment arm they have been assigned to (Moncrieff, Wessely, & Hardy, 1998). This may in turn boost response expectations. There are numerous studies showing strong effects of treatment outcome expectation on clinical outcomes within research on depression (Chen et al., 2011; Rutherford et al., 2017), anxiety (Colloca, Lopiano, Lanotte, & Benedetti, 2004) as well as pain (Bingel et al., 2011; Colloca et al., 2004). In SAD, response rates of 50-60% have been observed while approximately 40% respond to placebo (Baldwin et al., 2016).

This study directly addressed the question regarding how expectations, induced by verbal instructions, affects the clinical outcome by administering the SSRI escitalopram to two patient groups diagnosed with SAD. One group was given correct information regarding the pharmacological treatment and the other group was told they were given an active placebo in the form of a Neurokinin-1 (NK-1) receptor antagonist. Clinical outcomes between groups were compared, and to examine objective brain activation measures, fMRI and an emotional face matching task were used.

Methods

A total of 46 SAD patients were stratified by age and sex and subsequently assigned by the use of a random-number generator (RNG) to either a group given overt (n=24) or covert (n=22) treatment with an SSRI. All personnel, except the study psychiatrist who presented the cover story, was blinded to allocation, and patients had been instructed to not share information regarding their allocation. Participants assigned to the covert treatment group were told they were given a NK1-receptor antagonist which should have no effect on SAD symptoms but might elicit similar side effects, thereby being an active placebo to be used as a reference treatment in future research.

For all patients, treatment consisted of 9 weeks of 20 mg daily intake of escitalopram, with an initial week of 10mg daily. Patient compliance to treatment was assessed by analyzing levels of escitalopram metabolites in blood plasma drawn at last visit.

Participants filled out online LSAS-SR measurements, i.e. the main clinical outcome, at home biweekly during the treatment period and an additional time at post treatment.

The emotional face matching task created by Hariri et al., (2002) which was used during pre- and posttreatment fMRI-sessions, requires the participant to match emotional faces by clicking buttons on MRI-compatible hand-controllers. Elliptical shapes were used as non-emotional control stimuli. First level analysis compared the non-emotional BOLD-reactivity to the emotional BOLD signal, and after creating images with the pretreatment scan subtracted from the post treatment scan, the resulting difference images were subsequently used in second level analysis to compare treatment groups. Between group analyses of the change in BOLD were performed using two-sample t-tests with age and sex as covariates, and the statistical threshold set at $P_{FWE} < .05$.

Results

Overt and covert SSRI groups did not significantly differ on relevant baseline measurements, and, before randomization, the whole study sample deemed SSRI treatment more credible than NK1-R antagonist treatment ($t_{(42)} = 6.58$, $p < .001$). When examining reduction in LSAS-SR scores with 9 weeks of treatment, a statistically significant improvement was found both in the overt and covert treatment groups.

However, the groups were also significantly separated with overt treatment showing markedly fewer symptoms at post measurement (responders: overt, 50% ; covert, 14%)- see Figure 8 and Table 4, along with significantly larger improvements on most secondary measurements (MADRS; $p = .02$, $\eta^2 = 0.11$, SIAS; $p = .01$, $\eta^2 = 0.14$, QOLI; $p = .006$, $\eta^2 = 0.16$), but not all (SPSQ; $p = .09$, $\eta^2 = 0.06$ BAI; $p = .28$, $\eta^2 = 0.03$, STAI-S anticipatory anxiety; $p = .84$, $\eta^2 = 0.001$). Analyses indicated that groups were significantly differentiated on LSAS-SR scores after 3 weeks of treatment.

Table 4. *Changes in Liebowitz Social Anxiety Scale self-report after overt and covert treatment with escitalopram. Within and between group effects are listed.*

	Overt SSRI	**Covert SSRI**	**F(1, 44) (between)**	**p-value (between)**	**Partial η^2 (between)**
LSAS-SR			16.58	<.001	0.27
Pre (SD)	83.71 (20.13)	81.45 (17.82)			
Post (SD)	41.08 (17.92)	60.00 (20.17)			
Paired t-test, *p*-value	10.71, p<.001	6.58, p<.001			
Cohen's d	2.24	1.13			
SD change (within)	19.50	15.30			

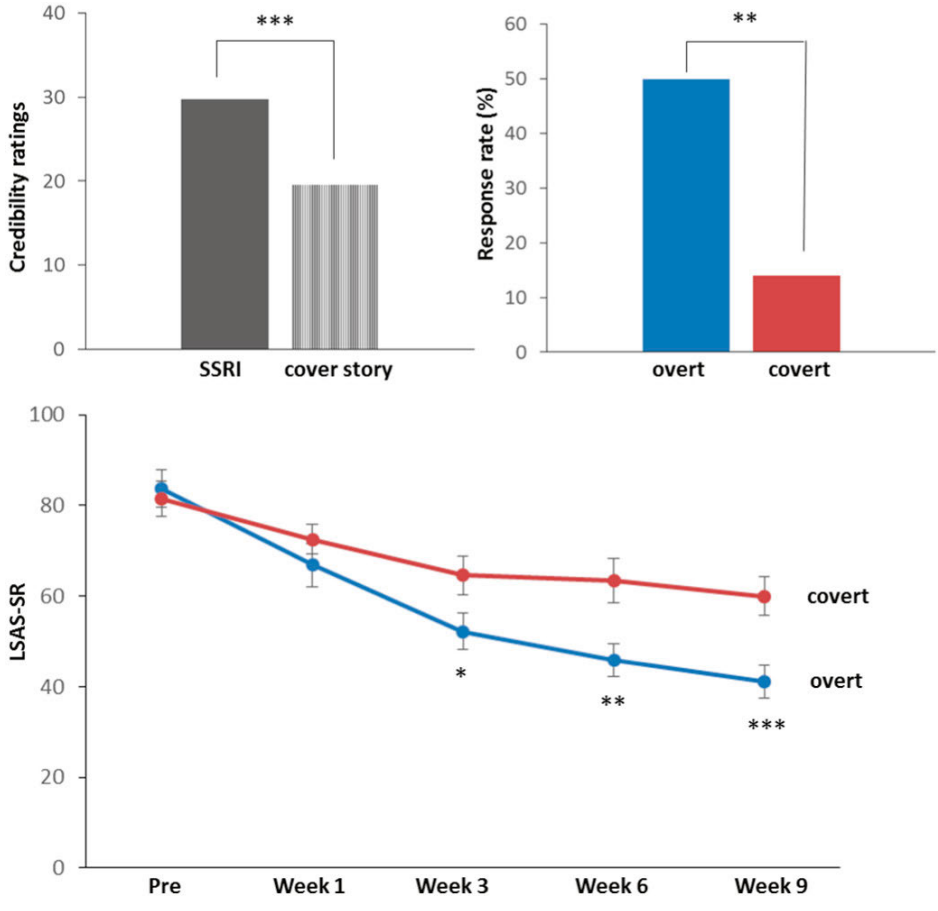

Figure 8. Top left panel depicts the full cohort difference in credibility ratings between SSRI treatment and NK1-R antagonist treatment. Bottom panel shows the attenuation and standard error (SE) of SAD symptoms progressively over the course of treatment for both overt and covert treatment. Top right panel illustrates the rate of responders in both treatment arms (Faria et al., 2017).

fMRI data analyses did not yield significant between group results when applying an amygdala ROI analysis, but exploratory analyses found bilateral increases in the posterior cingulate gyrus, left mid temporal gyrus and left inferior frontal gyrus in the overt group as compared to the covert group - see Figure 9 and Table 5. Regression using LSAS-SR pre-post difference scores as dependent variable and BOLD pre-post images as independent variable revealed that reductions of symptoms was related to attenuated right amygdala activation (MNI x, y, z; 33, -1, -29; Z = 2.70, Vol = 972 mm3, p = .003) and an inverse association was detected in the posterior cingulate/precuneus region (MNI x, y, z, -18,- 31, 34, Z = 3.24, vol = 216 mm3, *p* = .0006; 12, -46,

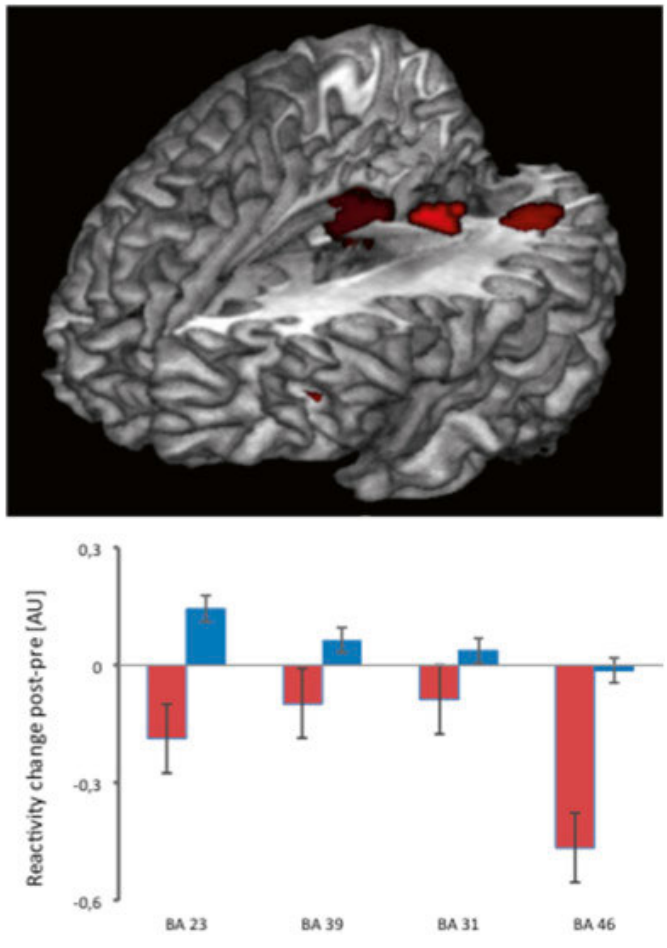

Figure 9. Brain image depicts the overt > covert contrast in bilateral posterior cingulate cortex, left mid temporal gyrus and inferior frontal gyrus, with relative increased BOLD to an emotional face matching task in the group receiving overt SSRI treatment as compared to covert treatment with the same drug. Bars show the relative changes (pre-post) and SE of the BOLD signal for overt (blue) and covert (red) treatment groups (Faria et al., 2017).

16, Z = 3.10, Vol = 2133 mm3, p = .001; -30, -58, 28, Z = 2.85, Vol = 486 mm3; p = .002). An overt vs. covert psychophysiological interaction (PPI) analysis with the amygdala (MNI; x, y, z; 33, 1, -29) peak voxel time-series data and emotional task (faces over shapes) entered as regressors along with their interaction. Covert treatment demonstrated increased amygdala and right dorsal posterior cingulate cortex (MNI; x, y, z; 21 -49 40) connectivity as compared to the overt group. Additionally, increased connectivity between amygdala and insula was found, also in the covert treatment groups- see Figure 10.

Table 5. *Differences in neural reactivity pre-post with either overt (N = 24) or covert (N = 22) SSRI treatment.*

	X[a]	Y[a]	Z[a]	Cluster Volume[b]	Z	*p*-value
Overt > covert						
R posterior cingulate gyrus	9	-28	28	3780	4.58	<.001
L mid temporal gyrus	-30	-58	25	513	3.93	<.001
L posterior cingulate gyrus	-21	-28	34	351	3.83	<.001
L inferior frontal gyrus	-36	35	1	270	3.69	<.001
Covert > overt						
n.s						

[a] MNI coordinates

[b] Cluster volume in mm^3

Discussion

The study results suggest that expectancies of improvement can affect clinical outcomes through the use of verbal instructions both on subjective (anxiety) and objective (fMRI) measures. The group difference in LSAS-SR scores after treatment was dramatic, the overt group exhibiting markedly lower anxiety scores. The responder rate was consequently almost fourfold in the overt group (55%) than in the covert (14%) treatment arm. Results are in line with earlier reports regarding differences in efficacy differences between covert and overt treatment (Bingel et al., 2011; Colloca et al., 2004), although doses were prolonged in the current study. The large differences in anxiety scores between groups demonstrates that SSRI treatment efficacy is highly influenced by how the treatment is presented to the patient. Still, the covert group did recover to some extent which was partly shrouded by the conservative responder rate methodology. Also, groups did not differ in improvement on anticipatory anxiety measurements before a speaking task and compared with waiting-list participants from earlier trials, covert treatment showed larger improvement. Given the stronger response to overt SSRI treatment in SAD, a biopsychosocial model is more coherent than a strict pharmacological, when trying to explain the full scope of the therapeutic effects of SSRIs.

With regards to fMRI-analyses, the expected change of amygdala reactivity after treatment was not detected, but social anxiety symptom improvement was associated with decreased amygdala BOLD signal, which is in line with earlier work from our group (Faria et al., 2014, 2012; Gingnell et al., 2016).

Further, the covert group exhibited increased connectivity between amygdala and regions important for emotion processing, a pattern earlier observed to be linked to perseverance of fear memories, whereas disruption of reconsolidation of fear memories weakens the link (Agren et al., 2012). Posterior

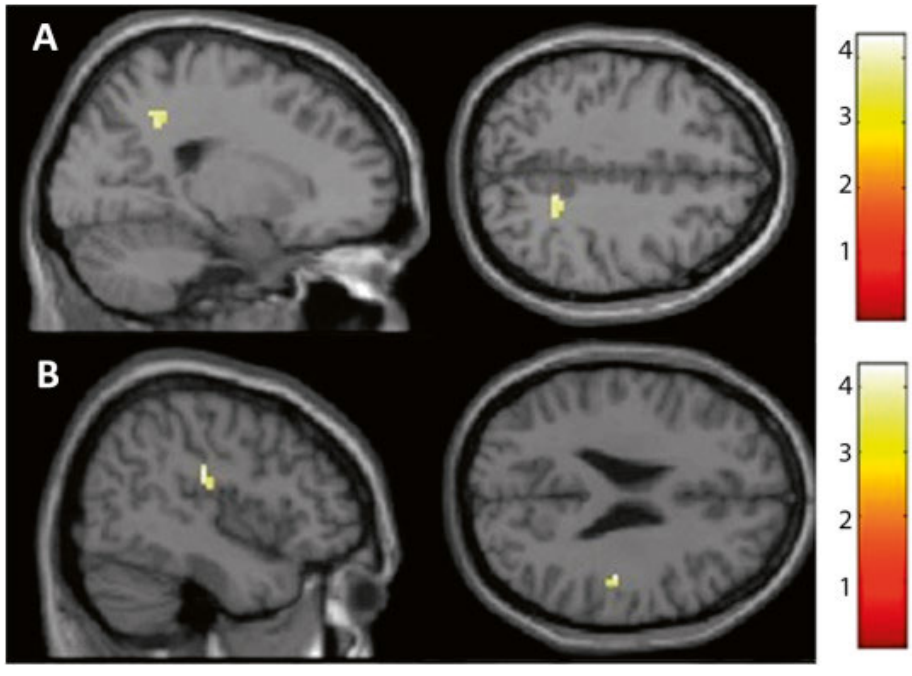

Figure 10. Panel rows show PPI analyses illustrating increased connectivity between amygdala and (**A**) the right dorsal posterior cingulate cortex and (**B**) insular cortex, in a SAD patient group administered covert SSRI treatment as compared to overt treatment (Faria et al., 2017).

cingulate is involved in cognitive processes but also receives emotional information and might be important for emotional-cognitive interactive processes as it is a key node in the default mode network together with the neighboring region, precuneus (Maddock, 1999; Pearson, Heilbronner, Barack, Hayden, & Platt, 2011). Although more research is needed it is possible that this region is involved in expectancy/anxiety interaction.

To our knowledge, this is the first fMRI study with a deception-placebo design, investigating clinical response to an SSRI. We also conclude that verbal instructions elicited expectancy effects of treatment success to such an extent that more research regarding the effects of how a treatment is presented needs more attention, due to the possible clinical implications.

Study III

Background and aim

As introduced in study II, the true contribution of pharmacology to the efficacy of SSRI treatment, and how large the effect of psychological factors is, are questions that has been widely discussed (Kirsch & Sapirstein, 1998; Kirsch et al., 2008; Quitkin et al., 1987). It has been suggested that the true pharmacological response cannot be captured by using standard double-blind designs with pill placebo due to pharmaceutical side-effects revealing the treatment arm assignment for participants in the drug arm, hence boosting the efficacy of treatment (Kirsch, 2000; Moncrieff et al., 1998). To better capture the true pharmacological effects of drugs, designs such as the balanced placebo design, include treatment arms where participants are deceptively administered the pharmaceutical under trial (Enck, Klosterhalfen, & Zipfel, 2011). The results from study II in this thesis, indicated stronger response to 9-weeks of escitalopram treatment if administration was overt as compared to covert. Groups also differed on fMRI BOLD measures.

Several lines of evidence support that serotonin and dopamine are involved in the etiology of SAD (Akimova, Lanzenberger, & Kasper, 2009; Bahi & Dreyer, 2018; Carpenter et al., 2012; Frick et al., 2015; Furmark et al., 2008; F. G. Graeff & Zangrossi, 2010; Hjorth et al., 2019; Moriyama et al., 2011; Tiihonen et al., 1997) and it is possible that SAD is characterized by monoamine dysregulation compromising aversive and appetitive motivation. Dopamine has also been suggested to govern placebo responses through expectation of reward, (de la Fuente-Fernández, 2001; de la Fuente-Fernández et al., 2002; Scott et al., 2008) which could feasibly be captured using neurochemical imaging techniques.

In the present study, we sought to get deeper insights into the neurophysiology of the placebo response in pharmacological anxiety treatment by employing multi-tracer PET targeting the SERT and DAT. The principal aim was to compare the effect of overt escitalopram treatment on serotonin- and dopamine transporter availability, to covert treatment.

Methods

In a PET subset of the sample in study II, 27 SAD patients (17 men) were randomized to 9 weeks of either overt or covert SSRI treatment. As described previously, patients in the covert group were told they received a NK1-R antagonist (active placebo), without effect on SAD symptoms but with similar side effects as the tested SSRI. The clinical effect of treatment was examined with a two-way ANOVA (Group×Time).

Both groups underwent PET before and after treatment, using the radiotracers [^{11}C]DASB, with 60 minutes scan time, and [^{11}C]PE2I with 80 minutes

scan time. Both ligands are highly specific to the SERT and DAT respectively. After co-registration to T1-images BP_{ND} images were retrieved using reference Logan for [^{11}C]DASB and RPM for [^{11}C]PE2I.

SERT pre-post treatment images were obtained by calculating the rate of SERTs occupied by the SSRI at post measurement as compared to initial pre-treatment levels. Hence, a high SSRI occupancy of the SERT is reflected by decreased BP_{ND}. DAT pre-post images were obtained by calculating the percentage change of DAT BP_{ND} meaning that an increased percentage change of DAT is reflected by an increase in DAT BP_{ND}.

Two-sample t-test were performed in SPM to compare overt and covert treatment on changes in DAT and SERT BP_{ND} separately. Associations between the change in LSAS-SR score and BP_{ND} changes for the separate transporters (occupancy for SERT) and their interaction were analyzed using multiple regression, with age and sex as additional regressors. Analyses were performed in MATLAB2018a.

Results

ANOVA revealed no significant Group effect but an effect of Time, both groups improving from pre- to posttreatment, as well as a Group×Time interaction indicating better improvement in the overt group according to LSAS-SR measurements - see Figure 11 and Table 6.

Table 6. *Overview of Liebowitz Social Anxiety Scale- Self Report version (LSAS-SR) measurements before and after overt or covert SSRI treatment.*

	Overt	Covert	Statistic	*p*-value
ANOVA			$F_{(1,25)}$[a] = 13.20	.001
Pre(SD)	86.64(20.39)	83.15(21.02)	$t_{(24.71)}$ = 0.44	.670
Post(SD)	39.57(20.08)	61.69(23.49)	$t_{(23.73)}$= 2.62	.015
Statistic	Paired $t_{(13)}$ = 9.16	Paired $t_{(12)}$ = 4.49		
p-value	<.001	.001		

[a] Interaction Group×Time

Both groups exhibited treatment relevant mean levels of escitalopram SERT occupancy (>80%) after 9 weeks of treatment – see Figure 12 for pre-post difference. No between group differences in SERT occupancy were detected and no associations between SERT occupancy and symptom severity was found in either group.

The covert treatment group showed increased percentage change DAT BP_{ND} after treatment in the bilateral pallidum, and groups also differed in the lateral left part of that cluster extending into the right putamen - see Table 7.

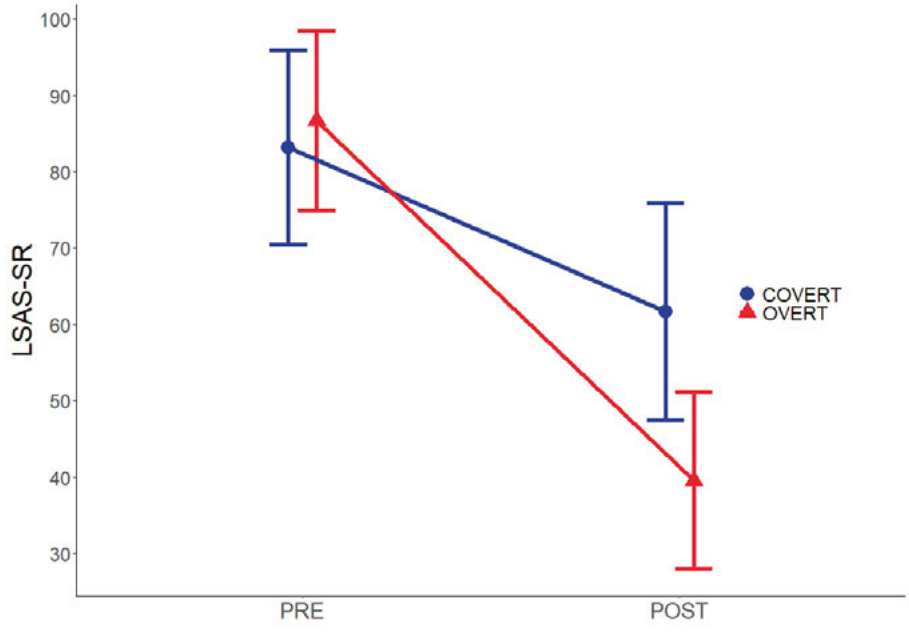

Figure 11. Changes in mean social anxiety symptoms, as measured by the LSAS-SR, after 9 weeks of overt (blue) or covert (red) escitalopram treatment. Bars are 95% CI.

Table 7. *Overt- and covert group differences in the percentage change of DAT ([^{11}C]PE2I) BP_{ND} and SERT ([^{11}C]DASB) occupancy after 9 weeks of treatment with escitalopram.*

	X[a]	Y[a]	Z[a]	Cluster volume	Z	p_{FWE}
DAT						
Overt Pre > Post						
R Amygdala	34	4	-20	8	2.94	.035
Covert Post > Pre						
L Hippocampus	-22	-36	4	8	3.27	.046
L Pallidum	-22	-2	-4	24	2.90	.041
R Pallidum	24	2	-4	8	2.80	.050
Covert > Overt						
R Putamen	22	8	-4	144	3.46	.017
R Pallidum	22	4	-2	72	3.10	.020
L Thalamus	-20	-30	4	56	3.40	.018
SERT						
n.s						

[a] MNI coordinates

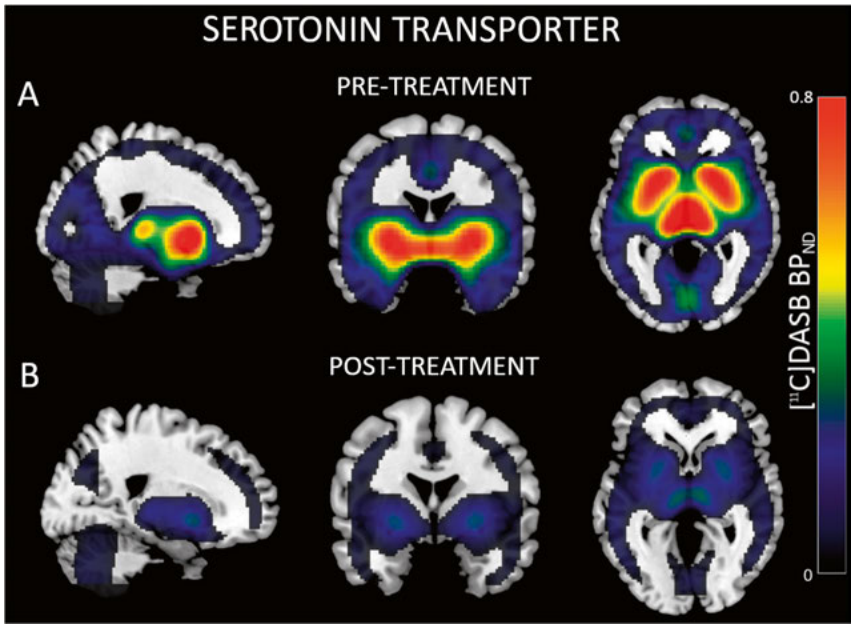

Figure 12. Serotonin transporter non-displaceable binding potential in the whole sample (**A**) before and (**B**) after 9 weeks of treatment with the SSRI escitalopram in the whole sample (N = 27).

These findings are further extended by multiple regression indicating a stronger association, in the overt group, between symptom reduction and decreased DAT BP_{ND} in this cluster (MNI; x, y, z, 28, -14, -2 Z = 3.72, Vol. = 168mm^3, P_{FWE} = .011). Further, the covert group exhibited a significant DAT BP_{ND} increase in the dorsal hippocampus while a significant decrease was evident in the right amygdala in the overt group, but between group analyses were only significant on a more lenient statistical threshold ($P_{uncorrected} < .05$). Initial SERT or DAT BP_{ND} levels did not predict treatment outcome for either group.

Correlations between SERT occupancy and the percentage change in DAT BP_{ND} were exclusively negative in the overt group and predominantly, positive in the covert treatment arm. Between group analyses revealed significant differences in putamen, pallidum thalamus and NAcc, the covert group exhibiting markedly higher Pearson correlation coefficients. Since increased SERT occupancy indicates lower estimates of bound serotonin, the overt group had a stronger association between decreased SERT BP_{ND} and decreased DAT BP_{ND}. Follow up logistic regressions indicated that the inclusion of the interaction term (SERT occupancy × DAT BP_{ND}) greatly increased the McFadden R^2 explained variance in all regions except right pallidum. (Main effect/Interaction; putamen = .02/.28; left pallidum = .03/.19, right pallidum = .27/.27, thalamus = .11/.41, NAcc = .03/.15).

Discussion

We here found differential effects on treatment response, concomitant with differential effects on serotonin- and dopamine transport, depending on participants expectations of improvement. The finding of superior clinical response of the overtly treated group as compared to the treatment arm with covert treatment is in line with the results of the full cohort (study II).

Escitalopram occupied the SERT at levels sufficient for therapeutic effect (Meyer et al., 2004; Voineskos et al., 2007), but groups did not differ in SERT occupancy, and no significant correlation between occupancy and symptom improvement was detected in neither overt nor covert treatment. This indicates that the difference in clinical effect can't be explained by differences in SERT occupancy. However, group differences in the change of DAT BP_{ND} was found in pallidum/putamen and thalamus, the overt group showing comparatively reduced DAT BP_{ND}, possibly indicating increased dopaminergic signaling. Further, associations between decreased DAT BP_{ND} in putamen and pallidum and reduced symptoms was found in the overt group. Moreover, interaction effects between the change of SERT and DAT BP_{ND} in NAcc, putamen, pallidum and thalamus significantly predicted group overt/covert group.

Since both groups significantly improved despite different expectancy of treatment success, results suggest that serotonin transporter blockade may be an important factor in successful treatment of SAD, but it is not sufficient for full treatment effect. Instead, data suggests that group dependent changes more likely have associations with changes in DAT and DAT/SERT interactions in the basal ganglia, thalamus and possibly amygdala, regions that are deeply involved in fear and reward processing. Results are consistent with a model where part of the anxiolytic effect of SSRI is due to SERT occupancy, while DAT and DAT/SERT interactions are involved in modulatory processes presumably needed for sufficient clinical improvement.

Designs including deception need to be extensively scrutinized from an ethical standpoint before being implemented, but when warranted, they enable the separation of pharmacological from psychological effects in a unique way. Results from study III demonstrate that response expectancies shape the efficacy of escitalopram both at the clinical and brain monoamine transporter level.

Study IV

Background and aim

SSRI and CBT are first line treatments of SAD, (Mayo-Wilson et al., 2014) and often combined, but the underlying biological mechanisms are not well understood. In addition, only 50-65% respond to available treatments (Leichsenring & Leweke, 2017) so deeper insights into the pathophysiological underpinnings of SAD are important for the development of new effective treatments. Mainly serotonergic (Davies et al., 2006; Frick et al., 2015; Furmark et al., 2016; Hjorth et al., 2019; Lanzenberger et al., 2006; van der Wee et al., 2008) but also dopaminergic (Bergman et al., 2014; Hjorth et al., 2019; Hood et al., 2010; Plavén Sigray et al., 2017; Schneier et al., 2009, 2000) involvement in the pathogenesis of SAD has been suggested. This multi-tracer ([^{11}C]DASB, [^{11}C]PE2I) PET study examined changes in serotonin and dopamine transport after 9 weeks of combined treatment of SAD with either escitalopram + ICBT or placebo + ICBT. Aims were to compare changes in DAT between groups, but also if SAD symptoms or changes in symptoms after treatment, could be predicted by SERT, DAT or their regional co-expression, across groups.

Methods

The study was a double-blind, randomized clinical trial, using a PET subsample (N=24, 12 women) of a larger clinical cohort assessed with fMRI (Gingnell et al., 2016). After screening patients were stratified by age and sex and subsequently randomized to either SSRI + CBT (N=12, 6 women) or placebo + CBT treatment (N = 12, 6 women). CBT was provided via internet and the program, based on the cognitive model of SAD by Clark & Wells (Clark & Wells, 1995) has been evaluated with positive results (Andersson et al., 2006; Carlbring et al., 2007; Furmark et al., 2009) LSAS and CGI were used to measure social anxiety symptoms.

PET was performed before and after treatment and at each occasion 22 [^{11}C]DASB images (during 60 min) and 22 [^{11}C]PE2I images (during 80 min) were retrieved per participant. ROIs were amygdala, hippocampus, ventral and dorsal striatum, pallidum and thalamus, with the ACC, raphe nuclei and insula also added in SERT-only analyses.

A Two-Way ANOVA (Group×Time) was applied to investigate changes in clinical outcome with the statistical threshold set at $P < .05$. Group differences in SERT and DAT BP_{ND} were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, University College London, www.fil.ion.ucl.ac.uk) t-tests with a statistical threshold of $P_{FWE} < .05$ and age

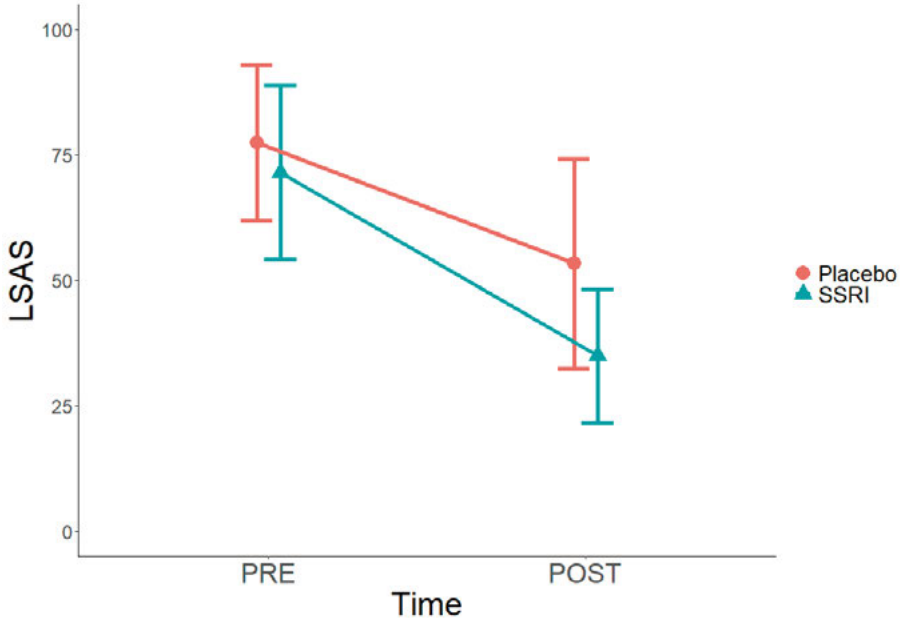

Figure 13. There was a trend towards SSRI+CBT being superior after 9-weeks of treatment as compared to placebo+CBT (p=.089).

and sex as covariates. SERT-DAT interactions were measured with partial Pearson's correlation coefficients and relations between DAT, SERT and their interaction, pre (co-expression), post and following treatment (Δ) with symptom improvement (LSAS) was analyzed with multiple regression ($P <. 05$) performed in Matlab2018a (Mathworks Inc., Nantucket, MA, USA).

Results

Whole sample analysis showed significant symptom improvement after 9 weeks of treatment ($F(1,22) = 43.12$, $P <.001$, Cohen's $d = 1.32$) but no group differences were detected (Group; ($F(1,22) = 1.51$, P=.232), Group×Time; ($F(1,1) = 1.83$, P =.186)) – see Figure 13. CGI-I response rates were, 10 (83%) in the SSRI+CBT group and 5 (42%) in the placebo + CBT group (Fisher's exact test: $P =.089$).

Mean SERT occupancy in the SSRI + CBT group was 83.3% and symptom improvement correlated with higher occupancy in right NAcc, left putamen and left ACC – see Figure 14. In the placebo + CBT group, increased SERT BP_{ND} was detected after treatment in the raphe nuclei. A trend was also found in the right amygdala, right putamen and the right NAcc.

Increased DAT BP_{ND} was observed in both groups after treatment in the amygdala, hippocampus, NAcc and putamen. However, in the SSRI+CBT

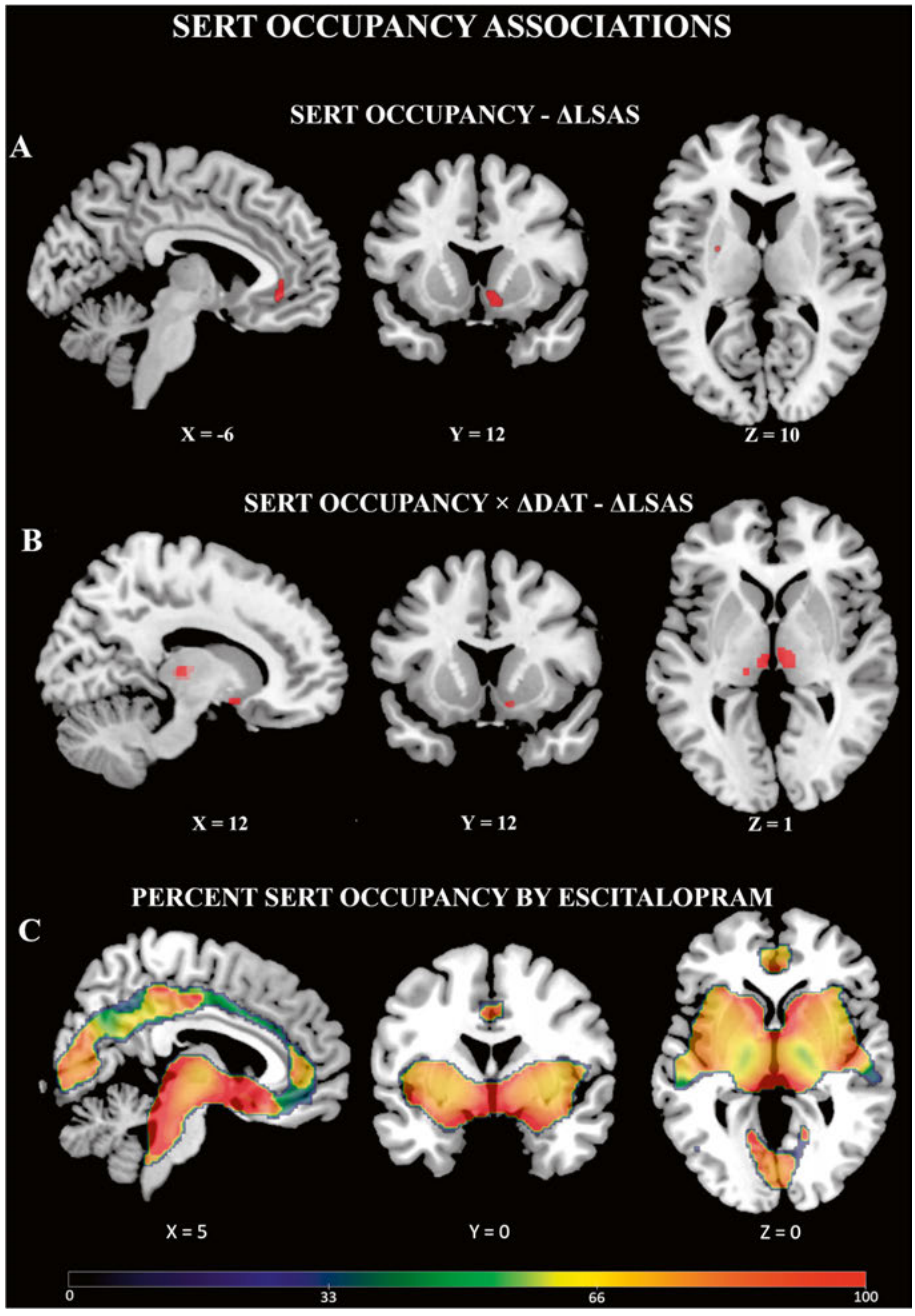

Figure 14. In the SSRI+CBT group SERT occupancy (**A**) in the right NAcc, the left ACC and left putamen was positively correlated with symptom improvement, and (**B**) the association between SERT occupancy and symptom improvement was moderated by the change in DAT BP_{ND} in the right NAcc. **C** depicts general SERT occupancy. The color gradient illustrates percent SERT occupancy.

group, a negative correlation between symptom improvement and increased DAT BP_{ND} was detected in the left amygdala and conversely, increased left NAcc DAT BP_{ND} in the placebo + CBT group was associated with larger symptom improvement and hence, groups differed significantly in this analysis – see Figure 15.

Significant pretreatment co-expression in the whole sample was observed in all ROIs, and higher co-expression in the left putamen and left thalamus was associated with increased symptom severity as measured with LSAS. Further, a moderation effect of the change in DAT BP_{ND} on the association between increased SERT-occupancy and greater symptom improvement was observed. In the placebo+CBT group, negatively correlated ΔDAT and ΔSERT BP_{ND} predicted symptom improvement. In the SSRI+CBT group, higher initial co-expression in the right NAcc, left putamen and right thalamus and lower co-expression in the right pallidum, predicted symptom reduction. With regards to the placebo+CBT group, symptom reduction was significantly predicted by lower pre-treatment co-expression in the right amygdala, bilateral hippocampus, left putamen and right pallidum, and higher co-expression in the bilateral thalamus.

Discussion

The study replicates previous findings from Study I regarding increased serotonin-dopamine co-expression in SAD in regions known to be involved in fear and reward. Extending on those findings, high initial co-expression was additionally associated with more severe SAD symptoms. Further, positive correlations between SERT occupancy in the NAcc and symptom improvement has not been observed before in SAD, and additionally the change in DAT BP_{ND} in NAcc moderated this effect. In the placebo+CBT group negative correlations between ΔSERT and ΔDAT BP_{ND} was associated with symptom improvement. Groups differed in the association between ΔDAT BP_{ND} and symptom improvement in the NAcc and left thalamus, the placebo + CBT group showing markedly higher correlation coefficient than the SSRI + CBT group. Results point towards different neurochemical mechanisms mediating the clinical response between treatments with the differential response in the NAcc implicating modulations of reward-aversion as important, yet different between treatment modalities.

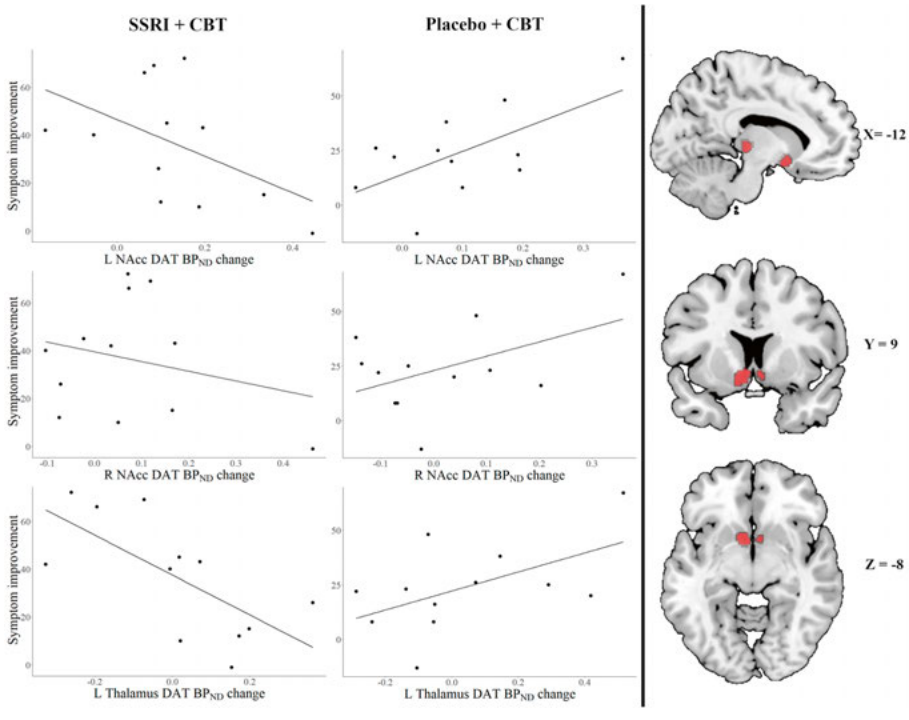

Figure 15. Scatterplots of brain regions where significant group differences were found in correlation coefficients between symptom improvement, as measured with the Liebowitz Social Anxiety Scale (LSAS), and the percentage change in DAT BP_{ND}. Regions with significant differences were the bilateral nucleus accumbens (NAcc) and left thalamus. Right panel illustrates the volume of significant voxels regions for the same analyses (P_{FWE}<.05).

Overall discussion

Main findings

The results of the present thesis can be summarized as follows:

- Patients with social anxiety disorder exhibit increased expression and co-expression of serotonin and dopamine transporters in limbic and striatal brain regions (*study I*).
- Expectancies have a large influence on SSRI efficacy in patients with SAD on subjective measures of anxiety, and also affect objective fMRI measures of brain activations (*study II*).
- Expectancies also exert marked effects on serotonin and dopamine transporters as measured with PET (*study III*).
- Pharmacologic (SSRI) and non-pharmacologic (CBT) treatment of SAD modulate brain serotonin and dopamine transporters in dissimilar ways (*study IV*).

Serotonin transporters

In Study I we demonstrated increased SERT BP_{ND} in NAcc and generally increased SERT BP_{ND} in SAD as compared to HC. Study III shows that serotonergic changes due to SSRI treatment may be of importance for clinical improvement, but that it's not sufficient for achieving full therapeutic effect as the overt and covert SSRI groups did not differ in SERT occupancy but differed significantly in clinical outcomes, as further verified in study II. SERT occupancy did not correlate with symptom reduction in study III, however this pattern was detected in Study IV. Further, increased SERT BP_{ND} was detected in the raphe nuclei after placebo + CBT treatment, also suggesting that SERT blockade is not a necessary condition for anxiety reduction.

Dopamine transporters

Throughout the studies DAT BP_{ND} in areas associated with fear and reward were found to moderate SAD symptom severity before treatment in Study I, and following treatment in Study III and IV. In Study III, DAT BP_{ND} effects differed depending on expectancy manipulations, while SERT occupancy did not. Decreased DAT BP_{ND} was detected in the amygdala in the overt treatment group, while the covert group exhibited increased DAT BP_{ND} in the hippocampus, and in a cluster in the pallidum extending into the putamen. Groups also differed in DAT BP_{ND} in this cluster, which could mean that the decreased DAT BP_{ND} in the overt group is reflected by increased dopaminergic signaling.

In Study IV, both groups exhibited increased DAT BP_{ND} in most ROIs with treatment. The placebo + CBT showed comparatively higher correlation coefficients between ΔDAT BP_{ND} in the NAcc and symptom improvement than the SSRI + CBT group. However, in SSRI-exposed individuals there was a

negative correlation between DAT change and symptom improvement, i.e. a correlation in the same direction as in the overt SSRI group in study II.

Serotonin-dopamine transporter interactions

High initial SERT-DAT co-expression in limbic and striatal areas differentiated SAD patients from controls in study I and was associated with more severe social anxiety symptoms before treatment in study IV. Overt SSRI treatment was associated with a higher negative correlation between SERT occupancy and the percentage change of DAT BP_{ND} in striatum, pallidum and thalamus, as compared to overt treatment. In study IV, the change in DAT BP_{ND} in the NAcc and thalamus moderated the association between SERT occupancy and symptom improvement. In the placebo + CBT group, a negative correlation between the change of SERT and DAT BP_{ND} in the putamen was associated with greater symptom improvement. Thus, there were different SERT-DAT functional interactions between a) SAD patients and controls before treatment, b) overt and covert SSRI groups differing in response expectancies and level of improvement and, c) patients exposed or not exposed to SSRIs when treated in com-bination with CBT.

Clinical outcomes

In study II, both medication groups exhibited statistical improvement, but overt SSRI-treatment was significantly superior to covert SSRI treatment. The same was true for the PET subsample from the same cohort reported in Study III. In study IV, treatments did not differ significantly on clinical outcome suggesting no additional benefit from combined treatment. However, there was a trend in favor of the SSRI+CBT combination, consistent with the treatment results in the larger cohort (Gingnell et al., 2016).

fMRI

In study II, symptom improvement was associated with attenuated amygdala activity with treatment, in line with previous studies (Faria et al., 2014, 2012; Gingnell et al., 2016). Also, connectivity between posterior cingulate cortex and amygdala was reduced in the overt treatment group as compared to covert treatment.

Discussion

This thesis has a broad scope that entails multi-tracer PET and MRI data, case-control and treatment designs using both double-blind and deception methodology. This discussion will touch upon all these different aspects of the included studies, what they can tell us, what their limitations are, and give a few pointers as to how further studies can answer the questions raised.

Firstly, the notion that the serotonin system is involved in SAD pathophysiology and treatment gains support by the studies in this thesis. Indices of increased serotonin reuptake as compared to controls were noted in study I and even if only a cluster in the NAcc survived correction for multiple comparisons, there was no region were SERT BP_{ND} was larger in the control group. This is in line with an earlier study from our group (Frick et al., 2015) and others (van der Wee et al., 2008) and in combination with findings of increased synthesis (Furmark et al., 2016), reduced inhibitory 5-HT_{1A} receptor binding and reduced synthesis after treatment (Frick et al., 2016), it has been suggested that SAD entails an overactive pre-synaptic system and possibly underactive postsynaptic inhibition. At least 80% occupancy of the SERT by SSRIs have been suggested to be an important threshold for therapeutic effect (Meyer et al., 2004) and no earlier studies on SAD has reported linear relationships between SERT occupancy and symptom improvement. This is also rarely seen in depression. This relationship was not detected in study III but conversely, in study IV, increased occupancy was associated with symptom improvement. Hence, the case is not closed regarding serotonin-symptom linearity. Further, SERT occupancy did not differ between overt, covert or double-blind treatment - i.e. similar rates in study III and study IV, which indicates that SERT occupancy cannot explain differences in treatment outcome between groups. This fact does not however exclude the possibility that other modalities of the serotonin system differed, but that issue requires further study. Another find that raises questions regarding the involvement of serotonin in SAD treatment is that SERT BP_{ND} in the placebo + CBT group increased in the raphe nuclei after treatment, and trends in the same direction were detected in the amygdala and striatum. This suggests that CBT treatment led to an increased SERT BP_{ND} from an already elevated state as compared to controls. It could be that CBT-treatment effects are not normalizing an altered state but are rather complementary, affecting other systems which indirectly increase SERT BP_{ND}, but it's difficult to correctly interpret main effects without a waiting-list group for comparison in this case.

Results regarding dopamine transport suggest a modulatory role of dopamine in SAD. DAT BP_{ND} in the amygdala, hippocampus and putamen correlated positively with symptom severity in study I. Dopaminergic innervation of amygdala has been shown to regulate amygdaloid inter-core communication, with D2 receptors exerting anxiolytic effects with regards to anxiety (de la Mora, Gallegos-Cari, Arizmendi-García, Marcellino, & Fuxe, 2010; Takahashi et al., 2010) and increased amygdala D2/D3 availability has been linked to increased amygdala reactivity to affective stimuli (Kobiella et al., 2010). Possibly increased receptor availability can be achieved by faster synaptic dopamine clearance by the DAT. In study III, verbally induced positive expectancies regarding treatment success did not affect the SERT, but was associated with relatively reduced DAT BP_{ND} in putamen, pallidum and thalamus in the overt treatment arm. A reduction that was also associated with

greater symptom improvement within that group. Putamen is mostly known for its involvement in voluntary movement but has been found to be involved in reward processing in both humans and monkeys (de la Fuente-Fernández et al., 2002; Schultz, 2016; Yoshimi, Kumada, Weitemier, Jo, & Inoue, 2015), dopamine mediated placebo response (Scott et al., 2008) and might be structurally enlarged in SAD (Bas-Hoogendam et al., 2017). Pallidum is also involved in reward processing, with its ventral part having a similar role as the NAcc (Berridge & Kringelbach, 2008). It also receives most of the output from the striatum, with some reciprocity (Voorn, 2010) and regulates information flow between striatum and the thalamus. A process that is mainly regulated by GABA but in part also by dopamine (Alexander & Crutcher, 1990). Further, the ventral pallidum is connected to the amygdala and increased pallidum dopamine signaling has been shown to decrease induced anxiety like behaviors in rodents (Chang & Grace, 2014). Dopamine probably also plays a role in compromised seeking and expectancies of reward as well as lowered motivational drive for social interactions – i.e. features that are prominent in social anxiety and related traits like risk aversion, anhedonia and introversion.

In study IV, DAT BP_{ND} increased in both limbic and striatal areas in both groups and the treatment arms could not be significantly separated in any region. In the placebo + CBT-group, increased DAT BP_{ND} in the NAcc was associated with symptom improvement, and the treatment modalities were significantly differentiated on this measure. Since both groups received CBT, the group difference is arguably driven by the SSRI.

DAT BP_{ND} has previously been found to be increased with SSRI-treatment (Kugaya et al., 2003; Pogarell et al., 2005; Rominger et al., 2015; Warwick et al., 2012) but in study III we only found this pattern in the covert treatment arm whereas overt treatment was associated with decreased DAT BP_{ND}. However, the radioligands used in earlier SPECT studies might not have been suitable to accurately describe serotonergic impact on DAT binding specifically. First, β-CIT DAT BP_{ND} has been shown to increase after acute SSRI administration in healthy subjects (de Win et al., 2005), and second, the similar ligand FP-CIT BP_{ND} has been show to both increase (Booij et al., 2007) and decrease due to SSRIs, whereas PE2I was not affected in a comparison study (Ziebell et al., 2010). A study in rodents also found decreased NAcc dopamine levels after acute administration of the SSRI fluoxetine, but no increase or decrease in base dopamine levels after chronic administration (Clark, Ashby, Dewey, Ramachandran, & Strecker, 1996). However, another animal study found increased dopamine levels in the prefrontal cortex and hypothalamus (Koch et al., 2002). This indicates that there is involvement of dopamine in the effects of SSRIs, but our results demonstrate that psychological factors, i.e. expectancies and CBT, also elicit dopaminergic responses.

SERT and DAT BP_{ND} co-expression was elevated in the SAD group as compared to healthy controls in Study I, and significant co-expression in the same ROIs was also seen in Study IV. This suggests that SAD is characterized

by an interaction between transporters, not seen to the same extent in healthy controls. Further, in Study III, increased SERT occupancy was associated with reduced DAT BP_{ND} in the overt group while an opposite pattern was detected in the covert group. It has been demonstrated that the DAT can also take up serotonin (Daws, 2009) and it can be hypothesized that serotonin diffusion would increase when SERT is blocked by SSRI, leading to a decrease in DAT BP_{ND}, and a further decrease could possibly be elicited by increased dopamine release induced by reward expectations. It has also been proposed by Zhou and coworkers (2005), that increased reuptake of serotonin by DAT leads to more VMAT2 competition and that the increased co-release leads to decreased dopaminergic signaling. This effect would also be counteracted by increased dopamine release. The enhanced clinical effect induced by higher expectancy of treatment success in the overt group could also initiate positive spirals with increased approach behaviors and more self-exposure further enhancing clinical effect and rewarding experiences.

In Study IV a moderation effect of increased DAT BP_{ND} with SSRI + CBT in the NAcc was found for the relationship between SERT occupancy and symptom improvement. Further, in the placebo + CBT group, a negative correlation between the changes in SERT and DAT BP_{ND} was associated with symptom improvement. This indicates that CBT and SSRI treatment exert their therapeutic effects by different transporter mechanisms.

In study II, symptom improvement correlated with reduced amygdala activity after treatment, which has been reported in earlier work from our group (Faria et al., 2014, 2012; Gingnell et al., 2016). Moreover, amygdala activity had weaker functional connectivity with emotion processing areas such as the insula and dorsal posterior cingulate cortex after overt treatment. Stronger amygdala-emotion processing network coupling has been related to more intact fear memories when reconsolidation is not disrupted (Agren et al., 2012). Posterior cingulate cortex activity is increased both by emotional and cognitive stimuli and has therefore been suggested to be a node for interaction between these modalities (Maddock, 1999). Changes in posterior cingulate BOLD has also been seen in placebo response to SSRI treatment in depression (Mayberg et al., 2002). When comparing the results from Study II and Study III, there was a correlation between symptom improvement and reduced DAT BP_{ND} in the right amygdala in the overt treatment group and similarly, in Study II, attenuated activity was associated with symptom improvement when examining the full sample. Note that no BOLD-BP_{ND} correlations has been performed.

There are some obstacles when comparing effects between Study III and Study IV. Firstly, there are no waiting-list conditions included in either study to more accurately delineate the main effects of treatment, but rather relative comparisons between conditions or linear changes within conditions within the separate studies are warranted. Secondly, the manipulations are different. Conditions in Study III are overt and covert SSRI treatment, whereas in Study

IV participants received CBT, half of them with concomitant SSRI treatment, in a double-blind design. Presumably, SSRIs given under double-blind conditions are not as effective as when administered overtly, as the effect sizes in study III and IV suggest. If dopamine has a modulatory role in SAD symptomology, then differential effects depending on treatment design could be expected.

Limitations

A very common problem in PET studies is small sample sizes, due to the large costs involved. In study I (N = 27) and full sample analyses in study IV (N = 24), the sample sizes are comparatively large as compared to common sample sizes within the field. Results from between group analyses in study III and Study IV however need to be interpreted with caution and replication is warranted to confirm the effects. The sample size for the between group fMRI analyses in study II also warrants some caution.

The difference in clinical effect reported in study II and III was measured with self-report questionnaires and could possibly be enhanced not only by boosted expectations in the overt treatment arm but also by underreporting of improvement in the covert group. However, the groups differed significantly on objective fMRI and PET measures, arguing against response biases of this kind.

None of the treatment studies included in the thesis had any waiting-list condition which makes the main effects of treatment more difficult to interpret, since uncontrolled factors could possibly have affected the PET-signal. This problem mainly affects study IV where between group analyses were limited to the DAT since occupancy of the SERT by the SSRI makes BP_{ND} differences between the pharmacological and non-pharmacological treatment arms almost infinite. A waiting-list could also have been beneficial to examine SERT changes in the placebo + CBT group. In Study III, differences between overt and covert treatment were the main issue of interest which reduces the need for waiting-list groups and since both groups received SSRI, information added by a waiting-list group would be limited to changes on the DAT. However, that information could have been useful in delineating the true interplay between expectancies and changes in SERT and DAT. For the same reason the value of adding the two extra conditions to complete the balanced placebo-design would be limited, even though it would add extra information regarding the clinical effect of escitalopram (Rohsenow & Marlatt, 1981). Adding a given placebo/told drug – condition also raises additional ethical concerns.

Another limitation is that we can't say if the changes in DAT and SERT BP_{ND} reflects an increase or a decrease in neural signaling. As earlier described, most data points toward an overactive presynaptic serotonin system, and hence, high SERT BP_{ND} has been interpreted as increased activity. Conversely, most animal studies indicate that, regarding social anxiety, a highly

active dopamine system is beneficial, which has, in the light of our data, led us to interpret low DAT BP_{ND} as reflecting increased activity. This interpretation becomes further intricate when accounting for the general increase in DAT BP_{ND} in Study IV. Furthermore, increased DAT BP_{ND} moderated the association between increased SERT occupancy and greater symptom improvement, indicating that an increase in DAT BP_{ND} is beneficial. One should keep in mind that the designs in Study III and Study IV are vastly different and between group analyses in Study III capture a very different variance than within-group regressions in Study IV. The designs of the current studies do not resolve these issues, and further study is needed.

Moreover, the techniques used does not allow for inferences about interactions at the neural signaling level, and interaction effects presented should always be interpreted as both transporters existing in the same volume, and not as a measure of neural interplay between the systems. There might be other systems that are not measured that could better explain the covariance between serotonin and dopamine. In reality, the interplay between neurotransmission systems in SAD and its treatments are likely to be very complex.

Future directions

The results from the studies included in this thesis suggest that both serotonin and dopamine could play important role in both the neuropathogenesis and treatment of SAD. One of the main issues that needs to be addressed is how transporter activity is related to receptor activity and synthesis to further delineate monoaminergic signaling in SAD. Considering the total amount of receptors in monoamine systems, and the fact that some of the receptors also work as autoreceptors, this is an intricate task. However, if focus is shifted to the most common receptors and groups of receptors with similar functions the number of targets could be narrowed down to a more manageable quantity. As have been described earlier, some work has already been performed on different parts of the serotonin system, but due to the common power issues in PET some work to replicate previous findings could be useful to verify identified effects on 5-HT_{1A} binding and serotonin synthesis. 5-HT_2 receptors is also likely to be involved in anxiety but studies characterizing the 5-HT_2 have demonstrated uneven patterns of effects (Quesseveur, Nguyen, Gardier, & Guiard, 2012).

Regarding dopamine, work on D2 receptors has yielded varying results with a cross-sectional study using [^{11}C]raclopride, showing no difference in binding between SAD and HC groups (Schneier et al., 2009), but increased extrastriatal D2 availability after CBT treatment has been associated with reduced anxiety using [^{11}C]FLB 457 (Cervenka et al., 2012). Since question marks remain, a multi-tracer PET study targeting both dopamine transport and receptor binding, could feasibly give more insight into synaptic activity of dopamine in SAD. Furthermore, only D2 receptors has been investigated in

SAD, and while there are available radiotracers for characterizing also the D1-receptor (Halldin et al., 1998; Halldin et al., 1986), no work on social anxiety patients has been performed targeting this receptor. However, one study has investigated D1-R binding in relation to the personality variables social desirability and aggression, which could be relevant to social anxiety, and found positive correlations between D1-R binding in striatum, amygdala and medial frontal cortex and social desirability, and an inverse pattern in the striatum for aggression (Plavén-Sigray et al., 2014). This makes the D1-receptor a possible target for delineating dopaminergic influence on SAD symptomology. Multi-tracer PET targeting D1 or D2 receptors in combination with DAT could possibly shed further light on the modulatory effects of dopamine which is an issue raised by the results from the studies included in this thesis. Furthermore, dopamine synthesis PET, which can be performed using the FDOPA or L-Dopa ligands, has not been targeted at all in previous PET studies with SAD samples. This makes it a possibly important new target for delineating dopaminergic influence on SAD symptoms. Using multi-tracer PET to investigate possible differences in serotonin and dopamine synthesis between SAD patients and controls could therefore be a feasible way to further characterize monoamine interactions in SAD. Additionally, combined PET-fMRI-scanners are becoming more commonly available which would allow simultaneous PET and MRI-scanning, which could be used to correlate ligand binding with the BOLD signal, opening for the possibility to delineate brain activity-neurochemistry interactions. In the long run, development of new fMRI contrast agents might make it possible to perform imaging of multiple monoamine systems simultaneously, rather than sequentially, which would increase the validity of estimated interactions between monoamine systems. There already exists a metalloprotein that binds selectively to dopamine, but so far it has only been tested in rats with transcranial injection and the hurdles of getting compounds safely across the blood-brain barrier has so far not been overcome (Bartelle, Barandov, & Jasanoff, 2016).

Regarding treatment the results from study II and III quite forcefully demonstrate the value of framing the administered drug as effective against SAD symptoms. It raises an ethical question regarding if clinicians should only provide information about the true drug effect of a prescribed drug, or provide information about how strong the combined drug + placebo effect is without mentioning that part of the effect is due to placebo, because that information would diminish the effect, reducing the benefit for the patient. I would argue that plausible placebo effects should not be revealed to patients under most circumstances, but that monitoring is important, because if treatment is not successful, the patient might experience emotions of failure. If taken to a more extreme, should we lie to patients if it was beneficial for them? My training begs me to say no, but where do we draw the perfect risk-benefit line?

Another way of identifying contributions of the different monoamine systems to SAD symptomology is to observe and compare behavioral changes after controlled administration of specific drugs. In the studies included in this thesis, the SSRI escitalopram was used, and the clinical effects of SSRIs are well described although the exact therapeutic mechanism is not identified. Other pharmacological treatments affecting the serotonin system that are currently being investigated in treatment resistant anxiety and depression are serotonergic psychedelics (5-HT_{2A}-agonists) such as lysergic acid diethylamide (LSD) and psilocin. Old studies are suffering from methodological issues, but more recent preliminary studies show promise of being effective. The pharmacological treatment is nowadays sometimes combined with psychotherapy and in the coming years we will get a better idea of the efficacy of these treatments, and hopefully clinical studies will be accompanied by PET-studies investigating putative accompanied neural alterations. Due to reported enhanced social cognition after treatment with serotonergic psychedelics, it could possibly be of interest to investigate these treatments in SAD patients.

Regarding drugs targeting the dopamine system, studies of the irreversible non-selective monoamine oxidase inhibitor phenelzine has shown large effect sizes in the treatment of SAD symptoms, but food restrictions have been seen as problematic. However, there are selective MAOB-inhibitors with much lower risk for severe side effects that could feasibly be interesting to investigate. There is only one small study performed on the MAOB-inhibitor selegiline (L-deprenyl) where 3 out of 9 patients responded to treatment (Simpson et al., 1998). However, the dose was very low (5mg) due to selegiline losing its MAOB specificity at doses over 10mg which means diet restrictions again become necessary. Now there are other drugs available such as rasagiline that do not lose specificity with dose. Rasagiline has not been investigated in social anxiety, but a small study on depression in de novo Parkinson's disease showed benefit of 2mg over 1mg on depressive symptoms. Given its safety, it could be used to investigate the effects of isolated increased dopaminergic signaling on SAD symptoms. Further, L-dopa administration has in some cases been reported to be anxiolytic, but contradictory effects have also been found. Just as serotonin can be taken up by the DAT, L-dopa can also be taken up by serotonin and norepinephrine cells and released as a "false" neurotransmitter (Eskow Jaunarajs, Angoa-Perez, Kuhn, & Bishop, 2011). Other low-risk pharmaceuticals that could be tested in adults with SAD are the commonly used ADHD-drug methylphenidate, a dopamine-norepinephrine reuptake inhibitor which has been reported to reduce SAD symptoms in addition to ADHD-symptoms (Golubchik, Sever, & Weizman, 2014), and also atomoxetine which selectively inhibits norepinephrine uptake has shown some effects on comorbid ADHD and SAD (Adler et al., 2009).

As earlier mentioned, even though there is likely a relationship between monoaminergic signaling and anxiety, the relationship is probably very com-

plex. This calls both for strategic, coherent planning of future studies following up on results from earlier studies, as well as testing completely new approaches to get new perspectives on the intricate neural underpinnings of anxiety. This is not a one-man job, nor is it a one-nation job, but international correspondence and exchange of data and experiences, ranging from clinicians to neuroscientists, will be needed for a long time ahead. This is important to further increase effect sizes of available treatments, but more importantly, find new effective treatments for the large proportion of patients that are not responding to what we can offer as alleviation today. Nuclear imaging research has given important contributions to our understanding of anxiety disorders, and I believe it will remain an important tool for investigating both functional and dysfunctional aspects of the human mind.

Acknowledgements

First, I want to thank my supervisors prof. Tomas Furmark and assistant prof. Andreas Frick who have given essential input in all parts of analysis and writing of the papers in this thesis. I am grateful I had two highly competent scholars helping me to scrutinize all results, and sometimes, helping me to remember them at all. Your contributions cannot be overstated, and have certainly given the articles a scientific quality much higher than what I could have produced on my own.

In addition, I also want to thank Andreas Frick for his essential role in creating an infrastructure for enhanced statistical work-flow which has saved me uncountable hours. In this regard, I also want to thank Malin Gingnell, Thomas Ågren, Jonas Engman and Iman Alaie. You have all contributed to how we perform analyses (or should I say some of you have made it possible for us to understand how to do it at all?), pre-processing and quality-control of data. I also want to thank you all for your work in creating study designs, and Vanda Faria deserves an extra mention in this regard for her crucial contributions to the design of study II and study III.

I also want to thank prof. Mats Fredrikson, especially for his self-imposed role as an internal negatively biased peer reviewer. Your ability to make the rest of us understand how our articles could be interpreted from multiple perspectives has been a true gift.

I also want to thank Johanna Motilla-Hoppe for giving a clinician's perspective on our research and academia as a whole, and Jörgen Rosén for being an in-house tech-support and for interesting discussions about research and society. Furthermore, I want to thank Johannes Björkstrand for letting me participate in your study and for showing me the basics of SPM-analyses.

Additionally, I want to thank My Jonasson and Mark Lubberink for letting me bother them with difficult PET-questions, and also the staff at the Uppsala university hospital PET-centre for performing the scanning procedures and for their professional reception of our participants, in particular our social anxiety patients. I also want to thank all co-authors for their unique contributions to our work, it would not have been possible without you!

Finally, I want to thank my family for being the best family one can possibly have, and for being so supportive and also very patient with me during work intensive times during these years, and in particular the last few months.

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