PhD Thesis

Sophia Armand

Affective cognition and brain serotonin in healthy individuals

The role of brain serotonin in cognitive-affective biases and amygdala response to threat

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Preface

The studies included in this PhD thesis were conducted over three years, from October 1st 2019, to October 31st 2022, at the PhD school at the Department of Psychology at the University of Copenhagen. The research was conducted at the Neurobiology Research Unit at Copenhagen University Hospitalet, Rigshospitalet, under the primary supervision of Associate Professor Dea Siggaard Stenbæk and co-supervision of Professor Gitte Moos Knudsen, Professor Barbara J. Sahakian and senior researcher Patrick Fisher. This work was funded by The Lundbeck Foundation (Grant no. R281-2017-4366 and R281-2018-131).

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2. Olsen, A. S., Lykkebo-Valløe, A., Ozenne, B., Madsen, M. K., Stenbæk, D. S., **Armand, S**., Mørup, M., Ganz, M., Knudsen, G. M., & Fisher, P. M. (2022). Psilocybin modulation of time-varying functional connectivity is associated with plasma psilocin and subjective effects. *NeuroImage*, 119716. https://doi.org/10.1016/j.neuroimage.2022.119716
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4. McCulloch, D. E.-W., Grzywacz, M. Z., Madsen, M. K., Jensen, P. S., Ozenne, B., **Armand, S**., … Stenbæk, D. S. (2022). Psilocybin-Induced Mystical-Type Experiences are Related to Persisting Positive Effects: A Quantitative and Qualitative Report. *Frontiers in Pharmacology*, *13*(March), 1–17. https://doi.org/10.3389/fphar.2022.841648
5. **Armand, S.,** Wagner, M. K., Ozenne, B., Verbunt, J., Sep, S. J. S., Berg, S. K., … Stenbæk, D. S. (2021). Acute Traumatic Stress Screening Can Identify Patients and Their Partners at Risk for Posttraumatic Stress Disorder Symptoms After a Cardiac Arrest. *Journal of Cardiovascular Nursing*, 394–401. https://doi.org/10.1097/jcn.0000000000000829
6. Tedeschi, E., **Armand, S**., Buyalskaya, A., Silston, B., & Mobbs, D. (2021). Fear in Groups: Increasing Group Size Reduces Perceptions of Danger. *Emotion*, *21*(7), 1499–1510. https://doi.org/10.1037/emo0001004

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A picture of most people at the Neurobiology Research Unit (NRU) from the NRU day 2021. Picture credit: a man passing by in the parc.

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Summary

Affective cognition is a research field studying the cognition processing of emotional stimuli, such as a happy face. Processing and interpreting facial expressions is an essential human function to, for example, understand the emotions and intentions of others. Research shows that certain domains within emotion processing are important for mental health, including cognitive-affective biases in emotion recognition (i.e., enhanced recognition of negative or positive facial expression over the other) and processing of threat in the amygdala, a critical brain region in emotion processing. Uncovering the neurobiology behind these cognitive-affective processes can advance our understanding of normal affective brain function and vulnerability to mental health issues, such as major depressive disorder (MDD). Within the affective cognitive research field, particular interest in the brain's serotonin (5-HT) system has been prompted because serotonergic pharmacological agents elicit antidepressant effects in clinical populations. These pharmacological agents include selective serotonin reuptake inhibitors (SSRIs) and the psychedelic agent psilocybin, agents that target the 5-HT transporter (5-HTT) and 5-HT 2A receptor subtype (5-HT_{2A}), respectively. The purpose of this PhD project was to advance our understanding of whether emotion processing is regulated by serotonergic neurobiology, particularly 5-HTT and 5-HT_{2A}. This was carried out by using cross-disciplinary research methods, including neuropsychological testing and brain imaging with Positron Emission Tomography (PET) and functional Magnetic Resonance (fMRI) combined with SSRI and psilocybin.

In study 1, we explored the association between cognitive-affective biases in emotion recognition and 5-HTT levels in the brain in 98 healthy individuals. We used PET with the radiotracer [^{11}]DASB to measure brain 5-HTT binding. We used the neuropsychological test, Emotional Face Identification task (EFIT) to measure cognitive-affective biases. In study 2, we investigated the

effects of sub-chronic administration (3-5 weeks) of the most selective SSRI, escitalopram, on amygdala and whole-brain responses to angry, fearful, and neutral faces using fMRI in a double-blind, placebo-controlled study with 64 healthy individuals. In study 3, we evaluated the acute effects of a medium-high dose of psilocybin on the amygdala response to angry, fearful, and neutral faces using fMRI in a single-blinded cross-over study with 26 healthy individuals.

Study 1 found that cognitive-affective biases can be coupled to brain 5-HTT levels. For example, we found that individuals with higher 5-HTT levels have a more negative affective bias, meaning they were relatively better at recognising sad faces than happy ones. In study 2, there was no evidence for sub-chronic administration of SSRI significantly affecting amygdala responses to angry, fearful or neutral faces compared to placebo. Our whole-brain, region-wise analyses identified frontal and occipital regions wherein the response to fear and angry was significantly decreased but increased to neutral faces in response to SSRI compared to placebo. In study 3, we found that the amygdala response to angry faces was significantly reduced during the acute effects of psilocybin compared to baseline, but no effect was observed for fearful and neutral faces.

Using advanced and highly-specialised methods, this PhD project bolsters the previously reported association between brain 5-HT and affective cognition in healthy individuals. Additionally, this work contributes with new knowledge of how affective cognition is shaped by a) 5-HTT levels, a key marker of endogenous 5-HT signalling, and b) serotonergic pharmacological agents with the potential to treat MDD, including sub-chronic SSRI and psilocybin. The implications of our results include that negative affective biases, a risk-marker for developing MDD, might result from excessive 5-HT clearance. Further, the antidepressant effect of sub-chronic SSRIs and psilocybin could stem from reduced brain responses or sensitivity to negative emotions.

Summary in Danish

Affektiv kognition er et forskningsområde, som undersøger kognitiv bearbejdning af følelsesmæssige stimuli, såsom følelser i ansigter. Kognitiv bearbejdning af følelser er en central funktion hos mennesker, som er afgørende for eksempelsiv at forstå andres følelser og intentioner. Nuværende forskning peger på, at visse funktioner indenfor den affektive kognition er involveret i risikoen for at udvikle depression. Disse funktioner inkluderer kognitiv affektive bias i følelses-genkendelse (dvs. bedre evne til enten at genkende negative eller positive ansigtsudtryk så som tristhed eller glæde) samt bearbejdning af følelser i amygdala, en del af hjernen som er central i følelses-bearbejdning generelt. Altså er affektiv kognition vigtig for at kunne navigere i en social verden samt for den mentale sundhed, og det er derfor vigtigt at forstå, hvordan disse funktioner reguleres i den raske befolkning. I nuværende undersøgelser af neurobiologiske underliggende faktorer for affektiv kognition har forskningen særligt været optaget af hjernens serotonin system, da farmakologiske præparater som påvirker serotonin lader til at have antidepressive egenskaber i kliniske populationer, såsom personer med depression. Disse farmakologiske præparater inkluderer serotonin-genoptagelseshæmmere (SSRI) som påvirker hjernens serotonin-transportere, samt psilocybin, som er et psykedelisk stof som påvirker hjernens serotonin 2A receptorer. Formålet med dette PhD projekt var at fremme vores forståelse af hvorvidt affektiv kognition er reguleret af hjernens serotonin-system med særlig fokus på serotonin-transportere og serotonin 2A receptorer. Denne undersøgelse blev udført ved brug af interdisciplinære forskningsmetoder, som indbefatter neuropsykologisk testning samt billeddannelse af hjernen ved brug af Positron Emission Tomography (PET) og funktionel Magnetic Resonance (fMRI) kombineret med SSRI og psilocybin.

I studie 1 undersøgte vi sammenhænge mellem niveauet af serotonin-transportere, målt med PET, og kognitiv affektiv bias i emotions-genkendelse, målt med en neuropsykologisk test, i et

udsnit af den danske raske befolkning på 98 personer. I studie 2 undersøgte vi effekten af SSRI, administreret over tre til fem uger, sammenlignet med placebo, på hjernes respons til vrede, angstfyldte og neutrale ansigter ved brug af fMRI i et udsnit af den danske raske befolkning på 64 personer. I studie tre undersøgt vi de akutte effekter af en medium til høj dosis af psilocybin sammenlignet med baseline (uden psilocybin), på amygdala respons til vrede, angstfyldte og neutrale ansigter ved brug af fMRI i et udsnit af den danske raske befolkning på 26 personer.

I studie 1 fandt vi, at der er en sammenhæng mellem niveauet af serotonin-transportere i hjernen og affektiv kognitive bias i følelses-genkendelse. Det betyder for eksempel, at personer med højere niveauer af serotonin-transportere har et mere negativt bias, hvilket betyder, at de er bedre til at genkende triste ansigter relativt til glade. I studie to fandt vi ingen evidens for, at SSRI påvirker amygdala respons til følelser i ansigter. Derimod viste vores udvidede analyser at hjernebarken responderede mindre på vrede og angstfyldte ansigter men højere på neutrale ansigter efter indtagelse af SSRI sammenlignet med placebo. I studie 3 fandt vi, at psilocybin reducerer amygdala respons til vrede ansigter, men vi fandt ingen ændring for angstfyldte eller neutral ansigter.

Ved brug af avancerede interdisciplinære forskningsmetoder, styrker dette PhD projekt tidligere forskning, som har påvist en sammenhæng mellem serotonin og affektiv kognition i raske personer. Samtidig bidrager projektet med ny viden om, at affektiv kognition er formet af a) serotonin-transportere, som er en central markør for endogen serotonin samt b) indtagelse af SSRI samt psilocybin. Disse resulter fortæller os blandt andet, at negative affektiv bias (risikomarkør for at udvikle depression), kan være et resultat at mindre serotonin i hjernen, og derudover, at mekanismen bag antidepressive effekter af SSRI og psilocybin potentielt kommer af at reducere hjernens bearbejdning af negative følelser.

Abbreviations and terminology

5-HT: Serotonin, 5-hydroxytryptamine

5-HTT: Serotonin Transporter

5-HT$_{2A}$: Serotonin 2A Receptor

ACC: Anterior cingulate cortex

BMI: Body Mass Index

BP$_{ND}$: Binding potential, the ratio at the equilibrium of specifically bound tracer to that of nondisplaceable tracer in tissue (Outcome measure for [^{11}C]DASB PET)

BOLD: blood-oxygen-level dependent

EFIT: The Emotional Identification Task

fMRI: functional Magnetic Resonance Imaging

FWER: family-wise error rate

HRRT: High-Resolution Research Tomograph

IQ: Intelligence quotient

LSD: Lysergic acid diethylamide

MDD: Major Depressive Disorder

MRI: Magnetic Resonance Imaging

MRTM2 Multilinear Reference Tissue Model

N: Number of participants in a sample

P-value: Probability value

PET: Positron Emission Tomography

PFC: Prefrontal cortex

PSS: Perceived Stress Scale

SD: Standard deviation

SSRI: Selective Serotonin Reuptake Inhibitor

ROI: Region of Interest

[^{11}C]DASB: PET-tracer for imaging the 5-HT transporter

I. Introduction

This PhD thesis examines how brain serotonin (5-HT) is involved in affective cognition in healthy humans. The methods used to examine these questions were neuropsychological testing and neuroimaging with positron emission tomography (PET) and pharmaco-functional magnetic resonance (fMRI) using sub-chronic selective serotonin reuptake inhibitors (SSRIs) and psilocybin, methods that will be described in the thesis' method section. In the present chapter, I will introduce affective cognition as a research field focusing on affective cognitive processes involved in the risk of developing affective disorders, including cognitive-affective biases and threat-related processing in the amygdala. Next, a brief introduction to the 5-HT system will be presented with a particular focus on the 5-HT transporter (5-HTT) and the 5-HT 2A receptor (5-HT_{2A}), representing two key markers of the 5-HT system. Next, I will introduce two pharmacological drugs, selective serotonin reuptake inhibitors (SSRIs) and the psychedelic drug psilocybin, that target the 5-HTT and 5-HT_{2A}, respectively. Finally, I will present a review of current literature investigating the role of 5-HT in affective cognition, focusing on how 5-HTT binding, SSRI, and psilocybin, modulate emotion processing.

In the thesis, I will present the background, aims, hypotheses, methods and results, and discussion points pertaining to the primary outcomes across the three papers. For secondary and post-hoc outcomes, I kindly refer to the full papers presented in the final part of the thesis.

I.I. Affective cognition

Affective cognition is a research field examining the interface between emotion and cognition. Although it is currently discussed and unclear whether cognition and emotion are ontologically

distinct phenomena or two sides of the same coin (e.g., Duncan & Barrett, 2007; E. A. Phelps, 2006), I define these separately in the current thesis for simplicity. *Cognition is the collection of mental processes and activities used in perceiving, remembering, thinking, and understanding, as well as the act of using those processes* (Radvansky & Ashcraft, 2014, p. 9). Affective cognition describes as *an interface at which emotional and cognitive processes are integrated to generate behavior* (Elliott et al., 2011, p. 154). The study of affective cognition is different from the study of emotional states, which is the examination of internal states such as the experience and expression of fear or happiness (Cowen, Sauter, Tracy, & Keltner, 2019), studied for centuries, for example, Charles Darwin in *The Expression of Emotions in Man and Animals* (1872) and William James in *What is an emotion?* (1884). Rather, affective cognition is a research field of the crossing between psychology and cognitive neuroscience, studying the cognitive processing of emotional stimuli using methods such as neuropsychological testing, fMRI and PET. These methods have allowed studying mental processes of emotions on behavioural as well as molecular and functional neural levels. These methods will be introduced in the method section.

While no consensus of nomenclature or precise delimitation of affective cognitive domains currently exists, three broad domains have been proposed. These include emotional processing (i.e., recognising or categorising emotions), motivation and reward, and social cognition (Bland et al., 2016; Dam et al., 2019). The current thesis focuses on emotional processing. Emotional processing describes the ability to perceive and infer emotional states and intentions of other individuals based on overt reactions such as facial or vocal expressions. These abilities represent fundamental human skills developed early in a child's development, essential for regulating emotions and engaging in social interaction (Klinnert, Emde, Butterfield, & Campos, 1986). Emotion processing scaffolds central social skills such as empathy and forming and maintaining social relationships and cooperation. Together, these skills represent central affective cognitive functions to

navigate a social and interpersonal world successfully. Facial expressions are a universal and rich source of emotional information (Cowen et al., 2019; Said, Haxby, & Todorov, 2011), which is why cognitive evaluations of emotional faces are commonly used to examine affective cognition in research. Typically, basic facial expressions are used in tasks evaluating basic emotional processing, including fear, anger, happy, surprise and disgust. These facial expressions are argued to be recognised across cultures and species (Ekman & Friesen, 2003). Section III.III.I.II. will elaborate on the use of facial expressions as a method.

The research field of affective cognition has revealed that certain patterns of affective cognitive functioning are observed within healthy populations, while different patterns of affective cognitive functions are observed within psychiatric populations, for example, in individuals with affective disorders such as major depressive disorder (MDD) (V. H. Dam et al., 2020; R. Elliott, Zahn, Deakin, & Anderson, 2011; Miskowiak & Carvalho, 2015). Affective cognitive domains particularly involved in the risk for developing affective disorders include cognitive-affective biases and threat-related amygdala reactivity, representing the focus of the current thesis and will be elaborated on next.

I.I.I. Cognitive-affective biases

Cognitive-affective biases describe the tendency to process and favour either negative information or positive information over the other. Affective biases distribute along a valence continuum ranging from positive biases (i.e., the priority of positive information) to negative biases (i.e., the priority of negative information). Healthy individuals typically display a neutral or a positive bias (V. H. Dam et al., 2020; Pool, Brosch, Delplanque, & Sander, 2016). For example, healthy individuals tend to be better and faster at recognising happy faces than sad ones (V. H. Dam et al., 2020). Furthermore, healthy individuals tend to be positively biased in thoughts and memories

about themselves, their past and their views of the future (Carver & Scheier, 2014; Taylor & Brown, 1994; Walker, Skowronski, & Thompson, 2003). Such positive affective bias is argued to reflect an inherently optimistic view associated with health-promoting behaviours (Carver & Scheier, 2014) and to represent a resilience factor in the development of MDD (Korn, Sharot, Walter, Heekeren, & Dolan, 2014). Conversely, negative affective biases have consistently been observed in individuals with MDD across a range of cognitive functions, including attention, perception and memory (Miskowiak & Carvalho, 2015). For example, compared to healthy individuals, individuals at risk for or within a depressive episode are better at recognising and display more processing of negative faces compared to happy faces (Bourke, Douglas, & Porter, 2010; Chan, Goodwin, & Harmer, 2007; Dalili, Penton-Voak, Harmer, & Munafò, 2015; Rubinow & Post, 1992). Further, individuals with MDD remember more negative events from their past than positive events (Brittlebank, Scott, Williams, & Ferrier, 1993) and have more pessimistic views on the future for themselves and others (Alloy & Ahrens, 1987; Zenger, Glaesmer, Höckel, & Hinz, 2011). Taken together, while positive affective biases are considered to reflect a resilience factor against developing MDD (Korn et al., 2014), negative affective biases are believed to play a major role in the aetiology of MDD (Beck, 2008; Clasen, Wells, Ellis, & Beevers, 2013; Roiser, Elliott, & Sahakian, 2012; van Oostrom et al., 2013). Notably, reducing negative biases by pharmacologically targeting the 5-HT system with SSRI has been proposed as a key mechanism in treating depressive symptoms (Beata R. Godlewska & Harmer, 2020). More research in affective biases can advance our understanding of resilience and vulnerability to MDD.

I.I.II. Affective processing in the amygdala

The amygdala is a brain structure composed of distinct subareas or nuclei (J. LeDoux, 2007). It is located in the bilateral medial temporal lobe at the anterior end of the hippocampus, commonly

recognised as a central part of the limbic system (see **Figure 1**). The amygdala has extensive and widespread connections to other brain regions such as the thalamus, hippocampal complex, hypothalamus, olfactory bulb and brain stem and cortical regions such as the prefrontal and temporal cortex and the insula. It receives sensory information, including visual, auditory, somatosensory, olfactory, and taste, allowing all sensory input to be processed by the amygdala (J. LeDoux, 2007). These amygdala input connections are the basis for detecting the salience and social relevance of emotional information in the environment (J. E. LeDoux, 2000; Sander, Grafman, & Zalla, 2003). The amygdala's output connections enable appropriate responses to perceived emotional information, including behaviours, such as escaping or avoiding the threat, and autonomic responses, such as hormonal secretion (Janak, Tye, Sciences, & Sciences, 2015; J. E. LeDoux & Gorman, 2001). For example, the amygdala projects to the brain stem to produce innate emotional reactions, such as freezing behaviour in response to a threat, and associated physiological responses, and to the striatum to produce actions such as running away from the threat (Janak et al., 2015). Overall, the human amygdala's primary function is argued to be to detect emotional salience and modulate the neural system that produces appropriate cognitive and behavioural responses (A. K. Anderson & Phelps, 2000). Research finds that the amygdala is involved in a range of affective processes such as memory, learning, attention, and sensory perception (E. A. Phelps, 2006), with visual perceptual emotional processing in the amygdala being the focus of the current thesis.

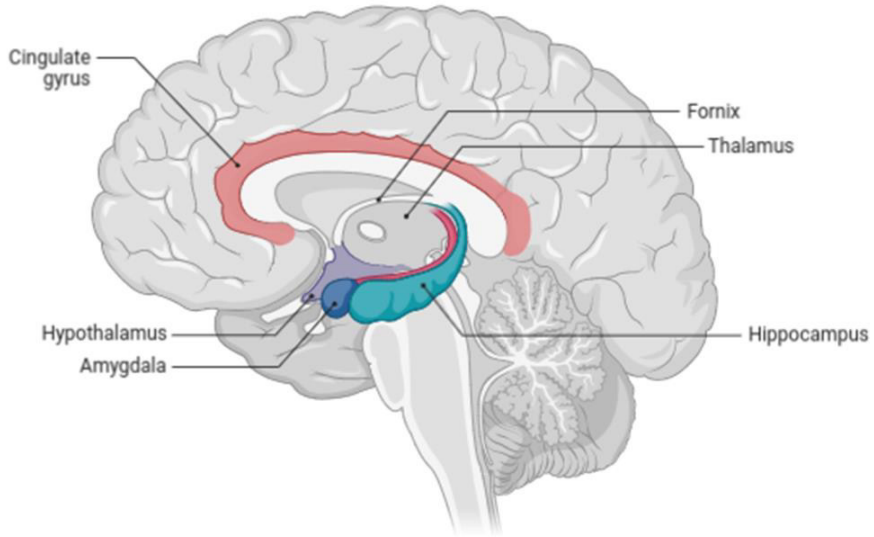

Figure 1. Illustration of the limbic system in a lateral view of the brain. Copied from Biorender.

Studies using fMRI show that the amygdala is activated during visual perception of basic emotional faces such as happiness, surprise, sadness, anger and fear, as well as neutrality (Adolphs, 2010; Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006; Fusar-Poli et al., 2009; Pessoa, 2010). However, converging evidence from fMRI studies suggests that the amygdala is particularly important for and sensitive to processing aversive and threat-related stimuli (Adolphs, 2008; M. Davis & Whalen, 2001; Fusar-Poli et al., 2009; Haxby, Hoffman, & Gobbini, 2002; J. E. LeDoux, 2000), supported by initial studies of lesions to the amygdala (Weiskrantz, 1956). In fact, the amygdala is commonly referred to as the brain's primary threat detector. For example, a meta-analysis of fMRI studies reported higher amygdala reactivity to fearful faces than to happy and sad faces (Fusar-Poli et al., 2009). Notably, the amygdala is activated during the processing of emotional stimuli both when presented during conscious awareness, but also when stimuli are given for such a short time that they do not reach conscious perception, signifying that the amygdala is both involved in the conscious and

unconscious perception of threat (Diano, Celeghin, Bagnis, & Tamietto, 2017; Morris, Öhman, & Dolan, 1998).

Research suggests that while some amygdala activity to threat is adaptive (Habel et al., 2007; Müller, Höhner, & Eickhoff, 2018; Schneider et al., 1997), exaggerated threat-related amygdala activity seems to be maladaptive and involved in affective disorders (R. Elliott et al., 2011; Miskowiak & Carvalho, 2015; Stuhrmann, Suslow, & Dannlowski, 2011). For example, some individuals with MDD or at risk for developing MDD exhibit overactive amygdala reactivity for the detection of threats and excessive amygdala reactivity during the processing of threats (Almeida, Versace, Hassel, Kupfer, & Phillips, 2010; da Cunha-Bang, Fisher, Hjordt, Holst, & Knudsen, 2019; R. Elliott et al., 2011; Catherine J. Harmer & Cowen, 2013; Maron et al., 2016), even during processing fear outside conscious awareness (Sheline et al., 2001). However, meta-analyses on this subject disagree with some finding altered brain responses during emotion processing in MDD (Stuhrmann et al., 2011), while others do not (Müller et al., 2017). Nonetheless, pharmacologically manipulating brain 5-HT with SSRI seems to attenuate hyperactive amygdala response to negative stimuli in individuals at risk for or within a current depressive episode (Bhagwagar, Cowen, Goodwin, & Harmer, 2004; B R Godlewska, Norbury, Selvaraj, Cowen, & Harmer, 2012; Beata R. Godlewska & Harmer, 2020; Catherine J Harmer, Goodwin, & Cowen, 2009; Sheline et al., 2001), however, this is not supported by all studies (Patrick M. Fisher et al., 2022). Further, targeting the 5-HT system with the psychedelic drug psilocybin links to reduced processing of negative scenes, which associates with an increase in positive mood in healthy individuals (Kraehenmann et al., 2015). As such, threat-related amygdala reactivity is linked to mental health, and it is, therefore, important to understand how it is regulated in healthy individuals, for example, by manipulating brain 5-HT.

I.I.III. Summary

Emotional processing is a central affective cognitive domain important for understanding the emotions and intentions of others. These processes are essential to navigate a social world, such as forming interpersonal relationships throughout life. Certain domains within emotion processing are seemingly particularly important for mental health, including cognitive-affective biases and threat-related amygdala reactivity. Notably, cognitive-affective biases and threat-related amygdala responses can be modulated by targeting the 5-HT system pharmacologically, indicating a link between these processes and 5-HT. Further uncovering molecular and functional serotonergic correlates of emotional processing can advance our understanding of normal affective brain functions and vulnerability to and treatment of affective disorders.

I.II. The serotonin system in the human brain

The serotonin (i.e., 5-hydroxytryptamine = 5-HT) system in the human brain is involved in a myriad of human functions such as sleep, eating behaviours, reproductive behaviours, stress, mood, and various cognitive processes (Jacobs & Azmitia, 1992; Lucki, 1998; Mendelsohn, Riedel, & Sambeth, 2009; Sharp & Barnes, 2020). For example, 5-HT projections to brain regions, including the amygdala, striatum, and prefrontal cortex, are reported to be critical for social and emotional cognition (Patrick M Fisher et al., 2011; Patrick Macdonald Fisher, Haahr, Jensen, & Frokjaer, 2015; Hornboll, Macoveanu, Rowe, & Elliott, 2015; Meyer et al., 2003; Roberts, Sahakian, & Robbins, 2020). Within the 5-HT system, there are seven classes of 5-HT receptors families, namely, the 5-HT_1 to 5-HT_7 (Hoyer et al., 1994; Sharp & Barnes, 2020), of which some have several receptor subtypes, such as the 5-HT_{2A} (Gray & Roth, 2001; Sharp & Barnes, 2020). All receptor families are G-protein-coupled except for the 5-HT_3 receptor, a ligand-gated cation channel. Brain 5-HT is

synthesised from the amino acid tryptophan in the soma of neurons mainly located in the raphe nuclei in the brain stem. From the raphe nuclei, the 5-HT neurons have ascending axons projecting to the entire cerebrum with a few descending axons projecting to the cerebellum (V. Beliveau et al., 2017; Charnay & Léger, 2010; Jacobs & Azmitia, 1992; Olivier, 2015) (see **Figure 2**). 5-HT is transported in synaptic vesicles to the presynaptic terminal, which, upon an action potential and calcium influx into the cell, fuses with the cell membrane to release 5-HT into the synaptic cleft (see **Figure 3**). Here, 5-HT stimulates available pre- and post-synaptic receptors, which typically activate coupled G-proteins resulting in downstream signalling cascades (Nichols & Nichols, 2008). Remaining extracellular 5-HT is then transported back into the pre-synaptic cell by the high-affinity 5-HTT, which prepares and reuses 5-HT for a new signal (Olivier, 2015; Torres, Gainetdinov, & Caron, 2003) (see **Figure 3**).

Synaptic 5-HT cannot be measured directly in the human brain, so neuroimaging of specific 5-HT binding sites is a way to establish biomarkers of the 5-HT system (Paterson, Tyacke, Nutt, & Knudsen, 2010). This thesis focuses on the 5-HTT and 5-HT_{2A}, as these are key biomarkers related to the pharmacological interventions used in the included studies. In particular, cerebral 5-HTT is argued to be a marker of the 5-HT tonus (Blakely, De Felice et al. 1994). Furthermore, interest in the 5-HTT and 5-HT_{2A} has been prompted by increasing evidence of their involvement in affective conditions (Bhagwagar et al., 2004; Meyer et al., 1999, 2003; Mintun et al., 2004; Spies, Knudsen, Lanzenberger, & Kasper, 2015).

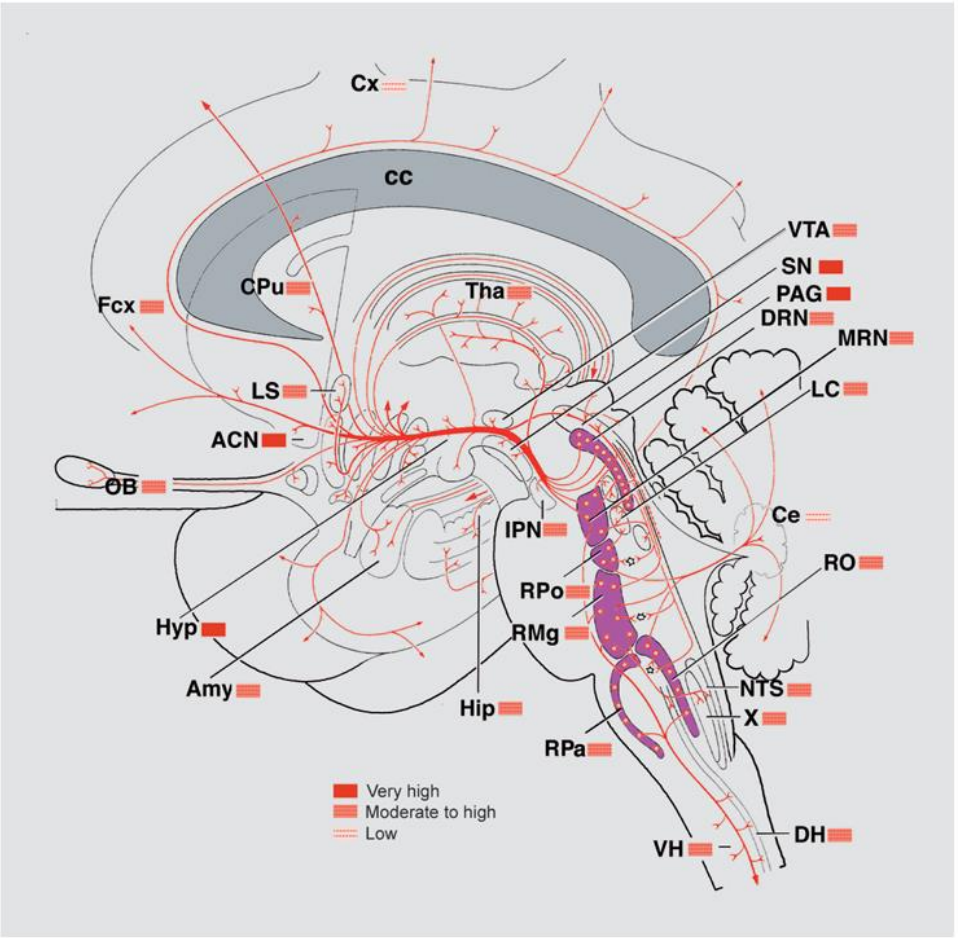

Figure 2. Illustration of the human serotonin (5-HT) system including 5-HT neurons in raphe (in pas well as ascending axons (in red) to the cerebrum and descending axons (in red) to the cerebellum. The densities of the axons are given by the coloured boxes ranging from very high to low. Abbreviations: X = dorsal motor nuclei of the vagus nerve; CAN = accumbens nuclei; ACN = accumbens nuclei; Amy = amygdala; cc = corpus callosum; Ce = cerebellum; Cpu = caudate-putamen; Cx = cortex; DH = dorsal horn spinal cord; DRN U dorsal raphe nuclei; Fcx = frontal cortex; Hip = hippocampus; Hyp = hypothalamus; IPN = interpeduncular nuclei; LC = locus coeruleus; LS = lateral septum; MRN = median raphe nuclei; NTS = nuclei of the solitary tract; OB = olfactory bulb; PAG = periaqueductal gray; RMg = raphe magnus nucleus; RO = raphe obscurus nuclei; Rpa = raphe pallidus; Rpo = raphe pontis nuclei; SN = subtantia nigra; Tha = thalamus; VH = ventral horn; VTA = ventral tegmental area. Figure copied from Nieuwenhuis R. Monoamines: Chemoarchitecture of the Brain. Berlin, Germany: Springer Verlag; 1985:33-4 in Charnay and Léger, 2010.

I.II.I. The serotonin transporter

The 5-HTT is a protein almost exclusively expressed in 5-HT neurons, both in the cell bodies of the raphe nucleus and in all 5-HT projections throughout the cerebrum (V. Beliveau et al., 2017; Torres et al., 2003). Neuroimaging studies with PET of healthy individuals show that there is high 5-HTT density within the raphe nucleus as well as in subcortical regions such as the striatum, thalamus and amygdala and lower density in cortical areas and cerebellar grey matter (V. Beliveau et al., 2017; Savli et al., 2012) (see **Figure 4**). 5-HTT is located at the plasma membrane on pre-synaptic terminals, away from the synaptic area (see **Figure 3**). The primary role of the 5-HTT is to regulate extracellular 5-HT concentrations through the reuptake of extracellular 5-HT into the synaptic terminal (Bel & Artigas, 1992). However, the relation between 5-HTT and 5-HT tonus has been widely discussed (Spies et al., 2015), and remains unsettled (see manuscript 1 for further elaboration on different hypotheses).

Taken together, as the 5-HTT is essential for regulating 5-HT concentrations and is located on 5-HT neurons and their widespread projections, it is argued that the 5-HTT is a marker of both 5-HT tonus and 5-HT cerebral architecture (Paterson et al., 2010). It is possible to image 5-HTT levels in the living human brain using PET with the radioligand [11C]DASB, a method that will be specified in section III.III.I.I. Quantifying brain 5-HTT levels using PET enables us to evaluate its direct association with phenotypic traits such as affective cognitive processes.

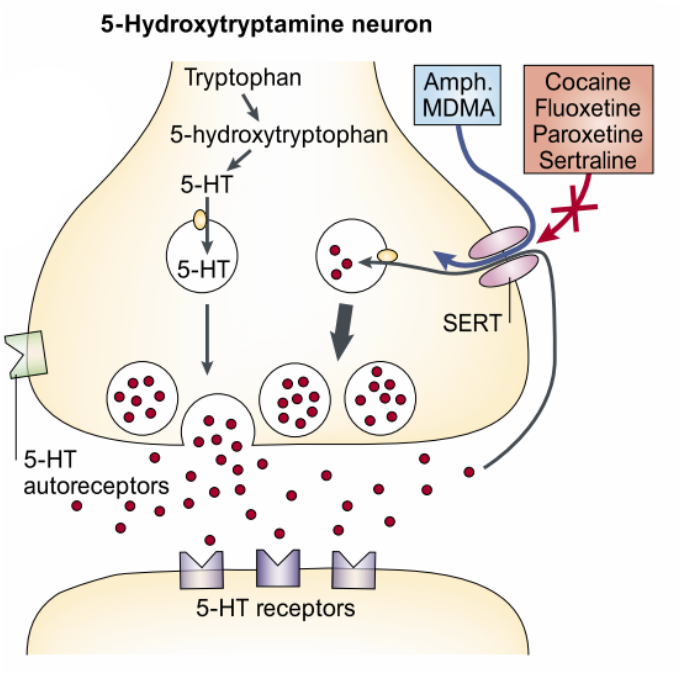

Figure 3. Illustration of serotonin (5-HT) synaptic terminals. The serotonin transporter 5-HTT is localised on presynaptic sites and are important for terminating 5-HT transmission and maintain presynaptic 5-HT storage. Several selective pharmacological agents acting at 5-HTT including SSRIs (i.e., sertraline, paroxetine and fluoxetine). Abbreviations: SERT = serotonin transporter, Amph = amphetamine; MDMA = (+)-3,4-methylenedioxymethamphetamine. Copied from Torres, Gainetdinov and Caron, 2003.

I.II.II. Selective serotonin reuptake inhibitors

The 5-HTT is a molecular target of SSRIs, a group of drugs used as the first-line pharmacological treatment of MDD and other psychiatric conditions. Escitalopram represents the most selective SSRI and is widely prescribed clinically (Kasper, Spadone, Verpillat, & Angst, 2006). SSRIs block the 5-HTT and thus inhibit its function of reuptake extracellular 5-HT into the pre-synaptic terminals (Nutt et al., 1999). It is argued that manipulating 5-HTT efficacy with SSRIs can alter the 5-HT tonus (Torres et al., 2003). After at least two weeks of treatment, SSRIs reduce clinical

depressive symptoms (Kasper et al., 2006) in at least half of the treated individuals with MDD (Trivedi et al., 2006). However, the underlying mechanism behind SSRIs efficacy remains unknown, but studies suggest that its clinical effects are mediated through changes in affective cognition, for example, by initially decreasing negative affective biases and threat-related amygdala reactivity evident after acute or short-term administration of SSRI (Beata R. Godlewska & Harmer, 2020; Catherine J. Harmer, Mackay, Reid, Cowen, & Goodwin, 2006; S. E. Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009). However, the effects of SSRI administration over a clinically relevant period of more than two weeks on affective cognition in healthy individuals remain sparsely investigated.

I.II.III. The serotonin 2A receptor

Studies using PET imaging show that the 5-HT_{2A} is widely distributed in the cortex, with higher levels in the prefrontal cortex, posterior cingulate cortex, precuneus, insula, visual cortex, and temporal cortices and with relatively lower levels in subcortical regions such as the striatum, amygdala, hippocampus and thalamus (X. V. Beliveau et al., 2020; Ettrup et al., 2016) (see **Figure 4**). A bulk of studies suggest that 5-HT_{2A} is involved in affective conditions (Bhagwagar, Hinz, Taylor, & Fancy, 2006; Meyer et al., 1999; Mintun et al., 2004; Yatham et al., 2000) and in the severity of negatively biased assumptions and beliefs within a depressive episode (Meyer et al., 2003). Notably, stimulating 5-HT_{2A} once with a medium to a high dose of a serotonergic psychedelic such as psilocybin within a therapeutic framework has been linked to a marked reduction in depressive symptoms (Carhart-Harris Robin L et al., 2016; A. K. Davis et al., 2021).

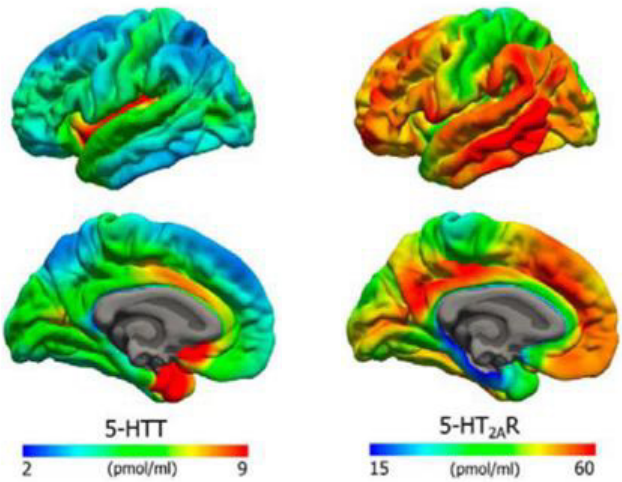

Figure 4. The distribution of and average density (Bmax) of serotonin transporters (5-HTT) and serotonin 2A receptors in a sample of healthy individuals. Cortical values are presented on the standard FreeSurfer surface (fsaverage, left hemisphere; lateral view, upper left and medial view, upper right)). The color scales were individually adjusted. Copied from Beliveau et al., 2017

I.II.IV. The psychedelic drug Psilocybin

Psilocybin is the principal psychoactive agent in the Psilocybe genus mushrooms (Nichols, 2016), perhaps better known as magic mushrooms. Once ingested, psilocybin gets metabolised into psilocin. Psilocin dose-dependently induces psychoactive effects by stimulating brain 5-HT_{2A} (Passie, Seifert, Schneider, & Emrich, 2002; Vollenweider, Vollenweider-Scherpenhuyzen, Bäbler, Vogel, & Hell, 1998) over four to eight hours (Stenbæk et al., 2020; Vollenweider & Preller, 2020), unfolding in three successive phases: ascent, peak and descent (Stenbæk et al., 2020). A medium to high dose (i.e., >0.2 mg/kg) of psilocybin can dose-dependently induce profound changes in consciousness. The drug recipient typically experiences vivid, altered perception and cognition, synaesthesia, enhanced affective states, altered sense of time, space and self, and increased attribution of meaning and personal significance (Vollenweider & Preller,

2020). Alongside other psychedelics, psilocybin is gaining renewed attention as a therapeutic. As psilocybin represents a new and less researched treatment, not yet FDA/EMA approved, results from studies of its therapeutic efficacy will be elaborated below.

Small clinical studies suggest that psilocybin is efficacious in treating a wide range of psychiatric conditions (B. T. Anderson et al., 2020; Bogenschutz et al., 2015, 2022; Garcia-Romeu et al., 2019; Grob et al., 2011; Matthew W Johnson, Hendricks, Barrett, & Griffiths, 2019; Moreno et al., 2015), including affective conditions (Robin L. Carhart-Harris et al., 2021, 2018; A. K. Davis et al., 2021; Roland R Griffiths et al., 2016; Ross et al., 2016), when the drug is administered to carefully prepared individuals with psychological support in safe settings (Robin L. Carhart-Harris et al., 2018; David Erritzoe & Richards, 2017; M. W. Johnson, Richards, & Griffiths, 2008). In addition to improving clinical symptoms in patients, healthy individuals who have undergone a psilocybin intervention generally report sustained positive psychological changes (Roland R. Griffiths et al., 2011; Vollenweider & Preller, 2020), including increased positive affect (Barrett, Doss, Sepeda, Pekar, & Griffiths, 2020), well-being (R R Griffiths, Richards, McCann, & Jesse, 2006; Kettner et al., 2021), mindfulness (Martin Korsbak Madsen et al., 2020; Smigielski, Scheidegger, Kometer, & Vollenweider, 2019; Søndergaard et al., 2022), and generally positive attitudes about life and with oneself (Smigielski et al., 2019).

Studies find that changes in emotional states during the acute effects of a psilocybin intervention are particularly important for its therapeutic effects (Roland R. Griffiths, Richards, Johnson, McCann, & Jesse, 2008; Matthew W. Johnson, Garcia-Romeu, & Griffiths, 2017; McCulloch et al., 2022; Roseman, Nutt, & Carhart-Harris, 2018; Søndergaard et al., 2022). According to retrospective self-reports, a psilocybin intervention acutely induces positive emotions (Kometer et al., 2012) such as deepfelt love and peacefulness (Barrett et al., 2020), blissful joy (Kraehenmann et al., 2015), as well as emotional excitation (Studerus, Kometer, Hasler, & Vollenweider, 2011), insights (A. K. Davis et al., 2021) and emotional breakthroughs

(R R Griffiths et al., 2006; Roseman et al., 2019, 2018), increased emotional empathy (Pokorny, Preller, Kometer, Dziobek, & Vollenweider, 2017) and recall and re-experiencing of autobiographical emotional memories (R. L. Carhart-Harris et al., 2012; Healy, 2021). In sum, compelling results find that individuals experience profound changes in their emotional state following the acute administration of psilocybin. However, it remains less investigated how acute effects of psilocybin modulate affective cognition.

I.II.V. Summary

The 5-HTT and 5-HT_{2A} are two key markers of the 5-HT system. Interest in these 5-HT binding sites has been prompted by increasing evidence of their involvement in affective conditions. In support, studies show that targeting 5-HTT and 5-HT_{2A} with the pharmacological agents SSRI and the psychedelic drug psilocybin elicit antidepressant and anxiolytic effects in clinical and healthy populations. However, it remains less clear whether the 5-HTT and 5-HT_{2A} are involved in modulating affective cognition.

I.III. Current knowledge of the role of serotonin in affective cognition

I.III.I. Scope of the examination

In current literature, the role of 5-HT in affective cognition in healthy individuals has been explored using a range of methods. These methods include dietary depletion or boosting of tryptophan (precursor of 5-HT), genetic studies of the 5-HTTLPR, serotonergic pharmacological drugs specifically targeting 5-HT, or PET imaging of 5-HT binding sites. The focus of this thesis

in terms of 5-HT targeting methods is on pharmacological drugs specifically targeting 5-HT, particularly SSRI and psilocybin, as well as 5-HT PET imaging. This methodological focus is reflected in the presented literature and the applied methods across the thesis studies. See **Table 1** for an overview of current literature using drugs specifically targeting 5-HT and 5-HT PET imaging to explore whether 5-HT is involved in emotion processing behaviourally and in the amygdala. Studies using dietary or genetic 5-HT targeting methods or drugs with an affinity for other neurotransmitters than 5-HT are beyond the scope of the current thesis and will therefore not be presented or discussed in the current dissertation (for more knowledge about these particular areas, we refer to R. Elliott et al., 2011; Jonassen & Landrø, 2014). Nor will this thesis cover 5-HT modulation of functional neural connectivity. Due to the thesis' particular interest in exploring the role of 5-HT in emotional processing, behaviourally and in the amygdala, using PET imaging of the 5-HTT as well as sub-chronic SSRI and psilocybin, current literature on these specific questions will be elaborated below.

I.III.II. The association between the serotonin transporter and emotional processing

Studying the relationship between 5-HTT as imaged with PET and affective cognition has only been done once in healthy individuals. Here, in a sample of 20 healthy individuals, researchers reported that higher 5-HTT was related to lower threat-related left amygdala reactivity (Rhodes et al., 2007). A related study in MDD investigating negatively biased assumptions and beliefs about oneself, the world, and the future using a questionnaire in individuals with MDD found that higher 5-HTT density was linked to more negatively biased assumptions and beliefs (Meyer et al., 2004). In sum, current spare literature suggests that 5-HTT is involved in emotional processing and

negatively biased assumptions. However, this has only been investigated in smaller samples, and it remains unknown how 5-HTT is related to cognitive-affective biases in healthy individuals.

I.III.III. Effects of selective serotonin reuptake inhibitors administration on affective cognition

Most studies using acute or short-term SSRI administration have reported a change in emotional processing, both behaviourally and in the amygdala, in individuals receiving SSRI compared to placebo (see **Table 1**) (I. M. Anderson et al., 2007; Bigos et al., 2008; Browning, Reid, Cowen, Goodwin, & Harmer, 2007; Cremers, Lee, Keedy, Phan, & Coccaro, 2016; Del-Ben et al., 2005; C. J. Harmer et al., 2003; Catherine J. Harmer et al., 2006; Catherine J. Harmer, Shelley, Cowen, & Goodwin, 2004; S. E. Murphy, Norbury, et al., 2009; S. E. Murphy, Yiend, Lester, Cowen, & Harmer, 2009; Selvaraj et al., 2018; Takahashi et al., 2005; Windischberger et al., 2010). To date, two studies have investigated how sub-chronic SSRI administration (i.e., over 18-21 days) modulates emotion processing in the amygdala in healthy individuals using BOLD fMRI. None of the studies found support for sub-chronic SSRI, compared to placebo, affecting threat-related processing in the amygdala or any other region of interest, including the medial prefrontal cortex, insula, ventral anterior cingulate cortex and the fusiform gyrus (Arce, Estibaliz, Simmons, Alan N. Lovero K. Stein, Murray B. Paulus, 2008; Patrick Macdonald Fisher et al., 2015). However, in a sub-analysis, one of the studies found a negative association between SSRI and threat-related amygdala responses in treatment-compliant participants, as confirmed by urinary drug concentration (Arce, Estibaliz, Simmons, Alan N. Lovero K. Stein, Murray B. Paulus, 2008), while the other study found that change in brain 5-HT_4 receptor levels, a putative marker of brain 5-HT tonus, was associated with a change in the amygdala response to threat within the SSRI group (Patrick Macdonald Fisher et al., 2015). The samples of these studies were relatively small,

consisting of 13 females and 32 males, respectively. A study with a relatively larger sample including both sexes is needed to clarify whether sub-chronic SSRI administration modulates threat-related amygdala reactivity. Such a study will advance our understanding of how sub-chronic SSRI modulates healthy affective brain function. Compared to the more common studies using acute and short-term SSRI administration, studying the effects of sub-chronic SSRI administration better mimics pharmacological treatment models of MDD and may, therefore, also be used as a model to shed light on how SSRI impacts the brain of individuals with MDD and potential treatment mechanisms of SSRIs.

I.III.IV. Effects of psilocybin on affective cognition

Two studies have used neuropsychological testing of emotion processing during acute exposure to psilocybin. They found that psilocybin attenuates the processing of negative facial emotional stimuli, an effect abolished by pre-treatment with ketanserin, a 5-HT_{2A} antagonist, while processing of positive and neutral facial stimuli remained unaffected (Kometer et al., 2012; Schmidt, Kometer, Bachmann, Seifritz, & Vollenweider, 2013). The only study that has investigated emotional processing in the amygdala during exposure to psilocybin found that the amygdala response was reduced to negative and neutral scenes (Kraehenmann et al., 2015), indicating generally reduced processing of both negative and neutral material during psilocybin. A previous study using lysergic acid diethylamide (LSD), another potent psychedelic with a high affinity for the 5-HT_{2A} (Nichols, 2016), found reduced processing of negative faces relative to neutral faces in the amygdala following acute administration of LSD (Mueller et al., 2017). For now, it has not been investigated whether psilocybin acutely modulates amygdala response to emotional faces. Particularly, it remains unclear whether psilocybin reduces emotion processing in the amygdala generally, including both negative and neutral emotions, as found by

Kraehenmann *et al.,* or rather specifically of negative emotions, as found behaviourally by Kometer *et al*.

I.III.V. Summary

Current literature suggests that 5-HT modulates emotional processing in healthy individuals. However, much remains unresolved about serotonergic modulation of affective healthy brain functions, including whether a) the 5-HTT is associated with cognitive-affective biases, b) sub-chronic SSRI administration modulates brain responses to negative and neutral faces using a large sample including both sexes, and c) psilocybin acutely modulates amygdala response to negative and neutral faces. Studies targeting these questions will advance our knowledge about healthy affective brain function and inform us whether 5-HT is involved in known cognitive risk factors for developing MDD.

Emotion processing assessed with neuropsychological testing

Researcher/ year	**Study design**	**5-HT intervention/PET study**	**Sample size**	**Outcome measure**	**Primary findings**
Browning et al., 2007	RCT	SSRI acute	32	FERT (i.e., d' for happiness, surprise, sadness, fear, anger, and disgust)	↑ fear, ⇿ all other emotions
Harmer et al., 2003	RCT	SSRI acute	24	FERT (i.e., hit-rate happiness, sadness, fearfulness, anger, and disgust)	↑ fear and happy, ⇿ all other emotions
Harmer et al., 2004	RCT	SSRI short-term	42	FERT (i.e., hit-rate happiness, surprise, sadness, fear, anger, and disgust)	↓ fear, angry, disgust and surprise, ⇿ all other emotions
Kometer et al., 2012	RCT	Placebo/ketanserin + psilocybin acute	17	FERT with eyes (i.e., hit-rate for positive, negative, and neutral)	Placebo + psilocybin: ↓ negative, ⇿ all other emotions; ketanserin + psilocybin: ⇿ all emotions
Schmidt et al., 2013	RCT	Psilocybin acute	21	FERT (i.e., d' for fear and happiness)	↓ fear, ⇿ happy
Dolder et al., 2016	RCT	LSD acute	24	FERT (i.e., hit-rate for happiness, sadness, anger, and fear)	↓ sad and fear, ⇿ all other emotions
Rocha et al., 2021	RCT	Ayahuasca acute	22	FERT (i.e., hit-rate for happiness, sadness, fear, disgust, anger, and surprise)	⇿ all emotions

Emotion processing in the amygdala assessed with fMRI

Researcher/year	**Study design**	**5-HT intervention/PET study**	**Sample size**	**Relevant outcome measure**	**Primary findings**
Selvaraj et al., 2018	RCT	SSRI acute	13	Fear vs neutral faces	↑ fear
Cremers et al., 2016	RCT	SSRI acute	14	Faces vs fixation point	↑ faces
Anderson et al., 2007	RCT	SSRI acute	12	Fear, angry and disgust vs neutral faces	↓ fear and disgust, ⇿ angry
Del-Ben et al., 2005	RCT	SSRI acute	12	Aversive (i.e., anger, disgust, and fear) vs neutral faces	↓ aversive
Murphy et al., 2009a	RCT	SSRI acute	26	Fear and happy vs. neutral faces	↓ fear, ⇿ happy
Takahashi et al., 2005	RCT	SSRI acute	13	Unpleasant vs neutral pictures	↓ unpleasant
Bigos et al., 2008	RCT	SSRI acute	8	Faces (i.e., angry, fearful, surprised, and neutral) vs shapes	↑ faces
Murphy et al., 2009b	RCT	SSRI short-term	42	Fear and happy vs. neutral (unmasked and masked attentional probe task)	↓ attentional vigilance to unmasked fear, ⇿ masked fear
Norbury et al., 2009	RCT	SSRI short-term	28	Fear and happy faces vs shapes	⇿ fear, ↑ happy
Harmer et al., 2006	RCT	SSRI short-term	24	Masked fear and happy vs fixation point	↓ fear, ⇿ happy
Windischberger et al., 2010	RCT	SSRI short-term	18	Faces (i.e., happiness, anger, fear, sadness, surprise, disgust and neutral) vs shapes	↓ faces

Maron et al., 2016	RCT	SSRI short-term	29	Fear vs baseline, Happy vs. Baseline	↓ fear, ⇿ happy
Arce et al., 2008	RCT	SSRI sub-chronic	13	Faces (i.e., angry, fearful, and happy) vs shapes	⇿ faces
Fisher et al., 2015	RCT	SSRI sub-chronic	32	Threat (i.e., fear and angry) vs neutral	⇿ threat
Kraehenmann et al., 2015	RCT	Psilocybin acute	25	Aversive and neutral scenes vs shapes	↓ aversive and neutral
Mueller et al., 2017	RCT	LCD acute	20	Fear vs neutral faces	↓ fear
Hornboll et al., 2013	Cross-sectional	Ketanserin acute	23	Threat (i.e., fear and angry) vs shapes	5-HT2AR blockade reduced OFC threat-related amygdala reactivity
Fisher et al., 2009	Cross-sectional	5-HT 2AR ([18F]altanserin)	35	Threat (i.e., fear and angry) vs neutral	Inverse association between mPFC 5-HT2A and threat-related right amygdala reactivity
Fisher et al., 2006	Cross-sectional	5-HT1AR ([11C]WAY100635)	20	Threat (i.e., fear and angry) vs shapes	Inverse association between DRN 5-HT1A threat-related amygdala reactivity
Rhodes et al., 2007	Cross-sectional	5-HTT ([11C]-DASB)	20	Threat (i.e., fear and angry) vs shapes	Inverse association between amygdala 5-HTT and threat-related left amygdala reactivity

Table 1. *Overview of current literature on the association between serotonin and emotional processing in healthy individuals. Here, I included studies assessing emotional processing in the amygdala using fMRI as well as behaviourally with neuropsychological tests. Studies using tasks of visual attentional or perceptual processing of faces or pictures were presented, while e.g., gambling and anticipation tasks were excluded. I present studies using drugs with high specifically targeting 5-HT sites including classic serotonergic psychedelic and therefore dietary and genetic studies as well as studies using drugs with affinity for other neurotransmitter systems (e.g., mirtazapin and MDMA) are beyond the scope of current literature review. I also included studies using PET imaging of 5-HT binding sites. However, I only attend to PET studies without a 5-HT intervention and do not attend to fMRI connectivity studies. Interventions with acute SSRI administration signifies assessment within hours, short-term within 7-10 days and sub-chronic extending beyond two weeks. Results from RCT studies corresponds to within or between subject design comparing the active 5-HT intervention with placebo. Abbreviations: 5-HT = serotonin, 5-HTT = serotonin transporter, 5-HT1AR/2AR = serotonin 1A/2A receptor, DRN = dorsal raphe nucleus, mPFC = medical prefrontal cortex, OFO =medial orbitofrontal cortex, d' = accuracy/false alarm rate, FERT = Facial expression recognition task.*

II. Aims and hypotheses

The overall aim of this PhD project across the three studies was to advance our understanding of the role of brain 5-HT in regulating affective cognition. This PhD focused on cognitive-affective biases and threat-related amygdala reactivity because of their central involvement in normal brain function and the risk of developing affective disorders. I will briefly describe the primary aims and hypotheses for the three studies making up the PhD.

II.I. The 5-HTT study (study 1)

The 5-HTT study aimed to evaluate the association between brain 5-HTT levels as imagined with PET in fronto-striatal and fronto-limbic regions and cognitive-affective biases as assessed with neuropsychological testing in 98 healthy individuals. We hypothesised that 5-HTT levels are inversely associated with affective biases, meaning that higher 5-HTT in fronto-striatal and fronto-limbic is associated with a more negative bias.

II.II. The SSRI study (study 2)

The SSRI study aimed to evaluate the effects of sub-chronic administration of a widely prescribed SSRI, escitalopram, on amygdala reactivity to angry, fearful and neutral faces using BOLD fMRI in a sample of 64 healthy individuals. We hypothesised that amygdala response to angry and fearful facial expressions would be lower but unchanged to neutral faces after sub-chronic administration of SSRI compared to placebo. Additionally, we evaluated the effects of escitalopram on regional whole-brain responses to emotional faces.

II.III. The psilocybin study (study 3)

In the psilocybin study, the aim was to evaluate the acute effects of a medium-high dose of psilocybin on the amygdala reactivity to angry, fearful and neutral faces using BOLD fMRI in a sample of 26 healthy individuals. We hypothesised that the amygdala response to angry and fearful faces but not to neutral faces is reduced during acute psilocybin intervention compared to without the drug.

III. Methods

The relationship between affective cognition and 5-HT signalling in healthy individuals is examined across three studies using multimodal methods. These methods include two different neuroimaging modalities (PET & fMRI), which contribute complementary information about in vivo neurobiology, and neuropsychological tests, which contribute to knowledge about behaviour. Sub-chronic SSRI and psilocybin are used to examine serotonergic pharmacological modulation of affective cognition. In this chapter, I will briefly outline the central theoretical, empirical and statistical aspects of the applied methods in the PhD studies. This chapter will also shortly introduce the study designs, participants and experimental procedures across the studies. Lastly, the statistical analyses will also be covered with the latent linear model in more detail as this is a newer statistical approach to analysing PET data.

III.I. Ethics and approvals

The Ethics Committee of the Capital Region, Copenhagen, Denmark, approved all studies. This included paper 1, counting all four studies from which data was pooled for secondary analysis (identifier: H-12010091, H-22010108, H- 12010085 and H-42011103), paper 2 (identifier: H-18038352, with amendments 71579, 73632 and 78565) and paper 3 (identifier: H-16028698, with amendments: 56023, 56967, 57974, 59673, 60437 and 62255). Paper 2 was also preregistered in ClinicalTrials.gov (identifier: NCT04239339), and paper 3 was approved by the Danish Medicines Agency (identifier: 2016-004000-61, with amendments: 2017014166, 2017082837 and 2018023295).

Across all studies, participants were recruited through advertisement. They were informed about the study verbally and in writing before giving their written informed consent to

participate (as described in the respective study protocols). A signed written informed consent was obtained from all participants before initiating the study. Recruitment, inclusion and all other study procedures were carried out in accordance with the Code of Ethics of the World Medical Association (i.e., The Declaration of Helsinki principles).

All sensitive personal information, including electronic information traceable to an identifiable person from the participants, was kept strictly confidential and only available to relevant staff working on the project following Danish laws ("Autorisationsloven", "Sundhedsloven", and the law on doctor-patient confidentiality). This sensitive personal information was stored in locked files or on password-protected computers behind secure "firewalls" following the GDPR. Accordingly, subject names have not and will never be used in scientific publications or presentations. The Danish Data Protection Agency has approved the CIMBI Database/Biobank (Capital Region protocol number 2012-58-0004 [local journal number 30-0291, I-suite: 00628]). Data collected for studies 2 and 3 have been transferred to the Center for Integrated Molecular Brain Imaging (Cimbi) Database/Biobank at the Neurobiology Research Unit (Knudsen et al., 2016) following updated approvals from the Danish Data Protection Agency.

III.II. Study designs, participants and procedures pertaining to the main outcomes

III.II.I. THE 5-HTT STUDY (STUDY 1)

Study 1 was a retrospective observational cross-sectional study. Data from 98 healthy individuals were pooled across four studies stored in the Cimbi database (Knudsen et al., 2016). This database contains multimodal neuroimaging data from healthy volunteers and patient populations collected over the past ~20 years and is currently the largest repository of brain PET scans characterizing features of the 5-HT system in the world. All participants in Study 1 had been PET scanned using [^{11}C]DASB, and their cognitive-affective bias was assessed with the Emotional Faces

Identification task (EFIT). Of the 98 participants, 19 were in the remitted phase of a Seasonal Affective Disorder (SAD) and were included to enrich the study sample to reflect better the general population with natural variations in vulnerability to MDD. For details on other specific in- and exclusion criteria, please see paper 1.

III.II.II. THE SSRI STUDY (STUDY 2)

Study 2 was an experimental semi-randomised controlled trial with an intervention conducted in a double-blinded manner. Participants were randomised based on age, sex and IQ (Fusar-Poli et al., 2009). The study investigated the effects of three to five weeks of a clinical dose of escitalopram (Rao, 2007) on neural emotional processing in healthy individuals. Participants were recruited from a list of individuals that expressed interest in participating in brain research. In total, 64 healthy participants were included and completed the study. On the last day of the intervention, participants were scheduled for an MRI scanning, where they completed the emotional faces paradigm during BOLD fMRI. The participants and the investigators involved in the data collection were blinded to intervention allocation until the completion of data analysis.

Drug intervention in study 2

All participants received written and verbal instructions from blinded medical personnel on how to take the drug capsules, including possible side effects. They were instructed to take one capsule (10 mg escitalopram in the active group) each morning for three days and two capsules (20 mg escitalopram in the active group) daily on the fourth day and until the last day of the intervention period. The participants could contact the project medical doctor at all times during the study in case of complications, side effects or other concerns. The medical personnel contacted the participants by phone to inquire about potential side-effects at two time points; half-way through the intervention period (i.e., around day 13) and on the last day of examination.

III.II.III. THE PSILOCYBIN STUDY (STUDY 3)

Data used in study 3 originated from a single-blinded, within-subject, cross-over intervention trial investigating the effects of a psychoactive dose of psilocybin (0.2-0.3 mg/kg) with psychological support and ketanserin (20 mg flat dose, 5-HT_{2A} antagonist) on brain function in 28 healthy individuals. In study 3, data pertaining to the psilocybin arm of the overall study was used. Participants were recruited from a list of volunteers who had signed up with an interest in participating in brain research. Participants were presented with the emotional faces paradigm during BOLD fMRI during baseline and, on a separate day, following acute administration of psilocybin.

The psilocybin intervention in study 3

A description of the psychological support model used to administer psilocybin will be detailed here. This description is prioritised specifically for the psilocybin intervention as this is not detailed in the research article.

We used a three-phase psychological support model for psilocybin administration comprising a) preparation for the experience, b) dosing of psilocybin, and c) integration of the experience, and adhered to current guidelines for safe administration of psychedelics (M. W. Johnson et al., 2008). Each participant was followed and supported by a lead therapist and an assistant therapist (i.e., therapist = psychologist and/or MSc psychology students) throughout their study participation. For the preparation phase, the participant met with the therapists who, in an open conversation, inquired about the participant's background, covered study information, affirmed informed consent, and made agreements about therapeutic touch, such as holding hands. The primary purpose of the preparation was to carefully prepare the participant for a high-dose psychedelic experience by giving practical information as well as building trust and rapport

between the therapist and the participant, to minimise risk for adverse events (M. W. Johnson et al., 2008; R. Murphy et al., 2022). For the dosing phase, before drug administration, the therapist checked in with the participant to explore their psychological state, inquired about potential thoughts and questions since the last time and took an intermediate stance between the participant and his/hers everyday life to avoid preoccupation with practical matters during the experience. When the therapist assessed the timing to be right, oral capsules of psilocybin were administered to the participant with a glass of water. During the session, the therapists used subtle vocal guidance, carefully selected music and therapeutic touch as agreed during preparation (Brennan, Jackson, MacLean, & Ponterotto, 2021; Kaelen et al., 2015; J. Phelps, 2017; Thal, Bright, Sharbanee, Wenge, & Skeffington, 2021). Once the subjective drug effects had weaned off around four to six hours after drug administration (M K Madsen et al., 2019; Stenbæk et al., 2020), the therapists conducted a debriefing of the experience, discussed plans for self-care (Earleywine, Low, Lau, & De Leo, 2022), informed about afterglow effects and made sure, in collaboration with the MD, that the participant was ready to leave the site. The day after the psilocybin intervention, the participant met with the two therapists to allow for the integration of the psychedelic experience (Earleywine et al., 2022). This session covered a) questions about basic post-intervention functions such as sleep, mood and appetite and potential side-effects such as headache, b) elicitation of the participants' experience, c) debriefing of potentially difficult experiences, d) relating the experience to everyday life, and e) self-care plans.

III.III. Main outcomes

III.III.I. THE 5-HTT STUDY (STUDY 1)

III.III.I.I. Imaging the serotonin transporter with Positron Emission Tomography

PET is a neuroimaging method that quantifies proteins such as receptors and transporters in the living brain (i.e., in vivo) (Heurling et al., 2017; Lammertsma, 2019). For the quantification, a radiotracer is used, which is a molecule with high affinity and specificity for the protein of interest labelled with a radioactive isotope, such as Carbon-11 ([^{11}C]). Imaging the 5-HTT is possible using the radiotracer [^{11}C]DASB, a highly selective radiotracer providing a valid and reliable quantitation of 5-HTT in brain tissue (Wilson, Ginovart, Hussey, Meyer, & Houle, 2002). In study 1, the participants were scanned using a Siemens ECAT high-resolution research tomography (HRRT) PET scanner operating in 3D-acquisition mode with an approximate in-plane resolution of 2 mm (Olesen et al., 2009). Following a transmission scan of six minutes, an intravenous bolus of [^{11}C]DASB was injected over 20 seconds that entered the brain via the bloodstream. In the brain, [^{11}C]DASB binds in a specific manner to the target protein, that is, the 5-HTT on the cell surface, and to surrounding tissue in a non-specific manner. The radiotracer's isotope [^{11}C] decays at a constant rate, which releases a positron. Once the positron meets an electron, its antiparticle, they annihilate and emit two gamma rays of 511 keV separated by approximately 180 degrees. The PET scanner records these opposing gamma rays, which serves as the foundation for detecting and localizing the 5-HTT in the brain. Starting at the time of injection, a dynamic 90-minute emission scan was acquired and binned into 36 frames (6×10 s, 3×20 s, 6×30 s, 5×60 s, 5×120 s, 8×300 s, 3×600 s). The PET images were reconstructed using a 3D-OP-OSEM iterative method (10 iterations and 16 subsets).

Brain image analysis and outcome parameters

Subject-level preprocessing of the PET data included an in-scan automatic image registration algorithm determining motion and realignment (Woods, Cherry, & Mazziotta, 1992). PET scans were smoothed using a 10-mm within-frame Gaussian filter before alignment. Subsequently, we estimated rigid translation/rotation parameters by aligning each PET frame to a single PET frame with sufficient structural information using the scaled least-squares cost function (frame 26: 20–25 min post-injection). Non-filtered PET images were resliced using these parameters. Participants had also been scanned on an MRI scanner (a Siemens Magnetom Trio 3T scanner (n=38) or a Siemens Verio 3T scanner (n=60) (Siemens, Erlangen, Germany)), from which a high-resolution T1-weighted structural MRI brain scan was acquired. The participants' high-resolution T1 was co-registered to the PET images using SPM, based on the mean of frames 10–26, corresponding to a flow-weighted image. Accurate co-registration was confirmed by visual inspection across all planes.

From the participants' high-resolution T1-weighted structural MRI brain scan, brain regions were automatically delineated using Pvelab (Svarer et al., 2005). From a dynamic PET scan, the radioactivity concentration for a region of interest (ROI) can be plotted over time as a so-called time-activity curve (TAC). TACs representing the mean of gray-matter voxels within each ROI was extracted and used for PET quantification using compartment models. In study 1, we used the multilinear reference tissue model 2 (i.e., MRTM2), a modified reference tissue model validated for quantifying [^{11}C]DASB (Ichise et al., 2016). For each participant, a fixed k2' was estimated using putamen, caudate, and thalamus as the high binding regions and cerebellar gray matter without vermis was used as the reference region.

From the MRTM2, [^{11}C]DASB non-displaceable binding potentials (BP_{ND}) were calculated (Ginovart, Wilson, Meyer, Hussey, & Houle, 2001), which is a measure of the binding

of the radiotracer in the regions of interest relative to the non-displaceable binding, described with the following equation:

$$BP_{ND} = f_{ND} * \frac{B_{avail}}{K_D}$$

In this equation, f_{ND} is the fraction of radiotracer in the non-displaceable compartment, and B_{avail} is the number of available 5-HTT in the brain to which [^{11}C]DASB can bind. K_D is the equilibrium dissociation constant. As f_{ND} and K_D are assumed to be constants, BP_{ND} is proportional to B_{avail}.

III.III.I.II. Assessing affective biases using cognitive behavioural testing

A standard method used to examine affective cognition is neuropsychological testing. These tasks follow information-processing principles where cognitive processes are operationalized as a sequence of processing stages, e.g. attention, recognition and memory, which can be measured using targeted and validated tests of selected processing stages (Radvansky & Ashcraft, 2014). In tests of affective cognition, emotional stimuli are typically presented, and research volunteers are asked to attend to, categories and/or remember the stimuli or to actively inhibit responses to certain stimuli, which can then be quantified into behavioural outcomes such as reaction time or accuracy. Facial expressions are a universal and rich source of such emotional information (Cowen et al., 2019; Said et al., 2011), which is why cognitive evaluations of emotional faces are commonly used to examine affective cognition. Typically, basic facial expressions are used, including anger, fear, sadness, disgust, happiness and surprise (Ekman & Friesen, 2003). In these tasks, research volunteers are typically presented with different emotional facial expressions and asked either to assess the emotional state (i.e., an explicit emotional task) or to match one face to another based on age, sex or facial similarity (i.e., an implicit emotional task). These tasks are argued not to elicit discernible changes in emotional

states, which would represent a confound when assessing affective cognition (R. Elliott et al., 2011). Variations of emotional face recognition tasks such as the one used in the present study (The Emotional Faces Identification Task: EFIT) are the most widely used cognitive test to assess affective biases in emotion recognition.

The Emotional Faces Identification Task

The EFIT is a task of perceptual processing and interpreting facial expressions, including happiness, anger, fear, sadness, disgust and neutrality (Hjordt et al., 2017). The basic task design of EFIT was initially published and validated by Young et al., 1997 demonstrating that facial expressions of basic emotions are recognised by their fit to distinct basic emotion categories. In our version of the EFIT, 172 facial images with different emotional expressions are presented on a black computer screen. The faces are presented at different intensities, from minimum to maximum emotional valence (morphed with neutral facial expressions). The participants are instructed to identify the given facial emotion with a cursor as fast and accurately as possible. Note that the EFIT does not limit the time the participant is shown the stimuli (unlike other emotion recognition tasks) and therefore does not include a perceptual attention component. The primary outcomes are the hit rate for each emotion calculated as the percentage of correctly identified trials for a given emotion. As we were only interested in affective biases, we only included the hit rates of happy and sad faces. The affective bias is calculated by subtracting the hit rate for sad faces (%) from the hit rate for happy faces (%); this means that a positive score indicates a positive affective bias while a negative score indicates a negative affective bias (Bland et al., 2016). See an example of the cognitive behavioural test Emotional Face Identification Task (EFIT) in **Figure 5**).

Figure 5. Illustration of the Emotional Face Identification Task (EFIT) with an example of a happy face. The EFIT is a test of the ability to correctly identify facial expressions of anger, disgust, fear, sadness and happiness at different intensities, or neutrality. On a computer screen, an image of a face was displayed on the top and a hexagon made of six circles with the word for each of the five emotional categories or neutral was shown was presented below. Volunteers were orally instructed by the tester to label the emotional or neutral facial expressions using the cursor as fast and accurate as possible. Copied from Hjordt et al., 2017.

III.III.II. THE SSRI STUDY (STUDY 2) AND THE PSILOCYBIN STUDY (STUDY 3)

III.III.II.I. BOLD fMRI

Magnetic resonance imaging (MRI) is a non-invasive imaging method used to obtain high-resolution structural images of the human brain and measure short-lived hemodynamic changes, reflecting patterns of brain activity. The biophysical basis of acquiring such images is the electromagnetic properties of atomic nuclei (Buxton, 2013). This thesis's main focus is fMRI using

a BOLD MRI signal, which will be covered here. BOLD fMRI was first demonstrated by researchers in 1990 (Ogawa, Lee, Kay, & Tank, 1990), a method that, since its discovery, has made significant contributions to our understanding of normal human brain function. BOLD fMRI is a measure of the metabolic correlates of neuronal activity: as neural activity increases, so does the metabolic oxygen demand, and an overcompensation of oxygenated blood is provided (i.e., the supply of oxygen exceeds the demand) to a population of neurons in question. Oxygenated (i.e., diamagnetic) and deoxygenated (i.e., paramagnetic) haemoglobin have different magnetic properties, which provides a contrast in the signal detected by the MR scanner. More specifically, oxygenated haemoglobin induces less distortion of the local magnetic field than deoxygenated haemoglobin. BOLD signal is based on distortions of a magnetic field, so the BOLD signal increases proportionally to the concentration of oxygenated haemoglobin (Arthurs & Boniface, 2002; Buxton, 2013). Essentially, the BOLD response is a measure of change in the total amount of oxygenated haemoglobin in the population of neurons in a voxel over time. This change is also referred to as a hemodynamic response (HR), which in study 3 was recorded using echo planar imaging (EPI) with a T2*-contrast weighted acquisition with the MRI-scanner (Handwerker, Gonzalez-Castillo, D'Esposito, & Bandettini, 2012).

Experimental fMRI paradigms can be leveraged to examine the BOLD response to a task of interest, such as processing emotional faces. This can be investigated using a simple block design wherein blocks (time periods) of the task of interest are interleaved with baseline and reference blocks. The resultant changes in the BOLD signal are then evaluated based on the hemodynamic response function (HRF), a model of the assumed structure of the HR based on experimental studies (Buxton, Uludağ, Dubowitz, & Liu, 2004). In, e.g. a block design of the task, the expected HRF profile is applied for each condition (e.g. response to angry, fearful and neutral faces, respectively) in a given voxel over time. These HRF profiles are used in the experimental design matrix as regressors in a generalized linear model (GLM) depicting a voxel time series in

BOLD signal Y by the design matrix X, including random error and other nuisance variables such as motion. If the variation in the BOLD response in a given voxel is significantly explained by the model, it is recognised as being activated by the task (see, **Figure 6**) (Handwerker et al., 2012). Typically, the functional brain response to emotional stimuli is assessed by contrasting the BOLD response to the emotional stimuli of interest (e.g. angry faces) with the BOLD response to a non-emotional reference stimulus (e.g. geometric shapes or a neutral face) across a group of individuals. This procedure will be further elaborated on in the next section.

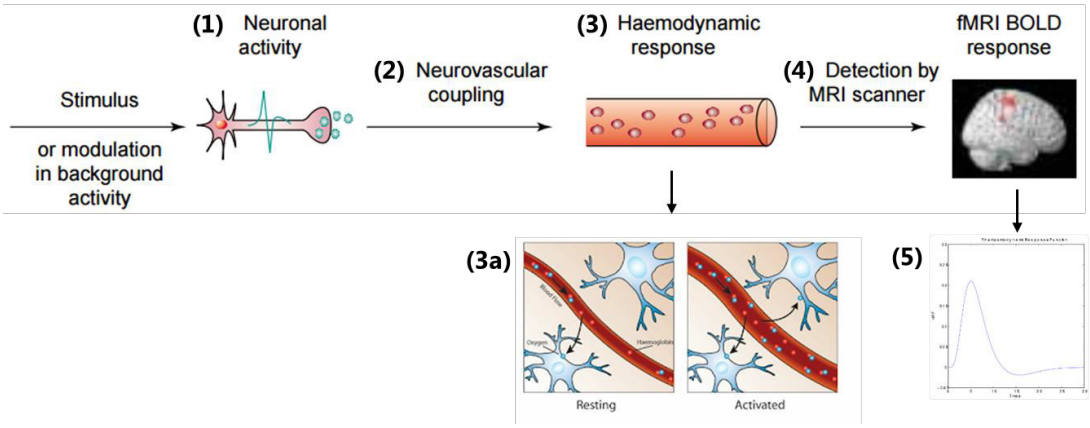

Figure 6. The hemodynamic response and fMRI BOLD signals. The BOLD signal has several components: (1) a presented stimulus evokes the neuronal response; (2) the neurovascular coupling; (3) the hemodynamic response, which is (3a) the overcompensated supply of oxygenated hemoglobin to neurons in question; (4) the MRI scanner detects a signal as oxyhemoglobin induces less local magnetic field distortion relative to deoxyhemoglobin, which affects the T2' (i.e., inhomogeneities in the magnetic field) and thereby T2*, and (5) The BOLD responses of interest are modelled applying the hemodynamic response function to the data, in e.g. SPM. Illustrations adapted from Arthurs & Boniface, 2002 and Huettel et al., 2014.

The emotional faces paradigm

A widely used method to examine neural correlates of emotional processing is using experimental fMRI paradigms that display visual stimuli of emotional faces (R. Elliott et al., 2011; Fusar-Poli

et al., 2009). In studies 2 and 3, we used the emotional faces paradigm (Patrick M. Fisher et al., 2022), a block design in which participants were asked to match the top stimulus to one of two stimuli presented below as fast and accurately as possible with a hand-controller (i.e., an implicit emotional task) (see **Figure 7**). This active motor task is not relevant to emotional processing but ensures attention to the task. In total, the paradigm took 6.5 minutes to complete. Four emotional blocks displaying a trio of faces expressing the same basic emotion (i.e., fearful, angry, surprised or neutral faces presented in random order across four versions) were presented on a screen: in the top centre displaying a target face and, on the bottom, left and right sides displaying two potential matching target faces. Each emotional block presented six face trios for four seconds, each interleaved by a fixation cross displayed with variable time intervals (i.e., two, four or six seconds) to minimise expectancy effects and habituation while maximising amygdala response (Patrick M. Fisher et al., 2022). Across five reference blocks, a trio of geometric shapes were presented: a top target shape and two potential matching shapes below. The fixation cross interleaving each of the six shape trios was displayed with a fixed time interval of two seconds. In studies 2 and 3, we only attended to the BOLD response to negative facial expressions (i.e., angry and fearful, respectively) and neutral faces, and all geometric shapes collapsed. The BOLD response to shapes was used to contrast the BOLD response to angry, fearful, and neutral faces, respectively. The emotional faces paradigm was implemented in E-prime, recording participants' responses with the hand-controller recording accuracy and reaction times. For pre-processing of the fMRI data, please see the manuscripts.

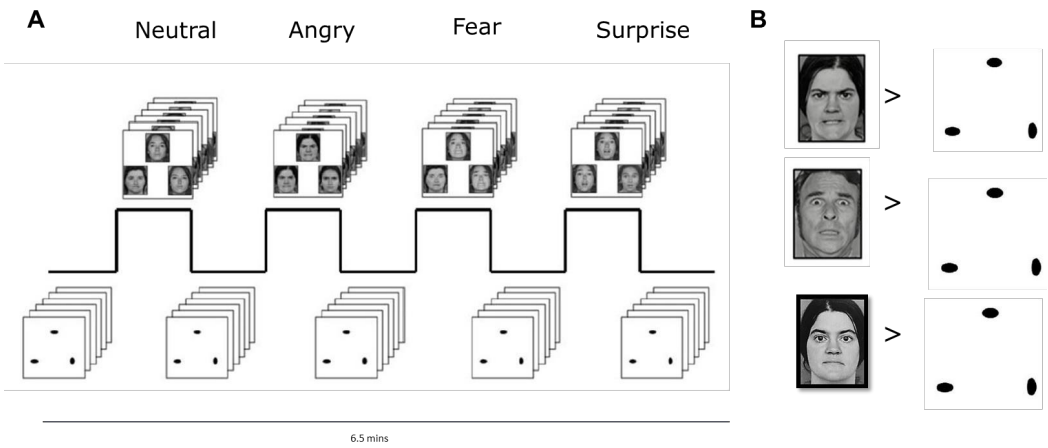

Figure 7. A) Illustration of the emotional faces paradigm during fMRI. Participants are instructed to match the target stimuli (in the top) to one of the two potential matching stimuli on the bottom left and right sides as fast and accurately as possible using a hand-controller pressing the left or right key. The entire paradigm consists of four blocks of emotional faces (i.e., fearful faces, angry faces, surprised faces, and neutral faces presented in random order across four versions) interleaved by five control blocks consisting of geometric shapes. Within each block, six face trios were displayed for four seconds, each interleaved by a fixation cross displayed with variable time intervals (i.e., two, four or six seconds) to minimise expectancy effects and habituation while maximising amygdala response throughout the paradigm. For shapes, fixation cross was displayed with a fixed time interval of two seconds, interleaving each of the six shape trios. The paradigm takes a total of 6.5 minutes to complete. B) In this study, we only attended to negative facial expressions (i.e. anger and fear) and neutral faces, as well as geometric shapes, which were used to contrast each facial expression. Copied from Armand et al., (submitted)

III.IIII. Primary statistical analysis

III.IV.I. THE 5-HTT STUDY (STUDY 1)

To analyse the association between 5-HTT BP_{ND} and cognitive-affective biases, we fitted a linear latent variable model (LVM) using the Lava package in R (Holst & Budtz-Jørgensen, 2013). The LVM is a structural equation model initially used to examine psychological constructs, typically with questionnaires. This statistical framework has proven to be suitable for handling molecular neuroimaging data because of the generally high inter-correlation between regional molecular measures, which was also the case for 5-HTT BP_{ND} (D Erritzoe et al., 2010). Using an LVM allows

fitting the high intercorrelation between regional 5-HTT BP_{ND} into a single latent variable capturing the shared variance in BP_{ND} between pre-selected brain ROIs. The advantage of the LVM statistical approach is that it reduces the risk of type II errors by summarising multiple variables into a single variable, thereby avoiding the need to conduct multiple statistical tests when evaluating associations across different regions in separate univariate analyses. In study 1, we modelled the 5-HTT latent variable (i.e., $5\text{-}HTT_{LV}$) from regions with a good signal-to-noise ratio implicated in affective biases: the fronto-striatal circuit(Eshel & Roiser, 2010) (i.e., frontal cortex, putamen, and caudate) and the fronto-limbic circuit (Robinson & Roiser, 2016) (i.e., frontal cortex, anterior cingulate cortex (ACC), and amygdala). Before modelling $5\text{-}HTT_{LV}$, we multiplied the regional 5-HTT BP_{ND} outcomes by 10 to display a more meaningful effect of $5\text{-}HTT_{LV}$ on the affective bias. Thereby, the effect of $5\text{-}HTT_{LV}$ on affective bias is reported as a 0.1 increase in $5\text{-}HTT_{LV}$. The frontal cortex was arbitrarily selected as a reference region for the LVM, so $5\text{-}HTT_{LV}$ values were expressed in 5-HTT frontal cortex units. Within the LVM, each regional 5-HTT BP_{ND} was separately adjusted for factors previously shown to influence 5-HTT BP_{ND} including sex, age, BMI, IQ, trait neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected mass of [11C]DASB, MR-scanner type, the 5-HTTLPR (i.e., alleles LL, SS, and SL) and group (i.e., healthy individuals with or without remitted SAD) (Mc Mahon et al., 2016; Spies et al., 2015; Tuominen et al., 2017). The procedure for estimating parameter extensions is described in paper 1. The p-value was considered statistically significant <.05.

III.IV.II. THE SSRI STUDY (STUDY 2)

We used linear regression models to examine the differences in the amygdala response to angry, fearful and neutral faces (contrasted with shapes) after three to five weeks of SSRI administration compared to placebo, with age and sex as covariates. Using the same model, we performed analyses of group differences in whole-brain regional responses to angry, fearful and neutral faces.

All analyses were carried out in R (v. 4.0.3). P-values are reported P_{UNC} and corrected for family-wise error rate (P_{FWER}) using the Bonferroni correction method for a family of three tests for the a priori amygdala analyses, and a family of 88 tests for the whole-brain analyses, reflecting the number of brain regions examined. P-values were considered significant at $P_{FWER} < 0.05$

III.IV.III. THE PSILOCYBIN (STUDY 3)

We fitted linear mixed-effect models (LMM) to evaluate the amygdala response to angry, fearful, and neutral faces (contrasted with shapes) during psilocybin intervention compared to baseline with MRI scanner was included as a covariate. In exploratory analyses using the same model, we assessed the effects of the emotional faces paradigm on brain-wide regions during psilocybin compared to baseline. Across all LMMs, an unstructured residual covariance pattern was modelled. The LMM was used to fit the data, as this model accounts for repeated measurements within participants, different residual variances and missing values. The analyses were carried out in in R (v. 4.0.3) using the packages *LMMstar* (Ozenne & Forman, 2021) (v. 0.7.6). P_{FWER} indicates correction for a family of three tests for the main amygdala analyses and, for exploratory whole brain analyses, P_{FWER} indicates correction for a family of 88 tests. P-values were considered statistically significant at $P_{FWER} < 0.05$.

IV. Results

This chapter will present a summary of the primary results across the three studies. The population in each study and secondary and post-hoc results are described in detail in the respective papers presented in the final part of the thesis.

IV.I. The 5-HTT study (study 1)

Using an LVM, we found that 5-HTT_{LV} BP_{ND} was inversely associated with cognitive-affective bias in emotion recognition as measured with EFIT; each 0.1 increase in 5-HTT BP_{ND} was associated with an 8% decrease in cognitive-affective bias, meaning a more negative bias (CI= -14% to -3%, p=.002), see **Figure 8** and **9.**

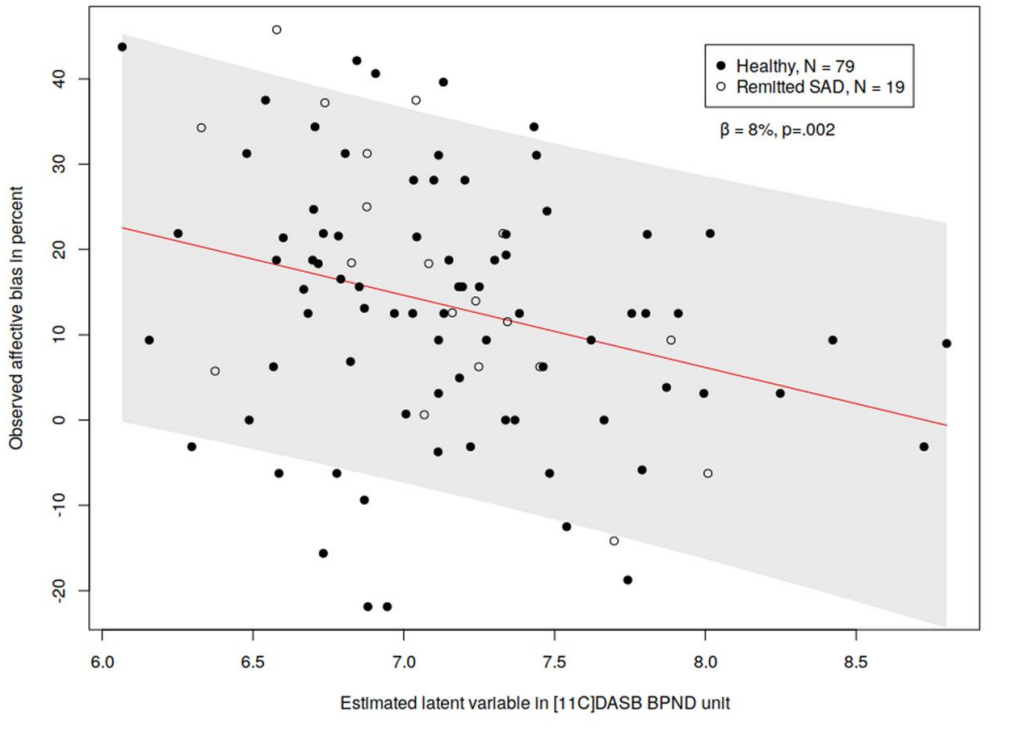

Figure 8. Plot of the estimated latent variable in units of [^{11}C]DASB PET (i.e. 5-HTT$_{LV}$) by observed affective bias in percent measured with The Emotional Identification Task. The effect of 5-HTT$_{LV}$ on affective bias is displayed as an increase of 0.1 in 5-HTT$_{LV}$. The red line corresponds to the estimated association between 5-HTT$_{LV}$ and affective biases (i.e. beta coefficient=8%) adjusted for covariates including sex, age, BMI, IQ, neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [^{11}C]DASB, MR-scanner type, the 5-HTTLPR and group (i.e. healthy individuals with or without a seasonal affective disorder (SAD). Copied from Armand et al., 2022.

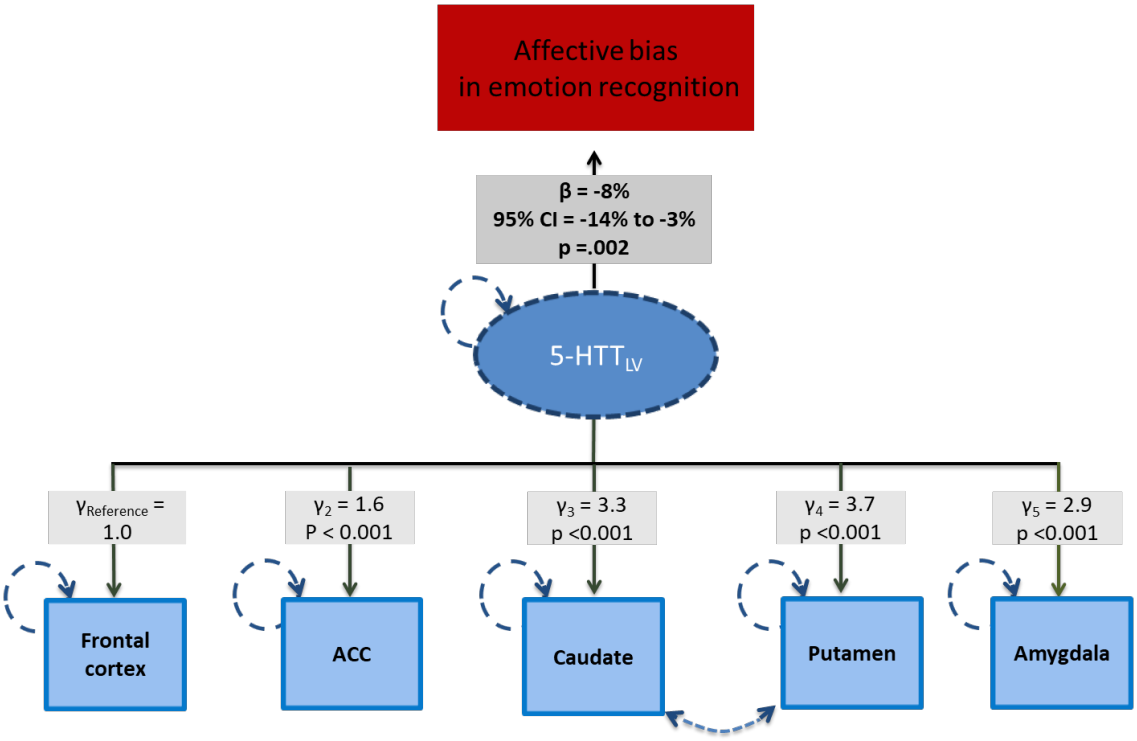

Figure 9. Illustration of the latent variable model (LVM). The red box represents the dependent variable affective bias in emotion recognition. The five brain regions of interest in the bottom blue boxes represents measured regional [11C]DASB BP_{ND} values used to define the latent variable (5-HTT_{LV}), which is represented in the blue oval. The effect of 5-HTT_{LV} on affective bias is displayed as increase of 0.1 in 5-HTT_{LV}. The hatched lines between caudate and putamen illustrates partial correlation included as covariance parameter. Circular blue hatched lines reflect variables estimated with error. Each regional [11C]DASB BP_{ND} was separately adjusted for sex, age, BMI, IQ, trait neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [^{11}C]DASB, MR-scanner type, the 5-HTTLPR (i.e. alleles LL, SS and SL) and group (i.e. healthy individuals with or without remitted SAD) (not illustrated). Abbreviations: β = point estimate for the regression coefficient of Emotional Face Identification Task (EFIT). γ = point estimate for the loadings onto 5-HTT_{LV}. ACC = anterior cingulate cortex. Copied from Armand et al., 2022.

IV.II. The SSRI study (study 2)

Using linear regression models, we found that the amygdala response to angry faces was numerically lower. In contrast, the responses to fearful and neutral faces were numerically higher in individuals administered with SSRI compared to placebo. However, none of these effects was statistically significant (angry: mean difference = -0.10, SE = 0.47, P_{FWER} = 1.00; fear: mean difference = -0.05, SE = 0.12, P_{FWER} = 1.00; neural: mean difference = -0.09, SE=0.17, P_{FWER} = 1.00). Males had a significantly higher amygdala response to fearful faces compared to females (mean difference = 0.33, SE = 0.12, P_{FWER} = 0.02), but we observed otherwise no sex differences (angry: mean difference = -0.01, SE = 0.21, P_{FWER} = 1.00; neutral: mean difference = -3.0, SE = 0.17, P_{FWER} = 0.26) or effects of age (angry: mean difference = -0.01, SE = 0.02, P_{FWER} = 1.00; fear: mean difference = 0.00, SE = 0.01, P_{FWER} = 1.00; neutral: mean difference = -0.01, SE = 0.01, P_{FWER} = 1.00). See **Figure 10** for an illustration of individual and average amygdala responses to each emotion, adjusting for the effect of sex and age.

The analyses of the effects of sub-chronic SSRI on whole-brain region-wise responses showed that responses to angry faces were significantly lower in the left opercular part of the inferior frontal gyrus (mean difference = -0.25, SE = 0.05, P_{FWER} <. 01), in the left triangular part of inferior frontal gyrus (mean difference = -0.21, SE = 0.06, P_{FWER} = 0.03) and in the right pallidum (mean difference = -0.16, SE = 0.04, P_{FWER} = 0.03) in the escitalopram group compared to placebo. For fearful faces, the responses were significantly lower in the left gyrus rectus (mean difference = -0.25, SE = 0.05, P_{FWER} < .01), in the left superior occipital gyrus (mean difference = -0.26, SE = 0.07, P_{FWER} < .01), in the left middle occipital gyrus (mean difference = -0.20, SE = 0.06, P_{FWER} = 0.04) and in the right inferior occipital gyrus (mean difference = -0.31, SE = 0.08, P_{FWER} < .01) in the escitalopram group compared to the placebo group. For neutral faces, the responses were significantly higher in the right orbital part of the inferior frontal gyrus (mean

difference = 0.27, SE = 0.06, $P_{FWER} < .01$) and in the right medial part of the superior frontal gyrus (mean difference = 0.23, SE = 0.06, $P_{FWER} = 0.03$). See the manuscript for an overview of region-wise estimates and p-values from the statistical models.

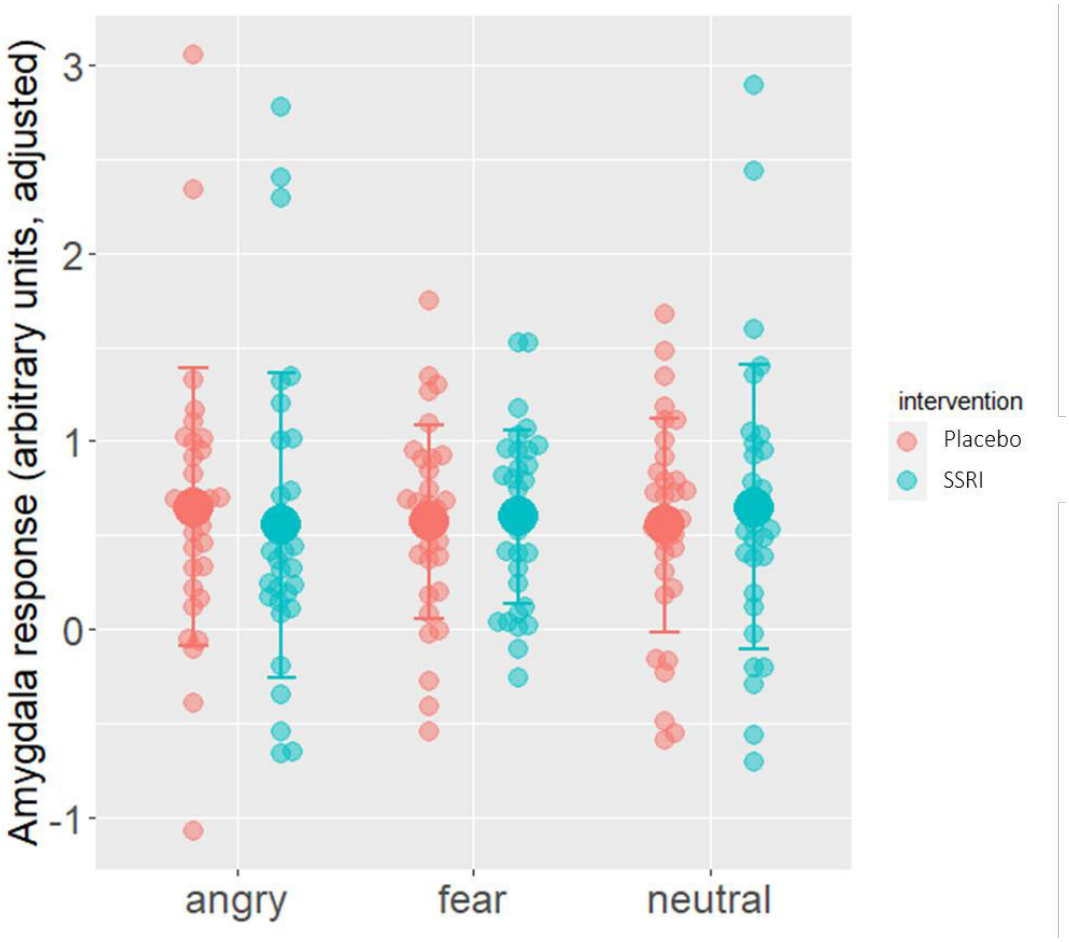

Figure 10. Bilateral amygdala response during BOLD fMRI to angry, fearful, and neutral faces (contrasted with geometric shapes) by group (SSRI, n=32; placebo, n=32), adjusted for effects of age and sex. Smaller transparent circles denote individual observed values and larger circles, and bars denote population Mean ± 1 SD. Copied from Armand et al., (in final prep).

IV.III. The psilocybin study (study 3)

Using LMMs, we found that the amygdala response to angry faces was significantly decreased during exposure to psilocybin compared to baseline (mean difference [95% CI]: -0.51 [-0.91; -0.12], $p_{FWER} = 0.04$). The amygdala response to fearful faces was numerically decreased during exposure to psilocybin; however, this effect was not statistically significant (mean difference [95% CI]: -0.18 [-0.47; 0.11], $p_{FWER} = 0.63$. The amygdala response to neutral faces was slightly numerically increased during exposure to psilocybin and was not statistically significant (mean difference [95% CI]: 0.02 [-0.27; 0.30], $p_{FWER} = 1.00$). **Figure 10** displays images of the average amygdala response to each emotional face, and **Figure 11** displays individual and average amygdala responses to emotional faces adjusting for the effect of the scanner.

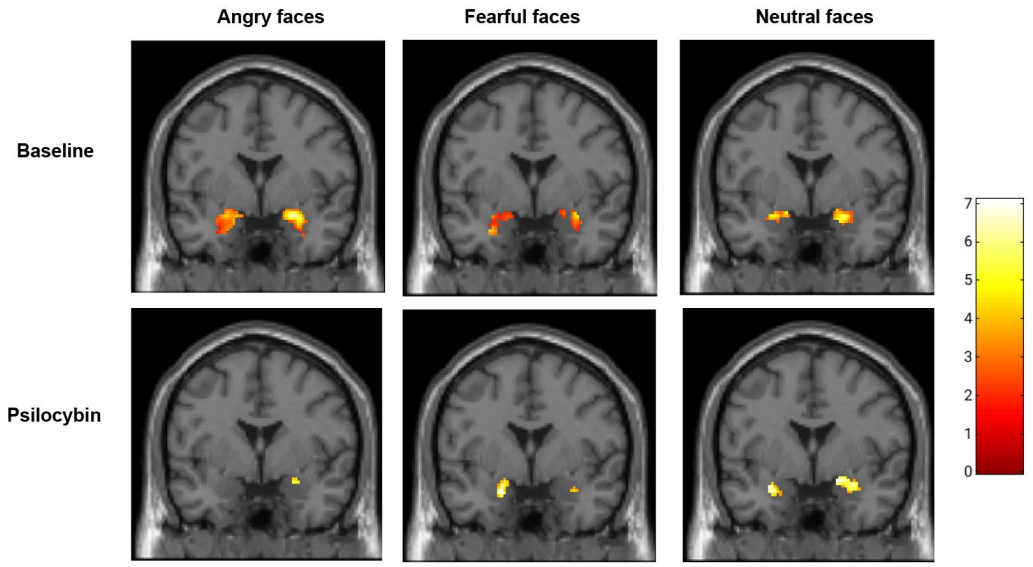

Figure 10. Illustration of coronal brain slices in standard space for the whole sample (baseline, n=26; psilocybin, n=20) during the presentation of the emotional faces paradigm during BOLD fMRI. Coloured areas represent a pronounced response in the amygdala to angry, fearful and neutral faces contrasted to geometric shapes (Punc < 0.05), during baseline and psilocybin intervention. SPM y coordinates across baseline and psilocybin were 0.0 for all emotions. The Colour bar indicates t-scores. Copied from Armand et al., submitted.

The exploratory analyses of whole-brain, region-wise responses to angry and fearful faces showed that almost all regional brain responses were numerically reduced during acute effects of psilocybin compared to baseline, but no effects were statistically significant after correction for multiple testing. For neutral faces, most brain regions were unchanged or numerically increased from baseline to psilocybin, but no effects of increase were statistically significant after controlling for multiple testing. See the manuscript for an overview of region-wise estimates and p-values from the statistical models.

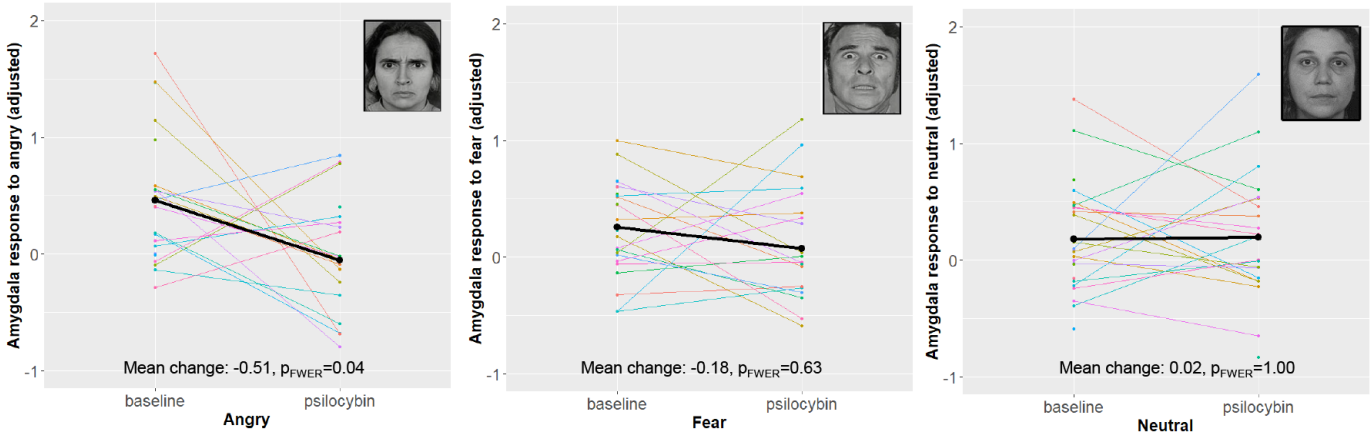

Figure 11. Plots of the partial residuals for the amygdala response (corrected for scanner) to angry faces (left), fearful faces (middle) and neutral faces (right), contrasted to geometric shapes at baseline (n=26) and during psilocybin intervention (n=20). Examples of face stimuli are portrayed in each corresponding plot. The black dots represent the means and coloured lines represent individual values. To evaluate emotional amygdala response during psilocybin compared to baseline, we used a linear mixed model for each emotion, which showed (mean difference [CI], p_{FWER}): Angry: -0.51 [-0.91; -0.12], p_{FWER}=0.04; fear: -0.18 [-0.47; 0.11], p_{FWER} = 0.63; neutral: 0.02 [-0.27; 0.30], p_{FWER} = 1.00. Copied from Armand et al., in final prep.

V. Discussion

Across three research studies of healthy individuals, we investigated the role of brain 5-HT in affective cognition, specifically in cognitive-affective biases and threat-related amygdala reactivity. This examination was carried out a) in study 1 by examining the association between cognitive-affective biases, assessed behaviourally using neuropsychological testing, and 5-HTT levels in the brain as imaged with PET, and b) in studies 2 and 3 by examining serotonergic modulation of amygdala response to angry, fearful, and neutral faces assessed using fMRI with the serotonergic drugs escitalopram and psilocybin, respectively.

In this chapter, I will present a discussion of the main findings. An empirical discussion of results will be presented, followed by a methodological discussion, including a critical view of the applied methods and our healthy population sample. The reader is referred to papers 1, 2 and 3 for more detailed interpretations and discussions of the results, including comparisons with existing literature.

V.I. Empirical discussion

V.I.I. The fundamental role of brain serotonin in affective cognition in healthy individuals

PET imaging of the 5-HT system to investigate emotion processing has rarely been done due to the method's complexity and cost (Beata R. Godlewska & Harmer, 2020). This method provides unique insight into whether endogenous 5-HT is involved in affective cognition. Our study of the association between brain 5-HTT levels and cognitive-affective biases was the first to investigate whether an in vivo molecular marker of the 5-HT system is involved in cognitive-affective biases carried out in a

large sample of healthy individuals. Our findings demonstrate that cognitive-affective biases are associated with 5-HTT levels, a key marker of endogenous 5-HT tonus. It remains debated how 5-HTT levels relate to extracellular 5-HT. One common hypothesis suggests that higher 5-HTT levels result in increased clearance of extracellular 5-HT (Mathews et al., 2004) based on animal studies reporting that boosting 5-HT concentrations decreases 5-HTT levels in the brain (Fritze, Spanagel, & Noori, 2017; Ginovart, Wilson, Meyer, Hussey, & Houle, 2003; Lundquist et al., 2007; Yamamoto, Onoe, Tsukada, & Watanabe, 2007). At the outset of this hypothesis, our findings indicate that lower extracellular 5-HT is associated with negative affective biases, a risk factor for developing affective disorders (Chan et al., 2007; van Oostrom et al., 2013). In accordance with our findings, a previous study reported that 5-HTT levels were associated with amygdala response to negative faces (Rhodes et al., 2007), suggesting that 5-HTT is involved in modulating response to emotional faces on a neural and behavioural level. Considering other PET studies, previous studies have likewise found an association between threat-related brain responses and other molecular serotonergic markers, including the 5-HT_{2A} (Patrick M. Fisher et al., 2009) and 5-HT_{1B} (P. M. Fisher, Meltzer, Ziolko, Price, & Hariri, 2006; Patrick M Fisher et al., 2011). So, evidence from basic scientific studies suggests that 5-HT is involved in both neural and behavioural emotion processing in healthy individuals. However, it remains unknown whether neural emotion processing in the amygdala underlies behavioural responses to emotions, representing an unresolved question that should be investigated in future studies.

V.I.II. Effects of serotonergic pharmacological interventions on affective cognition in healthy individuals

V.I.II.I. Selective serotonin reuptake inhibitors

Effects of serotonergic pharmacological interventions on emotion processing in healthy individuals have mainly been studied with SSRI, however rarely with administrations extending beyond ten days.

Our study represents one of few studies investigating the effects of sub-chronic SSRI administration on emotion processing, with our study being the largest to date. Our results did not support that sub-chronic SSRI modulates emotion processing in the amygdala compared to placebo, in accordance with two previous relatively smaller studies of sub-chronic SSRI on neural emotional processing (Arce, Estibaliz, Simmons, Alan N. Lovero K. Stein, Murray B. Paulus, 2008; Patrick Macdonald Fisher et al., 2015). However, our whole-brain analyses showed that sub-chronic SSRI significantly reduced brain responses to fear and angry emotions while it significantly increased response to neutral emotions cortical regions. Previous studies did not find any significant effects in cortical regions, perhaps explained by their relatively smaller sample sizes not being large enough to detect group differences. We speculate that our results of region-specific effects of sub-chronic SSRIs on emotional processing reflect region-wise molecular 5-HT changes in response to sub-chronic SSRIs. A previous study of healthy individuals from our lab reported region-specific increases in 5-HT tonus in response to sub-chronic SSRI administration (Haahr et al., 2014), as measured with PET imaging of 5-HT_4 receptors argued to be a marker of extracellular 5-HT tonus (Haahr et al., 2014; Mc Mahon et al., 2018). Our findings could indicate that region-specific increases in the 5-HT tonus result in less processing of negative faces and more of neutral faces in cortical regions. However, this hypothesis should be investigated in future studies of healthy individuals, for example, using a multimodal neuroimaging study with PET and fMRI investigating region-wise serotonergic molecular changes and functional emotional changes in response to sub-chronic SSRI administration.

Relative to the effects of sub-chronic SSRI administration, the impact of acute and short-term (i.e., 7-10 days) SSRIs on emotion processing have been widely studied. Using both neuropsychological testing and fMRI, these studies have generally reported that acute and short-term SSRIs modulate emotion processing, most consistently of threat-related emotions (see an overview of studies in **Table 1**). However, the results of the direction of the modulation of threat-related emotions have been mixed. While some studies report increased threat-related recognition and

amygdala response (Browning et al., 2007; Cremers et al., 2016; C. J. Harmer et al., 2003; Macoveanu, Rowe, Hornboll, Elliott, & Olaf, 2015; Selvaraj et al., 2018), other studies report a decreased threat-related recognition and amygdala response (I. M. Anderson et al., 2007; Del-Ben et al., 2005; Catherine J. Harmer et al., 2006; Maron et al., 2016; S. E. Murphy, Norbury, et al., 2009; Takahashi et al., 2005; Windischberger et al., 2010), compared to placebo. One study found no effect (Norbury et al., 2009). We speculate that these diverging findings could stem from diverse and opposing molecular changes in the 5-HT system in response to acute and short-term SSRI administration. A meta-analysis of the effects of SSRI administration on 5-HT tonus in animals found that 5-HT tonus increased within three days in some brain regions but dropped in the frontal cortex within the first week, whereafter 5-HT tonus gradually increased (Fritze et al., 2017). In healthy humans, studies suggest reduced 5-HT 1B receptors in the raphe nuclei but increased 5-HT 1B receptors in all 5-HT projection areas (Nord, Finnema, Halldin, & Farde, 2013). Meanwhile, another study points to large inter-individual differences in 5-HT tonus in response to acute SSRI intervention (da Cunha-Bang, Ettrup, et al., 2019). Taken together, these studies indicate dynamic and inter-individual molecular changes of the 5-HT system in response to acute and short-term SSRI administration, perhaps explaining the mixed results from studies investigating the effects of acute and short-term SSRIs on emotion processing.

V.I.II.II.. Psilocybin and other classic serotonergic psychedelics drugs

Another method to investigate serotonergic modulation of emotion processing is classic serotonergic psychedelics such as psilocybin, LSD and ayahuasca. Our study is the first to examine how psilocybin modulates amygdala response to emotional faces. Compared to placebo, we found that psilocybin significantly decreased amygdala response to angry faces, while no significant changes in amygdala responses to fearful or neutral faces were observed. Our result partially aligns with previous fMRI studies investigating amygdala response to emotional stimuli during exposure to psilocybin

(Kraehenmann et al., 2015) and LSD (Mueller et al., 2017). These studies reported that amygdala responses to negative stimuli were significantly lower during exposure the psilocybin as well as LSD, compared to baseline. However, Kraehenmann *et al.* reported that the amygdala response to neutral stimuli was also reduced, in contrast to our finding. This difference between results may reflect a divergence in the type of neutral stimuli used across studies, as we used faces while Kraehenmann *et al*. used scenes. In sum, evidence suggests that classic serotonergic psychedelics reduce the processing of specific negative emotions, both behaviourally and neurobiologically.

Our exploratory analyses revealed that almost all regional brain responses were numerically reduced to angry and fearful faces during exposure to psilocybin compared to baseline, while numerically unchanged or slightly increased to neutral faces. This aligns with Mueller *et al.*, and Kraehenmann *et al.,* who reported a significant effect of LSD and psilocybin on emotion processing in cortical regions. However, none of the effects from our exploratory analyses was statistically significant after correction for multiple testing, perhaps due to the poor reliability of the emotional faces fMRI paradigm (Plichta et al., 2012). This methodological consideration will be discussed later. In sum, current evidence suggests a general reduced brain sensitivity or attribution of salience to threat-related emotions following acute administration of psilocybin.

V.I.II.III. Implications for affective disorders

SSRIs are commonly used clinically to treat affective disorders (Nutt et al., 1999), while psilocybin-assisted-therapy has been shown in small clinical studies to be efficient in the treatment of MDD (Andersen, Carhart-Harris, Nutt, & Erritzoe, 2021; Breeksema, Niemeijer, Krediet, Vermetten, & Schoevers, 2020). Studying the effects of SSRI and psilocybin on neural emotion processing in healthy individuals may serve as a model to understand how these drugs work on the brain while shedding light on potential treatment mechanisms. As hyperactive amygdala response to threat is

involved in MDD (Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013), it is particularly relevant to understand how SSRIs and psilocybin modulate these disease-related cognitive processes. Notably, our results suggest that both drugs reduce neural processing of negative emotions, although in different brain regions, indicating reduced brain response or sensitivity to negative stimuli. So, it appears that SSRIs and psilocybin may have the potential to reduce dysfunctional exaggerated processing of negative emotions, processes argued to trigger and maintain depressive episodes, for example, by supporting negative thought patterns about one-self and the future (Beck, 2008; Bistrickv et al., 2011; Weightman, Air, & Baune, 2014; Zenger et al., 2011). These results are in line with the existing theory of antidepressant action, particularly for SSRIs (Beata R. Godlewska & Harmer, 2020). This theory suggests that SSRI-induced attenuated brain sensitivity to negative emotions allow individuals with MDD to engage more with positive emotions, such as noticing more positive stimuli in the environment or processing more positive autobiographical memories, which ultimately translate into a clinical antidepressant response (Bhagwagar et al., 2004; C. J. Harmer et al., 2003; Catherine J. Harmer et al., 2006; Hayward, Goodwin, Cowen, & Harmer, 2005; S. E. Murphy, Longhitano, Ayres, Cowen, & Harmer, 2006; Papadatou-Pastou, Miskowiak, Williams, Harmer, & Reinecke, 2012). For psilocybin-assisted therapy, a theory is that reduced processing of negative emotions during exposure to psilocybin facilitates trust in the therapeutic alliance and allows individuals to process previous painful experiences otherwise inaccessible. Such processes may lead to emotional breakthroughs and insights, which, along with the therapeutic alliance (R. Murphy et al., 2022), mediate the reduction of clinical symptoms (Roseman et al., 2018; Yaden & Griffiths, 2021).

An important limitation of using a healthy brain model to undercover how the effects of SSRI and psilocybin on neural emotion processing might contribute to their therapeutic efficacy is the absence of clinical depressive mood in healthy populations. An important outstanding question is how changes in neural emotion processing relate to changes in mood across SSRI and psilocybin.

Current evidence suggests that mood changes only emerge after at least two weeks on SSRIs in MDD (Kasper et al., 2006). However, changes in emotional processing are evident acutely (Beata R. Godlewska & Harmer, 2020). Conversely, during acute effects of psilocybin, changes in emotion processing and mood are consistently observed. One hypothesis (Kometer et al., 2012) suggests that mood changes are mediated through 5-HT_{2A}, which is stimulated acutely during psilocybin, while only sub-chronic, and not acute, SSRI administration modulates 5-HT_{2A} density. Future studies should investigate how neural emotion processing changes relate to mood changes.

V.I.II.IV. Summary and perspectives

Most fMRI studies investigating the effects of acute and short-term SSRI and serotonergic psychedelics, including psilocybin, on emotion processing, suggest that these drugs modulate amygdala response to emotional stimuli, mainly by reducing the processing of specific negative emotions. Conversely, there is no evidence to suggest that sub-chronic SSRI modulates emotion processing in the amygdala; instead, sub-chronic SSRI seems to reduce the processing of negative emotions while increasing the processing of neutral emotions in cortical regions. So, modulation of brain responses to emotional stimuli appears to differ depending on whether serotonergic psychedelics or SSRIs have been administered, including whether SSRIs are administered over short periods or more chronically. One speculation is that acute SSRIs and psilocybin target bottom-up emotional processing through modulation of amygdala response to emotional stimuli, while sub-chronic SSRI modulates top-down control of emotions through modulation of cortical regions. Future studies should compare how psilocybin and SSRI impact emotional processing by using tasks specifically targeting top-down and bottom-up emotion processing, such as masked and unmasked emotional stimuli.

V.II. Methodological discussion

This thesis' studies have several strengths, including using an integrative scientific approach by combining a) a validated neuropsychological test allowing for an objective assessment of emotion processing behaviorally and b) multimodal brain imaging with PET and pharmaco-fMRI contributing with complimentary knowledge about in-vivo molecular and functional neurobiology. The cross-sectional database study allowed us to investigate how a key marker of brain 5-HT with PET relates to cognition in the largest healthy cohort to date, while the two pharmacological studies were large blinded studies using either placebo or cross-over controls to minimize expectancy effects. However, the results from the studies must be interpreted under some methodological limitations, which will be elaborated on next.

V.II.I. Neuropsychological testing

EFIT measures the perception and interpretation of facial expressions of basic emotions, as there was no time constraint in the presentation of faces. However, it may be an oversimplification that the task only measures perception and interpretation as task performance relies on additional supporting cognitive functions such as attention. So, it should be investigated whether our findings can be replicated using other types of tasks of emotional face perception. It would furthermore be interesting to examine whether affective biases in other cognitive domains, such as reinforcement sensitivity, which is also linked to MDD (Katz, Matanky, Aviram, & Yovel, 2020), is modulated by brain 5-HT. The EFIT measures individuals' discrete perceptual responses to unfamiliar faces isolated from the context in controlled settings. Such task setup does not resemble how humans perceive and interpret faces in everyday life. Therefore, the task's ecological validity is limited, representing a common issue for neuropsychological tests (Dawson & Marcotte, 2017). So, individuals' affective biases in perception and interpretation as measured on the EFIT might not be representative of how they would perform in everyday situations, for example, under stress or

during the interpretation of emotional expressions in familiar faces. Future studies should explore the converging validity of cognitive-affective biases as measured with EFIT by examining its association with other measures of affective biases, for example, in behaviour as measured with self-report questionnaires.

V.II.II. Brain imaging with PET and fMRI

Brain imaging with PET and fMRI can provide unique insight into brain function and molecular organisation in the living human brain. However, the interpretation of PET and fMRI data should be made with consideration to certain limitations. The pre-processing pipeline for PET raw data may vary from lab to lab (Nørgaard et al., 2019). Notably, our lab demonstrated that different pre-processing pipelines for [11C]DASB-PET lead to different results (Nørgaard et al., 2020), which challenges between-study comparisons. Furthermore, studies have reported that 5-HTT levels in healthy individuals are significantly lower during the second compared to the first scan indicating test-retest issues for this particular PET marker (Kim & Robert, 2006; Nørgaard et al., 2019). The difference is hypothesised to be due to higher stress levels during the first compared to the second scan, which may influence the 5-HT system. Future studies should consider controlling for state stress levels during scanning. Across all types of emotional face fMRI paradigms, poor within-subject reliability has been demonstrated (Plichta et al., 2012), which is a common problem for task-related fMRI paradigms (M. L. Elliott et al., 2020). One hypothesis is that the poor reliability stems from so-called "off-task time" in simple task design, such as the faces paradigm, where individuals engage in other cognitive processes while presenting the target stimuli (Plichta et al., 2012). We speculate that the poor reliability of the emotional faces paradigm has constrained our ability to detect significant group differences in studies 2 and 3 in regions with apparent numeric group differences. Developing and validating fMRI paradigms that reliably measure brain

responses to salient emotional stimuli is imperative to better resolve alterations in neural emotion processing in clinical cohorts and response to pharmacological challenges.

V.II.II. Pharmacological interventions

There is a risk of incompliance with drug intake for pharmacological interventions over long periods, such as our sub-chronic SSRI study. However, all participants in our study were compliant based on satisfactory serum escitalopram levels halfway through the intervention and at follow-up. Further, participants' capsule containers were inspected at each visit and their medication logbook was verified at the follow-up visit. So, we do not suspect that the lack of group differences in amygdala response to emotional stimuli was due to incompliance in the treatment group. Five participants dropped out due to side effects, of whom four received SSRI. Assuming that this group had an overall greater psychoactive response to SSRI, missing these data can have biased our findings towards under-estimating effects of SSRI on amygdala responses to emotional faces. Participants receiving SSRI correctly guessed their group assignment was around the chance level (i.e., 53%), but the frequency of guessing SSRI assignment in the active group was significantly higher than in the placebo group (i.e., 16%) (see analysis in paper 2). Perceptual differences influencing participant guess may have been similarly associated with our observed group differences in emotion-processing cortical regions.

In the psilocybin study, we did not include a placebo group. We can, therefore, not exclude that factors other than pharmacological effects of psilocin may have influenced the observed effects on the brain, such as psychological factors including expectancy, inter-personal support and music (Robin L. Carhart-Harris et al., 2018; Haijen et al., 2018; Kaelen et al., 2015). In fact, we speculate that the observed effects of reduced amygdala reactivity to angry faces might have been a mood-congruent effect, which may have looked differently if the drug had been taken

in unsafe settings without interpersonal support. Future studies should employ a placebo group. However, the most appropriate placebo control is widely discussed in the context of psychedelics studies, as the prominent psychoactive effects of psilocybin unblind participants and facilitating therapists.

V.II.III. Research with healthy individuals

Across the three studies, our sample of healthy individuals was, on average, relatively young with average IQ scores in the upper range of normal variation, and they had no history of psychiatric disorder (except in study 1, where 19% of the healthy individuals were in the remitted phase of a seasonal affective disorder). So, our sample represents a particular group of the healthy population; therefore, caution should be taken when generalising our findings to the general population, for example, to more vulnerable groups recovered from psychiatric conditions (except for study 1) or with first-degree relatives with psychiatric conditions. Further, our overall sample consisted of more females than males; however, the sex distribution is comparable to that of depressed populations where the majority are females, allowing for comparing our results to future studies in MDD. Finally, our sample consisted of a North European population, limiting our results' generalisability to other populations. Overall, future studies should investigate whether our results can be replicated in more diverse populations regarding age, educational levels, ethnicity, and in groups with higher vulnerability to psychiatric conditions.

VI. Conclusion

Our main findings demonstrate that brain 5-HT is involved in affective cognitive processes in healthy individuals, both on a behavioural and neural level. In our first study, we find that 5-HTT levels in the fronto-striatal and fronto-limbic brain regions are inversely associated with affective biases in emotion recognition, meaning that higher 5-HTT levels are associated with a priority of negative faces over positive ones. As the primary mechanism of 5-HTT is to clear extracellular 5-HT, our results could reflect that negative biases link to excessive clearance of extracellular 5-HT. Following this speculation, results from our second study suggest that sub-chronic SSRI, presumably increasing extracellular 5-HT, decreases the processing of negative faces in cortical regions compared to placebo. Along the same line, results from our third study suggest that psilocybin, a new promising treatment for MDD, reduces the processing of angry faces in the amygdala. This could reflect that psilocybin targets bottom-up affective processing while sub-chronic SSRI targets top-down affective processing, perhaps reflecting these drugs' antidepressive effects. However, this hypothesis deserves to be investigated in future studies with clinical populations.

By using advanced cross-disciplinary methods, including neuropsychological testing combined with multimodal neuroimaging, this line of studies bolsters the previously reported association between 5-HT and affective cognition in healthy individuals. This PhD project contributes with new knowledge of how affective cognition is shaped by a) a key marker of endogenous 5-HT signalling and b) 5-HT modulating drugs, comprising sub-chronic SSRI and psilocybin, shedding light on possible treatment mechanisms behind their antidepressant effects.

VII. Perspectives

Results from this PhD project have stimulated further questions, and the most critical research perspective are highlighted below:

- Based on study 1, we suggest future studies investigate whether excessive 5-HT clearance, as measured with [^{11}]DASB PET, underlies negative affective biases in a large sample of individuals with MDD. It would further be interesting to explore whether the strength of the potential association between 5-HTT and affective biases is related to the severity of the depressive state.
- Based on study 2 and previous studies of the effects of acute and short-term SSRI on emotional processing, one suggestion for future studies would be simultaneous to explore region-wise serotonergic molecular changes as well as functional emotional changes in response to sub-chronic SSRI administration by using multimodal neuroimaging with PET and fMRI in a longitudinal study design. Such a comprehensive study would shed light on whether the region-wise functional effects of SSRI on emotion processing map to region-wise molecular 5-HT changes over time.
- Based on study 3, an important next step would be to investigate whether emotion processing is altered in individuals with MDD following acute administration of psilocybin and whether potential alterations relate to clinical outcomes.
- With the outset in results from study 2 and 3, a suggestion for future research would be to compare how sub-chronic SSRIs and psilocybin affects emotion processing in healthy and clinical populations. A pending hypothesis is that sub-chronic SSRIs target top-down affective processes, while psilocybin acutely targets bottom-up affective processes, a hypothesis that can be investigated using paradigms specifically targeting different levels of cognitive-affective processing.

- Based on the results across the thesis' three studies, future studies of healthy individuals should investigate whether emotional processing in the amygdala underlies behavioural cognitive-affective biases; one hypothesis could be that exaggerated threat-related amygdala reactivity underlies negative cognitive-affective biases.
- Based on findings across all the studies, future studies should explore how 5-HT relates to other emotional processes involved in MDD, such as reinforcement sensitivity, which would clarify whether the role of 5-HT is specific for emotion processing or may be generalised to affective cognition more broadly.
- Based on results across all the studies, future studies should investigate how cognitive-affective biases in emotion recognition and amygdala responses to emotional faces are related to mood across healthy and clinical populations.
- Future studies should look into developing and validating fMRI paradigms assessing emotion processing more reliably than currently available paradigms, as it is imperative to resolve better how 5-HT modulates emotion processing across healthy and clinical populations, including elucidating possible mechanisms of pharmacological treatments of affective disorders.

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Appendices

Paper 1: Brain serotonin transporter is associated with cognitive-affective biases in healthy individuals.

Paper 2: Functional brain responses to emotional faces after three weeks intake of selective serotonin reuptake inhibitor or placebo in healthy individuals.

Paper 3: Amygdala response to emotional faces following acute administration of psilocybin in healthy individuals.

Paper 1

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RESEARCH ARTICLE

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Brain serotonin transporter is associated with cognitive-affective biases in healthy individuals

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Abstract

Cognitive affective biases describe the tendency to process negative information or positive information over the other. These biases can be modulated by changing extracellular serotonin (5-HT) levels in the brain, for example, by pharmacologically blocking and downregulating the 5-HT transporter (5-HTT), which remediates negative affective bias. This suggests that higher levels of 5-HTT are linked to a priority of negative information over positive, but this link remains to be tested in vivo in healthy individuals. We, therefore, evaluated the association between 5-HTT levels, as measured with [^{11}C]DASB positron emission tomography (PET), and affective biases, hypothesising that higher 5-HTT levels are associated with a more negative bias. We included 98 healthy individuals with measures of [^{11}C]DASB binding potential (BP_{ND}) and affective biases using The Emotional Faces Identification Task by subtracting the per cent hit rate for happy from that of sad faces ($EFIT_{AB}$). We evaluated the association between [^{11}C]DASB BP_{ND} and $EFIT_{AB}$ in a linear latent variable model, with the latent variable ($5\text{-}HTT_{LV}$) modelled from [^{11}C]DASB BP_{ND} in the fronto-striatal and fronto-limbic networks implicated in affective cognition. We observed an inverse association between $5\text{-}HTT_{LV}$ and $EFIT_{AB}$ ($\beta = -8\%$ $EFIT_{AB}$ per unit $5\text{-}HTT_{LV}$, CI $= -14\%$ to -3%, $p = .002$). These findings show that higher 5-HTT levels are linked to a more negative bias in healthy individuals. High 5-HTT supposedly leads to high clearance of 5-HT, and thus, a negative bias could result from low extracellular 5-HT. Future studies must reveal if a similar inverse association exists in individuals with affective disorders.

KEYWORDS
attentional bias, cognition, emotions, healthy volunteers, latent variable modelling, mood disorders, positron emission tomography, serotonin, serotonin transporter

1 | INTRODUCTION

Affective cognition describes the interplay between emotion and cognition in humans. A well—documented aspect of affective cognition that is important for mental health is cognitive-affective biases, which describe the tendency to process negative information or positive information over the other (Elliott et al., 2011). Simply phrased, do you see the glass as half-full or half-empty? Affective biases distribute

along a valence continuum ranging from extremely positively biased cognition (processing positive over negative information) to extreme negatively biased cognition (processing negative over positive information). Healthy individuals generally display a neutral or a slightly positive bias (Dam et al., 2020; Korn et al., 2014), while individuals with depression generally display a negative bias (Dam et al., 2020; Miskowiak & Carvalho, 2015). During a depressive episode, individuals tend to exhibit negative biases across domains of cognitive processes, including attention, perception, reward processing and memory, reliably demonstrated using cognitive tests (Leppänen, 2006; Miskowiak & Carvalho, 2015; Roiser et al., 2012). In tests of attention to and perception of basic emotions with faces, individuals with depression consistently allocate more attention to and more accurately recognise sad faces compared to happy faces (Bourke et al., 2010; Dalili et al., 2015) and miscategorise neutral faces as sad (Leppänen et al., 2004). In memory tests, they preferentially recall more negative words than positive words (Matt et al., 1992). Although healthy nondepressed individuals generally show neutral or positive affective biases, a subset display negative biases (Pool et al., 2016; Dam et al., 2020) indicating an increased risk of developing depression (van Oostrom et al., 2013). Negative biases in how one views the self, the world and the future seem to predispose, trigger and maintain depressive episodes (Beck, 2008; Clasen et al., 2013; Hammen, 2018; Roiser et al., 2012), while positive biases may be protective against developing depressive episodes (Korn et al., 2014). Therefore, it is important to understand what might drive these affective biases to better inform preventive and treatment initiatives for depression.

It is well established that we can alter affective biases by changing the level of extracellular serotonin (5-HT) in the brain. A commonly used research method to reduce brain 5-HT is through dietary depletion of the 5-HT precursor tryptophan, which acutely elicits negative biases in healthy individuals (Firk & Markus, 2008; Robinson et al., 2012; Rogers et al., 2003) including those remitted from depression (Booij et al., 2005; Hayward et al., 2005; Munafò et al., 2006) generally without affecting mood. Conversely, boosting brain 5-HT with dietary tryptophan elicits a positive bias in healthy individuals (Meyer et al., 2003; Murphy et al., 2006). Brain 5-HT can be modulated more directly by pharmacologically targeting the 5-HT transporter (5-HTT), a protein located on 5-HT neurons in the midbrain and projection sites throughout the brain. The 5-HTT is the primary mechanism by which 5-HT is cleared from the extracellular space and hence critically involved in controlling the duration and magnitude of 5-HT signalling (Charnay & Léger, 2010). Blocking the 5-HTT pharmacologically with a *Selective Serotonin Reuptake Inhibitor* (SSRI), the first-line pharmacological treatment for depression, leads to increased extracellular 5-HT acutely in several brain areas (Bel & Artigas, 1992; Fritze et al., 2017), which is followed by gradual downregulation of 5-HTT levels over time resulting in a further increase in extracellular 5-HT (Benmansour et al., 2002). SSRIs remediate negative biases by inducing a cognitive shift towards positive information observed in healthy individuals (Browning et al., 2006; Harmer et al., 2003; Harmer et al., 2004) and individuals with depression (Bhagwagar et al., 2004), suggesting a link between 5-HTT levels and affective biases. However, whether 5-HTT levels are associated with affective biases in healthy individuals not stimulated by dietary or pharmacological supplements remain unknown.

Imaging 5-HTT levels in the living human brain is possible using positron emission tomography (PET) with the radioligand [^{11}C]DASB. Quantifying brain 5-HTT levels using PET enables us to evaluate its direct association with affective biases. As 5-HTT levels are proposed to be a surrogate marker of extracellular 5-HT concentrations (Paterson et al., 2010), associating brain 5-HTT to affective biases further allows us to infer how effective biases may relate to extracellular 5-HT concentrations. From previous studies, we know that 5-HT modulates fronto-striatal (Eshel & Roiser, 2010) and fronto-limbic circuits (Robinson et al., 2013), both key neural circuits involved in affective bias processing (Godlewska & Harmer, 2020; Roiser et al., 2012). Mapping the association between affective biases and 5-HTT levels in fronto-striatal and fronto-limbic regions will enable us to understand the underlying neurobiology of affective biases better.

This study evaluates the association between brain 5-HTT levels in fronto-striatal and fronto-limbic regions and cognitive affective in emotion recognition biases in 98 healthy individuals. We hypothesise that 5-HTT levels are inversely associated with affective biases so that higher 5-HTT in fronto-striatal and fronto-limbic is associated with a more negative bias.

2 | MATERIALS AND METHODS

2.1 | Participants and study design

We included data from 98 healthy individuals with a [11C]DASB PET scan and a measure of affective biases using *the Emotional Face Identification Task* (EFIT). Data were pooled across four studies stored in the Cimbi database at the Neurobiology Research Unit, Rigshospitalet (Knudsen et al., 2016), collected between 2010 and 2012. Part of the data have been published elsewhere (Hjordt et al., 2017; Mc Mahon et al., 2016; Mc Mahon et al., 2018). While all participants were without primary psychiatric disease at the time of testing and scanning, 19 participants were in their remitted phase of a seasonal affective disorder (SAD). These participants remitted from SAD were included to assess a more representative sample of the healthy population, which naturally also includes healthy individuals at-risk for depression. They had not received psychotropic drugs or bright light therapy as a treatment in the past year, and a trained psychiatrist had assessed them during the summer and excluded the presence of psychiatric disease at the time of cognitive testing and the PET scan (described in detail elsewhere (Mc Mahon et al., 2016)). All participants were recruited through advertisement and gave their written informed consent to participate as described in the respective study protocols, all approved by *The Ethics Committee of the capital region, Copenhagen, Denmark* (protocol numbers for H-12010091, H-22010108, H-12010085, H-42011103). Although inclusion criteria varied slightly across studies, all participants were healthy and without (1) primary psychiatric illness (besides participants remitted from SAD), (2) past or

current substance or drug abuse, and (3) neurological or severe systemic disease, based on both a general somatic and a neurological examination, together with a self-reported medical history. Educational attainment scores for all participants were measured using a five-point Likert scale from 1 (no vocational degree) to 5 (>4 years of higher learning at the university level). We assessed their intelligence quotient (IQ) with the *Reynolds Intellectual Screening Test* (Reynolds & Kamphaus, 2011). Depression scores for all participants were measured with the major depression inventory (MDI) with a range from 0 to 50, where >21 indicates a depressed mood (Bech et al., 2015). Trait neuroticism was measured using the Danish version of the NEO Personality Inventory-Revised (NEO-PI-R) (Costa Jr. & McCrae, 2002), reported as nonstandardised raw scores. A saliva sample was collected for genotyping of the serotonin transporter-linked polymorphic region (5-HTTLPR) using a previously described method (Fisher et al., 2015).

2.2 | Outcome measures

2.2.1 | The emotional faces identification task

EFIT is the most widely used cognitive test to assess affective biases in emotion recognition. The test validly measures the ability to correctly identify facial emotions, including happiness, anger, fear, sadness, disgust, and neutrality (Hjordt et al., 2017; Young et al., 1997). A total of 172 facial images with emotional expressions are presented on a black computer screen at different intensities, from minimum to maximum emotional valence (morphed with neutral facial expressions). Subjects are instructed to identify the presented facial emotion with a mouse as fast and accurate as possible. The primary outcomes are the hit rate for each emotion, calculated as the percentage of correctly identified emotions. The affective bias is calculated by subtracting the per cent hit rate for sad faces from the per cent hit rate for happy faces so that a score above zero indicates a positive affective bias while a score less than zero indicates a negative affective bias. This is a standard method to calculate affective biases(Bland et al., 2016; Dam et al., 2019) found to be particularly sensitive for depression (Dam et al., 2020), which is related to 5-HTT functioning (Gryglewski et al., 2014). As we were only interested in affective biases, we did not attend to hit rates of other emotions than happy and sad.

2.2.2 | Neuroimaging

Magnetic resonance imaging

A high-resolution T1-weighted structural brain scan was acquired for each participant and used to segment and delineate brain regions. Participants were scanned on one of two magnetic resonance imaging (MRI) scanners, a Siemens Magnetom Trio 3 T scanner ($n = 38$) or a Siemens Verio 3T scanner (Siemens, Erlangen, Germany) ($n = 60$).

[^{11}C]DASB PET imaging

Using [^{11}C]DASB to measure 5-HTT binding is advantageous as it binds to 5-HTT with high affinity and high selectivity, with a good ratio of specific binding relative to free and nonspecific binding while showing reliable measurement in multiple brain regions (Wilson et al., 2002). We quantified [^{11}C]DASB as the ratio at the equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in the brain tissue (BP_{ND}) (Ginovart et al., 2001). All participants were scanned using a Siemens ECAT high-resolution research tomography (HRRT) scanner operating in 3D-acquisition mode with an approximate in-plane resolution of 2 mm (Olesen et al., 2009). Following a transmission scan of 6 min, an intravenous bolus of [^{11}C]DASB was injected over 20 s. Post-injection, the imaging protocol consisted of a dynamic 90-min emission scan acquired over 36 frames (6×10 s, 3×20 s, 6×30 s, 5×60 s, 5×120 s, 8×300 s, 3×600 s). The dynamic PET images were reconstructed using an iterative OP-OSEM 3D method with resolution modelling (10 iterations and 16 subsets).

Brain image analysis and outcome parameters

Preprocessing the PET data at the subject level included an in-scan automatic image registration algorithm to determine motion and realignment (Woods et al., 1992). PET scans were smoothed using a 10-mm within-frame Gaussian filter before alignment. Subsequently, we estimated rigid translation/rotation parameters aligning each PET frame to a single-PET frame with sufficient structural information using the scaled least-squares cost function (frame 26: 20–25 min post-injection). Nonfiltered PET images were resliced using these parameters. Co-registration of high-resolution MR and PET images was performed using SPM, based on the mean of frames 10–26, corresponding to a flow-weighted image. Accurate co-registration was confirmed by visual inspection across all planes.

From the participants' structural MRI scans, brain regions were automatically delineated using Pvelab (Svarer et al., 2005). Time-activity curves were determined, reflecting the mean of grey-matter voxels within each region. We did not apply partial volume correction, as the participants were generally young (mean age: 25.5 ± 6.2 years, range: 18–45 years). Kinetic modelling of regional time-activity curves determined our primary outcome, the regional BP_{ND}, in PMOD (Zurich, Switzerland), using the multilinear reference tissue model 2, a modified reference tissue model, validated for quantifying [^{11}C]DASB (Ichise et al., 2016). Here, a fixed k2′ was estimated for each individual using putamen, caudate, and thalamus as the high-binding regions, while cerebellar grey matter without vermis was used as the reference region.

2.3 | Statistical analyses

We examined the associations between 5-HTT BP_{ND} and affective biases in emotion recognition assessed with the EFIT using a linear latent variable model (LVM) with the lava-package version 1.6.10 in R version 4.1.2 (see Appendix S1 for further detail) (Holst & Budtz-Jørgensen, 2012). The LVM enables us to model the large correlation in 5-HTT BP_{ND} across brain regions (Erritzoe et al., 2010) into a single latent variable, 5-HTT_{LV}. Here, we modelled 5-HTT_{LV} from brain

regions with a good signal-to-noise ratio involved in affective bias processing, including regions in the fronto-striatal circuit (Eshel & Roiser, 2010) (i.e., frontal cortex, putamen, and caudate) and fronto-limbic circuit (Robinson et al., 2013) (i.e., frontal cortex, anterior cingulate cortex [ACC], and amygdala). The frontal cortex was arbitrarily selected as a reference region for the LVM, so 5-HTT_{LV} values were expressed in 5-HTT frontal cortex units. To display a more meaningful effect of 5-HTT_{LV} on the affective bias, we multiplied the regional 5-HTT BP_{ND} outcomes by 10 prior to modelling 5-HTT_{LV}. The effect of 5-HTT_{LV} on the affective bias is thus reported as a 0.1 increase in 5-HTT_{LV} throughout the manuscript. Within the LVM, each regional [11C]DASB BP_{ND} was separately adjusted for sex, age, BMI, IQ, trait neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [^{11}C]DASB, MR-scanner type, the 5-HTTLPR (i.e., alleles LL, SS, and SL) and group (i.e., healthy individuals with or without remitted SAD), which are variables that may influence 5-HTT BP_{ND} (Mc Mahon et al., 2016; Spies et al., 2015; Tuominen et al., 2017). Although the brain-derived neurotrophic factor has been found to impact 5-HTT BP_{ND} (Fisher et al., 2017) it was not included as a covariate as the data was not available for all participants. All one-parameter extensions of the LVM were considered based on score tests of improvement in model fit. If the smallest false discovery rate (FDR)-adjusted *p*-value was below 0.05, the corresponding parameter was added to the model. The procedure was repeated until no additional parameter was found relevant. Possible extensions included additional covariance parameters but not nonlinear or interaction effects for the mean structure. To visualise the association between 5-HTT_{LV} and EFIT, we computed the estimated 5-HTT_{LV} value for each individual based on their regional 5-HTT BP_{ND}. In addition to the LVM, we used a post hoc region-specific statistical approach to verify our LVM results, namely univariate multiple linear regression models, to test the associations between affective biases and 5-HTT BP_{ND} in the five brain regions of interest together with 5-HTT in the midbrain and whole brain (i.e., 5-HTT BP_{ND} in occipital-, orbitofrontal-, parietal-, and sensory-motor cortex, anterior-, and posterior cingulate cortex, entorhinal cortex, middle inferior frontal-, and middle inferior temporal gyri, superior frontal- and superior temporal gyri, insula, caudate, putamen, hippocampus, amygdala, and thalamus). These analyses are presented in Table 2.

To examine whether a potential association between 5-HTT levels and affective biases was group dependent, and thus, whether pooling the two groups could be justified, we carried out group-wise sensitivity analyses. For healthy individuals without a history of depression ($n = 79$), we evaluated the association between 5-HTT_{LV} BP_{ND} and affective biases using the same LVM as in the primary analysis. For healthy individuals in the remitted period of their SAD, due to the smaller group size ($n = 19$) and lack of convergence in the LVM, we evaluate the association between frontal cortex 5-HTT BP_{ND}, our reference region, and affective biases in a linear regression model. All additional models included the same covariates as in the LVM, except for the MR scanner in the model for healthy individuals in the remitted period of their SAD, as all were scanned on the same MR. Model assumptions (e.g., normality of residuals, QQ-plots and influential cases) were considered and showed no evidence of model violations.

P-values <.05 (two-sided) were considered statistically significant. For the post hoc analyses, we also presented FDR-corrected values. Results are reported with parameter estimates (e.g., *r* for the Pearson correlation coefficient) and a 95% confidence interval (CI) for the estimates.

TABLE 1 Descriptive information for the 98 healthy individuals in the study

Categorical variables	Number	Percentage
Healthy Individuals with/without SAD	19/79	19%/81%
Female/male	74/24	76%/24%
5-HTTLPR genotype LL/LS /S	35/37/26	36%/38%/26%
Continuous variables	**Mean ± SD**	**Median [Min ; Max]**
Age in years	25.5 ± 6.2	22.9 [18.4; 45.3]
IQ	107.7 ± 7.6	108 [93; 126]
Body mass index	23.3 ± 3.1	22.9 [17.7; 32.9]
Neuroticism	82.5 ± 20.7	82.5 [38; 148]
MDI	5.3 ± 3.2	5 [0; 15]
Daylight minutes	778 ± 231	808 [428; 1052]
Injected DASB mass pr kg	0.027 ± 0.031	0.013 [0.004; 0.158]
DASB BPND in brain regions	**Mean ± SD**	**Median [Min; Max]**
Frontal cortex BPND	0.41 ± 0.07	0.41 [0.23; 0.58]
ACC BPND	0.63 ± 0.09	0.61 [0.43; 0.95]
Caudate BPND	1.92 ± 0.31	1.92 [1.33; 3.07]
Putamen BPND	2.26 ± 0.33	2.22 [1.63; 3.43]
Amygdala BPND	1.86 ± 0.31	1.82 [1.28; 3.04]
Midbrain BPND	2.09 ± 0.26	2.07 [1.33; 2.71]
Whole-brain BPND	0.51 ± 0.07	0.50 [0.34; 0.73]
The emotional face identification task	**Mean ± SD**	**Median [Min; Max]**
Happy face, % hit rate	85% ± 8%	88% [58%; 100%]
Sad face, % hit rate	72% ± 13%	75% [32%; 94%]
Affective bias	13% ± 15%	13% [−22%; 46%]

Note: Outcomes: Affective bias is calculated as happy minus sad hit rate in the emotional face identification task (EFIT) and DASB BPND is displayed as raw data (not multiplied by 10).
Abbreviations: ACC, anterior cingulate cortex; BPND, binding potential nondisplaceable, IQ, intelligence quotient; MDI, major depression inventory; SAD, seasonal affective disorder.

3 | RESULTS

3.1 | Baseline characteristics

The study population's demographics, psychometrics, PET parameters, and cognitive test outcomes are reported in Table 1. MDI scores confirmed the absence of depression by cut-off > 21 in the study population (Bech et al., 2015) (score range = 0–15), and the mean IQ was slightly higher compared to the average of 100 in the general population (mean IQ ± SD = 107.7 ± 7.6). The time between [^{11}C]DASB PET scan and EFIT was a maximum of 1 week for 81 participants and up to 1 month for 14 participants and up to 147 days for the remaining three participants (median days between PET scan and EFIT for all participants = 0 days).

3.2 | 5-HTT BP_{ND} intercorrelation across brain regions

Figure 1 visualises *the distribution of 5-HTT BP_{ND} in a representative healthy individual* from our sample. High 5-HTT BP_{ND} intercorrelation between brain regions was confirmed by correlation analyses (i.e., Pearson's *r*: frontal cortex and ACC = 0.9, caudate = 0.5, putamen = 0.5, amygdala = 0.5; ACC and caudate = 0.5, putamen = 0.5, amygdala = 0.5; caudate and putamen = 0.7, amygdala = 0.5), and the midbrain 5-HTT BP_{ND} was also highly correlated with the regions of interest (i.e., *r*: frontal cortex = 0.5, caudate = 0.3, putamen = 0.4, ACC = 0.5, amygdala = 0.4) and likewise for whole-brain 5-HTT BP_{ND} (i.e., *r*: with frontal cortex = 0.9, ACC = 0.8, caudate = 0.6, putamen = 0.6, amygdala = 0.6).

3.3 | The association between 5-HTT BP_{ND} and cognitive-affective biases

An LVM structure of 5-HTT BP_{ND} was supported by the strong loading of regional 5-HTT BP_{ND} onto the latent variable, 5-HTT_{LV} (loadings from all regions: point estimate range = 1.6–3.7, $p < .001$), as presented in Figure 2. Tests of improvement in model fit with a FDR of $p < .05$ supported adding one covariance between caudate and putamen, which had no impact on the estimate or *p*-value for the association between 5-HTT_{LV} and affective bias.

Within our LVM, 5-HTT_{LV} BP_{ND} was inversely associated with affective bias in emotion recognition as measured with EFIT; each 0.1 increase in 5-HTT_{LV} BP_{ND} was associated with an 8% decrease in affective bias, that is, a more negative bias (CI = −14% to −3%, $p = .002$), see Figure 3. In line with the results from the LVM, univariate region-specific analyses showed a significant inverse association between affective biases and 5-HTT BP_{ND} across the fronto-striatal and fronto-limbic regions of interests (i.e., frontal cortex, caudate, putamen, and ACC), and as well as 5-HTT BP_{ND} in the midbrain and the whole brain, whose FDR adjusted *p*-values were below 5% except

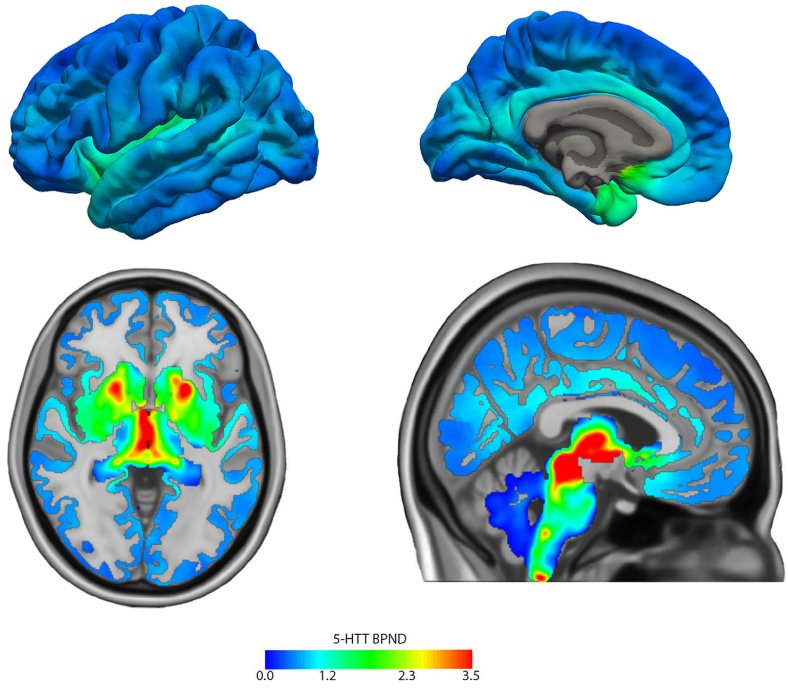

FIGURE 1 The distribution of 5-HTT BPND in a representative healthy individual from the study sample. Cortical values are presented on the standard FreeSurfer surface (fsaverage, left hemisphere; lateral view, upper left and medial view, upper right) and subcortical values are presented in the standard MNI152 space (transverse view, bottom left, and sagittal view, bottom right)

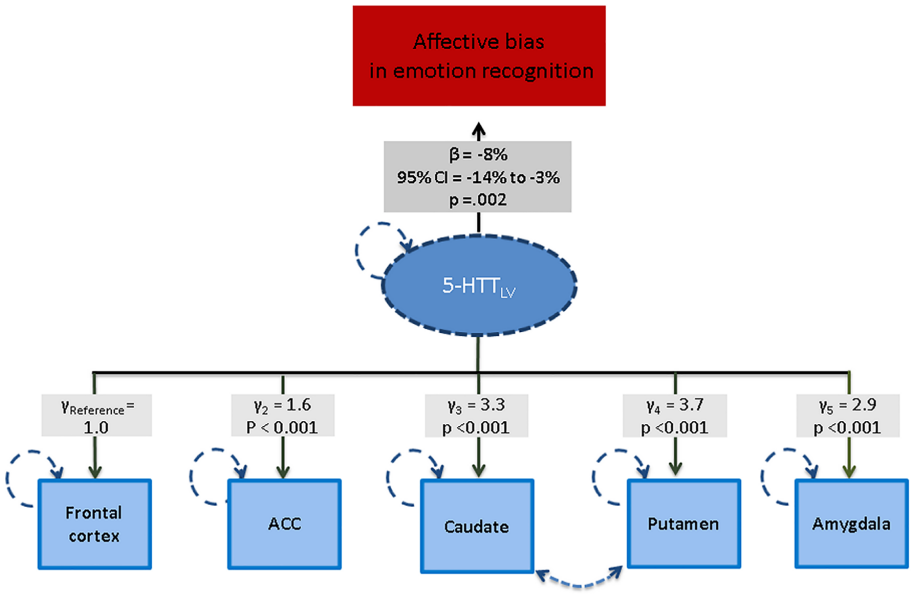

FIGURE 2 An illustration of the latent variable model (LVM). The red box represents the dependent variable affective bias in emotion recognition. The five brain regions of interest in the bottom blue boxes represents measured regional [11C]DASB BPND values used to define the latent variable (5-HTTLV), which is represented in the blue oval. The effect of 5-HTTLV on affective bias is displayed as increase of 0.1 in 5-HTTLV. The hatched lines between caudate and putamen illustrates partial correlation included as covariance parameter. Circular blue hatched lines reflect variables estimated with error. Each regional [11C]DASB BPND was separately adjusted for sex, age, BMI, IQ, trait neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [11C]DASB, MR-scanner type, the 5-HTTLPR (i.e., alleles LL, SS and SL) and group (i.e. healthy individuals with or without remitted SAD) (not illustrated). β, point estimate for the regression coefficient of emotional face identification task (EFIT). γ, point estimate for the loadings onto 5-HTTLV. ACC, anterior cingulate cortex

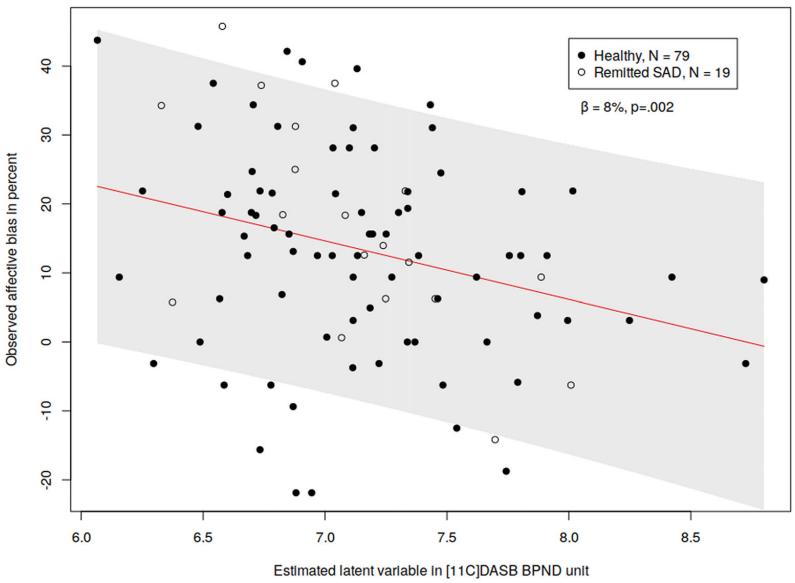

FIGURE 3 A plot of the estimated latent variable in units of [11C]DASB PET (i.e., 5-HTTLV) by observed affective bias in per cent measured with the emotional identification task. The effect of 5-HTTLV on affective bias is displayed as an increase of 0.1 in 5-HTTLV. The red line corresponds to the estimated association between 5-HTTLV and affective biases (i.e., beta coefficient = 8%) adjusted for covariates including sex, age, BMI, IQ, neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [11C]DASB, MR-scanner type, the 5-HTTLPR and group (i.e., healthy individuals with or without a seasonal affective disorder (SAD)

TABLE 2 The association between serotonin transporter binding and affective bias using univariate multiple linear regression models with the covariates sex, age, BMI, IQ, trait neuroticism, number of daylight minutes at PET-scan, weight-adjusted injected masse of [11C]DASB, MR-scanner type, the 5-HTTLPR (i.e., alleles LL, SS, and SL) and group (i.e., healthy individuals with or without remitted SAD)

Affective bias in emotion recognition						
Brain region	**Estimate (%)**	**SE B (%)**	**P**	**FDR**	**95% CI**	
Frontal cortex	−6.7	2.4	0.007	0.031	−11.6	−1.9
ACC	−4.1	1.6	0.013	0.031	−7.3	−0.9
Caudate	−1.1	0.5	0.047	0.054	−2.1	0.0
Putamen	−1.1	0.5	0.028	0.043	−2.1	−0.1
Amygdala	−0.1	0.5	0.874	0.874	−1.1	1.1
Midbrain	−1.4	6.4	0.031	0.043	−2.7	−0.1
Whole-brain	−6.1	2.3	0.012	0.031	−10.5	−1.3

Note: Affective bias in emoting recognition as measured with the emotional face identification task (EFIT) ($n = 98$) is reported in percent. The effect of regional 5-HTT BPND on affective bias is displayed as increase of 0.1 in 5-HTT BPND. Estimate is the unstandardised beta.
Abbreviations: ACC, anterior cingulate cortex, FDR, 5% false discovery rate correction; SE B, standard error for unstandardised beta; P, unadjusted significance level; 95% CI, 95% confidence interval.

for the caudate. However, we did not find a significant association between affective biases and 5-HTT BP_{ND} in the amygdala (see Table 2 for region-specific posthoc analyses and Figure S1 for visualisations).

For healthy individuals without a history of depression, the sensitivity analysis showed a significant inverse association between $5\text{-}HTT_{LV}$ and affective biases ($n = 79$, $\beta = -7\%$, CI $= -13\%$ to -6%, $p = .03$). Likewise, for healthy individuals with a remitted SAD, the sensitivity analysis showed a significant inverse association between frontal cortex 5-HTT BP_{ND} and affective biases ($n = 19$, $\beta = -16\%$, CI $= -22\%$ to -11%, $p < .001$). The estimated associations in both groups had a similar statistical significance level indicating that the result from the primary analysis was not an artefact of pooling the groups.

4 | DISCUSSION

This is the first study to investigate the association between in vivo brain 5-HTT levels and affective biases in a large sample of healthy individuals. Consistent with our hypothesis, we find that individuals with higher 5-HTT levels, as indexed by PET [^{11}C]DASB BP_{ND}, are relatively better at recognising sad faces than happy faces: For every 0.1 increase in [^{11}C]DASB BP_{ND} there was an 8% more negative bias. Consistent with previous studies, the healthy individuals in our study distributed along the continuum of affective biases, ranging from positive to negative biases (Dam et al., 2020). Critically, we demonstrate that this affective bias continuum (positive to negative) can be coupled to in vivo brain 5-HTT levels, a key marker of 5-HT brain signalling.

Only one previous study has investigated the association between 5-HTT levels, and affective bias carried out in a sample of 20 medication-free individuals with depression and 20 matched healthy individuals (Meyer et al., 2004). In line with our results, the study found that higher 5-HTT levels were correlated with more negatively biassed self-reported attitudes in individuals with current depression; however, no association was found in healthy individuals (Meyer et al., 2004). The latter negative finding could be ascribed to lack of power as the study's sample size was considerably smaller than ours and thus perhaps not big enough to detect an association. Furthermore, the study used a self-reported measure of negatively biassed attitudes, exclusively capturing the negative side of the affective bias continuum. Measuring the full continuum of affective biases represents a major strength of our study of healthy individuals, especially given previous observations that healthy individuals display an average positive affective bias (Dam et al., 2020). A further strength of our study is the use of a standardised cognitive test which is not subject to response bias to the same extent as self-reported affective bias. We separately examined healthy individuals without a history of depression and healthy individuals in the remitted phase of SAD. We found a significant inverse association between 5-HTT levels and affective biases in both groups. Notably, in individuals in the remitted phase of SAD, the effect of 5-HTT on affective biases was relatively larger and with minimal overlap in confidence interval across the groups. Although different statistical models were used, this could indicate greater importance of 5-HT on cognitive-affective processing in individuals with a vulnerability to depression, a finding consistent with the hypothesis that 5-HT is involved in vulnerability to depression (Frokjaer et al., 2009). Whether a stronger association between 5-HTT and affective biases is a risk-marker for depression and whether it ultimately influences the severity of the depressive state should be investigated in longitudinal clinical studies. Taken together, our results of an inverse association between 5-HTT levels and affective biases apply to healthy populations with and without a history of depression, and since a similar association was found in a depressed population (Meyer et al., 2004), the link between 5-HTT and affective biases seems to be independent of psychiatric risk profile and current mood.

It has been proposed that 5-HTT, here indexed with [^{11}C]DASB BP_{ND}, could serve as a surrogate marker of extracellular 5-HT concentrations (Paterson et al., 2010), but it remains debated how 5-HTT and 5-HT are related. One model proposes that low 5-HT

concentrations lead to 5-HTT downregulation (Milak et al., 2005; Rothman et al., 2003), while high 5-HT concentrations restrict 5-HTT internalisation leading to higher 5-HTT density (Steiner et al., 2008). An opposing model suggests that high 5-HTT levels lead to increased clearance of extracellular 5-HT (Mathews et al., 2004), and that high 5-HTT levels may therefore be a marker of low extracellular 5-HT concentrations. This remains open to debate, but some evidence favours the latter model as boosting 5-HT concentrations decreases 5-HTT availability in the brain of rats (Fritze et al., 2017; Lundquist et al., 2007), cats (Ginovart et al., 2003) and nonhuman primates (Lundquist et al., 2007; Yamamoto et al., 2007). Accordingly, we find that higher 5-HTT levels are related to a more negative bias in emotion recognition, consistent with the hypothesis that lower 5-HT concentration is involved in increased attention to and processing of threat-related stimuli and negative information (Godlewska & Harmer, 2020). In further support of this hypothesis, behavioural studies report that depleting 5-HT leads to negative biases (Booij et al., 2005; Firk & Markus, 2008; Hayward et al., 2005; Munafò et al., 2006; Robinson et al., 2012; Rogers et al., 2003), while boosting the 5-HT lead to positive biases in humans (Bhagwagar et al., 2004; Browning et al., 2006; Harmer et al., 2003; Murphy et al., 2006), and rodents (Bari et al., 2010; Stuart et al., 2013). Another model of how 5-HTT is regulated proposes that 5-HTT levels may reflect an early-life wiring of 5-HT brain architecture more broadly, as 5-HTT sits on both serotonergic neurons and projections throughout the brain (Gaspar et al., 2003). This is consistent with findings of an association between early-life wiring of 5-HT and later affective cognition and risk of depression (Ansorge et al., 2004). Importantly, these different models of how 5-HTT is regulated may not exclude one another but complement each other. Future mechanistic studies will help elucidate the dynamics of how 5-HTT and 5-HT interact in the human brain. While much is still unresolved, we hope that understanding how different aspects of 5-HT signalling are involved in affective cognition may ultimately lead to novel preventive initiatives and treatment strategies urgently needed for individuals with depression or at high risk of depression.

4.1 | Methodological considerations

This is the first PET study to examine the association between an imaging marker of 5-HT signalling and affective biases in healthy individuals in a large sample. Considering our sample, a small proportion had a history of depression ($n = 19$) who were included to examine a more representative sample of the general healthy population with natural variations in vulnerability to depression. Sensitivity analyses revealed that the inverse relation between 5-HTT and affective biases was present in healthy individuals with and without a history of depression, which we believe justifies pooling the groups. We did not have an equal sex distribution in our sample, as 74% were women; however, the sex distribution is comparable to that of depressed populations where the majority are women (Dam et al., 2020), allowing for comparing our results to future studies exploring affective biases in depression. This study was conducted with a North European sample of younger age, limiting our results' generalisability to other populations. It remains to be investigated whether our results can be replicated in more diverse populations.

5 | CONCLUSION

In healthy individuals, we found that 5-HTT levels in the fronto-striatal and fronto-limbic brain regions are inversely associated with affective biases in emotion recognition. This finding bolsters the previously reported association between 5-HT and affective biases using a key in vivo marker of serotonin signalling. Although the relationship between 5-HTT levels and 5-HT signalling remains debated, our finding could reflect that negative biases result from excessive clearance of extracellular 5-HT. Whether excessive 5-HT clearance underlies the negative bias typically observed in individuals with affective disorders should be investigated in large depressed populations.

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CONFLICT OF INTEREST

Vibe G. Frøkjær declares that she serves as a lecturer for Sage Therapeutics and Lundbeck Pharma A/S. Gitte M. Knudsen served as a consultant and lecturer for Sage Therapeutics/Biogen and Sanos. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Cimbi database. Restrictions apply to the availability of these data, which were used under license for this study. Data are available in the Cimbi database managed by Peter S. Jensen with the permission of Cimbi Steering Group.

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Paper 2

Functional brain responses to emotional faces after three weeks intake of selective serotonin reuptake inhibitor or placebo in healthy individuals

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Conflicts of Interest

SA, AJ, CL do not have any conflicts of interest, including financial, consultant, institutional or other relationships, which could lead to bias or conflict of interest. GMK has received honoraria as a speaker for Sage and for H. Lundbeck and as advisor for Sanos. BJS consulted for Cambridge Cognition.

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Abstract

Acute and few days intake of selective serotonin reuptake inhibitors (SSRIs) modulate threat-related amygdala responses in healthy individuals. However, it is less clear whether longer-term SSRI intake also modulates threat-related amygdala responses. In a randomised, double-blind, placebo-controlled study of 64 healthy individuals (41 females), we examined the effect of 3-5 weeks intake of the SSRI escitalopram on whole-brain, region-wise responses to angry, fearful and neutral faces using blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI). A priori, we hypothesised that SSRI would attenuate amygdala responses to angry and fearful faces but not neutral ones. We also explored whether SSRI modulates the correlations between amygdala responses to emotional faces and negative mood states, as assessed with the Profile of Mood States questionnaire. We found that compared to placebo, 3-5 weeks of escitalopram intake did not significantly affect the processing of angry, fearful, or neutral faces in the amygdala (|Cohen's d| < 0.2, $P_{FWER} = 1.00$). Whole-brain, region-wise analyses revealed significant differences in frontal (|Cohen's d| < 0.6, $P_{FWER} < .01$) and occipital regions (|Cohen's d| < 0.5, $P_{FWER} < .01$). Escitalopram did not modulate correlations between amygdala responses to emotional faces and negative mood states ($P_{FWER} = 0.19$). In conclusion, we do not find evidence that a 3-5 week intake of escitalopram is associated with amygdala activity in response to emotional stimuli in healthy individuals. Rather, our results suggest that SSRI modulated activity in cortical regions during emotion processing. Taken together, our findings indicate that 3-5 week escitalopram intake particularly affects cortical processing of emotional stimuli, which may relate to the associated therapeutic effects.

1. Introduction

Emotional cognition in humans describes the cognitive processing of emotional information in the environment. It is important for successfully navigating a social and interpersonal world, e.g., accurately perceiving other people's emotions and intentions based on facial expressions.[1,2] Facial expressions represent a universal and rich source of emotional information,[3,4] with threat-related expressions, e.g., angry or fearful facial expressions, being important cross-cultural emotional cues that guide human behaviour, e.g., avoiding danger.[1,2] The amygdala is a critical brain region for detecting salience and social relevance of emotional stimuli,[5–7] especially for threat-related salience.[2,8–11] There is evidence to suggest that some activity in the amygdala in response to threat is good and adaptive,[12–14] but that hyperactive amygdala reactivity to threat is maladaptive and involved in Major Depressive Disorder (MDD).[15–18] In addition to the amygdala, meta-analyses have established a functional atlas of emotional face processing, including visual, limbic, temporal-parietal, and prefrontal areas, the putamen, and the cerebellum.[6] These regions have, in some studies,[19] but not all,[20] been reported to be activated abnormally during emotional face processing in patients with MDD.

Serotonin (5-HT) is a highly relevant neural signalling mechanism that shapes various behavioural phenotypes.[21,22] Serotonergic projections to areas including the amygdala, striatum, and prefrontal cortex are critical for social and emotional cognition.[23–27] The 5-HT transporter (5-HTT) is a protein critically involved in controlling the duration and magnitude of 5-HT signalling as it clears 5-HT from the extracellular space.[28] The 5-HTT seems particularly important for regulating emotional processing across healthy populations[29–31] and individuals with depression.[32] The 5-HTT is the main target of selective serotonin reuptake inhibitors (SSRIs), a group of drugs prescribed as the first-line pharmacological treatment of MDD. Clinically, SSRIs are administered to attenuate depressive symptomology, such as mood

disturbances, with clinical effects typically becoming evident after two weeks or more.[33] It has been suggested that the clinical effects are mediated by effects on emotional cognition, for example, by attenuating amygdala response particularly to threat,[34,35] although this was not supported in a recent study in MDD.[36] It remains unclear whether several weeks of SSRI intake modulates threat-related responses in healthy individuals' amygdala and other brain regions involved in emotion processing and whether it relates to mood states.

Combining SSRI administration with functional Magnetic Resonance Imaging (fMRI) is a strategy to investigate serotonergic modulation of relevant brain function, e.g., neural correlates of emotional processing. fMRI studies of healthy individuals evaluating the effects of acute (i.e., within hours) and short-term (i.e., 7-10 days) intake of SSRIs compared to placebo have generally reported an altered amygdala response to emotional stimuli,[37–40] with the majority reporting reduced amygdala response to threat.[41–46] Effects of short-term SSRI intake on emotional face processing have also been observed in other brain regions, e.g., the frontal cortex.[47] Studying the effects of longer-term intake of SSRI better represents the pharmacological treatment of MDD. To date, only two studies have investigated how SSRI intake modulates emotional processing in the amygdala in healthy individuals over a clinically relevant time, i.e., more than two weeks. One study found decreased amygdala response to threat was associated with higher escitalopram urine concentrations after 18 days of SSRI intake.[48] The other study, from our lab, reported a numerically decreased amygdala response to threat, but not statistically significant, following 21 days of SSRI intake compared to placebo.[25] However, change in brain 5-HT 4 receptor levels, a putative marker of brain 5-HT tonus, was associated with a change in the amygdala response to threat within the SSRI group. Both studies were relatively small and sex-specific, including 13 females and 32 males, respectively. Examining the effects of SSRI intake on changes in emotion processing in the brain over a clinically relevant time frame would benefit from including a relatively larger

sample with equal sex distribution, as MDD is prevalent in both males and females, and they show different clinical responses to SSRIs.[49] It further remains to be investigated how threat-related amygdala responses after weeks of SSRI intake correlate with mood states.

Here, we evaluated the effects of 3-5 weeks intake of the most selective SSRI, escitalopram, versus placebo on amygdala response to angry, fearful, and neutral faces using fMRI in a double-blind study in 64 healthy individuals. We hypothesised that the amygdala response to angry and fearful facial expressions would be lower but unchanged to neutral faces in response to SSRI compared to placebo. Additionally, we evaluated the effects of escitalopram on regional whole-brain responses to facial expressions. Finally, we explored whether 3-5 weeks intake of SSRI moderated the correlation between amygdala responses to facial expressions and negative mood states in healthy controls.

2. Methods and material

2.1. Ethics

Before obtaining written informed consent, participants were informed about the study, including potential side effects and risks. The study was approved by the Danish ethics committee for the capital region of Copenhagen (journal ID: H-18038352, with amendments 71579, 73632 and 78565) and was preregistered at ClinicalTrials.gov (identifier: NCT04239339).

2.2. Participants

In total, 64 healthy volunteers (mean age in years (SD) [range]: 25 (6) [19–45], 41 females) completed the study. The volunteers were recruited from a list of individuals who expressed interest in participating in brain research. From an initial group of 73 included volunteers who

started the intervention, five participants dropped out due to self-reported side effects (of which four participants received SSRI and one received placebo), and two participants did not complete the follow-up assessment due to sickness or pain during MRI-scanning. One participant was excluded following an incidental finding of severe brain atrophy on MRI (see **Figure 1** for a flow diagram of the inclusion of research volunteers and reasons for missing data). After participants gave written informed consent to participate in our study, they underwent screening for somatic illness including a medical examination, blood screening for somatic disease, an ECG, and the presence of psychiatric conditions. The exclusion criteria were: 1) Current or former primary psychiatric disorder as classified in DSM-V or WHO ICD-11, 2) Current or former neurological disease or severe somatic disease, 3) Head injury or concussion resulting in loss of consciousness for more than two min., 4) Current use of psychoactive medication, 5) Drug use other than tobacco and alcohol within the last 30 days, 6) Alcohol or drug abuse, 7) Use of cannabis more than 50 times, 8) Use of illegal psychoactive drugs more than ten times for each drug, 9) Use of any drugs likely to influence the test results, 10) Nicotine addiction, 11) Allergy to the ingredients in the administered drug, 12) Abnormal ECG, such as prolonged QT syndrome, 13) Dizziness when changing from supine to upright position (e.g. postural orthostatic tachycardia syndrome), 14) Mild hypotension (blood pressure below 100/70 mmHg) or hypertension (blood pressure above 140/90 mmHg), 15) Contraindications for MRI such as a pacemaker or soft-tissue metal, 16) Pregnancy or breast-feeding, 17) Current or past learning disability, 18) Non-fluent in Danish, 19) Pronounced visual or auditory impairments, 20) Severe physical impairments affecting eyesight or motor performance.

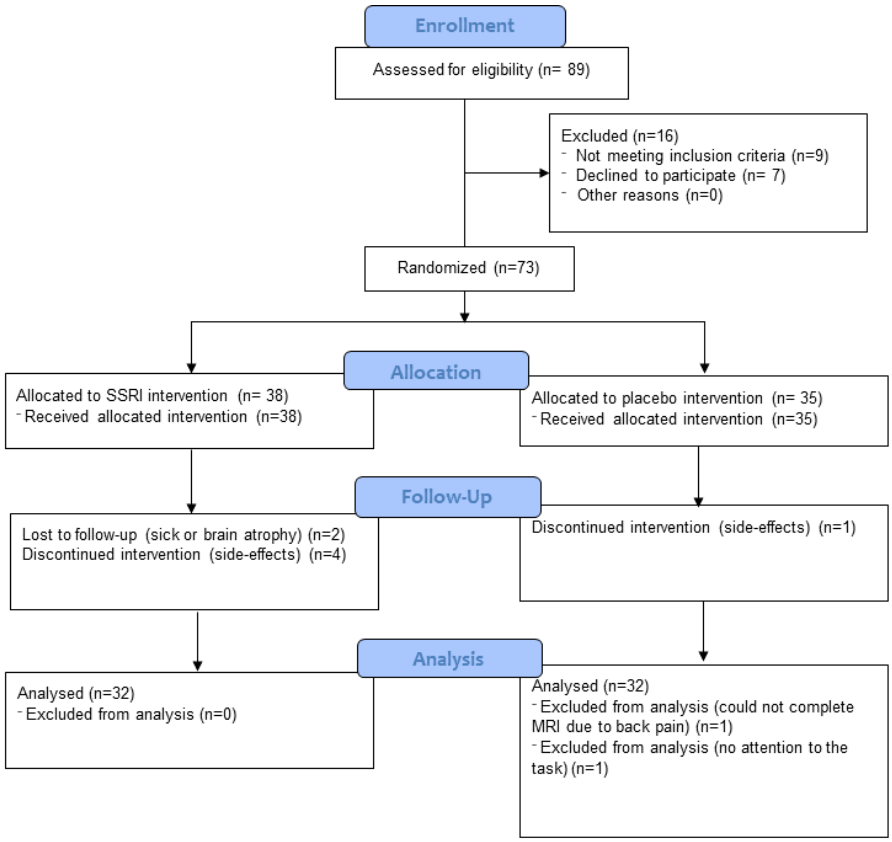

Figure 1. Consort Flow Diagram presenting number of research volunteers enrolled, allocated to intervention, completed the study to follow-up and were included in the data analysis

2.3. Experimental design and drug administration

In a double-blinded design, participants were semi-randomised to receive a clinically relevant dose of escitalopram (20 mg daily in capsules of 10 mg)[50] or placebo in identical capsules for 3-5 weeks, representing a clinically relevant time period of SSRI intake.[33] Capsules were manufactured and distributed by the Capital Region Pharmacy. A staff member who was not in contact with the participants nor participated in data collection or analysed data randomised participants stratified with respect to sex, age, and IQ. Participants received oral and written instructions for taking escitalopram, including possible side effects. They were instructed to take one capsule (10 mg escitalopram in the active group) each morning for three days and two capsules (20 mg escitalopram in the active group) daily on the fourth day and until the last day

of examination when the participants were scanned. Participants were asked to fill in a daily medication logbook, recording the time and number of capsules taken. This logbook served as a daily reminder to participants to take the capsules and provided additional information about participant compliance. To objectively verify intervention compliance, a blood sample was taken halfway through the intervention period and again at follow-up, typically in the morning before MRI. Further, participants' capsule containers were inspected at each visit and their medication logbook was verified at the follow-up visit. On blood sampling days, participants were instructed to take the capsule after the blood sample. During the intervention period, side effects were monitored weekly. If participants experienced unusual side effects or other complications during the study period, they could contact the project physician. Following the intervention period (3-5 weeks), participants completed an MRI scan session. Prior to MRI scanning, participants completed self-report questionnaires to evaluate their psychological state. Participants and investigators involved in data acquisition and analysis were blinded to intervention type until completion of data analysis. Once participants had completed the study, we assessed efficacy of blinding by asking the participants whether they believed they had received active drug or placebo.

2.4. Study measures

2.4.1. Psychometrics

Before participants commenced the intervention, we measured their intelligence quotient (IQ) using the Reynolds Intellectual Screening Test,[51] body mass index (BMI), and administered questionnaires to assess levels of depressive symptoms using the Beck's Depression Inventory II (BDI-II) (range: 0–63, where a score above 20 indicates a dysphoric or depressed mood),[52] levels of state anxiety using the State-Trait Anxiety Inventory (STAI)[53,54] (range: 0–80, higher levels indicating more anxiety, but no cut-off adapted to indicate severe clinical anxiety) and

stress level using the Cohen's Perceived Stress Scale (PSS) (range: 0–40, higher levels indicating more stress, but no cut-off adapted to indicate severe clinical stress).[55]

Both at baseline and follow-up (i.e., on the same day as MRI scanning), and typically in the morning, we assessed participants' level of negative mood states using the validated self-report questionnaire, the Profile of Mood States (POMS).[56,57] The questionnaire was developed to measure transient mood states from 65 items using a 5-point Likert scale ranging from 0 (not at all) to 4 (extremely), from which a total composite score indicates the level of negative mood states (i.e., tension/anxiety, depression/dejection, anger/hostility, fatigue/inertia, and confusion/bewilderment versus vigour/activity). Total score ranges from -32 to 200, with higher scores indicating a more negative or disturbed mood but no cut-off indicative of severe mood disturbance.[57]

2.4.2. Emotional faces fMRI paradigm

The emotional faces paradigm consisted of four blocks of emotional faces (i.e., fear, anger, surprise, and neutral blocks, presented in a randomised order) interleaved with five control blocks of geometric shapes (i.e., circles and vertical/horizontal ellipses) and lasted 6.5 minutes.[36] A trio of stimuli was presented with two on the bottom of the screen, and one on the top, and participants were then instructed to identify which of the two stimuli on the bottom matched the one on top as quickly and accurately as possible. For the faces blocks, a trio of stimuli were presented, including two similar faces to match and one odd face, all expressing the same emotion. The matching task was intended to ensure that participants attended to the stimuli. Written instructions (i.e., "match faces") were displayed (2 s) at the beginning of each faces block. Within each face block, six face trios were presented for four seconds each, interleaved with a fixation cross ("+") presented for two, four or six seconds, mean = four seconds, to minimise expectancy effects and habituation. The control block followed a similar procedure, written instructions (i.e., "match shapes") were displayed for two seconds, and the

fixation cross was displayed for fixed two-second periods. The paradigm, as well as participants' accuracy and reaction times, were recorded in E-prime (Psychological Software Tools, Pittsburgh, USA).

2.4.3. Magnetic Resonance Imaging

At the end of the intervention period, participants completed an MRI scan on a 3T Siemens Magnetom Prisma scanner (Erlangen, Germany) using a 32-channel head coil. We acquired a high-resolution, whole-brain, T1-weighted MPRAGE structural scan (inversion time = 972 ms, repetition time = 2000 ms, echo time = 2.58 ms, flip angle = 8°, in-plane matrix = 256x256 mm, in-plane resolution = 0.9x0.9 mm, 224 slices, slice thickness = 0.9 mm), followed by BOLD fMRI scans acquired during the emotional faces paradigm using a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, flip angle = 70^{o}, in-plane matrix = 76x76 mm, in-plane resolution=3x3 mm, 35 slices (thickness = 3.0 mm, gap between slices= 0.6 mm), number of volumes acquired = 195).We acquired a gradient field map to ameliorate spatial distortions in the BOLD fMRI acquisition.

2.5. Data analysis

2.5.1. Pre-processing and analyses of fMRI data

MRI images were pre-processed and analysed in Statistical Parametric Mapping 12 (SPM12) (https://www.fil.ion.ucl.ac.uk/ spm/software/spm12/). Single-subject functional volumes were corrected for slice-timing, unwarped, realigned to a subject-specific mean functional image, and smoothed using a 4 mm FWHM Gaussian filter. Functional images were kept in subject space for further analysis. The Automatic Anatomical Label (AAL) atlas was used to define regions of interest (ROIs).[58] The T1-weighted structural image was co-registered with the functional images and then normalised into Montreal Neurological Institute standard space.

The subject-specific inverse deformation map was applied to warp the AAL atlas into subject space and then resliced to match the functional images (both procedures applied nearest-neighbour interpolation to retain region labels). Artefact Detection Tool (ART; https://www.nitrc.org/projects/artifact_detect/) was applied to identify individual functional volumes with excess motion (>2 mm) and/or signal variability (>4 SD), which were censored from task-related analyses.

We analysed data from each subject in general linear models (GLMs) by employing a canonical hemodynamic response function to the smoothed functional images to estimate task-specific BOLD activity (i.e., beta images). A high-pass filter (128 s) was applied to control for slow-frequency fluctuations. Motion parameters and censored volumes were included as covariates. The GLMs were used to generate contrast images (linear combination of task-specific effects) for our effect of interest (i.e., fearful, angry, and neutral faces contrasted to geometric shapes). Next, group-level analyses were used to determine population-level brain responses to fear, angry, and neutral faces in the SSRI group compared to the placebo group. The amygdala, our primary ROI, and all other brain areas were defined using the WFU PickAtlas v3.0.3.[59,60] For each participant, we extracted mean signal values from the bilateral amygdala for the main analyses, and for hemisphere-specific, whole-brain regions for secondary analyses, which were evaluated further in R version 4.0.3 (https://cran.r-project.org/).

2.5.2. Quantification of serum escitalopram concentration

We analysed the concentration of serum escitalopram (nmol/L) using ultra-high performance liquid chromatography/tandem-mass spectrometry (UPLC-MS/MS) (Filadelfia Epilepsy Hospital, Dianalund, Denmark).

2.5.3. Missing data

Two participants were excluded based on poor behavioural task performance (i.e., accuracy and reaction time), indicating no attention to the emotional face task.

2.5.4. Statistical analysis

From the emotional faces paradigm, we only included data for angry, fearful, and neutral faces and geometric shapes. Blocks of geometric shapes were collapsed and used to contrast each facial expression (e.g., BOLD response to angry faces versus BOLD response to shapes). We used linear regression models to evaluate differences in the amygdala response to angry, fearful, and neutral faces between SSRI and placebo groups. Age and sex were included as covariates. Using the same model, we performed analyses of group differences in whole-brain regional responses to angry, fearful, and neutral faces.

We used linear regression models to evaluate whether amygdala responses to each emotional face were correlated with negative mood states, as measured with POMS on the same day, and whether SSRI modulated such potential correlation using an interaction term between amygdala responses and group. Age and sex were included as covariates. We computed post-hoc group-wise partial correlations between emotional amygdala responses and negative mood states with age and sex as covariates. Across all tests, p-values are reported uncorrected (P_{unc}) and corrected for family-wise error rate (P_{FWER}) using the Bonferroni correction method. For the a priori amygdala analyses, we controlled for a family of three tests and the whole-brain analyses for a family of 88 tests. P-values were considered significant at $P_{FWER} < 0.05$. Effects sizes are reported as Cohen's *d*.

We used one-sample t-tests, including the whole sample, to examine whether the emotional faces paradigm induced a significant response in the amygdala. We used chi-square or two-sample t-tests to evaluate any differences between the groups on baseline measures, including sex, age, IQ, BMI, BDI-II, STAI, PSS and POMS, as well as POMS, censored

volumes during fMRI, and participants' blinding at follow-up. For the emotional faces paradigm, accuracy rates were positively skewed, so we evaluated group differences using a two-sample permutation test with 10000 Monte Carlo replications for each emotion. Group differences in reaction time for each emotion were assessed using t-tests. P-values are reported uncorrected (P_{unc}).

3. Results

3.1. Sample characteristics

Participant demographics, psychometrics, serum escitalopram levels, and emotional amygdala responses as assessed with the emotional faces paradigm and its behavioural outcomes are reported in **Table 1**. BDI scores confirmed the absence of dysphoric or depressed mood (i.e., < 20; score range: active group = 0-9, placebo group = 0-12).[52] STAI and PSS levels across groups were aligned with typical scores in a healthy cohort (STAI, score range: active group = 20-39, placebo group = 20-36;[44] PSS score range: active group = 2-20, placebo group = 1-19).[52] The participants' mean IQ was in the normal upper range, i.e., higher than the average 100 IQ point of the general population, across both the active group (mean ± SD = 110 ± 8) and the placebo group (mean ± SD = 108 ± 7). Overall, there were no significant differences in baseline characteristics between the escitalopram and placebo groups (see **Table 1** for group-wise descriptive data and estimate and p-values from statistical tests of group differences).

BASELINE						
	Placebo group (N=32)		SSRI group (N=32)		Group difference	
	Frequency	Percent	Frequency	Percent	Estimate	Punc
Female / male	20 / 12	63% / 37%	21 / 11	66% / 34%	1.12	0.29
	Mean (SD)	Median [Min, Max]	Mean (SD)	Median [Min, Max]	Estimate	Punc
Age (years)	25.8 (5.9)	23.9 [19.9, 45.6]	24.8 (5.6)	22.7 [19.3, 41.9]	0.2	0.49
IQ	108 (6.9)	110 [90.0, 118]	110 (8.07)	107 [97.0, 129]	-0.3	0.27
BMI (kg/m2)	24.0 (3.9)	23.1 [18.9, 40.3]	23.9 (3.8)	23.1 [17.5, 32.8]	0.0	0.87
Days of intervention	25.8 (2.8)	26.0 [21.0, 36.0]	26.1 (2.8)	25.0 [22.0, 33.0]	-0.1	0.56
Depression (BDI-II)	3.2 (3.3)	2.5 [0, 12.0]	2.4 (2.6)	2.00 [0, 9.0]	-0.1	0.29
State anxiety (STAI)	25.0 (4.5)	24.0 [20.0, 36.0]	24.7 (4.1)	23.0 [20.0, 39.0]	0.1	0.82
Stress (PSS)	9.03 (4.4)	9.00 [1.00, 19.0]	8.3 (3.9)	8.00 [2.00, 20.0]	0.2	0.47
Mood disturbance (POMS)	1.5 (12.2)	-0.5 [-14.0, 39.0]	-0.7 (10.4)	-2.00 [-17.0, 32.0]	0.1	0.44
MID-INTERVENTION						
Mid-term serum escitalopram (nmol/L)	0 (0)	0 [0, 0]	82.9 (59.6)	69.0 [28.0, 338]	-	-
POST-INTERVENTION						
	Placebo group (N=32)		SSRI group (N=32)		Group difference	
	Frequency	Percent	Frequency	Percent	Estimate	Punc
Patient guess of intervention (active/placebo)	5 / 26*	16% / 81%*	17 / 15	53% / 47%	4.13	0.04
	Mean (SD)	Median [Min, Max]	Mean (SD)	Median [Min, Max]	Estimate	Punc
Mood disturbance (POMS)	8.1 (22.2)	1 [-23.0, 81.0]	3.7 (15.4)	3.5 [-17.0, 58.0]	0.2	0.37
Follow-up serum escitalopram (nmol/L)	0 (0)	0 [0, 0]	82.7 (49.8)	67.5 [28.0, 263]	-	-
	Mean (SD)	Median [Min, Max]	Mean (SD)	Median [Min, Max]	Estimate	Punc
Amygdala response to angry faces	0.38 (0.74)	0.40 [-1.4, 2.8]	0.29 (0.81)	0.15 [-1.09, 2.42]	0.1	0.63
Amygdala response to fearful faces	0.31 (0.51)	0.35 [-0.83, 1.31]	0.33 (0.47)	0.37 [-0.71, 1.30]	-0.1	0.68
Amygdala response to neutral faces	0.28 (0.61)	0.29 [-1.03, 1.37]	0.39 (0.74)	0.31 [-0.92, 2.66]	-0.2	0.59
Accuracy for angry faces (%)	0.97 (0.08)	1.00 [0.67, 1.00]	0.98 (0.05)	1.00 [0.83, 1.00]	-0.2	0.78
Accuracy for fearful faces (%)	0.97 (0.08)	1.00 [0.67, 1.00]	0.97 (0.06)	1.00 [0.83, 1.00]	0.0	1.00
Accuracy for neutral faces (%)	0.98 (0.05)	1.00 [0.83, 1.00]	0.98 (0.06)	1.00 [0.83, 1.00]	0.0	0.95
Accuracy for shapes (%)	0.96 (0.06)	0.97 [0.73, 1.00]	0.93 (0.1)	0.97 [0.73, 1.00]	0.3	0.61
Reaction time for angry faces (ms)	909 (174)	870 [639, 1330]	888 (173)	849 [519, 1330]	0.1	0.64
Reaction time for fearful faces (ms)	861 (166)	852 [622, 1260]	833 (184)	785 [553, 1260]	0.2	0.52
Reaction time for neutral faces (ms)	875 (159)	874 [650, 1390]	856 (147)	809 [599, 1150]	0.1	0.62
Reaction time for shapes (ms)	817 (120)	799 [591, 1030]	805 (135)	791 [561, 1140]	0.1	0.71

Table 1. Descriptive information for participants in each group along with group differences. Group differences on age, IQ, BMI, BDI-II, STAI, PSS and POMS both at baseline and follow-up as well as reaction times in the emotional faces paradigm, were assessed using two-sample t-tests with estimate represting Cohen's *d*. Group differences in sex and blinding were assed using chi-square test with the estimate represting $\chi 2$. As accuracy rates were positively skewed, group differences were assesed using two-sample permutation test with 10000 Monte Carlo replications for each emotion, with estimate representing Cohen's *d*. Group differences in amygdala response to angry, fearful and neutral faces were assessed using linear regression models with age and sex as covariates with estime representing Cohen's *d*. * = one missing. Abbreviations: PSS = Cohen's Perceived Stress Scale, BDI-II = Beck Depression Index II, POMS = Profile of Mood States ms = milliseconds, SD = standard deviation, IQ= intelligence quotient, BMI = body mass index, SSRI = selective serotonin reuptake inhibitors administered sub-chronically, Punc = uncorrected p-value.

3.3. Treatment compliance and assessment of blinding

The serum escitalopram concentrations, as measured approximately halfway through the intervention period (mean ± SD: 12 ± 2 days) and at follow-up, typically on the same day as MRI (mean ± SD: 26 ± 3 days), confirmed that all participants in the escitalopram group complied with the drug-intervention. Although the fraction of participants in the escitalopram group who guessed that they got escitalopram was at a chance level (53%), the number was significantly higher than in the placebo group (χ^2= 4.13, df = 1, P_{unc} = 0.04). See **Table 1** for descriptive data and estimate and p-values from statistical tests of group differences.

3.4. Amygdala responses to the emotional faces paradigm

Across the placebo and escitalopram groups, we observed a significant response of the amygdala to angry (mean ± SD: 0.333 ± 0.772, $P_{unc} = 0.001$), fearful (mean ± SD: 0.316 ± 0.479, $P_{unc} < 0.001$), and neutral faces (mean ± SD: 0.332 ± 0.670, $P_{unc} < 0.001$). There was no evidence of significant differences between groups in accuracy or reaction time (see **Table 1**). Further, there were no significant group differences in censored volumes ($P_{unc} = 0.81$).

The amygdala response to angry faces was numerically lower whereas the response to fearful and neutral faces was numerically higher in the SSRI group compared with the placebo group, but none of these effects were statistically significant (angry: mean difference = -0.10, SE = 0.47, $P_{FWER} = 1.00$; fear: mean difference = -0.05, SE = 0.12, $P_{FWER} = 1.00$; neural: mean difference = -0.09, SE=0.17, $P_{FWER} = 1.00$). Evaluation of sex differences showed that, across treatment groups, males had a significantly higher amygdala response to fearful faces than females (mean difference = 0.33, SE = 0.12, $P_{FWER} = 0.02$). Otherwise, no sex differences were observed (angry: mean difference = -0.01, SE = 0.21, $P_{FWER} = 1.00$; neutral: mean difference = -3.0, SE = 0.17, $P_{FWER} = 0.26$). Nor age effects were observed (angry: mean difference = -0.01, SE = 0.02, $P_{FWER} = 1.00$; fear: mean difference = 0.00, SE = 0.01, $P_{FWER} = 1.00$; neutral: mean difference = -0.01, SE = 0.01, $P_{FWER} = 1.00$). See **Table 1 for** group-wise descriptive data as well as Cohen's *d* and p-values from the statistical test of group differences, **Table A** in the supplementary material for detailed estimates and corrected p-values from the statistical models assessing the effect of escitalopram on amygdala response to emotional faces, and **Figure 2** for individual and average amygdala responses to each emotion adjusting for the effect of sex and age.

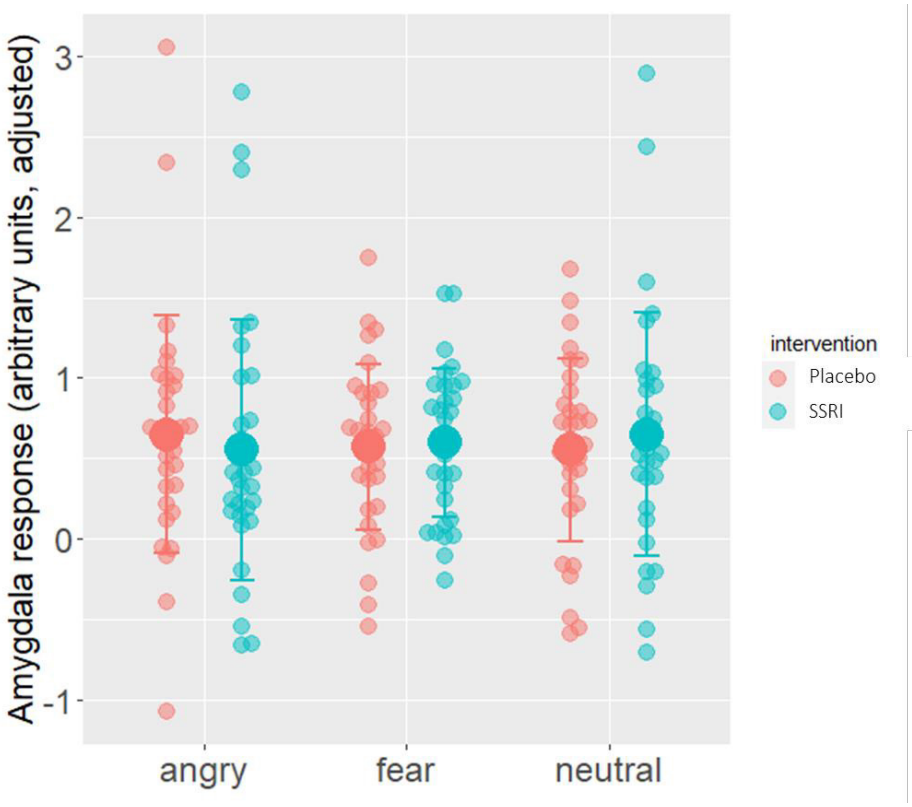

Figure 2. Bilateral amygdala response during BOLD fMRI to angry, fearful and neutral faces (contrasted with geometric shapes) by group (SSRI, n=32; placebo, n=32), adjusted for effects of age and sex. Linear regression models revealed no significant differences between groups on amygdala response to emotional faces (angry: d = 0.1, P_{FWER} = 1.00; fear: d = -0.1, P_{FWER} = 1.00; neutral: d = -0.2, P_{FWER} = 1.00). Smaller transparent circles denote individual observed values and larger circles and bars denote population Mean ± 1 SD.

3.5. Whole-brain responses to the emotional faces paradigm

Analyses of effects of 3-5 weeks intake of escitalopram on whole-brain regional responses revealed that responses to angry faces were significantly lower in the left opercular part of inferior frontal gyrus (mean difference = -0.25, SE = 0.05, P_{FWER} <. 01), in the left triangular part of inferior frontal gyrus (mean difference = -0.21, SE = 0.06, P_{FWER} = 0.03) and in the right pallidum (mean difference = -0.16, SE = 0.04, P_{FWER} = 0.03) in the escitalopram group

compared to placebo. For fearful faces, the responses were significantly lower in the left gyrus rectus (mean difference = -0.25, SE = 0.05, $P_{FWER} < .01$), in the left superior occipital gyrus (mean difference = -0.26, SE = 0.07, $P_{FWER} < .01$), in the left middle occipital gyrus (mean difference = -0.20, SE = 0.06, $P_{FWER} = 0.04$) and in the right inferior occipital gyrus (mean difference = -0.31, SE = 0.08, $P_{FWER} < .01$) in the escitalopram group compared to the placebo group. For neutral faces, the responses were significantly higher in the right orbital part of the inferior frontal gyrus (mean difference = 0.27, SE = 0.06, $P_{FWER} < .01$) and in the right medial part of the superior frontal gyrus (mean difference = 0.23, SE = 0.06, $P_{FWER} = 0.03$). See **Table C**, **D, and E** in the supplementary material for region-wise estimates and p-values from the statistical models.

3.6. Negative mood states

There was no significant group difference in negative mood states at baseline or at follow-up (see **Table 1**). We found no significant correlations between amygdala responses to angry, fearful, or neutral faces and negative mood states in the placebo group (angry, $P_{FWER} = 0.33$; fear, $P_{FWER} = 0.12$; neutral, $P_{FWER} = 0.07$) or for a modulatory effect of escitalopram (angry, $P_{FWER} = 0.49$; fear, $P_{FWER} = 0.29$; neutral, $P_{FWER} = 0.19$). For an overview of the results, see **Table 2**. See Table B in the supplementary material for an overview of all group-wise partial correlations.

Estimated model coefficients when regressing amygdala response to angry and negative mood states				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	13.93	11.35	0.225	
Amygdala response to angry	7.24	4.47	0.111	0.332
SSRI	-1.64	5.03	0.746	1.000
Amygdala response to angry*SSRI	-8.63	6.12	0.164	0.493
Age	-0.18	0.41	0.659	1.000
Sex	-10.82	4.86	0.030	0.090
Estimated model coefficients when regressing amygdala response to fear and negative mood states				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	18.41	10.82	0.094	
Amygdala response to fear	-14.15	6.71	0.039	0.118
SSRI	-9.60	5.39	0.080	0.241
Amygdala response to angry*SSRI	16.27	9.65	0.097	0.292
Age	-0.08	0.41	0.836	1.000
Sex	-10.78	4.96	0.034	0.102
Estimated model coefficients when regressing amygdala response to neutral and negative mood states				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	6.47	12.00	0.592	
Amygdala response to neutral	13.45	5.70	0.022	0.065
SSRI	-0.89	5.10	0.863	1.000
Amygdala response to angry*SSRI	-13.18	7.30	0.076	0.189
Age	0.07	0.42	0.864	1.000
Sex	-10.96	4.74	0.024	0.073

Table 2. The models explore whether emotional amygdala responses is correlated with negative mood states and whether this correlation is modulated by 3-5 weeks' SSRI intake, controlled for covariates age and sex. Sex is the marginal effect of men relative to women. The amygdala response to angry, fearful and neutral faces (contrasted with geometric shapes) are assesed using BOLD fMRI. Mood disturbances are measured with the self-report questionnaire Profile of Mood States (POMS), with higher values indicating more disturbance. Estimate is the unstandardised beta. Abbreviations: SE B = standard error for unstandardised beta, Punc = unadjusted significance level, SSRI = selective serotonin reuptake inhibitors. All p-values were corrected for family-wise multiple testing using the Bonferroni method (i.e. all p-values multiplied by three), displayed under PFWER.

4. Discussion

In this study, we evaluated functional brain responses to angry, fearful, and neutral faces following 3–5 weeks intake of either the SSRI escitalopram or placebo in the largest such study to date in healthy individuals. Inconsistent with our hypothesis, SSRI did not significantly affect amygdala responses to faces compared to placebo. However, whole-brain, region-wise analyses identified frontal and occipital brain regions wherein the response to emotional faces was significantly different following SSRI intake compared to placebo. Our results do not support a modulatory effect of SSRIs on the correlations between amygdala responses to emotional faces and negative mood states. Our findings contrast with evidence for acute and short-term SSRI intake changing the amygdala response to emotional faces in healthy individuals, but implicate the modulation of a broader set of brain regions, including the frontal and occipital cortex in response to SSRI intake over a clinically relevant period.

A primary region of interest in our analyses was the amygdala considering previous related studies. We observed a pronounced and significant amygdala response to the emotional face task and a high degree of accuracy across both the SSRI and placebo groups, comparable to previous reports.[36] As such, the lack of a significant effect of SSRI on emotion processing in the amygdala does not appear to stem from a weak or atypical neural response to the task. Two previous studies evaluating SSRI effects over a similar time frame also report a limited effect on the amygdala response to threat-related or happy faces.[25,48] However, in a sub-analysis, one of the previous studies reported a negative effect of SSRI on amygdala responses to threat in treatment-compliant participants, as confirmed by urinary drug concentration.[48] The authors suggested that the lack of a significant group difference could be attributed to non-compliance with the treatment. We verified treatment compliance for our participants in the current study via serum SSRI concentration and self-report daily medication logbook. These findings suggest that study non-compliance is not the cause of the non-significant results

observed in our current study. Taken together, current evidence does not support that SSRI intake over a clinically relevant time period significantly modulates amygdala response to emotional faces across healthy populations. Future studies should investigate effects of over a clinically relevant time period of SSRI intake on other emotional processes involved in MDD such as reinforcement sensitivity.

Our whole-brain, region-wise analyses did show significantly lower brain responses to angry and fearful faces following SSRI intake compared to placebo in cortical areas including frontal and occipital regions, brain regions implicated via meta-analyses in emotional face processing[6] and MDD.[19] Previous studies did not find a significant effect of 3 weeks' SSRI on emotion processing in cortical regions,[25,48] perhaps explained by their relatively smaller sample sizes. Our study is the first to evaluate neutral faces processing in response to SSRIs; we observed significantly higher responses to neutral faces in frontal regions compared to placebo. Increased brain responses to happy faces in healthy controls following short-term SSRI intervention have previously been reported.[62] Accordingly, we speculate whether the higher response to neutral faces could reflect that SSRI induces or bolsters positive processing biases, which is a tendency to misinterpret neutral faces as being happy. The frontal cortex is involved in top-down cognitive control of the amygdala,[63,64] as such it is not surprising that SSRI has neuro-modulatory effects on emotional face processing in frontal regions already identified as being critical for this task in previous publications.[6] In sum, our findings suggest that 3-5 weeks intake of SSRI does influence brain responses to emotional face processing, but in brain regions outside the amygdala, particularly in frontal regions. Future studies using positron emission tomography (PET) could identify the serotonergic receptors involved in this neuro-modulation of emotional face processing.

4.1. Methodological consideration

This study is not without limitations. The emotional faces paradigms have been shown to have low within-subject test-retest reliability.[65,66] Although this mitigates the lack of a baseline scan in estimating drug effects, it nevertheless represents a noise source that may challenge our capacity to detect group differences. Developing and validating paradigms that more reliably measure neural responses to emotionally salient stimuli is challenging but imperative for the field to better resolve alterations in clinical cohorts and associated effects of drug interventions.

Five participants dropped out due to side effects, of whom four received SSRI. Assuming that this group had an overall greater psychoactive response to SSRI, these missing data may have biased our results towards underestimating SSRI effects on amygdala reactivity.

In this study, we examined only brain responses. Future studies should investigate alternative modelling of these data, e.g., brain connectivity, which may capture relevant neural correlates associated with SSRI intervention. It also deserves to be explored whether connectivity between the amygdala and frontal regions during emotion processing, argued to be important for emotion regulation,[67] might relate to mood states in healthy individuals rather than brain responses per se, which our results did not support.

Participants receiving SSRI guessed their group assignment correctly at chance level, but the frequency of guessing SSRI group assignment was significantly higher than those in the placebo group (i.e., 16%). This contrasts with our previous study in healthy males with the SSRI fluoxetine, where nearly all participants guessed that they received a placebo.[68] The reason for the observed group discrepancy and whether it reflects an influential confound is not apparent, but its presence is notable. It is possible that perceptual differences influencing participants' guesses could be similarly associated with our observed exploratory group differences in frontal and occipital brain responses.

4.1.Conclusion

We did not find evidence that 3-5 weeks intake of SSRI significantly affected amygdala response to threat-related or neutral faces in a large cohort of healthy individuals. However, whole-brain analyses revealed significant effects in frontal and occipital regions, implicating serotonergic modulation of top-down emotional processing.

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SUPPLEMENTARY MATERIAL

Functional brain responses to emotional faces after three weeks intake of selective serotonin reuptake inhibitor or placebo in healthy individuals

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Estimated model coefficients when regressing amygdala response to angry faces				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	0.65	0.47	0.18	
SSRI	-0.10	0.20	0.63	1.000
Age	-0.01	0.02	0.93	1.000
Sex	-0.01	0.02	0.55	1.000
Estimated model coefficients when regressing amygdala response to fearful faces				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	0.13	0.28	0.65	
SSRI	0.05	0.12	0.68	1.000
Age	0.00	0.01	0.86	1.000
Sex	0.33	0.12	0.01	0.023
Estimated model coefficients when regressing amygdala response to neutral faces				
	Estimate	SE	p_{unc}	p_{FWER}
Constant	0.65	0.40	0.11	
SSRI	0.09	0.17	0.59	1.000
Age	-0.01	0.01	0.51	1.000
Sex	-0.30	0.17	0.08	0.255

Table A. The effect of SSRI (n=32), relative to placebo (n=32), on amygdala response to angry, fearfull and nautral faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using mixed linear effect models. Covariates included age and sex. Sex is the marginal effect of men relative to women. Estimate is the mean differences between groups. Abbreviations: SE = standard error for unstandardised beta, Punc = unadjusted significance level, 95% CI = 95% confidence interval, SSRI = selective serotonin reuptake inhibitors. All p-values were corrected for family-wise multiple testing using the Bonferroni method (i.e. all p-values multiplied by three), displayed under PFWER.

Correlation between amygdala response to angry faces and mood disturbances					
	Estimate	SE	p_{unc}	95% CI	
Placebo	0.24	0.19	0.211	-0.15	0.56
SSRI	-0.12	0.19	0.542	-0.47	0.27
Correlation between amygdala response to fearful faces and mood disturbances					
	Estimate	SE	p_{unc}	95% CI	
Placebo	-0.32	0.19	0.092	-0.621	0.06
SSRI	-0.03	0.19	0.886	-0.40	0.35
Correlation between amygdala response to neutral faces and mood disturbances					
	Estimate	SE	p_{unc}	95% CI	
Placebo	0.41	0.19	0.033	0.04	0.68
SSRI	0.11	0.19	0.568	-0.28	0.47

Table B. Partial correlations between amygdala response to angry/fearful/neutral faces and mood disturbances adjusted for covariates age and sex. The amygdala response to angry, fearfull and neutral faces (contrasted with geometric shapes) are assesed using BOLD fMRI. Mood disturbances are measured with the self-report questionnaire Profile of Mood States (POMS), with higher values indicating more disturbance. Estimate is rho. SE = standard error, Punc = unadjusted significance level, 95% CI = 95% confidence interval, SSRI = selective serotonin reuptake inhibitors, which was administered sub-chronically.

Estimated model coefficients when regressing whole-brain responses to angry faces							
Region	**Estimate**	**SE**	**lower**	**upper**	**p_{unc}**	**p_{FWER}**	**Cohen's d**
Precentral (L)	-0.08	0.05	-0.19	0.02	0.12	1.000	-0.2
Precentral (R)	-0.08	0.05	-0.18	0.02	0.13	1.000	-0.2
Frontal Sup (L)	-0.05	0.06	-0.16	0.07	0.43	1.000	-0.1
Frontal Sup (R)	-0.02	0.06	-0.14	0.10	0.76	1.000	0.0
Frontal Sup Orb (L)	-0.09	0.06	-0.20	0.02	0.11	1.000	-0.2
Frontal Sup Orb (R)	0.01	0.06	-0.11	0.13	0.87	1.000	0.0
Frontal Mid (L)	-0.10	0.06	-0.22	0.01	0.08	1.000	-0.2
Frontal Mid (R)	-0.07	0.06	-0.20	0.05	0.25	1.000	-0.1
Frontal Mid Orb (L)	0.00	0.12	-0.23	0.24	0.97	1.000	0.0
Frontal Mid Orb (R)	-0.05	0.08	-0.20	0.10	0.53	1.000	-0.1
Frontal Inf Oper (L)	**-0.25**	**0.05**	**-0.36**	**-0.15**	**0.00**	**<0.001**	**-0.6**
Frontal Inf Oper (R)	-0.20	0.07	-0.34	-0.06	0.01	0.466	-0.3
Frontal Inf Tri (L)	**-0.21**	**0.06**	**-0.32**	**-0.10**	**0.00**	**0.025**	**-0.5**
Frontal Inf Tri (R)	-0.13	0.06	-0.26	-0.01	0.04	1.000	-0.3
Frontal Inf Orb (L)	-0.13	0.06	-0.25	0.00	0.05	1.000	-0.2
Frontal Inf Orb (R)	-0.02	0.06	-0.14	0.10	0.76	1.000	0.0
Rolandic Oper (L)	-0.12	0.06	-0.24	0.00	0.05	1.000	-0.2
Rolandic Oper (R)	-0.11	0.07	-0.24	0.02	0.11	1.000	-0.2
Supp Motor Area (L)	-0.05	0.06	-0.17	0.08	0.46	1.000	-0.1
Supp Motor Area (R)	-0.04	0.05	-0.15	0.06	0.39	1.000	-0.1
Olfactory (L)	0.04	0.09	-0.14	0.21	0.66	1.000	0.1
Olfactory (R)	-0.08	0.07	-0.22	0.06	0.25	1.000	-0.1
Frontal Sup Medial (L)	-0.13	0.09	-0.30	0.05	0.15	1.000	-0.2
Frontal Sup Medial (R)	0.00	0.07	-0.15	0.14	0.97	1.000	0.0
Rectus (L)	0.01	0.08	-0.15	0.17	0.91	1.000	0.0
Rectus (R)	0.07	0.06	-0.06	0.19	0.30	1.000	0.1
Insula (L)	-0.16	0.05	-0.27	-0.06	0.00	0.236	-0.4
Insula (R)	-0.13	0.07	-0.26	-0.01	0.04	1.000	-0.3
Cingulum Ant (L)	-0.13	0.08	-0.29	0.04	0.13	1.000	-0.2
Cingulum Ant (R)	-0.04	0.06	-0.16	0.07	0.44	1.000	-0.1
Cingulum Mid (L)	-0.06	0.07	-0.21	0.08	0.40	1.000	-0.1
Cingulum Mid (R)	-0.05	0.06	-0.17	0.07	0.39	1.000	-0.1
Cingulum Post (L)	-0.06	0.10	-0.25	0.14	0.56	1.000	-0.1
Cingulum Post (R)	0.00	0.07	-0.14	0.14	0.98	1.000	0.0
Hippocampus (L)	-0.07	0.06	-0.19	0.05	0.23	1.000	-0.1
Hippocampus (R)	-0.07	0.04	-0.16	0.02	0.10	1.000	-0.2
ParaHippocampal (L)	-0.06	0.07	-0.19	0.07	0.34	1.000	-0.1
ParaHippocampal (R)	-0.03	0.06	-0.14	0.09	0.67	1.000	-0.1
Amygdala (L)	-0.01	0.10	-0.21	0.20	0.96	1.000	0.0

Amygdala (R)	-0.17	0.09	-0.36	0.01	0.06	1.000	-0.2
Calcarine (L)	-0.17	0.08	-0.34	-0.01	0.04	1.000	-0.3
Calcarine (R)	-0.15	0.06	-0.26	-0.03	0.01	1.000	-0.3
Cuneus (L)	-0.02	0.08	-0.19	0.14	0.77	1.000	0.0
Cuneus (R)	-0.11	0.07	-0.24	0.02	0.11	1.000	-0.2
Lingual (L)	-0.11	0.07	-0.25	0.04	0.16	1.000	-0.2
Lingual (R)	-0.09	0.07	-0.24	0.06	0.23	1.000	-0.2
Occipital Sup (L)	-0.07	0.06	-0.19	0.05	0.25	1.000	-0.1
Occipital Sup (R)	-0.08	0.06	-0.20	0.04	0.19	1.000	-0.2
Occipital Mid (L)	-0.10	0.05	-0.20	-0.01	0.03	1.000	-0.3
Occipital Mid (R)	-0.10	0.05	-0.20	0.00	0.06	1.000	-0.2
Occipital Inf (L)	-0.13	0.06	-0.26	-0.01	0.04	1.000	-0.3
Occipital Inf (R)	-0.11	0.09	-0.30	0.07	0.22	1.000	-0.2
Fusiform (L)	-0.02	0.06	-0.14	0.11	0.76	1.000	0.0
Fusiform (R)	-0.04	0.07	-0.17	0.09	0.52	1.000	-0.1
Postcentral (L)	-0.08	0.05	-0.19	0.03	0.15	1.000	-0.2
Postcentral (R)	-0.18	0.05	-0.28	-0.08	0.00	0.051	-0.4
Parietal Sup (L)	-0.16	0.08	-0.31	0.00	0.04	1.000	-0.3
Parietal Sup (R)	-0.19	0.10	-0.39	0.00	0.05	1.000	-0.2
Parietal Inf (L)	-0.20	0.06	-0.33	-0.08	0.00	0.139	-0.4
Parietal Inf (R)	-0.09	0.08	-0.24	0.06	0.24	1.000	-0.1
SupraMarginal (L)	-0.18	0.07	-0.31	-0.06	0.01	0.409	-0.3
SupraMarginal (R)	-0.12	0.07	-0.25	0.01	0.08	1.000	-0.2
Angular (L)	-0.13	0.08	-0.30	0.04	0.12	1.000	-0.2
Angular (R)	-0.07	0.08	-0.23	0.09	0.41	1.000	-0.1
Precuneus (L)	-0.10	0.09	-0.28	0.08	0.25	1.000	-0.1
Precuneus (R)	-0.07	0.08	-0.22	0.08	0.37	1.000	-0.1
Paracentral Lobule (L)	0.04	0.06	-0.09	0.16	0.56	1.000	0.1
Paracentral Lobule (R)	0.07	0.06	-0.05	0.19	0.28	1.000	0.1
Caudate (L)	-0.14	0.07	-0.27	-0.01	0.03	1.000	-0.3
Caudate (R)	-0.19	0.06	-0.30	-0.07	0.00	0.102	-0.4
Putamen (L)	-0.19	0.06	-0.30	-0.08	0.00	0.080	-0.4
Putamen (R)	-0.14	0.05	-0.23	-0.05	0.00	0.214	-0.4
Pallidum (L)	-0.08	0.04	-0.17	0.00	0.06	1.000	-0.2
Pallidum (R)	**-0.16**	**0.04**	**-0.24**	**-0.07**	**0.00**	**0.025**	**-0.5**
Thalamus (L)	-0.03	0.06	-0.14	0.08	0.61	1.000	-0.1
Thalamus (R)	-0.05	0.05	-0.15	0.06	0.38	1.000	-0.1
Heschl (L)	-0.13	0.07	-0.27	0.01	0.08	1.000	-0.2
Heschl (R)	-0.14	0.08	-0.30	0.02	0.08	1.000	-0.2
Temporal Sup (L)	-0.08	0.07	-0.21	0.05	0.24	1.000	-0.1
Temporal Sup (R)	-0.15	0.05	-0.26	-0.04	0.01	0.475	-0.3
Temporal Pole Sup (L)	-0.11	0.08	-0.26	0.05	0.17	1.000	-0.2

Temporal Pole Sup (R)	-0.19	0.07	-0.34	-0.05	0.01	0.555	-0.3
Temporal Mid (L)	-0.12	0.05	-0.22	-0.02	0.02	1.000	-0.3
Temporal Mid (R)	-0.10	0.06	-0.22	0.01	0.08	1.000	-0.2
Temporal Pole Mid (L)	0.00	0.05	-0.10	0.10	0.94	1.000	0.0
Temporal Pole Mid (R)	-0.10	0.05	-0.20	0.00	0.06	1.000	-0.2
Temporal Inf (L)	-0.08	0.04	-0.17	0.01	0.08	1.000	-0.2
Temporal Inf (R)	-0.09	0.06	-0.20	0.02	0.11	1.000	-0.2

Table C. The effect of SSRI (n=32), relative to placebo (n=32), on regional brain responses to angry faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using linear regression models with age and sex as covariates. The estimate is expressed as the marginal effect of SSRI relative to placebo. Abbreviations: SE = standard error, Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

Estimated model coefficients when regressing whole-brain responses to fearful faces							
Region	**Estimate**	**SE**	**lower**	**upper**	**p_{unc}**	**p_{FWER}**	**Cohen's d**
Precentral (L)	0.01	0.05	-0.09	0.11	0.88	1.000	0.0
Precentral (R)	-0.01	0.05	-0.10	0.08	0.84	1.000	0.0
Frontal Sup (L)	0.02	0.05	-0.07	0.12	0.62	1.000	0.1
Frontal Sup (R)	0.04	0.06	-0.07	0.16	0.46	1.000	0.1
Frontal Sup Orb (L)	-0.04	0.05	-0.14	0.05	0.36	1.000	-0.1
Frontal Sup Orb (R)	0.04	0.06	-0.07	0.15	0.52	1.000	0.1
Frontal Mid (L)	0.04	0.06	-0.08	0.16	0.54	1.000	0.1
Frontal Mid (R)	0.06	0.08	-0.09	0.22	0.43	1.000	0.1
Frontal Mid Orb (L)	-0.23	0.09	-0.41	-0.06	0.01	0.617	-0.3
Frontal Mid Orb (R)	-0.11	0.05	-0.22	0.00	0.05	1.000	-0.2
Frontal Inf Oper (L)	0.04	0.06	-0.08	0.17	0.47	1.000	0.1
Frontal Inf Oper (R)	0.04	0.08	-0.12	0.20	0.62	1.000	0.1
Frontal Inf Tri (L)	0.00	0.06	-0.11	0.12	0.97	1.000	0.0
Frontal Inf Tri (R)	-0.03	0.07	-0.16	0.10	0.63	1.000	-0.1
Frontal Inf Orb (L)	-0.06	0.05	-0.16	0.04	0.24	1.000	-0.1
Frontal Inf Orb (R)	-0.13	0.06	-0.25	-0.01	0.03	1.000	-0.3
Rolandic Oper (L)	0.03	0.05	-0.07	0.13	0.53	1.000	0.1
Rolandic Oper (R)	0.06	0.06	-0.06	0.18	0.34	1.000	0.1
Supp Motor Area (L)	-0.01	0.07	-0.14	0.12	0.89	1.000	0.0
Supp Motor Area (R)	0.01	0.05	-0.09	0.11	0.81	1.000	0.0
Olfactory (L)	-0.05	0.06	-0.18	0.07	0.38	1.000	-0.1
Olfactory (R)	-0.11	0.06	-0.22	0.01	0.07	1.000	-0.2
Frontal Sup Medial (L)	-0.11	0.07	-0.25	0.04	0.15	1.000	-0.2
Frontal Sup Medial (R)	-0.03	0.06	-0.15	0.09	0.66	1.000	-0.1
Rectus (L)	**-0.25**	**0.05**	**-0.36**	**-0.15**	**0.00**	**<0.001**	**-0.6**
Rectus (R)	-0.08	0.04	-0.16	-0.01	0.02	1.000	-0.3
Insula (L)	0.08	0.05	-0.02	0.18	0.12	1.000	0.2
Insula (R)	0.00	0.06	-0.11	0.12	0.96	1.000	0.0
Cingulum Ant (L)	-0.11	0.07	-0.24	0.02	0.09	1.000	-0.2
Cingulum Ant (R)	-0.04	0.05	-0.13	0.05	0.38	1.000	-0.1
Cingulum Mid (L)	-0.09	0.07	-0.22	0.04	0.17	1.000	-0.2
Cingulum Mid (R)	-0.01	0.06	-0.12	0.10	0.86	1.000	0.0
Cingulum Post (L)	-0.05	0.08	-0.22	0.11	0.53	1.000	-0.1
Cingulum Post (R)	0.01	0.07	-0.12	0.14	0.93	1.000	0.0
Hippocampus (L)	-0.05	0.05	-0.14	0.04	0.32	1.000	-0.1
Hippocampus (R)	-0.01	0.04	-0.09	0.07	0.84	1.000	0.0
ParaHippocampal (L)	0.02	0.04	-0.06	0.11	0.60	1.000	0.1
ParaHippocampal (R)	-0.07	0.05	-0.16	0.02	0.11	1.000	-0.2
Amygdala (L)	0.03	0.06	-0.10	0.15	0.67	1.000	0.1

Amygdala (R)	0.05	0.06	-0.07	0.17	0.44	1.000	0.1
Calcarine (L)	-0.26	0.09	-0.43	-0.08	0.00	0.316	-0.4
Calcarine (R)	-0.18	0.07	-0.31	-0.05	0.01	0.514	-0.3
Cuneus (L)	-0.22	0.08	-0.38	-0.05	0.01	0.786	-0.3
Cuneus (R)	-0.20	0.08	-0.35	-0.04	0.01	0.932	-0.3
Lingual (L)	-0.19	0.08	-0.34	-0.04	0.01	0.961	-0.3
Lingual (R)	-0.14	0.07	-0.28	0.00	0.04	1.000	-0.3
Occipital Sup (L)	**-0.26**	**0.07**	**-0.40**	**-0.13**	**0.00**	**0.008**	**-0.5**
Occipital Sup (R)	-0.17	0.06	-0.29	-0.04	0.01	0.754	-0.3
Occipital Mid (L)	**-0.20**	**0.06**	**-0.31**	**-0.09**	**0.00**	**0.042**	**-0.4**
Occipital Mid (R)	-0.16	0.06	-0.27	-0.04	0.01	0.729	-0.3
Occipital Inf (L)	-0.19	0.07	-0.34	-0.05	0.01	0.676	-0.3
Occipital Inf (R)	**-0.31**	**0.08**	**-0.46**	**-0.16**	**0.00**	**0.004**	**-0.5**
Fusiform (L)	-0.13	0.06	-0.25	-0.02	0.02	1.000	-0.3
Fusiform (R)	-0.14	0.05	-0.25	-0.04	0.01	0.698	-0.3
Postcentral (L)	0.02	0.05	-0.08	0.12	0.74	1.000	0.0
Postcentral (R)	0.05	0.04	-0.04	0.13	0.26	1.000	0.1
Parietal Sup (L)	-0.09	0.08	-0.24	0.07	0.26	1.000	-0.1
Parietal Sup (R)	0.08	0.08	-0.08	0.24	0.30	1.000	0.1
Parietal Inf (L)	0.07	0.08	-0.07	0.22	0.33	1.000	0.1
Parietal Inf (R)	0.21	0.09	0.04	0.38	0.02	1.000	0.3
SupraMarginal (L)	0.06	0.07	-0.08	0.19	0.42	1.000	0.1
SupraMarginal (R)	0.16	0.07	0.02	0.30	0.02	1.000	0.3
Angular (L)	-0.05	0.08	-0.21	0.10	0.51	1.000	-0.1
Angular (R)	0.03	0.09	-0.15	0.21	0.73	1.000	0.0
Precuneus (L)	-0.11	0.07	-0.25	0.03	0.12	1.000	-0.2
Precuneus (R)	-0.01	0.07	-0.15	0.12	0.84	1.000	0.0
Paracentral Lobule (L)	-0.08	0.06	-0.19	0.03	0.17	1.000	-0.2
Paracentral Lobule (R)	0.02	0.07	-0.11	0.15	0.79	1.000	0.0
Caudate (L)	-0.03	0.06	-0.15	0.10	0.69	1.000	0.0
Caudate (R)	-0.04	0.05	-0.14	0.06	0.39	1.000	-0.1
Putamen (L)	0.03	0.05	-0.07	0.13	0.53	1.000	0.1
Putamen (R)	0.04	0.04	-0.05	0.12	0.39	1.000	0.1
Pallidum (L)	0.05	0.04	-0.02	0.12	0.19	1.000	0.2
Pallidum (R)	0.09	0.04	0.02	0.17	0.01	0.818	0.3
Thalamus (L)	0.02	0.05	-0.08	0.12	0.67	1.000	0.1
Thalamus (R)	0.03	0.05	-0.07	0.12	0.56	1.000	0.1
Heschl (L)	0.08	0.06	-0.04	0.19	0.18	1.000	0.2
Heschl (R)	-0.02	0.07	-0.15	0.12	0.80	1.000	0.0
Temporal Sup (L)	0.02	0.06	-0.10	0.13	0.77	1.000	0.0
Temporal Sup (R)	0.00	0.05	-0.10	0.10	0.98	1.000	0.0
Temporal Pole Sup (L)	-0.06	0.06	-0.18	0.06	0.34	1.000	-0.1

Temporal Pole Sup (R)	-0.03	0.07	-0.16	0.09	0.60	1.000	-0.1
Temporal Mid (L)	-0.02	0.04	-0.10	0.07	0.70	1.000	0.0
Temporal Mid (R)	-0.10	0.05	-0.19	0.00	0.06	1.000	-0.2
Temporal Pole Mid (L)	-0.11	0.04	-0.19	-0.03	0.01	0.523	-0.3
Temporal Pole Mid (R)	-0.04	0.04	-0.13	0.05	0.38	1.000	-0.1
Temporal Inf (L)	-0.05	0.04	-0.13	0.03	0.19	1.000	-0.2
Temporal Inf (R)	-0.05	0.05	-0.14	0.05	0.35	1.000	-0.1

Table D. The effect of SSRI (n=32), relative to placebo (n=32), on regional brain responses to fearful faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using linear regression models with age and sex as covariates. The estimate is expressed as the marginal effect of SSRI relative to placebo. Abbreviations: SE = standard error, Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

Estimated model coefficients when regressing whole-brain responses to neutral faces

Region	Estimate	SE	lower	upper	p_{unc}	p_{FWER}	Cohen's d
Precentral (L)	-0.06	0.06	-0.18	0.06	0.31	1.000	-0.1
Precentral (R)	-0.09	0.06	-0.20	0.02	0.12	1.000	-0.2
Frontal Sup (L)	0.00	0.05	-0.11	0.11	0.99	1.000	0.0
Frontal Sup (R)	0.08	0.05	-0.02	0.17	0.12	1.000	0.2
Frontal Sup Orb (L)	0.11	0.05	0.02	0.21	0.02	1.000	0.3
Frontal Sup Orb (R)	0.10	0.06	-0.01	0.22	0.07	1.000	0.2
Frontal Mid (L)	0.05	0.06	-0.06	0.17	0.35	1.000	0.1
Frontal Mid (R)	0.08	0.07	-0.05	0.21	0.21	1.000	0.2
Frontal Mid Orb (L)	0.09	0.09	-0.10	0.27	0.36	1.000	0.1
Frontal Mid Orb (R)	0.11	0.06	-0.02	0.23	0.09	1.000	0.2
Frontal Inf Oper (L)	-0.03	0.06	-0.15	0.09	0.65	1.000	-0.1
Frontal Inf Oper (R)	0.06	0.07	-0.08	0.20	0.40	1.000	0.1
Frontal Inf Tri (L)	-0.04	0.06	-0.16	0.08	0.54	1.000	-0.1
Frontal Inf Tri (R)	0.10	0.06	-0.02	0.23	0.10	1.000	0.2
Frontal Inf Orb (L)	0.13	0.06	0.01	0.25	0.03	1.000	0.3
Frontal Inf Orb (R)	**0.27**	**0.06**	**0.14**	**0.39**	**0.00**	**0.004**	**0.5**
Rolandic Oper (L)	-0.12	0.06	-0.24	0.00	0.05	1.000	-0.2
Rolandic Oper (R)	-0.23	0.07	-0.37	-0.10	0.00	0.055	-0.4
Supp Motor Area (L)	0.00	0.07	-0.13	0.14	0.97	1.000	0.0
Supp Motor Area (R)	-0.03	0.05	-0.13	0.08	0.62	1.000	-0.1
Olfactory (L)	0.17	0.08	0.02	0.32	0.03	1.000	0.3
Olfactory (R)	0.14	0.06	0.02	0.27	0.03	1.000	0.3
Frontal Sup Medial (L)	0.19	0.08	0.02	0.35	0.02	1.000	0.3
Frontal Sup Medial (R)	**0.23**	**0.06**	**0.11**	**0.36**	**0.00**	**0.027**	**0.5**
Rectus (L)	-0.01	0.06	-0.13	0.11	0.86	1.000	0.0
Rectus (R)	0.09	0.05	0.00	0.18	0.05	1.000	0.2
Insula (L)	-0.04	0.05	-0.14	0.07	0.48	1.000	-0.1
Insula (R)	0.02	0.06	-0.10	0.14	0.73	1.000	0.0
Cingulum Ant (L)	0.00	0.08	-0.15	0.15	0.99	1.000	0.0
Cingulum Ant (R)	0.06	0.05	-0.04	0.16	0.23	1.000	0.1
Cingulum Mid (L)	0.01	0.07	-0.13	0.15	0.90	1.000	0.0
Cingulum Mid (R)	-0.04	0.06	-0.15	0.07	0.49	1.000	-0.1
Cingulum Post (L)	0.08	0.08	-0.07	0.23	0.31	1.000	0.1
Cingulum Post (R)	0.09	0.06	-0.03	0.21	0.13	1.000	0.2
Hippocampus (L)	0.06	0.06	-0.05	0.18	0.28	1.000	0.1
Hippocampus (R)	0.02	0.05	-0.07	0.11	0.66	1.000	0.1
ParaHippocampal (L)	0.04	0.05	-0.05	0.14	0.36	1.000	0.1
ParaHippocampal (R)	0.09	0.06	-0.02	0.20	0.12	1.000	0.2

Amygdala (L)	0.07	0.10	-0.12	0.26	0.46	1.000	0.1
Amygdala (R)	0.14	0.07	0.00	0.29	0.05	1.000	0.2
Calcarine (L)	-0.07	0.09	-0.26	0.12	0.46	1.000	-0.1
Calcarine (R)	-0.11	0.07	-0.25	0.03	0.14	1.000	-0.2
Cuneus (L)	0.03	0.09	-0.14	0.20	0.74	1.000	0.0
Cuneus (R)	0.04	0.07	-0.10	0.17	0.57	1.000	0.1
Lingual (L)	-0.08	0.08	-0.24	0.08	0.35	1.000	-0.1
Lingual (R)	-0.10	0.09	-0.27	0.07	0.25	1.000	-0.1
Occipital Sup (L)	-0.02	0.06	-0.15	0.10	0.74	1.000	0.0
Occipital Sup (R)	0.06	0.06	-0.06	0.18	0.34	1.000	0.1
Occipital Mid (L)	0.04	0.05	-0.07	0.15	0.45	1.000	0.1
Occipital Mid (R)	0.07	0.06	-0.05	0.18	0.26	1.000	0.1
Occipital Inf (L)	-0.03	0.08	-0.18	0.12	0.66	1.000	-0.1
Occipital Inf (R)	0.07	0.10	-0.12	0.26	0.45	1.000	0.1
Fusiform (L)	0.09	0.07	-0.04	0.22	0.19	1.000	0.2
Fusiform (R)	-0.01	0.07	-0.16	0.13	0.85	1.000	0.0
Postcentral (L)	-0.15	0.07	-0.28	-0.02	0.02	1.000	-0.3
Postcentral (R)	-0.11	0.06	-0.23	0.00	0.06	1.000	-0.2
Parietal Sup (L)	-0.01	0.08	-0.18	0.15	0.87	1.000	0.0
Parietal Sup (R)	-0.11	0.09	-0.28	0.07	0.22	1.000	-0.2
Parietal Inf (L)	-0.02	0.07	-0.16	0.12	0.81	1.000	0.0
Parietal Inf (R)	0.05	0.09	-0.12	0.22	0.58	1.000	0.1
SupraMarginal (L)	-0.04	0.06	-0.16	0.09	0.54	1.000	-0.1
SupraMarginal (R)	-0.06	0.07	-0.19	0.08	0.40	1.000	-0.1
Angular (L)	0.14	0.07	0.00	0.27	0.05	1.000	0.2
Angular (R)	0.17	0.07	0.02	0.31	0.02	1.000	0.3
Precuneus (L)	0.04	0.08	-0.12	0.19	0.65	1.000	0.1
Precuneus (R)	-0.01	0.07	-0.15	0.13	0.88	1.000	0.0
Paracentral Lobule (L)	0.04	0.07	-0.10	0.19	0.54	1.000	0.1
Paracentral Lobule (R)	0.02	0.07	-0.11	0.14	0.82	1.000	0.0
Caudate (L)	0.05	0.07	-0.08	0.18	0.47	1.000	0.1
Caudate (R)	0.09	0.06	-0.02	0.20	0.11	1.000	0.2
Putamen (L)	0.01	0.05	-0.09	0.12	0.81	1.000	0.0
Putamen (R)	-0.02	0.05	-0.12	0.07	0.64	1.000	-0.1
Pallidum (L)	-0.04	0.04	-0.12	0.05	0.39	1.000	-0.1
Pallidum (R)	-0.06	0.04	-0.13	0.02	0.16	1.000	-0.2
Thalamus (L)	-0.02	0.05	-0.12	0.09	0.73	1.000	0.0
Thalamus (R)	0.02	0.05	-0.08	0.11	0.73	1.000	0.0
Heschl (L)	-0.17	0.07	-0.30	-0.04	0.01	1.000	-0.3
Heschl (R)	-0.07	0.08	-0.24	0.09	0.37	1.000	-0.1
Temporal Sup (L)	-0.04	0.08	-0.20	0.11	0.60	1.000	-0.1
Temporal Sup (R)	-0.01	0.06	-0.14	0.11	0.85	1.000	0.0

Temporal Pole Sup (L)	0.04	0.07	-0.11	0.18	0.59	1.000	0.1
Temporal Pole Sup (R)	0.00	0.07	-0.13	0.14	0.95	1.000	0.0
Temporal Mid (L)	0.03	0.05	-0.07	0.13	0.55	1.000	0.1
Temporal Mid (R)	0.13	0.05	0.02	0.23	0.02	1.000	0.3
Temporal Pole Mid (L)	0.06	0.04	-0.02	0.15	0.14	1.000	0.2
Temporal Pole Mid (R)	0.02	0.04	-0.06	0.10	0.58	1.000	0.1
Temporal Inf (L)	0.05	0.05	-0.04	0.14	0.27	1.000	0.1
Temporal Inf (R)	0.01	0.05	-0.10	0.12	0.84	1.000	0.0

Table E. The effect of SSRI (n=32), relative to placebo (n=32), on regional brain responses to neutral faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using linear regression models with age and sex as covariates. The estimate is expressed as the marginal effect of SSRI relative to placebo. Abbreviations: SE = standard error, Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

Paper 3

Amygdala response to emotional faces following acute administration of psilocybin in healthy individuals

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Abstract

Background: The serotonergic psychedelic psilocybin acutely induces changes in emotional states. However, it remains unresolved whether psilocybin modulates emotion processing in the amygdala, a brain region critically involved in processing emotions. Aims and methods: Using functional magnetic resonance imaging (fMRI), we examine in 26 healthy individuals whether amygdala responses to angry, fearful and neutral faces differ between acute exposure to psilocybin and baseline without drug. We also evaluate whether plasma psilocin levels (PPL) and subjective drug intensity (SDI) during psilocybin are related to amygdala responses to emotional faces. Results: We find that amygdala response to angry faces is significantly reduced during exposure to psilocybin as compared to baseline (mean difference = -0.51, P_{FWER}=0.04), while no significant changes in amygdala responses to fearful or neutral faces were observed. We also find that amygdala response to fearful faces is significantly negatively associated with SDI (slope = -0.18, P_{FWER}=0.02), while no significant association with PPL was observed. Conclusion: Our findings indicate that psilocybin attenuates amygdala reactivity to angry faces and that a more intense subjective psilocybin response (SDI) is associated with attenuated amygdala reactivity to fearful faces, in accordance with previously reported results. Future studies should investigate whether exposure to psilocybin acutely changes emotion processing in individuals with depression and whether such changes are related to therapeutic outcomes.

1. Introduction

Psilocybin is a psychedelic compound gaining renewed attention as a therapeutic to treat various psychiatric conditions when combined with psychological support (Studerus *et al.*, 2011; Grob *et al.*, 2011; Bogenschutz *et al.*, 2015; Moreno *et al.*, 2015; Bogenschutz *et al.*, 2022; Ross *et al.*, 2016; Griffiths *et al.*, 2016; Carhart-Harris *et al.*, 2018, 2021; Garcia-Romeu *et al.*, 2019; Johnson *et al.*, 2019; Anderson *et al.*, 2020; Vollenweider and Preller, 2020; Davis *et al.*, 2021). Once ingested, psilocybin is metabolised into psilocin, which dose-dependently induces psychoactive effects by stimulating brain serotonin (5-HT) 2A receptors (5-HT_{2A}R) (Vollenweider *et al.*, 1998; Passie *et al.*, 2002; Madsen *et al.*, 2019) over the course of four to eight hours (Stenbæk *et al.*, 2020). The unfolding of the psychoactive effects can be described in three successive phases: ascent, peak and descent (Stenbæk *et al.*, 2020), as measured with subjective drug intensity (SDI). SDI is highly correlated with plasma psilocin levels (PPL), and both SDI and PPL map on to 5-HT_{2A}R occupancy (Madsen *et al.*, 2019). Together, SDI and PPL represent two feasible metrics of acute behavioural and neurobiological effects of psilocybin (Madsen *et al.*, 2019; Stenbæk *et al.*, 2020; Madsen and Knudsen, 2021).

A medium to high dose (i.e., >0.2 mg/kg) of psilocybin can dose-dependently induce profound changes in consciousness, such as changes in perception and cognition, sense of self, time and space and emotional state (Studerus *et al.*, 2011; Kometer *et al.*, 2012; Vollenweider and Preller, 2020). According to retrospective self-reports from patients and healthy individuals, psilocybin can occasion emotions such as deepfelt love and peacefulness (Griffiths *et al.*, 2006), emotional breakthroughs (Griffiths *et al.*, 2006; Roseman, Nutt and Carhart-Harris, 2018; Roseman *et al.*, 2019), emotional acceptance rather than avoidance (Watts *et al.*, 2017), increased emotional empathy (Pokorny *et al.*, 2017), as well as re-experiencing of autobiographical emotional memories (Carhart-Harris *et al.*, 2012; Healy, 2021). Thus, from self-reports it seems clear that psilocybin has the capacity to acutely change one's emotional

state, but it remains unclear in what way psilocybin may modulate processing of emotions in the brain. Investigating the neural correlates of emotional processing under the influence of psilocybin using functional Magnetic Resonance Imaging (fMRI), a high-resolution in-vivo brain imaging method, may contribute to our understanding of how the brain processes emotional stimuli during psilocybin intervention.

Emotion processing is a cognitive function essential for successfully navigating a social world which is involved in mental health (Elliott *et al.*, 2011). The amygdala is a neural structure critical for emotion processing (LeDoux, 2000; Davis and Whalen, 2001; Rhodes *et al.*, 2007; Janak *et al.*, 2015). In particular, the amygdala is important for detecting the salience and social relevance of information in the environment (Sander, Grafman and Zalla, 2003; Pessoa, 2010), especially for negative and threat-related information such as fearful or angry facial expressions (Davis and Whalen, 2001; Haxby, Hoffman and Gobbini, 2002; Fusar-Poli *et al.*, 2009). Amygdala reactivity to threat-related faces is modulated by brain 5-HT signalling (Robinson *et al.*, 2013; Fisher *et al.*, 2015; Bocchio *et al.*, 2016), and associated with 5-HT_{2A}R levels (Fisher *et al.*, 2009, 2011; Hornboll *et al.*, 2015). Only one fMRI study has investigated emotional processing in the amygdala during psilocybin exposure compared to placebo, reporting that the amygdala response was lower when viewing negative and neutral scenes (Kraehenmann *et al.*, 2015). Facial expressions are argued to be clear and pronounced sources of valanced emotional information (Carrera-Levillain and Fernandez-Dols, 1994), representing a prototypic stimuli to investigate emotional processing (Said, Haxby and Todorov, 2011; Cowen *et al.*, 2019). A previous study using lysergic acid diethylamide (LSD), another potent psychedelic with a high affinity for the 5-HT_{2A}R (Nichols, 2016), found reduced processing of negative faces relative to neutral faces in the amygdala following acute administration of LSD (Mueller *et al.*, 2017). It remains to be investigated how psilocybin modulates amygdala reactivity to facial expressions.

Here, we evaluate the acute effects of a medium-high dose of psilocybin on the amygdala response to angry, fearful and neutral faces. We also examine whether the amygdala response to angry, fearful and neutral faces is related to PPL and SDI. We hypothesised that 1) amygdala response to angry and fearful faces but not to neutral faces is reduced during acute psilocybin intervention compared to without the drug, 2) PPL and SDI are negatively associated with the amygdala response to angry and fearful faces, but not to neutral faces.

2. Methods and materials

2.1. Participants

Data were collected from 28 healthy individuals in the study. Data presented here was part of a single-blind cross-over study design wherein participants received either a single dose of psilocybin or ketanserin (5-HT_{2A}R antagonist) on two separate intervention days. Here, we only present procedures and results regarding the effects of the psilocybin intervention. Participants were recruited from a list of volunteers interested in participating in neuroscientific psychedelic research. Before obtaining their written informed consent, the participants were informed about the study, including side effects and risks. All participants underwent a screening procedure comprising a screening for neurological or significant somatic illness and a screening interview for present or previous psychiatric conditions using a Danish translation of the Mini-International Neuropsychiatric Interview, version 6.0.0. (Sheehan *et al.*, 1998). The exclusion criteria were: 1) past or current primary psychiatric disease (DSM axis 1 or WHO ICD-10 diagnostic classifications) or in first-degree relatives, 2) past or current neurological disease or significant somatic disease, 3) past or current substance or drug abuse, 4) non-fluent Danish language skills, 5) vision or hearing impairment, 6) past or current learning disability, 7) intake of drugs suspected to influence test results, 8) allergy to test drugs, 9) intake of QT-prolonging medication or electrocardiogram (ECG) results indicative of heart disease, 10)

blood donation less than three months before project participation, 11) bodyweight lower than 50 kg, 12) low plasma ferritin levels (< 12 μg/L), 13) pregnancy or breastfeeding, 14) MRI contraindications, 15) significant exposure to radiation within the past year.

The ethics committee approved this study for the Capital Region of Copenhagen (journal identifier: H-16028698, amendments: 56023, 56967, 57974, 59673, 60437, 62255) and the Danish Medicines Agency (EudraCT identifier: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295). The study was conducted according to the World Medical Association Declaration of Helsinki and to the Committee on Publication Ethics' International Standards for Authors.

2.2 Experimental design

At baseline, all participants completed the emotional faces paradigm during blood oxygen level dependent (BOLD) fMRI (see detailed description of study outcomes below). We also assessed the participants' baseline intelligence quotient (IQ) using the Reynolds Intellectual Screening Test (Reynolds and Kamphaus, 2011), body mass index (BMI), mood using the Major Depression Inventory (MDI) (range from 0–50, where > 21 indicates a depressed mood) (Bech *et al.*, 2015), sleep quality using the Pittsburgh Sleep Quality Index (PSQI) (range: 0–21, where 45 indicates sleep disturbances) (Buysse *et al.*, 1989) and stress level using the Cohen's Perceived Stress Scale (PSS) (range: 0–40, no cut-off adapted to indicate stress).

Before the day of the psilocybin intervention, participants met with two assisting psychological staff members to prepare for the psilocybin experience. The same staff members facilitated the participant's psilocybin experience with psychological support during the intervention. On the psilocybin intervention day, before dosing, we obtained urine samples from the participants testing for common drug of abuse (Rapid Response, BTNX Inc., Markham, Canada) and the PSQI, MDI and PSS were completed. In a private room next to the

scanner, the participants were given oral psilocybin in gelatine capsules, with a glass of water (Mean ± SD, range: 0.26 ± 0.04, range: 0.19-0.30 mg/kg oral psilocybin). Around three hours after drug administration, SDI and PPL were obtained and the emotional faces paradigm was repeated during BOLD fMRI. When the psychoactive effects had decreased (i.e., at the end of the day), participants completed the 11-dimension Altered States of Consciousness questionnaire (Studerus, Gamma and Vollenweider, 2010) (11D-ASC). The day after the intervention, participants met with the psychological staff members to facilitate integration of the experience. The median number of days between the baseline BOLD fMRI scan and psilocybin intervention was 33 days (range = 5 to 152 days).

2.3 Study outcomes

2.3.1. Emotional faces paradigm during BOLD fMRI

In the emotional faces paradigm, participants were presented with a trio of faces expressing the same emotions on a screen: one target face in the top centre and two potential matching targets on the bottom left and right sides. Participants were instructed to match the target faces as fast and accurately as possible using a hand controller pressing the left or right key, ensuring attention to the stimuli. The entire paradigm consisted of four blocks of emotional faces (i.e., fearful faces, angry faces, surprised faces and neutral faces presented in random order across four versions) interleaved by five control blocks consisting of geometric shapes (i.e., blocks of circles and vertical and horizontal ellipses), see **Figure 4** in the supplementary material. Within each block, six face trios were displayed for four seconds, each interleaved by a fixation cross displayed with variable time intervals (i.e., two, four or six seconds) to minimise expectancy effects and habituation while maximising amygdala response throughout the paradigm. The geometric shape block followed the same procedure, but here, participants were initially instructed to "match shapes" while the fixation cross was displayed with a fixed time interval

of two seconds, interleaving each of the six shape trios. The paradigm took a total of 6.5 minutes to complete. In this study, we only attended to negative facial expressions, that is, anger and fear, and neutral faces, as well as all geometric shapes collapsed, which were used to contrast each facial expression (see section 2.4.1. for details). We also recorded participants' accuracy and reaction times. The paradigm was presented using E-Prime (Psychological Software Tools, Pittsburgh, USA), through which we recorded accuracy and reaction time.

2.3.2. Magnetic resonance imaging acquisition parameters

Participants were scanned on one of two 3T Siemens Magnetom Prisma scanners (Erlangen, Germany) using either a 64-channel head/neck coil (MRI_1) or a 32-channel head coil (MRI_2) (see participant distribution across scanners in **Table 1**). All participants completed their two MRI scans on the same scanner. High-resolution, whole-brain, T1-weighted MPRAGE structural scans were acquired in all participants (MRI_1: inversion time = 900 ms, repetition time = 1900 ms, echo time = 2.58 ms, flip angle = 9°, in-plane matrix = 256x256 mm, in-plane resolution = 0.9x0.9 mm, 224 slices, slice thickness = 0.9 mm; MRI_2: inversion time = 920 ms, repetition time = 1810 ms, echo time = 2.41 ms, flip angle = 9°, in-plane matrix = 288x288 mm, in-plane resolution = 0.8x0.8 mm, 224 slices, slice thickness = 0.8 mm). BOLD fMRI scans were acquired during the emotional faces paradigm using a T2*-weighted gradient echo-planar imaging (EPI) sequence (MRI_1: TR = 2000 ms, TE = 30 ms, flip angle = 90^o, in-plane matrix = 64x64 mm, in-plane resolution=3.6x3.6 mm, 32 slices (interleaved, bottom-up) slice thickness = 3.0 mm, gap between slices= 0.75 mm, number of volumes acquired = 195; MRI_2: TR = 800 ms, TE = 37 ms, flip angle = 52^o, in-plane matrix = 104x104 mm, in-plane resolution = 2x2 mm, 72 slices (interleaved, bottom-up) slice thickness = 2 mm, no gap, number of volumes acquired = 488, multi-band acceleration factor = 8).

2.3.3. Plasma psilocin levels and subjective drug effects during psilocybin intervention

Immediately before completing the emotional faces paradigm, we asked participants to verbally rate their SDI ("How intense is your experience right now?") on a Likert scale from 0-10 (i.e., from 0 = "not at all intense" to 10 = "very intense"). Similarly, we acquired a blood sample from an intravenous catheter to measure free unconjugated psilocin (μg/L) plasma level.

2.4. Missing data

Some individuals had missing or invalid data for the emotional faces paradigm. This included two participants' data at baseline (n=1 due to poor vision; n=1 due to claustrophobia at baseline only) and eight participants' data at the psilocybin intervention (n=1 due to poor vision; n=2 due to technical errors; n=4 due to nausea and/or challenges regarding the psychological state; n=1 participant reported closed eyes during the paradigm). Therefore, data from 26 participants were included in the final analyses. One participant had a 50% accuracy (chance) on the emotional faces paradigm during psilocybin intervention. We included data from this participant as it appeared this person had processed the emotional faces, despite not performing the task as instructed. For PPL, two participants had missing data just before the emotional faces paradigm; in those cases, we used the PPL measure acquired immediately after the paradigm.

2.5. Data analysis

2.4.1. Pre-processing and data analyses of fMRI data

Functional images were pre-processed and analysed in SPM12. Single-subject functional volumes were corrected for slice-timing (MRI_1 data only), unwarped and realigned to a subject-

specific mean functional image. Functional images were smoothed using a 4 mm FWHM Gaussian filter and kept in subject-space for further analysis. We used the Automatic Anatomical Label (AAL) atlas to define regions of interest (Tzourio-Mazoyer *et al.*, 2002). The AAL atlas was warped into subject-space by co-registering the high-resolution T1-weighted structural image with the functional images. We then normalised the T1-weighted image into Montreal Neurological Institute (MNI) standard space and applied the inverse warping map to the AAL atlas in MNI space (using nearest neighbour interpolation to maintain region labels) to generate an AAL atlas in subject space. The subject-space AAL atlas was resliced to match the voxel dimensions of the functional images. Only the 90 cortical/subcortical regions were included in analyses. We used the artefact detection tool (ART; https://www.nitrc.org/projects/artifact_detect/) to identify individual functional volumes with excess motion (>2mm) and signal variability (>4 SD).

In single-subject general linear models (GLMs), we employed a canonical hemodynamic response function to the smoothed functional images to estimate task-specific BOLD activity (i.e., beta images), including motion parameters and censored volumes. The GLMs were used to generate contrast images for our effects of interest (i.e., angry, fearful and neutral faces versus geometric shapes). For each participant, mean BOLD fMRI response to each face condition was extracted from a bilateral amygdala region of interest (ROI).

2.4.2. Quantification of plasma psilocin level

Plasma psilocin level (PPL) was quantified using ultra-performance liquid chromatography and tandem mass spectrometry (measuring free and unconjugated psilocin), as previously described (Madsen *et al.*, 2019). PPL was reported in µg/L.

2.4.3. Statistical analysis

To evaluate the amygdala response to angry, fearful, and neutral faces during psilocybin intervention compared to baseline (i.e., scan type: baseline vs. psilocybin intervention), we fitted a linear mixed model (LMM) for each emotion type with an unstructured residual covariance pattern using the packages *LMMstar* in R (v. 4.0.3). The LMM accounts for repeated measurements within participants and different residual variances at baseline and during psilocybin intervention. MRI scanner was included as a covariate due to differences in acquisition parameters between MRI_1 and MRI_2, as described in section 2.3.3. Age, sex and censored volumes (accounting for motion) were considered covariates. However, due to our small sample size and lack of evidence for associations between amygdala response and age, sex, and censored volumes, these covariates were excluded from final models. In sensitivity analyses using the same model, we assessed effects of the emotional faces paradigm on whole-brain regions during psilocybin compared to baseline.

To evaluate the associations between amygdala response to angry, fearful, and neutral faces and PPL and SDI, respectively, we fitted LMMs for each emotion, again with an unstructured residual covariance pattern and MRI scanner as a covariate. Two additional mean parameters were introduced in the LMM encoding the association between (i) scan time, i.e., baseline vs. psilocybin, and (ii) mean-centered PPL/SDI with amygdala response. Mean-centered values were computed by setting baseline PPL/SDI values at 0, while the mean PPL/SDI was subtracted from individual PPL/SDI values from the psilocybin MRI. These parameters can be interpreted as (i) the change in amygdala response for a person with mean PPL/SDI and (ii) how the amygdala response changes along with PPL/SDI.

We used LMMs (with an unstructured residual covariance pattern) to examine whether there were differences in accuracy and reaction time for the emotional faces paradigm as well as in censored volumes for scans acquired during baseline compared to during the psilocybin intervention.

P-values are reported both uncorrected (p_{unc}) and corrected for family-wise error rate (P_{FWER}) using the Bonferroni correction method for a family of three tests (i.e., angry, fear and neutral) (Dunn, 1961), as indicated. P-values were considered statistically significant at $P_{FWER} < 0.05$. P-values for tests of differences in accuracy and reaction time across scan sessions were corrected for a family of four tests (i.e., angry, fear, neutral and shapes). For sensitivity whole brain analyses, p-values were corrected for 88 tests, reflecting the number of brain regions examined.

3. Results

3.1. Baseline characteristics

Demographics, psychometrics, PPL and SDI at the time of the emotional faces paradigm and behavioural outcomes are summarised in **Table 1**. MDI scores showed low levels of depressive symptoms at baseline (score range=2-12) and psilocybin intervention (score range=0-11), the PSQI showed an absence of sleep disturbances by cut-off >45 at baseline (score range=2-8) and psilocybin intervention (score range=2-6). The PSS showed low or average stress levels at baseline (score range=1-16) and psilocybin intervention (score range=1-13) (Cohen and Janicki-Deverts, 2012). The participants' mean IQ was slightly higher than the average of 100 in the general population (mean ± SD = 113±5). At baseline, we observed a pronounced response of the amygdala to each of the emotional faces contrasted with geometric shapes (see **Table 1**).

Categorical variables	Frequency	Percent	Frequency	Percent
N	26		20	
Female / male	9 / 17	35% / 65%	9 / 11	45% / 55%
MRI1 / MRI2	12 / 14	46% / 54%	8 / 12	40% / 60%
Continuous variables	**Mean ± SD**	**Median [Min ; Max]**	**Mean ± SD**	**Median [Min ; Max]**
Age (years)	33 ± 8	31 [23 ; 58]	32 ± 8	29 [23 ; 58]
Body mass index (kg/m2)	23 ± 3	23 [19 ; 29]	23 ± 3	23 [19 ; 29]
Psychometrics	**Mean ± SD**	**Median [Min ; Max]**	**Mean ± SD**	**Median [Min ; Max]**
IQ	113 ± 5	112 [107 ; 125]	-	-
Percieved stress scale	9 ± 4	9 [1 ; 16]	8 ± 3	8 [1 ; 13]
Depressive symptoms	5 ± 3	4 [2 ; 12]	5 ± 3	5 [0 ; 11]
Sleep quality	4 ± 2	4 [2 ; 8]	4 ± 2	3 [2 ; 6]
Altered state of consciousness	-	-	38 ± 15	41 [9 ; 59]
Emotional face paradigm	**Mean ± SD**	**Median [Min ; Max]**	**Mean ± SD**	**Median [Min ; Max]**
Amygdala response to angry faces	0.56 ± 0.55	0.47 [-0.29 ; 1.87]	0.05 ± 0.59	0.07 [-1.30 ; 0.93]
Amygdala response to fearful faces	0.31 ± 0.40	0.30 [-0.31 ; 1.1]	0.19 ± 0.54	0.13 [-0.89 ; 1.3]
Amygdala response to neutral faces	0.26 ± 0.47	0.13 [-0.46 ; 1.5]	0.28 ± 0.56	0.17 [-0.70 ; 1.6]
Accuracy for angry faces	0.99 ± 0.03	1 [0.83 ; 1]	0.96 ± 0.12	1 [0.5 ; 1]
Accuracy for fearful faces	1.00 ± 0.00	1 [1 ; 1]	0.97 ± 0.12	1 [0.5 ; 1]
Accuracy for neutral faces	0.98 ± 0.05	1 [0.83 ; 1]	0.94 ± 0.15	1 [0.5 ; 1]
Accuracy for shapes	0.97 ± 0.03	0.97 [0.9 ; 1]	0.96 ± 0.11	1 [0.5 ; 1]
Reaction time for angry faces (ms)	1053 ± 190	999 [780 ; 1483]	1339 ± 322	1319 [899 ; 1824]
Reaction time for fearful faces (ms)	970 ± 192	936 [611 ; 1365]	1213 ± 321	1181 [831 ; 1909]
Reaction time for neutral faces (ms)	1000 ± 163	993 [678 ; 1379]	1399 ± 437	1312 [697 ; 2293]
Reaction time for shapes (ms)	936 ± 113	942 [708 ; 1258]	1175 ± 313	1106 [606 ; 2101]
Timing of paradigm (min. since drug administration)	-	-	169 ± 24	182 [131 ; 207]
Plasma psilocin level in µg/L	-	-	12.0 ± 4.2	11.4 [4.9 ; 20.9]
Subjective drug intensity	-	-	7.15 ± 2.5	8 [0 ; 10]

Table 1. Descriptive information for the study sample. Outcomes: IQ and body mass index were measured upon inclusion. On both MR scan days before initiating scanning, we measured the participants' perceived stress scale with Cohen's Perceived Stress Scale, major depressive disorder with Major Depression Inventory and sleep quality with Pittsburgh Sleep Quality Index. Amygdala responses to faces is constrasted to geometric shapes. At the end of the psilocbyin intervnetion day, we measuerd the participants' altered state of consciousness with the 11-dimension Altered States of Consciousness questionnaire. Abbreviations: ms = milliseconds, SD = standard deviation, IQ= intelligence quotient, mcg/L = microgram per liter.

3.2. Change in amygdala and behavioural response to the emotional faces paradigm

Figure 1 displays images of the average amygdala response to each emotion and **Figure 2** displays individual and average amygdala responses adjusting for effect of scanner. Estimated mean parameters are reported in **Table 2**. The amygdala response to angry faces was significantly decreased during psilocybin intervention compared to baseline (mean difference [95% CI]: -0.51 [-0.91; -0.12], $p_{FWER} = 0.04$). The amygdala response to fearful faces was numerically decreased during psilocybin intervention, but this effect was not statistically significant (mean difference [95% CI]: -0.18 [-0.47; 0.11], $p_{FWER} = 0.63$. Amygdala response to neutral faces was only slightly numerically increased during psilocybin intervention and was not statistically significant (mean difference [95% CI]: 0.02 [-0.27; 0.30], $p_{FWER} = 1.00$).

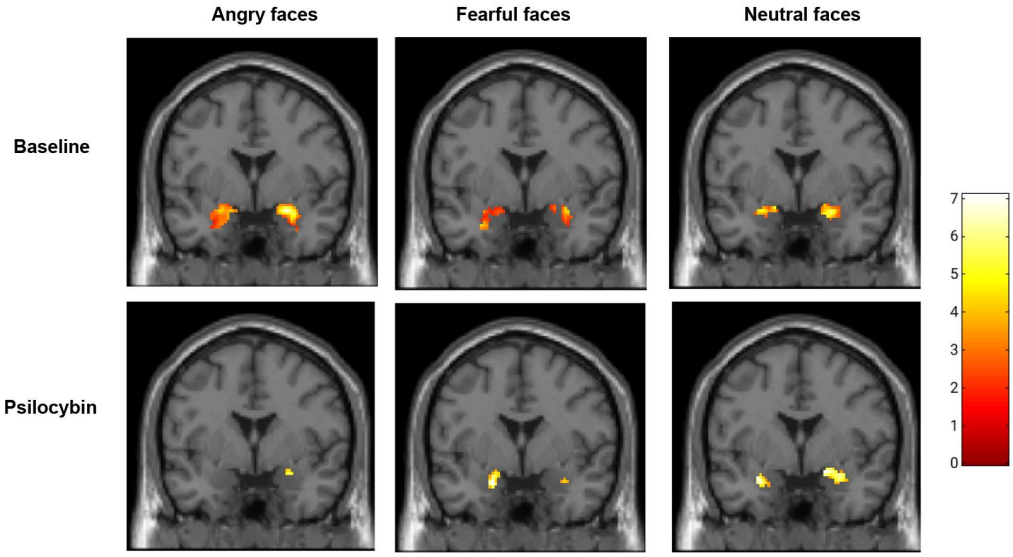

Figure 1. Illustration of coronal brain slices in standard space for the whole sample (baseline, n=26; psilocybin, n=20) during the presentation of the emotional faces paradigm during BOLD fMRI. Coloured areas represent a pronounced response in the amygdala to angry, fearful and neutral faces contrasted to geometric shapes (Punc < 0.05), during baseline and psilocybin intervention. SPM y coordinates across baseline and psilocybin were 0.0 for all emotions. The Colour bar indicates t-scores.

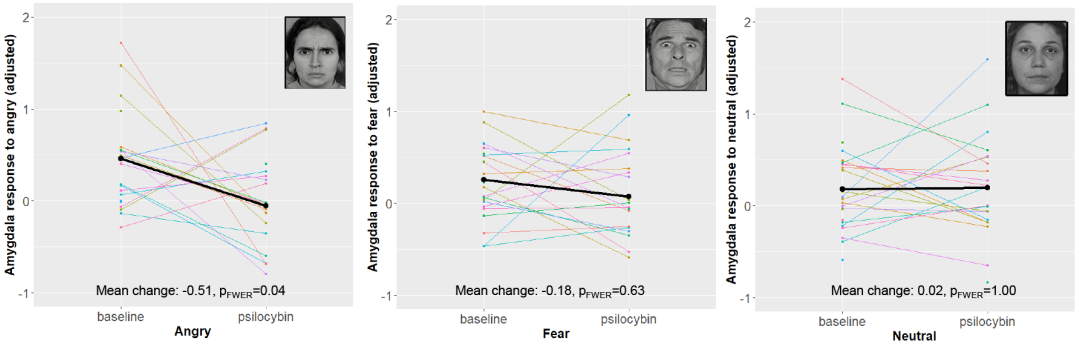

Figure 2. Plots of the partial residuals for the amygdala response (corrected for scanner) to angry faces (left), fearful faces (middle) and neutral faces (right), contrasted to geometric shapes at baseline (n=26) and during psilocybin intervention (n=20). Examples of face stimuli are portrayed in each corresponding plot. The black dots represent the means and coloured lines represent individual values. To evaluate emotional amygdala response during psilocybin compared to baseline, we used a linear mixed model for each emotion, which showed (mean difference [CI], p_{FWER}): Angry: -0.51 [-0.91; -0.12], p_{FWER}=0.04; fear: -0.18 [-0.47; 0.11], p_{FWER} = 0.63; neutral: 0.02 [-0.27; 0.30], p_{FWER} = 1.00.

The accuracy for the emotional faces paradigm task did not differ between exposure to psilocybin and baseline (angry: $p_{FWER} = 0.34$; fearful: $p_{FWER} = 0.98$; neutral: $p_{FWER} = 0.39$; shapes: $p_{FWER} = 0.96$). Reaction time was numerically slower across all stimuli types during exposure to psilocybin, statistically different for angry and neutral faces (mean difference [95% CI], p_{FWER}: angry: 273 ms [149 ; 398], $p_{FWER} < 0.001$; neutral: 370 ms [138 ; 602], $p_{FWER} = 0.01$), but not for fearful faces and shapes (mean difference [95% CI]: fearful: 182 ms [-11 ; 376], $p_{FWER} = 0.25$; shapes: 192 ms [13 ; 372, $p_{FWER} = 0.15$).

Change in amygdala response to angry faces during psilocybin compared to baseline						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.46	0.13	< 0.001		0.53	1.74
Psilocybin	-0.51	0.19	0.013	0.040	-0.91	-0.12
MRI	0.15	0.13	0.256	0.769	-0.12	0.43
Change in amygdala response to fearful faces during psilocybin compared to baseline						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.26	0.11	0.026		0.03	0.48
Psilocybin	-0.18	0.14	0.209	0.628	-0.47	0.11
MRI	0.15	0.14	0.288	0.862	-0.14	0.44
Change in amygdala response to neutral faces during psilocybin compared to baseline						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.18	0.13	0.175		-0.09	0.45
Psilocybin	0.02	0.14	0.911	1.000	-0.27	0.30
MRI	0.13	0.17	0.448	1.000	-0.22	0.48

Table 2. The effect of the psilocybin intervention (n=20), relative to baseline (n=26), on amygdala response to angry, fearfull and nautral faces (each contrasted with geometric shapes) during BOLD fMRI, was asseded using mixed linear effect models. MRI scanner type was included as covariate and MRI is the marginal effects of MRI1 relative to MRI2. Estimate is the unstandardised beta. Abbreviations: SE B = standard error for unstandardised beta, P = unadjusted significance level, 95% CI = 95% confidence interval, punc = uncorrected p-values. All p-values were corrected for family-wise multiple testing using the Bonferroni method (i.e. all p-values multiplied by three), displayed under PFWER.

3.3. Associations between amygdala response, plasma psilocin level and subjective drug intensity

We found no evidence of a dose-response effect of PPL on amygdala response to angry faces (p_{FWER}=0.93), fearful faces (p_{FWER}=0.88) or neutral faces (p_{FWER}=0.23). We did see a significant negative association between SDI and amygdala response to fearful faces (slope [95% CI]: -0.13 [-0.22; -0.04], p_{FWER} = 0.02), see **Figure 3** displaying the association between SDI/PPL and amygdala response to emotional faces and see **Table B** in the supplementary material for the estimates of the LMM. We did not find evidence for an intensity-response effect for angry (p_{FWER}=0.71) or neutral faces (p_{FWER}=0.63), see **Figure 3** (second row, first and third column).

3.4. Sensitivity analysis: change in whole-brain response to the emotional faces paradigm

Sensitivity analyses of whole-brain responses to angry and fearful faces, showed that almost all 88 regional brain responses were numerically reduced during acute effects of psilocybin compared to baseline, but no effects were statistically significant after correction for multiple testing (**Table C and Table D** in supplementary material). For neutral faces, most brain regions were unchanged or numerically increased from baseline to psilocybin, but no effects of increase were statistically significant after controlling for multiple testing (**Table E** in the supplementary material).

3.5. Difference in censored volumes

We found that there were significantly more censored volumes for scans acquired during psilocybin compared to baseline (mean difference in % of volumes censored [95% CI]: 7.5 [3 ; 11], p_{unc} <.001).

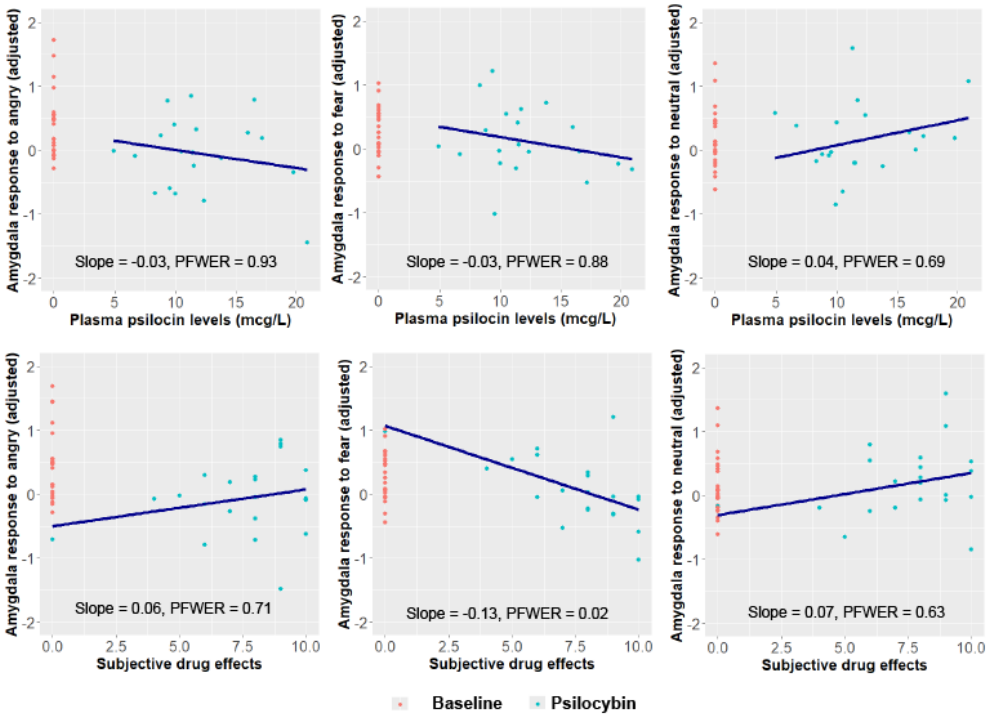

Figure 3. Plots of the amygdala response to angry, fearful, and neutral faces (contrasted to geometric shapes) by plasma psilocin levels (PPL)/subjective drug intensity (SDI). Associations were estimated using linear mixed effects models with MRI scanner as a covariate. In the LMM, two additional mean parameters were introduced, encoding the association between (i) scan time, i.e., baseline vs psilocybin, and (ii) mean-centred PPL/SDI with amygdala response (i.e., baseline PPL/SDI values were set to 0 and, while the mean PPL/SDI was subtracted from individual PPL/SDI values from the psilocybin MRI). These parameters can be interpreted as (i) the change in amygdala response for a person with mean PPL/SDI and (ii) how the amygdala response changes along with PPL/SDI. Red dots correspond to PPL/SDI at baseline, and blue dots correspond to PPL/SDI at psilocybin. The blues lines correspond to the estimated association between amygdala responses and PPL/SDI. PFWER = corrected for family-wise error rate using the Bonferroni method with three tests.

4. Discussion

Our main finding shows that the amygdala response to angry faces was significantly reduced during acute effects of psilocybin compared to baseline. There were no significant changes in

the amygdala responses to fearful or neutral faces although amygdala response to fearful faces was numerically reduced compared to baseline. We observed a significant negative association between SDI and amygdala response to fearful faces, meaning that a more intense psychedelic experience associate with greater reduction in fear processing. Our findings show that neural emotion processing changes during acute effects of psilocybin, findings which bolster previous results from self-reports describing substantial changes in emotional orientation during a psilocybin experience.

As we hypothesised, the amygdala response to angry faces was significantly reduced during psilocybin intervention, whereas amygdala responses to fearful and neutral faces were not statistically significantly affected. We did find, however, that the mean amygdala response to fearful faces was numerically reduced which was not the case for neutral faces. Our result partially aligns with a previous fMRI study investigating amygdala responses to negative and neutral scenes during psilocybin intervention (Kraehenmann *et al.*, 2015). This study found that amygdala responses to both negative and neutral scenes (e.g., neutral pictures of humans, animals and activities of daily living contrasted to shapes) during psilocybin intervention were significantly lower compared to placebo (Kraehenmann *et al.*, 2015). Neutral stimuli can be emotionally ambiguous and hence the difference between Kraehenmann *et al*. and our results may reflect divergence in the type of neutral stimuli used in the studies. Another fMRI study investigated amygdala processing of emotional faces following acute administration LSD (Mueller *et al.*, 2017) and found that, LSD significantly reduced amygdala responses to fearful faces relative to neutral faces, as compared to placebo. In sum, the attenuation of amygdala responses to angry and fearful faces and negative scenes suggests a reduced sensitivity or attribution of salience to negative stimuli during psilocybin intervention which aligns with previously reported results from studies using cognitive testing (Kometer *et al.*, 2012), EEG (Schmidt *et al.*, 2013) and self-report questionnaires (Studerus *et al.*, 2011).

Taken together, reduced processing of negative emotions seems to be a consistent effect of psilocybin intervention across all current studies.

Our post-hoc analyses revealed that there was a general numeric reduction of response to angry and fearful faces in almost all brain regions during psilocybin, while responses to neutral faces were generally similar or numerically increased during psilocybin compared to baseline. Although none of these effects were statistically significant after controlling for multiple testing, these results point to an overall reduced brain response to negative emotions during exposure to psilocybin. We speculate that the lack of statistical significance might be due to properties of the emotional faces paradigm as a measurement tool, including unsatisfactory signal-to-noise ratio resulting in noisy estimates of neural activity during emotional processing, in line with previous reports of poor test-retest reliability of the emotional faces paradigm (Plichta *et al.*, 2012). Future studies evaluating effects of psychedelics on emotional processing should consider applying or developing paradigms with a better signal-to-noise ratio to more accurately estimate neural activity during emotional processing.

As hypothesised, we found a negative association between SDI and the amygdala response to fearful faces. In line with our results, a previous LSD study of emotional processing reported that higher subjective drug effects were associated with reduced amygdala response to fearful faces (Mueller *et al.*, 2017). Previous studies have found that SDI during acute effects of psilocybin is significantly associated with resting-state brain network integrity and segregation (Madsen *et al.*, 2021), baseline 5-HT_{2A}R (Stenbæk *et al.*, 2020) and 5-HT_{2A}R psilocin occupancy (Madsen *et al.*, 2019). Our current observations reinforce that SDI is an informative, yet simple and feasible tool with minimal disruption to the participants' psychedelic experience, which can be used to obtain a measure of subjective effects in real time during psychedelic exposure.

4.1. Methodological considerations and limitations

For the emotional faces task, we observed a pronounced amygdala response to the task (i.e., to each emotion of interest) at baseline, confirming that the task induced the anticipated neural response. The amygdala responses were comparable to other healthy individuals in the same environment (Fisher *et al.*, 2022). Participants generally completed the task with a high degree of accuracy across all task conditions and scan times, suggesting that the reduced amygdala response is not a result of less engagement in the task during psilocybin intervention. We found higher reaction times across emotions during the psilocybin intervention, in line with previous findings (Carter *et al.*, 2005; Kraehenmann *et al.*, 2015; Mueller *et al.*, 2017). Higher reaction times are argued to be due to greater cognitive demands related to the completion of tasks during the psychedelic experience. The time of scanning (~ 3 hours after drug administration), represents the turn of peak to decent phase for most of the participants, which is also reflected in the relatively lower PPL and SDI levels in the current study compared to previously reported levels during peak (Madsen *et al.*, 2019; Stenbæk *et al.*, 2020). Consequently, we might have underestimated the effects of psilocybin on emotional face processing, which may be of greater magnitude during peak as seen with other psilocybin-induced neural changes (Madsen *et al.*, 2021).

In our analyses of association between PPL and amygdala responses to emotional faces, we did not observe significant effects for any emotion. However, we used a two-parameter statistical model, which we speculate might have been a too conservative model considering our smaller sample size. Future studies should examine dose-response effects in a placebo-controlled study design to establish that PPL does not modulate emotional amygdala responses.

We had significantly more censored volumes for scans acquired during psilocybin exposure, compared to baseline, in line with previous reports of increased motion during

psilocybin (Madsen *et al.*, 2021). However, effects of motion on amygdala responses were adjusted for in single-subject GLMs and across all our fitted statistical models, we found that number of censored volumes was not significantly associated with amygdala responses.

4.2. Conclusion

As hypothesised, we found that amygdala response to angry faces was significantly reduced but remained unchanged to neutral faces using BOLD fMRI following acute administration of psilocybin in healthy individuals. Consistent with our hypothesis, we also find that amygdala response to fearful faces is negatively association with SDI. This is the first study to establish that psilocybin acutely modulates amygdala response to angry faces and that the amygdala response to fearful faces is associated with subjective drug intensity. Future studies should investigate whether emotion processing is altered in individuals with depression following acute administration of psilocybin, and whether such alterations are related to long-term therapeutic and clinical outcomes.

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6. Declaration of conflicting interests

MKM has received an honorarium as a speaker for Lundbeck Pharma and the Lundbeck Foundation. KHP is currently an employee of Boehringer-Ingelheim GmbH & Co KG. GMK has received honoraria as a consultant for Sanos and as a speaker for Sage-Biogen. DSS has received an honorarium as a speaker for the Lundbeck Foundation. The remaining authors have disclosed that they have no potential conflicts, including financial, consultant, institutional or other relationships, which could lead to bias or conflict of interest.

7. Data availability statement

The data from the study are available upon request from the Cimbi database (www.cimbi.dk/db) (Knudsen *et al.*, 2016).

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SUPPLEMENTARY MATERIAL

Amygdala response to emotional faces following acute administration of psilocybin in healthy individuals

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Assocation between plasma psilocin levels and amygdala response to angry faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.46	0.13	0.001		0.72	0.72
Psilocybin	-0.51	0.20	0.015	0.044	-0.11	-0.11
Plasma psilocin level	-0.03	0.03	0.324	0.928	0.03	0.03
MRI-scanner	0.15	0.14	0.282	0.847	-0.14	0.44
Assocation between plasma psilocin levels and amygdala response to fearful faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.27	0.11	0.018		0.05	0.49
Psilocybin	-0.15	0.14	0.310	0.931	-0.44	0.15
Plasma psilocin level	-0.03	0.03	0.293	0.880	-0.09	0.03
MRI-scanner	0.12	0.14	0.375	1.000	-0.16	0.41
Assocation between plasma psilocin levels and amygdala response to neutral faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.17	0.13	0.220		-0.11	0.44
Psilocybin	-0.01	0.14	0.953	1.000	-0.29	0.28
Plasma psilocin level	0.04	0.03	0.230	0.691	-0.03	0.11
MRI-scanner	0.16	0.18	0.381	1.000	-0.21	0.52

Table A. Effects of plasma psilocybin levels in mcg/L(PPL) (n=20), relative to baseline (n=26), on amygdala response to angry and fearfull faces (each contrasted with geometric shapes) during BOLD fMRI, were asseded using mixed linear effect models. PPL during psilocybin was centered and the effect of PPL on amygdala response is displayed as an increase of 0.001 in PPL. Covariates included intervention type (i..e baseline or psilocybin) and MR-scanner type. MRI is the marginal effects of MRI1 relative to MRI2. Estimate is the unstandardised beta. Abbreviations: SE B = standard error for unstandardised beta, P = unadjusted significance level, 95% CI = 95% confidence interval, punc = uncorrected p-values. All p-values were corrected for family-wise multiple testing using the Bonferroni method (i.e. all p-values multiplied by three), displayed under PFWER.

Association between subjective drug intensity and amygdala response to angry faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.45	0.12	0.001		0.19	0.70
Psilocybin	-0.52	0.19	0.123	0.037	-0.91	-0.12
Subjective drug intensity	0.06	0.05	0.235	0.706	-0.04	0.16
MRI	0.18	0.13	0.179	0.537	-0.09	0.46
Association between subjective drug intensity and amygdala response to fearful faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.27	0.11	0.017		0.05	0.48
Psilocybin	-0.18	0.12	0.155	0.465	-0.43	0.07
Subjective drug intensity	-0.13	0.04	0.006	0.017	-0.22	-0.04
MRI	0.13	0.13	0.335	1.000	-0.14	0.40
Association between subjective drug intensity and amygdala response to neutral faces						
	Estimate	SE B	p_{unc}	p_{FWER}	95% CI	
Constant	0.17	0.13	0.206		-0.10	0.44
Psilocybin	0.02	0.13	0.904	1.000	-0.26	0.30
Subjective drug intensity	0.07	0.05	0.208	0.625	-0.04	0.17
MRI	0.15	0.17	0.407	1.000	-0.21	0.05

Table B. Effects of subjective drug intensities (n=20), relative to baseline (n=26), on amygdala response to angry and fearfull faces (each contrasted with geometric shapes) during BOLD fMRI, were asseded using mixed linear effect models. Covariates included intervention type (i..e baseline or psilocybin) and MRI scanner type. MRI is the marginal effects of MRI1 relative to MRI2. Estimate is the unstandardised beta. Abbreviations: SE B = standard error for unstandardised beta, P = unadjusted significance level, 95% CI = 95% confidence interval, punc = uncorrected p-values. All p-values were corrected for family-wise multiple testing using the Bonferroni method (i.e. all p-values multiplied by three), displayed under PFWER.

Effect of psilocybin in regional brain responses to angry faces

Region	Estimate	lower	upper	p_{unc}	p_{FWER}
Precentral (L)	-0.18	-0.52	0.17	0.301	1.000
Precentral (R)	-0.05	-0.43	0.32	0.778	1.000
Frontal Sup (L)	-0.21	-0.55	0.13	0.213	1.000
Frontal Sup (R)	-0.09	-0.40	0.21	0.535	1.000
Frontal Sup Orb (L)	-0.19	-0.50	0.13	0.230	1.000
Frontal Sup Orb (R)	0.14	-0.15	0.44	0.328	1.000
Frontal Mid (L)	-0.19	-0.57	0.19	0.318	1.000
Frontal Mid (R)	-0.06	-0.44	0.32	0.737	1.000
Frontal Mid Orb (L)	-0.43	-0.89	0.02	0.062	1.000
Frontal Mid Orb (R)	-0.28	-0.68	0.12	0.167	1.000
Frontal Inf Oper (L)	-0.13	-0.44	0.18	0.409	1.000
Frontal Inf Oper (R)	-0.09	-0.49	0.31	0.645	1.000
Frontal Inf Tri (L)	-0.29	-0.68	0.11	0.145	1.000
Frontal Inf Tri (R)	-0.21	-0.57	0.16	0.253	1.000
Frontal Inf Orb (L)	-0.33	-0.66	0.01	0.055	1.000
Frontal Inf Orb (R)	-0.14	-0.52	0.23	0.447	1.000
Rolandic Oper (L)	-0.06	-0.44	0.32	0.760	1.000
Rolandic Oper (R)	0.11	-0.32	0.54	0.615	1.000
Supp Motor Area (L)	-0.11	-0.54	0.32	0.599	1.000
Supp Motor Area (R)	-0.01	-0.38	0.37	0.977	1.000
Olfactory (L)	-0.25	-0.70	0.20	0.259	1.000
Olfactory (R)	-0.25	-0.58	0.08	0.127	1.000
Frontal Sup Medial (L)	-0.36	-0.87	0.15	0.157	1.000
Frontal Sup Medial (R)	-0.38	-0.79	0.03	0.069	1.000
Rectus (L)	**-0.41**	**-0.66**	**-0.15**	**0.003**	**0.288**
Rectus (R)	-0.22	-0.48	0.04	0.097	1.000
Insula (L)	-0.08	-0.37	0.21	0.587	1.000
Insula (R)	-0.07	-0.43	0.29	0.685	1.000
Cingulum Ant (L)	-0.25	-0.62	0.11	0.161	1.000
Cingulum Ant (R)	-0.17	-0.45	0.11	0.214	1.000
Cingulum Mid (L)	-0.18	-0.50	0.14	0.248	1.000
Cingulum Mid (R)	-0.20	-0.49	0.10	0.188	1.000
Cingulum Post (L)	-0.36	-0.79	0.07	0.099	1.000
Cingulum Post (R)	-0.32	-0.66	0.02	0.063	1.000
Hippocampus (L)	-0.27	-0.54	0.00	0.054	1.000
Hippocampus (R)	-0.24	-0.49	0.02	0.065	1.000
ParaHippocampal (L)	**-0.33**	**-0.58**	**-0.07**	**0.014**	**1.000**
ParaHippocampal (R)	**-0.31**	**-0.60**	**-0.02**	**0.038**	**1.000**
Amygdala (L)	**-0.57**	**-0.99**	**-0.14**	**0.012**	**0.979**

Amygdala (R)	**-0.43**	**-0.84**	**-0.03**	**0.036**	**1.000**
Calcarine (L)	-0.08	-0.62	0.47	0.778	1.000
Calcarine (R)	-0.20	-0.62	0.22	0.343	1.000
Cuneus (L)	-0.13	-0.68	0.42	0.622	1.000
Cuneus (R)	-0.26	-0.72	0.19	0.247	1.000
Lingual (L)	-0.23	-0.65	0.18	0.259	1.000
Lingual (R)	-0.25	-0.67	0.18	0.245	1.000
Occipital Sup (L)	-0.17	-0.68	0.35	0.509	1.000
Occipital Sup (R)	-0.16	-0.56	0.24	0.407	1.000
Occipital Mid (L)	-0.20	-0.61	0.22	0.334	1.000
Occipital Mid (R)	-0.31	-0.77	0.16	0.183	1.000
Occipital Inf (L)	-0.41	-0.88	0.07	0.092	1.000
Occipital Inf (R)	**-0.54**	**-1.03**	**-0.06**	**0.030**	**1.000**
Fusiform (L)	**-0.39**	**-0.65**	**-0.12**	**0.006**	**0.488**
Fusiform (R)	**-0.33**	**-0.57**	**-0.08**	**0.012**	**0.979**
Postcentral (L)	-0.10	-0.50	0.29	0.595	1.000
Postcentral (R)	0.07	-0.36	0.51	0.727	1.000
Parietal Sup (L)	-0.24	-0.68	0.20	0.265	1.000
Parietal Sup (R)	-0.18	-0.62	0.26	0.400	1.000
Parietal Inf (L)	-0.17	-0.65	0.31	0.466	1.000
Parietal Inf (R)	-0.06	-0.50	0.38	0.793	1.000
SupraMarginal (L)	-0.04	-0.50	0.42	0.861	1.000
SupraMarginal (R)	0.11	-0.32	0.54	0.613	1.000
Angular (L)	-0.03	-0.57	0.51	0.901	1.000
Angular (R)	-0.19	-0.60	0.23	0.361	1.000
Precuneus (L)	-0.28	-0.72	0.16	0.195	1.000
Precuneus (R)	-0.17	-0.60	0.25	0.405	1.000
Paracentral Lobule (L)	-0.08	-0.50	0.33	0.677	1.000
Paracentral Lobule (R)	-0.03	-0.59	0.54	0.922	1.000
Caudate (L)	-0.27	-0.62	0.08	0.123	1.000
Caudate (R)	-0.28	-0.63	0.08	0.118	1.000
Putamen (L)	-0.10	-0.41	0.20	0.489	1.000
Putamen (R)	-0.10	-0.41	0.21	0.502	1.000
Pallidum (L)	-0.09	-0.36	0.18	0.509	1.000
Pallidum (R)	0.02	-0.24	0.28	0.862	1.000
Thalamus (L)	-0.22	-0.46	0.01	0.063	1.000
Thalamus (R)	-0.15	-0.43	0.12	0.268	1.000
Heschl (L)	-0.26	-0.72	0.21	0.263	1.000
Heschl (R)	-0.24	-0.79	0.32	0.395	1.000
Temporal Sup (L)	-0.15	-0.57	0.26	0.453	1.000
Temporal Sup (R)	-0.10	-0.49	0.28	0.589	1.000
Temporal Pole Sup (L)	**-0.48**	**-0.82**	**-0.15**	**0.006**	**0.546**

Temporal Pole Sup (R)	**-0.37**	**-0.73**	**-0.01**	**0.044**	**1.000**
Temporal Mid (L)	-0.19	-0.49	0.11	0.194	1.000
Temporal Mid (R)	-0.24	-0.57	0.09	0.147	1.000
Temporal Pole Mid (L)	**-0.35**	**-0.61**	**-0.08**	**0.013**	**1.000**
Temporal Pole Mid (R)	**-0.29**	**-0.53**	**-0.06**	**0.017**	**1.000**
Temporal Inf (L)	-0.26	-0.55	0.03	0.072	1.000
Temporal Inf (R)	-0.21	-0.51	0.09	0.157	1.000

Table C. The effect of the psilocybin intervention (n=20), relative to baseline (n=26), on regional brain responses to angry faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using mixed linear effect models. The estimate (psilocybin vs. baseline) is expressed as the marginal effect of psilocybin. Abbreviations: Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

Effect of psilocybin in regional brain responses to fearful faces

Region	Estimate	lower	upper	p_{unc}	p_{FWER}
Precentral (L)	-0.25	-0.60	0.09	0.143	1.000
Precentral (R)	**-0.31**	**-0.58**	**-0.04**	**0.026**	**1.000**
Frontal Sup (L)	-0.22	-0.56	0.11	0.182	1.000
Frontal Sup (R)	-0.15	-0.44	0.15	0.313	1.000
Frontal Sup Orb (L)	-0.18	-0.45	0.08	0.169	1.000
Frontal Sup Orb (R)	-0.11	-0.44	0.22	0.497	1.000
Frontal Mid (L)	-0.16	-0.52	0.21	0.392	1.000
Frontal Mid (R)	-0.24	-0.62	0.13	0.191	1.000
Frontal Mid Orb (L)	**-0.51**	**-0.96**	**-0.07**	**0.025**	**1.000**
Frontal Mid Orb (R)	-0.28	-0.63	0.06	0.102	1.000
Frontal Inf Oper (L)	-0.06	-0.46	0.33	0.743	1.000
Frontal Inf Oper (R)	-0.23	-0.63	0.18	0.264	1.000
Frontal Inf Tri (L)	-0.07	-0.44	0.30	0.681	1.000
Frontal Inf Tri (R)	-0.26	-0.65	0.13	0.179	1.000
Frontal Inf Orb (L)	-0.04	-0.43	0.34	0.814	1.000
Frontal Inf Orb (R)	-0.13	-0.54	0.28	0.514	1.000
Rolandic Oper (L)	-0.20	-0.52	0.12	0.205	1.000
Rolandic Oper (R)	-0.25	-0.60	0.09	0.144	1.000
Supp Motor Area (L)	-0.15	-0.51	0.21	0.391	1.000
Supp Motor Area (R)	-0.15	-0.44	0.15	0.307	1.000
Olfactory (L)	-0.08	-0.48	0.31	0.666	1.000
Olfactory (R)	-0.04	-0.43	0.35	0.835	1.000
Frontal Sup Medial (L)	-0.38	-0.92	0.15	0.153	1.000
Frontal Sup Medial (R)	-0.17	-0.57	0.22	0.376	1.000
Rectus (L)	-0.21	-0.52	0.11	0.187	1.000
Rectus (R)	-0.08	-0.36	0.21	0.579	1.000
Insula (L)	-0.03	-0.33	0.27	0.827	1.000
Insula (R)	-0.09	-0.42	0.23	0.553	1.000
Cingulum Ant (L)	-0.16	-0.56	0.25	0.439	1.000
Cingulum Ant (R)	-0.03	-0.38	0.32	0.869	1.000
Cingulum Mid (L)	**-0.37**	**-0.70**	**-0.05**	**0.026**	**1.000**
Cingulum Mid (R)	-0.23	-0.49	0.03	0.077	1.000
Cingulum Post (L)	**-0.47**	**-0.91**	**-0.03**	**0.036**	**1.000**
Cingulum Post (R)	**-0.31**	**-0.58**	**-0.03**	**0.030**	**1.000**
Hippocampus (L)	-0.10	-0.29	0.10	0.308	1.000
Hippocampus (R)	**-0.24**	**-0.42**	**-0.05**	**0.014**	**1.000**
ParaHippocampal (L)	-0.06	-0.27	0.15	0.560	1.000
ParaHippocampal (R)	-0.08	-0.25	0.09	0.343	1.000
Amygdala (L)	-0.13	-0.45	0.19	0.414	1.000

Amygdala (R)	-0.16	-0.44	0.12	0.251	1.000
Calcarine (L)	-0.60	-1.26	0.07	0.078	1.000
Calcarine (R)	-0.46	-1.02	0.10	0.100	1.000
Cuneus (L)	-0.69	-1.41	0.03	0.060	1.000
Cuneus (R)	-0.67	-1.34	0.00	0.051	1.000
Lingual (L)	-0.48	-0.97	0.01	0.053	1.000
Lingual (R)	-0.56	-1.14	0.01	0.053	1.000
Occipital Sup (L)	-0.73	-1.65	0.19	0.113	1.000
Occipital Sup (R)	-0.66	-1.35	0.03	0.060	1.000
Occipital Mid (L)	**-0.66**	**-1.31**	**-0.01**	**0.047**	**1.000**
Occipital Mid (R)	-0.68	-1.44	0.09	0.079	1.000
Occipital Inf (L)	**-0.86**	**-1.59**	**-0.12**	**0.025**	**1.000**
Occipital Inf (R)	**-1.11**	**-1.83**	**-0.39**	**0.004**	**0.375**
Fusiform (L)	**-0.44**	**-0.78**	**-0.11**	**0.012**	**1.000**
Fusiform (R)	**-0.40**	**-0.72**	**-0.09**	**0.015**	**1.000**
Postcentral (L)	-0.33	-0.70	0.03	0.071	1.000
Postcentral (R)	-0.35	-0.72	0.03	0.067	1.000
Parietal Sup (L)	-0.60	-1.22	0.01	0.053	1.000
Parietal Sup (R)	-0.60	-1.22	0.01	0.055	1.000
Parietal Inf (L)	-0.41	-0.95	0.14	0.137	1.000
Parietal Inf (R)	-0.29	-0.72	0.14	0.176	1.000
SupraMarginal (L)	-0.36	-0.82	0.10	0.119	1.000
SupraMarginal (R)	-0.36	-0.85	0.13	0.140	1.000
Angular (L)	-0.52	-1.18	0.15	0.121	1.000
Angular (R)	-0.51	-1.06	0.05	0.070	1.000
Precuneus (L)	**-0.62**	**-1.13**	**-0.11**	**0.018**	**1.000**
Precuneus (R)	**-0.54**	**-0.96**	**-0.12**	**0.013**	**1.000**
Paracentral Lobule (L)	-0.16	-0.57	0.25	0.427	1.000
Paracentral Lobule (R)	-0.35	-0.82	0.12	0.139	1.000
Caudate (L)	-0.25	-0.62	0.12	0.179	1.000
Caudate (R)	-0.18	-0.51	0.14	0.255	1.000
Putamen (L)	-0.05	-0.29	0.18	0.642	1.000
Putamen (R)	-0.11	-0.33	0.10	0.291	1.000
Pallidum (L)	-0.09	-0.32	0.14	0.438	1.000
Pallidum (R)	0.02	-0.17	0.22	0.805	1.000
Thalamus (L)	-0.20	-0.45	0.06	0.123	1.000
Thalamus (R)	**-0.26**	**-0.51**	**-0.01**	**0.041**	**1.000**
Heschl (L)	-0.17	-0.50	0.16	0.287	1.000
Heschl (R)	-0.33	-0.74	0.08	0.111	1.000
Temporal Sup (L)	-0.29	-0.61	0.04	0.080	1.000
Temporal Sup (R)	**-0.32**	**-0.61**	**-0.02**	**0.039**	**1.000**
Temporal Pole Sup (L)	-0.17	-0.48	0.15	0.283	1.000

Temporal Pole Sup (R)	**-0.30**	**-0.58**	**-0.01**	**0.044**	**1.000**
Temporal Mid (L)	-0.23	-0.54	0.08	0.130	1.000
Temporal Mid (R)	**-0.38**	**-0.73**	**-0.02**	**0.039**	**1.000**
Temporal Pole Mid (L)	-0.13	-0.34	0.07	0.198	1.000
Temporal Pole Mid (R)	-0.20	-0.41	0.01	0.057	1.000
Temporal Inf (L)	-0.20	-0.44	0.04	0.092	1.000
Temporal Inf (R)	-0.24	-0.49	0.00	0.052	1.000

Table D. The effect of the psilocybin intervention (n=20), relative to baseline (n=26), on regional brain responses to fearful faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using mixed linear effect models. The estimate is the marginal effect of psilocybin compared to baseline. Abbreviations: Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

Effect of psilocybin in regional brain responses to neutral faces

Region	Estimate	lower	upper	p_{unc}	p_{FWER}
Precentral (L)	0.17	-0.13	0.46	0.252	1.000
Precentral (R)	0.06	-0.17	0.30	0.590	1.000
Frontal Sup (L)	0.19	-0.13	0.50	0.230	1.000
Frontal Sup (R)	0.10	-0.17	0.37	0.450	1.000
Frontal Sup Orb (L)	0.12	-0.13	0.37	0.338	1.000
Frontal Sup Orb (R)	0.25	-0.12	0.61	0.179	1.000
Frontal Mid (L)	0.13	-0.18	0.45	0.398	1.000
Frontal Mid (R)	0.28	-0.01	0.56	0.057	1.000
Frontal Mid Orb (L)	0.14	-0.15	0.44	0.327	1.000
Frontal Mid Orb (R)	0.05	-0.23	0.33	0.704	1.000
Frontal Inf Oper (L)	0.16	-0.14	0.46	0.284	1.000
Frontal Inf Oper (R)	0.19	-0.14	0.51	0.247	1.000
Frontal Inf Tri (L)	-0.05	-0.44	0.33	0.784	1.000
Frontal Inf Tri (R)	0.14	-0.15	0.42	0.330	1.000
Frontal Inf Orb (L)	0.00	-0.27	0.28	0.976	1.000
Frontal Inf Orb (R)	0.29	-0.02	0.59	0.062	1.000
Rolandic Oper (L)	0.17	-0.11	0.44	0.226	1.000
Rolandic Oper (R)	0.13	-0.18	0.44	0.402	1.000
Supp Motor Area (L)	0.02	-0.27	0.31	0.884	1.000
Supp Motor Area (R)	0.07	-0.15	0.29	0.533	1.000
Olfactory (L)	0.17	-0.31	0.64	0.473	1.000
Olfactory (R)	0.07	-0.49	0.63	0.799	1.000
Frontal Sup Medial (L)	0.26	-0.11	0.64	0.165	1.000
Frontal Sup Medial (R)	0.19	-0.23	0.61	0.357	1.000
Rectus (L)	0.13	-0.20	0.45	0.419	1.000
Rectus (R)	0.04	-0.23	0.31	0.787	1.000
Insula (L)	0.15	-0.05	0.35	0.132	1.000
Insula (R)	0.20	-0.06	0.46	0.132	1.000
Cingulum Ant (L)	0.13	-0.28	0.53	0.524	1.000
Cingulum Ant (R)	0.05	-0.29	0.40	0.748	1.000
Cingulum Mid (L)	0.11	-0.13	0.34	0.354	1.000
Cingulum Mid (R)	0.08	-0.11	0.27	0.373	1.000
Cingulum Post (L)	0.27	-0.18	0.71	0.229	1.000
Cingulum Post (R)	0.16	-0.17	0.50	0.315	1.000
Hippocampus (L)	-0.03	-0.19	0.12	0.670	1.000
Hippocampus (R)	0.00	-0.17	0.18	0.970	1.000
ParaHippocampal (L)	0.00	-0.23	0.23	0.983	1.000
ParaHippocampal (R)	-0.02	-0.23	0.20	0.881	1.000
Amygdala (L)	0.05	-0.29	0.38	0.773	1.000

Amygdala (R)	0.01	-0.25	0.27	0.919	1.000
Calcarine (L)	0.05	-0.40	0.50	0.818	1.000
Calcarine (R)	0.11	-0.26	0.48	0.542	1.000
Cuneus (L)	0.18	-0.45	0.82	0.556	1.000
Cuneus (R)	0.24	-0.39	0.87	0.441	1.000
Lingual (L)	-0.08	-0.36	0.20	0.581	1.000
Lingual (R)	0.04	-0.31	0.39	0.813	1.000
Occipital Sup (L)	0.14	-0.57	0.84	0.693	1.000
Occipital Sup (R)	0.13	-0.46	0.73	0.649	1.000
Occipital Mid (L)	0.18	-0.30	0.66	0.448	1.000
Occipital Mid (R)	0.26	-0.40	0.92	0.426	1.000
Occipital Inf (L)	-0.02	-0.46	0.42	0.918	1.000
Occipital Inf (R)	0.11	-0.54	0.76	0.734	1.000
Fusiform (L)	-0.04	-0.24	0.16	0.694	1.000
Fusiform (R)	-0.02	-0.30	0.25	0.880	1.000
Postcentral (L)	0.21	-0.06	0.48	0.125	1.000
Postcentral (R)	0.09	-0.22	0.41	0.546	1.000
Parietal Sup (L)	0.39	-0.07	0.84	0.092	1.000
Parietal Sup (R)	0.26	-0.19	0.71	0.254	1.000
Parietal Inf (L)	**0.49**	**0.03**	**0.96**	**0.040**	**1.000**
Parietal Inf (R)	**0.57**	**0.14**	**1.01**	**0.011**	**0.985**
SupraMarginal (L)	**0.44**	**0.08**	**0.80**	**0.018**	**1.000**
SupraMarginal (R)	**0.52**	**0.09**	**0.95**	**0.019**	**1.000**
Angular (L)	0.34	-0.18	0.87	0.191	1.000
Angular (R)	0.41	-0.19	1.02	0.171	1.000
Precuneus (L)	0.13	-0.31	0.57	0.555	1.000
Precuneus (R)	0.08	-0.35	0.51	0.699	1.000
Paracentral Lobule (L)	-0.08	-0.37	0.21	0.573	1.000
Paracentral Lobule (R)	-0.20	-0.50	0.09	0.174	1.000
Caudate (L)	0.19	-0.02	0.40	0.075	1.000
Caudate (R)	0.14	-0.04	0.32	0.128	1.000
Putamen (L)	0.17	-0.06	0.39	0.138	1.000
Putamen (R)	**0.24**	**0.04**	**0.44**	**0.023**	**1.000**
Pallidum (L)	0.11	-0.05	0.26	0.164	1.000
Pallidum (R)	0.12	-0.06	0.30	0.175	1.000
Thalamus (L)	0.07	-0.14	0.28	0.474	1.000
Thalamus (R)	0.08	-0.10	0.26	0.364	1.000
Heschl (L)	0.26	-0.07	0.58	0.117	1.000
Heschl (R)	0.17	-0.22	0.56	0.379	1.000
Temporal Sup (L)	0.16	-0.10	0.43	0.217	1.000
Temporal Sup (R)	0.14	-0.15	0.42	0.330	1.000
Temporal Pole Sup (L)	0.18	-0.09	0.45	0.185	1.000

Temporal Pole Sup (R)	0.09	-0.16	0.35	0.471	1.000
Temporal Mid (L)	0.15	-0.08	0.37	0.183	1.000
Temporal Mid (R)	0.21	-0.09	0.51	0.165	1.000
Temporal Pole Mid (L)	0.08	-0.07	0.23	0.258	1.000
Temporal Pole Mid (R)	0.03	-0.18	0.24	0.755	1.000
Temporal Inf (L)	0.10	-0.08	0.27	0.262	1.000
Temporal Inf (R)	0.15	-0.09	0.39	0.206	1.000

Table E. The effect of the psilocybin intervention (n=20), relative to baseline (n=26), on regional brain responses to neutral faces (contrasted with geometric shapes) during BOLD fMRI, was asseded using mixed linear effect models. The estimate is the marginal effect of psilocybin compared to baseline. Abbreviations: Punc = unadjusted significance level, lower and upper = 95% confidence interval for the estimate, punc = uncorrected p-values. All p-values were corrected for multiple testing using the Bonferroni-Holm method displayed under Pfwer.

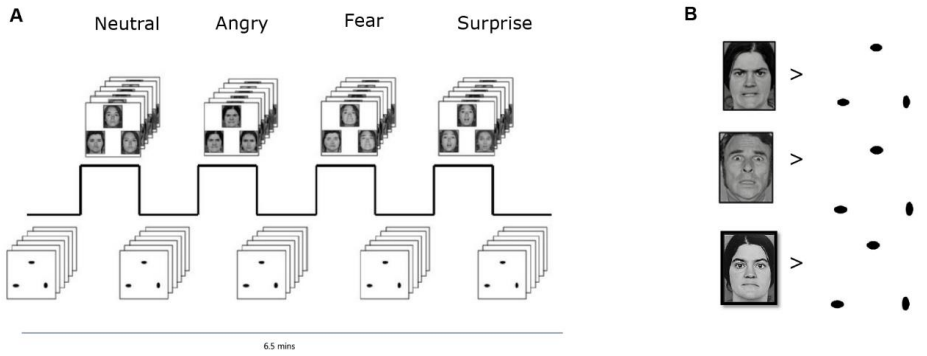

Figure 4. Illustration of the Emotional Face Identification Task (EFIT) with an example of a happy face. The EFIT is a test of the ability to correctly identify facial expressions of anger, disgust, fear, sadness and happiness at different intensities, or neutrality. On a computer screen, an image of a face was displayed on the top and a hexagon made of six circles with the word for each of the five emotional categories or neutral was shown was presented below. Volunteers were orally instructed by the tester to label the emotional or neutral facial expressions using the cursor as fast and accurate as possible. Copied from Hjordt et al., 2017.

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Article/paper/chapter/manuscript
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*Title	Brain serotonin transporter is associated with cognitive-affective biases in healthy individuals
*Author(s)	Sophia Armand, Brice Ozenne, Nanna Svart, Vibe G. Frokjaer, Gitte M. Knudsen, Patrick M. Fisher & Dea S. Stenbæk
Journal	Human Brain Mapping
Volume (no)	doi: 10.1002/hbm.25946
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What was the role of the PhD student in designing the study?

I was involved in conceptualising the research questions and specifying the necessary data to answer this question.

How did the PhD student participate in data collection and/or development of theory?

I wrote an application to the Cimbi database (Knudsen et al., 2016) in which I stated our research questions, hypotheses and requested data to explore our hypotheses, as well as a timeline for data analysis, writing and publishing the paper.

Which part of the manuscript did the PhD student write or contribute to?

All parts of the paper including abstract, introduction, methods, results, discussion and conclusion.

Did the PhD student read and comment on the final manuscript?

Yes, as well as submitting it to the Human Brain Mapping journal and proof reading final version of the manuscript.

Signatures

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By their signature, the authors agree that the article/paper/chapter/manuscript will be included as a part of the PhD thesis made by the PhD student mentioned above.

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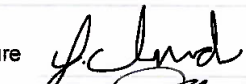
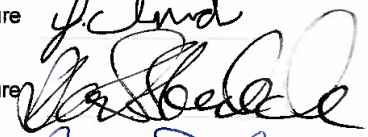
Date 14-06-22 Name Sophia Armand Signature 

Date 24/10 22 Name Dea S. Stenbæk (corr. & senior author) Signature 

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B. drafting the work or revising it critically for important intellectual content, and
C. final approval of the version to be published, and
D. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved."

Article/paper/chapter/manuscript
This co-authorship declaration applies to the following:

*Title: Whole-brain responses to emotional faces after three-to-five weeks intake of selective serotonin reuptake inhibitor or placebo in healthy individuals

Author(s): Sophia Armand, Christelle Langley, Annette Johansen, Brice Ozenne, Oliver O. Overgaard, Peter S. Jensen, Gitte M. Knudsen, Barbara J. Sahakian, Dea S. Stenbæk & Patrick M. Fisher*

Journal: Planned submission to Neuropsychopharmacology

Volume (no):

Start page:

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I was involved in conceptualising the research questions and specifying the necessary data to answer questions in current manuscript.

How did the PhD student participate in data collection and/or development of theory?

I was involved in the setting up the study measurements including cognitive behavioural tests, MRI protocol, questionnaires and practicalities regarding data-collection and following GDPR. I was involved in the data-collection including recruitment, cognitive behavioural testing, MRI-scanning and administration of questionnaires. Along the study period, I was the day-to-day project leader and involved in supervising students contributing the data-collection.

Which part of the manuscript did the PhD student write or contribute to?

All parts of the paper including abstract, introduction, methods, results, discussion and conclusion.

Did the PhD student read and comment on the final manuscript?

Yes, and I will be submitting it to a relevant journal.

Signatures

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PhD student Sophia Armand

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minimum three authors, which is comprised of the first author, the corresponding author, the senior author, and 1-2 authors (preferably international/non-supervisor authors).

By their signature, the authors agree that the article/paper/chapter/manuscript will be included as a part of the PhD thesis made by the PhD student mentioned above.

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B. drafting the work or revising it critically for important intellectual content, and
C. final approval of the version to be published, and
D. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved."

Article/paper/chapter/manuscript
This co-authorship declaration applies to the following:

*Title	Amygdala response to emotional faces following acute administration of psilocybin in healthy individuals
*Author(s)	Sophia Armand, Martin K. Madsen, Brice Ozenne, Katrin H. Preller, Gitte M. Knudsen, Patrick M. Fisher & Dea S. Stenbæk
Journal	Planned submission to Journal of psychopharmacology
Volume (no)	
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I was involved in conceptualising the research questions and specifying the necessary data to answer this question.

How did the PhD student participate in data collection and/or development of theory?
I participated in the data-collection by preparing participants for a psilocybin experiences, facilitating the psilocybin session using psychological support and met with the participants the day after to allow for integration of the experience. I also collected specific measure throughout the psilocybin intervention.

Which part of the manuscript did the PhD student write or contribute to?
All parts of the paper including abstract, introduction, methods, results, discussion and conclusion.

Did the PhD student read and comment on the final manuscript?
Yes, and I will be submitting it to a relevant journal.

Signatures

If an article/ paper/chapter/manuscript is written in collaboration with three or less researchers (including the PhD student), all researchers must sign the statement. However, if an article has more than three authors the statement may be signed by a representative sample, cf. article 12, section 4 and 5 of the Ministerial Order No. 1039, 27 August 2013. A representative sample consists of minimum three authors, which is comprised of the first author, the corresponding author, the senior author, and 1-2 authors (preferably international/non-supervisor authors).

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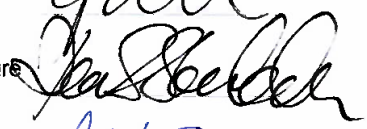
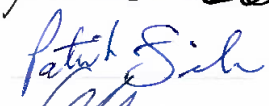
Date	24/10-22	Name	Dea S. Stenbæk	Signature	
Date	24/10 - 2022	Name	Patrick M. Fisher	Signature	
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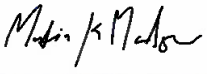
Date	25-08-22	Name	Martin K. Madsen	Signature	
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