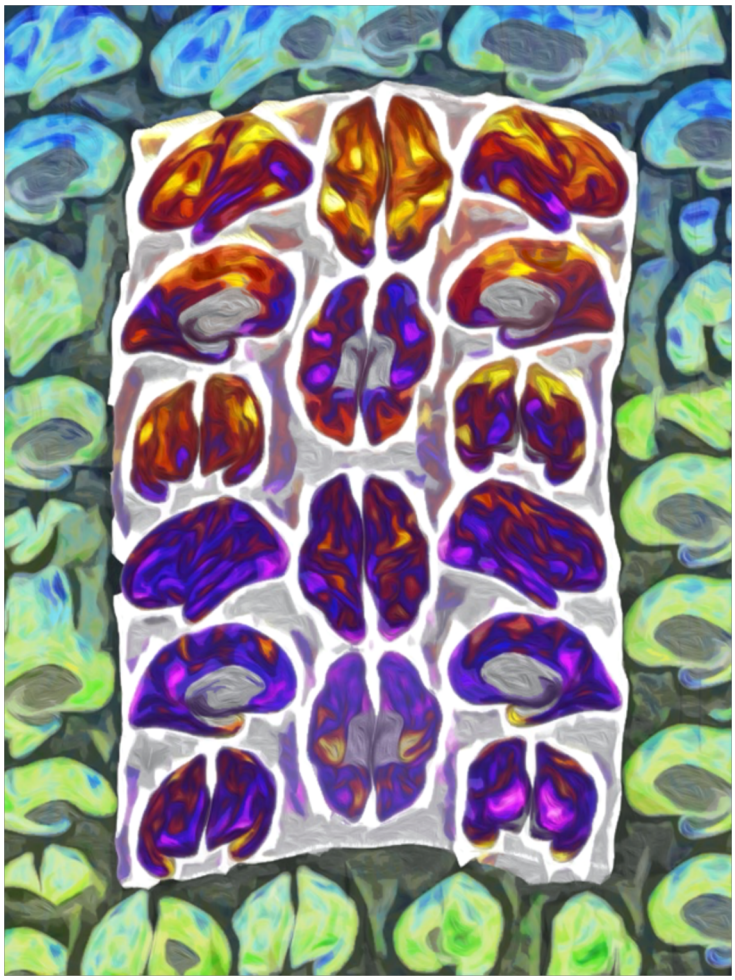
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

PhD thesis

Martin Korsbak Madsen

Neurobiological effects of 5-HT2AR modulation



Supervisor: Prof. Gitte Moos Knudsen

Co-supervisor: Dr. Patrick MacDonald Fisher

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Name of department: Neurobiology Research Unit, Neuroscience Center, Copenhagen University Hospital Rigshospitalet, Denmark

Author: Martin Korsbak Madsen, M.D.

Title: Neurobiological effects of 5-HT2AR modulation

Institutions: Faculty of Health and Medical Sciences, University of Copenhagen & Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet

Principal supervisor: Prof. Gitte Moos Knudsen, Neurobiology Research Unit, Neuroscience Center, Copenhagen University Hospital Rigshospitalet, Denmark
Co-supervisor: Dr. Patrick MacDonald Fisher, Neurobiology Research Unit, Neuroscience Center, Copenhagen University Hospital Rigshopitalet, Denmark
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Assessment committee:
Prof. Steen Hasselbalch, University of Copenhagen, Rigshospitalet, Denmark (chair)
Prof. Morten Kringelbach, Aarhus University, Denmark & University of Oxford, United Kingdom
Ass. Prof. Boris Quednow, University of Zürich, Switzerland

Frontpage: Psychedelic-like rendition of unthresholded whole-brain statistical GCOR (top) and LCOR (bottom) maps of associations with plasma psilocin level (included in Appendix Study 3); warm colors: positive association, cool colors: negative association.

Preface

The present PhD thesis is based on my work at the Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, which commenced Oct. 1, 2016. My main supervisor is Prof. Gitte Moos Knudsen, and my co-supervisor is Patrick MacDonald Fisher, PhD.

List of manuscripts included in this thesis:

I. **Madsen, M. K.,** Fisher P.M., Burmester D., Dyssegaard A., Stenbæk D.S., Kristiansen S., Johansen S.S., Lehel S., Linnet K., Svarer C., Erritzoe D., Ozenne B., Knudsen G.M. (2019). Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology* **44**, 1328–1334.

II. **Madsen, M. K**., Fisher P.M., Stenbæk D.S., Kristiansen S., Burmester D., Lehel S., Páleníček, T., Kuchař M., Svarer C., Ozenne B., Knudsen G.M. A single psilocybin dose is associated with long-term increased mindfulness, preceded by a proportional change in neocortical 5-HT2A receptor binding (2020). *Eur. Neuropsychopharmacol.* **33**, 71–80.

III. **Madsen, M.K**., Stenbæk D.S., Arvidsson A., Armand S., Marstrand-Jørgensen M., Johansen S.S., Linnet K., Ozenne B., Knudsen G.M. Fisher P.M. Integrity and segregation of macroscale cerebral functional networks correlate with plasma psilocin level and psychedelic experience. Manuscript.

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Summary (English)

The serotonin (5-HT) 2A receptor (5-HT2AR) is a highly expressed cortical 5-HT receptor. It is important for psychedelic effects of psilocybin, a classic serotonergic psychedelic compound. The present PhD thesis sought to improve the understanding of psilocybin's neuropsychopharmacology and the role of 5-HT2ARs in brain function. Study 1 evaluated relations between acute subjective effects, measured using subjective drug intensity (SDI) ratings, plasma levels of psilocybin's active metabolite psilocin (PPL) and 5-HT2AR occupancy, measured with [^{11}C]Cimbi-36 positron emission tomography (PET). Study 2 assessed protracted effects of a single dose of psilocybin on personality and mindfulness and on 5-HT2AR binding measured with [^{11}C]Cimbi-36 PET. Study 3 evaluated associations of PPL and SDI with functional magnetic resonance imaging (fMRI) resting state functional connectivity (RSFC).

Study 1 showed close positive correlations between SDI, PPL and 5-HT2AR occupancy, indicating that PPL is a key determinant for subjective effects and can be used as an objective measure of both target engagement and overall drug effects.

Study 2 found increased mindfulness and personality trait Openness at three-months follow-up and stable 5-HT2AR levels across individuals at the PET rescan one week after psilocybin. Individual changes in mindfulness and 5-HT2AR levels correlated negatively, suggesting a possible mechanism (changed 5-HT tonus and/or regulation of 5-HT2AR levels) by which mindfulness changes could occur.

Study 3 observed that PPL and SDI correlated negatively with default mode network (DMN) RSFC and with RSFC across networks. PPL and SDI correlated positively with average between-network RSFC. These findings suggest that psilocin reduces network integrity and segregation and implicate the expression of network integrity and segregation in psychedelic experience and normal consciousness.

In conclusion, the present work expands the current understanding of psilocybin's neuropsychopharmacology and the role of the 5-HT2AR in brain function. It demonstrates that psilocin is a key determinant for psilocybin effects and also exemplifies how a combination of molecular and functional neuroimaging methods coupled with measurement of plasma drug concentration and subjective experience can provide knowledge beneficial to drug development and the understanding of the brain.

Resume (Dansk)

Serotonin-2A-receptoren (5-HT2AR) er en receptor for signalstoffet serotonin og forekommer i særlig høj koncentration i hjernebarken. Receptoren formidler psykedeliske effekter af stoffet psilocybin, der kendes fra ”magic mushrooms” (f.eks. spids nøgenhat). Psilocybin omdannes i kroppen til stoffet psilocin, som er det psykedelisk aktive stof. Nærværende ph.d.-afhandling bygger på tre hjernescanningsforsøg i raske forsøgspersoner og undersøger hvordan psilocin påvirker hjernens funktion akut og mere langvarigt. Det undersøges også hvilken rolle 5-HT2AR spiller. I studie 1 undersøges sammenhængen mellem den selvoplevede intensitet af den psykedeliske oplevelse (SDI), koncentration af psilocin i blodet (PPL) og 5-HT2AR-okkupans målt med [^{11}C]Cimbi-36 positron-emissions-tomografi (PET) hjernescanning. I studie 2 undersøges virkningerne af en enkelt dosis psilocybin på hjernens 5-HT2AR-niveauer målt med [^{11}C]Cimbi-36 PET samt længerevarende effekter på personlighed og *mindfulness*. I studie 3 undersøges forbindelser mellem PPL, SDI og *resting state* funktionel konnektivitet (RSFC), som måler hjernenetværks kommunikation. RSFC måles her med funktionel magnetisk resonansscanning (fMRI).

Studie 1 viste en nøje positiv sammenhæng mellem SDI, PPL og 5-HT2AR okkupans, hvilket viser at psilocin i høj grad binder til 5-HT2AR, og at stofeffekter afhænger af PPL. Således kan PPL bruges som et objektivt mål for 5-HT2AR stimulering og overordnede effekter af psilocybin.

Studie 2 fandt at *mindfulness* og personlighedstrækket Åbenhed var øget tre måneder efter psilocybin. Mens der ikke kunne påvises en forskel i 5-HT2AR-niveauer målt før og en uge efter psilocybin, fandtes en negativ sammenhæng mellem den enkelte persons forandring i *mindfulness* og 5-HT2AR-binding i hjernen. Det er muligt, at små ændringer i 5-HT2AR niveauer eller i hjernens serotoninkoncentration kan være en mekanisme, hvorved psilocybin kan bevirke vedvarende ændringer i *mindfulness*.

Studie 3 fandt, at under påvirkning af psilocybin korrelerer PPL og SDI negativt med RSFC i hjernenetværket *default mode network* og med gennemsnitlig RSFC for alle hjernenetværk. PPL og SDI korrelerer positivt med gennemsnitlig RSFC mellem hjernens netværk. Dette tyder på, at psilocin forbigående hæmmer funktionen i hjernens

netværk, og at disse fungerer mindre adskilt. Det taler også for, at netværkenes kommunikation spiller en rolle i bevidsthed.

Disse forsøg bidrager med vigtig ny viden om psilocybins neurobiologiske virkninger og påviser, at psilocinstimulering af 5-HT2AR er vigtig for den psykedeliske oplevelse såvel som hjernefunktion. Som helhed udgør studierne et eksempel på, hvordan man hos det levende menneske kan opnå ny viden om et lægemiddel ved at kombinere forskellige billeddannelsesmetoder af hjernen (her PET og fMRI) med måling af stofkoncentration i blodet og undersøgelser af subjektive oplevelser.

Abbreviations

1WRS – One-week rescan
3MFU – three-months follow-up
5-HT – serotonin
5-HT1AR – serotonin 1A receptor
5-HT2BR – serotonin 2B receptor
5-HT2AR – serotonin 2A receptor
5-HT2CR – serotonin 2C receptor
ACC – anterior cingulate cortex
ACTH – adrenocorticotropic hormone
AIPS – anterior intraparietal sulcus
ALAT – alanine transaminase
AN – auditory network
ASAT – aspartate amino transferase
ASC – Altered States of Consciousness
ASL – arterial spin labelling
B_{avail} – receptors available for binding
BBB – blood-brain barrier
BOLD – blood-oxygen-level dependent
BP_{ND} – non-displaceable binding potential
C_{max} – maximum concentration of drug in blood
C_P – concentration of drug in plasma
CSF – cerebrospinal fluid
D2R – dopamine 2 receptor
DAN – dorsal attention network
DMN – default mode network
DPAT - 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol
EC_{50} – C_P corresponding to 50% occupancy
ECN – executive control network
EDI – Ego-Dissolution Inventory
EDTA - Ethylenediaminetetraacetic acid
EEG – electroencephalography
EPI – echo planar imaging
EPS – excitatory post-synaptic potential
fMRI – functional magnetic resonance imaging
f_{ND} – fraction of unbound radioligand in ND compartment
FWER – family-wise error rate
FPN – frontoparietal (control) network
GCOR – Global correlation
GGT – gamma glutamyltransferase
GM – grey matter
GPCR – G-protein-coupled receptor
HR – heart rate
HRRT – high-resolution research tomography
ITG – inferior temporal gyrus
K_D – Dissociation constant
keV – kiloelectron volt
L - ligand
LCOR – Local correlation
LD_{50} – median lethal dose
LFP – local field potential
LP – lateral parietal
LSD – lysergic acid diethylamide
MAAS – Mindful attentive awareness scale
MBq – mega becquerel (number of radioactive decays per second)
MBSR – mindfulness-based stress reduction
MDD – major depression disorder
MEG – magnetoencephalography
MEQ – Mystical Experiences Questionnaire
MPFC – medial prefrontal cortex
MRI – magnetic resonance imaging
NEO PI-R – Neuroticism-Extraversion-Openness Personality Inventory Revised
nM – nanomolar
NRU – Neurobiology Research Unit
Occ_{max} – maximum occupancy
PCC – posterior cingulate cortex
PET – positron emission tomography
PIPS – posterior intraparietal sulcus
PLA_2 – phospholipase-A2
PLC – phospholipase-C
PKC – protein kinase C
PPI – prepulse inhibition
PPL – plasma psilocin level
R – receptor
ROI – region of interest
rs – resting state
RSFC – resting state functional connectivity
SAN – salience network
SERT – Serotonin transporter
SMN – sensorimotor network
$t_{1/2}$ – half-life (time duration until 50% of drug is left in blood)
TAC – Time-activity curve
t_{max} – time at which C_{max} occurs
μL – microliter
VAN – ventral attention network
VN – visual network
VOI – volume of interest
WM – white matter

"When we started our isolation studies, we had no idea to what group of chemical substances the active principle would belong… We therefore tested the fractions of our extracts on animals, selecting mice and cats for the purpose. These animals ate nearly all of the very rare material without showing any specific signs. On testing the fractions upon ourselves, however, the results were clear."

A. Hofmann, 1959
Acta Phys et Pharm Neer (8)

Background

The present PhD thesis leverages two different neuroimaging modalities (PET & fMRI), which yield complementary information about *in vivo* neurobiology. These techniques are used to gain knowledge about how modulation of the serotonin 2A receptor, using the psychedelic compound psilocybin, affects brain function. Thus, in the present chapter, these two imaging modalities are presented along with key background information of relevance to this thesis, including aspects of psilocybin's neuropsychopharmacology.

The serotonin 2A receptor

The serotonin (5-HT) 2A receptor (5-HT2AR) is one of 14 5-HT receptors identified in the human brain (Vilaró *et al*, 2020). It is a membrane-bound G-protein-coupled receptor (GPCR), meaning that upon stimulation by a ligand (e.g., the endogenous ligand 5-HT), the receptor interacts with a G-protein, which leads to activation of one or more downstream signaling pathways (**Fig. 1**). 5-HT2AR-linked signaling pathways include $G_{q/11}$ proteins, which activate phospholipase-C (PLC), resulting in increased inositol phosphate and intracellular Ca^{2+} levels (Blaazer *et al*, 2008; Conn and Sanders-Bush, 1984; López-Giménez and González-Maeso, 2018). $G_{i/o}$ proteins constitute another signaling pathway that activate phospholipase-A2 (PLA_2), leading to arachidonic acid build-up (Kurrasch-Orbaugh *et al*, 2003).

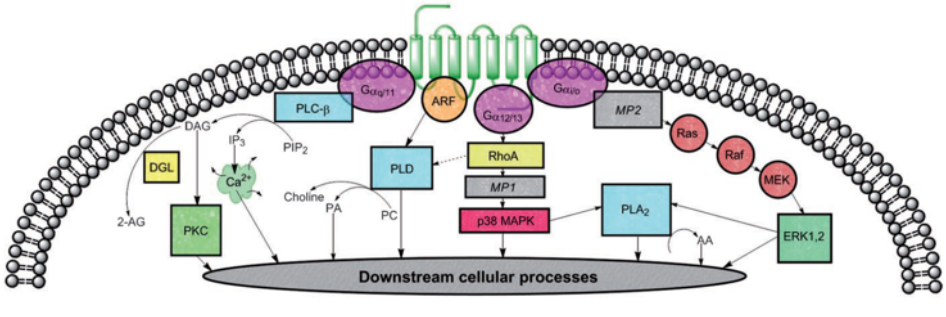

Fig. 1. Overview of 5-HT2AR signaling pathways. 5-HT2AR is linked to different G-proteins, which upon stimulation of the receptor are activated, resulting in a down-stream signaling cascade. The figure is reproduced from Blaazer et al. 2008 with permission from the publisher.

Many 5-HT2AR agonists share common effects such as psychedelic experiential phenomenology in humans (Glennon *et al*, 1984; Nichols, 2016), head-twitch response in mice (González-Maeso *et al*, 2007; Halberstadt *et al*, 2020) and excitatory neuronal electrophysiological response (Aghajanian and Marek, 1999; Araneda and Andrade, 1991; González-Maeso *et al*, 2007). Although many 5-HT2AR agonists share these effects, agonists differentially activate 5-HT2AR-linked down-stream pathways—a phenomenon known as biased-signaling (López-Giménez and González-Maeso, 2018), agonist-directed trafficking (Berg *et al*, 1998; Kurrasch-Orbaugh *et al*, 2003) and functional selectivity (Roth, 2012). This phenomenon is exemplified by the divergent transcriptomic (González-Maeso *et al*, 2003, 2007) and downstream messenger profiles (Kurrasch-Orbaugh *et al*, 2003) of the non-psychedelic 5-HT2AR agonist lisuride and the psychedelic 5-HT2AR agonists psilocin and lysergic acid diethylamide (LSD). Thus, the phenomenon of functional selectivity should be kept in mind as a possible limitation when generalizing an effect observed for one 5-HT2AR agonist to another 5-HT2AR agonist.

Microanatomical animal studies showed high 5-HT2AR expression on apical dendrites of cortical layer V neurons (Cornea-Hébert *et al*, 1999) and on pyramidal neurons in layers II, III, V and VI (Jakab and Goldman-Rakic, 1998). Post-mortem human autoradiography studies reported high cortical levels of 5-HT2ARs in layers III and V, but lower levels in subcortical regions and cerebellum where 5-HT2ARs are almost absent (Pazos *et al*, 1987; Varnäs *et al*, 2004).

Interestingly, a microscopy study found that a large proportion of 5-HT2ARs were located intracellularly (Cornea-Hébert *et al*, 1999), which may be related to rapid internalization of 5-HT2ARs following stimulation as observed *in vitro* (Berry *et al*, 1996; Bhattacharyya *et al*, 2002; Karaki *et al*, 2014). In addition, prolonged stimulation can result in 5-HT2AR down-regulation (i.e., a decrease in membrane-bound receptors) (Buckholtz *et al*, 1988, 1990).

The macroanatomical distribution of 5-HT2ARs in the human brain has been investigated using post-mortem autoradiography (Pazos *et al*, 1987; Varnäs *et al*, 2004) and PET imaging (Beliveau *et al*, 2017; Ettrup *et al*, 2016). These studies consistently demonstrate high levels of 5-HT2AR expression in the cortex with particularly high binding in medial prefrontal cortex (MPFC), dorsolateral prefrontal cortex (DLPFC), posterior cingulate cortex (PCC), precuneus, insula, visual cortex, and temporal

cortices, but lower 5-HT2AR expression in subcortical regions such as striatum, amygdala, hippocampus and thalamus (Beliveau *et al*, 2017; Ettrup *et al*, 2016) (**Fig. 2**).

Several studies suggest that 5-HT2AR agonism constitutes an important mode of action of serotonergic psychedelics. For instance, Glennon and colleagues reported a close correlation between 5-HT2 affinity and estimated active oral dose of psychedelic compounds (Glennon *et al*, 1984). This is further supported by experimental human studies, showing that pretreatment with the 5-HT2A/2CR antagonist ketanserin blocks or substantially attenuates subjective effects of subsequently administered serotonergic psychedelics, including psilocybin (Kometer *et al*, 2012; Vollenweider *et al*, 1998), LSD (Preller *et al*, 2017) and ayahuasca (Valle *et al*, 2016). Further, an important study by González-Maeso and colleagues demonstrated that behavioral LSD effects were absent in 5-HT2AR knock-out mice but present in mice with restored cortical 5-HT2ARs (González-Maeso *et al*, 2007). This finding was subsequently extended to psilocin by Halberstadt and coworkers (Halberstadt and Geyer, 2011). Together, these studies show that 5-HT2AR agonism is a deciding factor for psychedelic effects of known serotonergic psychedelics such as LSD and psilocin.

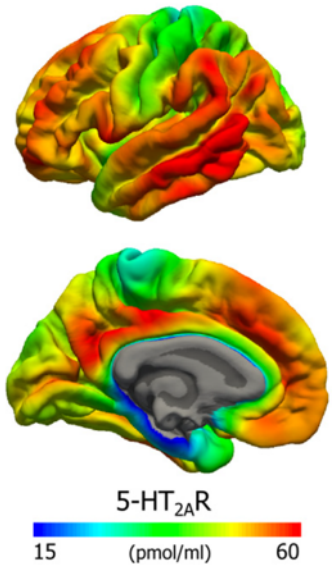

Fig. 2. Cerebral 5-HT2AR levels. Left hemisphere cortical surface density map of 5-HT2ARs. From Beliveau et al. 2016.

A growing corpus of research suggests 5-HT2AR involvement in several neuropsychiatric disorders, including schizophrenia (Girgis *et al*, 2019; Rasmussen *et al*, 2010, 2011), depression (Attar-Lévy *et al*, 1999; Bhagwagar *et al*, 2006; Biver *et al*, 1997; Meyer *et al*, 1999, 2001, 2003; Mintun *et al*, 2004; Yatham *et al*, 2000), anxiety (Benekareddy *et al*, 2010, 2011; Weisstaub, 2006) and neurodegeneration (Hasselbalch *et al*, 2008; Marner *et al*, 2012; Meltzer *et al*, 1999). In addition, 5HT2AR levels have also been linked to personality conveying vulnerability for mood disorders (Erritzoe *et al*, 2009; Frokjaer *et al*, 2008, 2010). Thus, better insight into the role 5-HT2ARs play in brain function may lead to improved understanding of both healthy brain function as well as disease-related processes, and this may ultimately help inform treatment of brain disorders.

Positron emission tomography imaging

Positron emission tomography (PET) is a quantitative imaging technique that allows for imaging of functional aspects of the brain (Heurling *et al*, 2017; Lammertsma, 2019). PET imaging makes use of a radioligand, which is a molecule labelled with a radioactive atomic isotope. When a proton of the isotope undergoes β^+ decay, it turns into a neutron and in the same process releases a positron. The positron travels a short distance in the tissue until it meets its antiparticle, an electron, which results in an annihilation. In the annihilation reaction, a pair of gamma rays of 511keV are released at an approximate angle of 180 degrees. This emission can be recorded by crystal detectors in the PET scanner. Based on the recorded radioactive decay, reconstruction algorithms can estimate the location in space from where the coincident decay occurred. The radioactivity (measured in mega Becquerel (MBq) per volume tissue) over time for a brain volume of interest (VOI) is termed a time-activity curve (TAC). The TAC describes the kinetics of the radioligand and is used in compartmental modelling of PET data, which includes applying differential equations that describe the rate of radioligand transfer between compartments (**Fig 3**). When solving these differential equations, it is possible to estimate model parameters that are linked to the concentrations of the target (e.g., a receptor).

In PET neuroimaging, it is possible to combine the brain TACs with the corresponding plasma radioligand concentration data from arterial blood to quantify volumes of distribution, which can be used for within- or between-subject comparison. However, arterial blood sampling can be obviated, if there is an appropriate reference region (a region devoid of specific binding to the molecular target). In this case, a reference-tissue model, such as the simplified reference-tissue model (SRTM), can be used. By means of reference tissue models, the non-displaceable binding potential, BP_{ND}, can be computed. BP_{ND} is a unitless measure of binding in a VOI compared to the non-displaceable compartment (i.e., the reference region) (Lammertsma and Hume, 1996). Assumptions of the SRTM are that 1) the contribution of blood to the signal is negligible, 2) the reference region is devoid of specific binding, 3) kinetics can be described by a one-tissue compartmental model, and 4) non-displaceable binding is the same in reference and target regions. For the 5-HT2AR agonist radioligand [^{11}C]Cimbi-

36 PET neuroimaging, the cerebellum is an appropriate reference region (Ettrup *et al*, 2014).

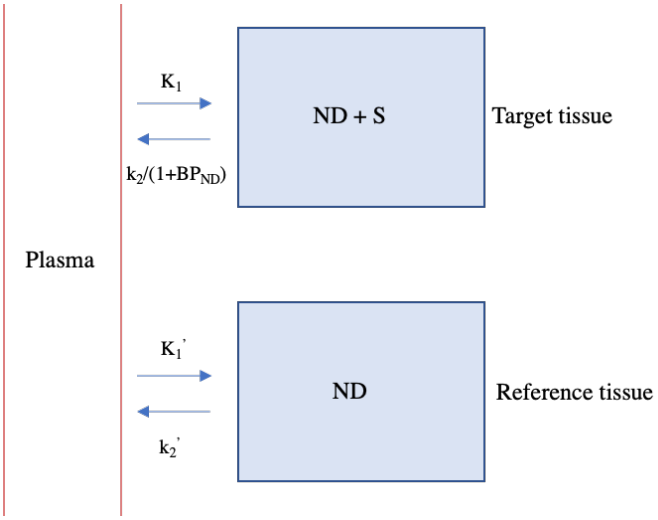

Fig. 3. Compartmental model of the SRTM. Adapted from (Lammertsma, 2019). K_1 and k_2 designate rate of transfer between plasma and target tissue compartment. In the simplified reference tissue model (SRTM), the transfer from the non-displaceable (ND) compartment to the specific compartment (S) is assumed to occur very quickly, and the two theoretical compartments are collapsed into one. K_1' and k_2' designate the rate of transfer between plasma and the reference tissue compartment (the reference tissue).

BP_{ND} is described by the following equation, where f_{ND} is the fraction of unbound radioligand in the non-displaceable compartment, and B_{avail} is the number of receptors available for binding (Innis *et al*, 2007):

$$BP_{ND} = f_{ND} * \frac{B_{avail}}{K_D} \quad \text{(I)}$$

B_{avail} is affected by the number of membrane-bound receptors and the concentration of endogenous ligand (5-HT), since the endogenous ligand will compete with the radioligand for the receptor binding site.

K_D is the dissociation constant and is derived from the law of mass action. It describes how well a given ligand, L, binds to a target, R.

$$K_D = \frac{[L]*[R]}{[LR]} \quad \text{(II)}$$

PET imaging of 5-HT2ARs with [^{11}C]Cimbi-36

The PET radioligand [^{11}C]Cimbi-36 (also known in its unlabeled form as 25B-NBOMe) is a highly selective 5-HT2A/2C agonist (Ettrup *et al*, 2011, 2013). The agonist activity of [^{11}C]Cimbi-36 at 5-HT2ARs is also evidenced by the unlabeled compound's use as a psychedelic street-drug (Halberstadt, 2017). [^{11}C]Cimbi-36 has been validated in both non-human primates (Finnema *et al*, 2014) and in humans (Beliveau *et al*, 2017; Ettrup *et al*, 2014, 2016). 5-HT2AR binding measured with [^{11}C]Cimbi-36 correlates closely with other well-established 5-HT2AR antagonist radioligands including [^{18}F]Altanserin (Ettrup *et al*, 2016) and [^{11}C]MDL 100907 (Finnema *et al*, 2014). It also correlates positively with 5-HT2AR levels measured in post-mortem human brain tissue (Beliveau *et al*, 2017), supporting that [^{11}C]Cimbi-36 is a good radioligand for quantification of cerebral 5-HT2ARs. [^{11}C]Cimbi-36 has a low test-retest variability (Ettrup *et al*, 2014), is metabolized into two fractions: M1 (small polar) and M2 (glucoronidated) (Johansen *et al*, 2017) and has an advantageous dosimetry profile (Johansen *et al*, 2019).

In terms of kinetic modelling, it is possible to quantify cerebral [^{11}C]Cimbi-36 binding with both full arterial input modelling and cerebellum-reference tissue modelling (Ettrup *et al*, 2014; Finnema *et al*, 2014; Varnäs *et al*, 2004).

According to the ternary complex receptor model, G-protein-coupled receptors, such as 5-HT2ARs, can be in a high- or a low-affinity state. Agonists bind preferentially to receptors in high-affinity states (Egan *et al*, 2000; Kenakin, 2002; Leff, 1995; Paterson *et al*, 2013; Titeler *et al*, 1987). Because [^{11}C]Cimbi-36 is an agonist, [^{11}C]Cimbi-36 PET imaging should thus provide a more physiologically relevant measure of 5-HT2ARs than antagonist radioligands, which theoretically bind with similar affinity to both high- and low-affinity receptors.

An additional beneficial property of agonist radioligands is that, compared to antagonist radioligands, they may be more sensitive to competition with the endogenous ligand (here, 5-HT). Thus, [^{11}C]Cimbi-36 has been used to assess 5-HT release in pigs (Jørgensen *et al*, 2016), in a non-human primate model (Yang *et al*, 2017) and in healthy human individuals (da Cunha-Bang *et al*, 2019) as well as MDD patients (Erritzoe *et al*, 2019). Together, these studies show that [^{11}C]Cimbi-36 is sensitive to competition with 5-HT at the 5-HT2AR.

Measuring receptor occupancy using PET

An attractive feature of PET imaging is the ability to measure both the penetration of a ligand across the blood-brain barrier (BBB) and its target engagement. Related to this, the drug occupancy of a target can be calculated as the percent reduction in BP_{ND} after a pharmacological intervention (Takano *et al*, 2016; Zhang and Fox, 2012):

$$Occupancy = \frac{BP_{ND_{baseline}} - BP_{ND_{Intervention}}}{BP_{ND_{baseline}}} * 100\% \text{ (III)}$$

The relationship between occupancy, predicted maximum attainable occupancy (Occ_{max}) and drug concentration (C_P) is described by:

$$Occupancy = \frac{Occ_{max} * C_P}{K_D + C_P} \text{ (IV)}$$

PET 5-HT2AR occupancy studies have been performed using as radioligand [^{11}C]NMSP (a dopamine 2 receptor (D2R) and 5-HT2AR antagonist radioligand) to evaluate occupancy of the selective 5-HT2AR antagonist volinanserin (Grunder *et al*, 1997) and the selective 5-HT2A/2CR inverse agonist pimavanserin (Nordstrom *et al*, 2008). The two studies predicted a maximum occupancy of 88% and 78%, respectively, and demonstrate the feasibility of conducting 5-HT2AR PET imaging occupancy studies using the SRTM.

A particular field of PET occupancy studies deserves mentioning, since it exemplifies the method's utility. An important mechanism of action for atypical antipsychotic drugs is the blocking of dopamine receptors. It has long been known that both treatment response and extrapyramidal side-effects (EPS) are shaped by dopamine receptor antagonism (van Rossum, 1966). Interestingly, a series of human [^{11}C]raclopride PET-studies demonstrated an antipsychotic D2R occupancy "sweet spot" of 60-80%: Side-effects were more likely to occur at receptor occupancies above 80%, and unsatisfactory treatment effect was more likely to occur at below 60% occupancy (Farde *et al*, 1992; Kapur *et al*, 2000; Nordström *et al*, 1993). Thus, with appropriate radioligand choice, PET occupancy studies coupled with measures of behavioral or subjective effects can illuminate important aspects of a pharmaceutical compound's neuropsychopharmacological profile.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) relies on the electromagnetic properties of atomic nuclei, most commonly of hydrogen protons (Buxton, 2013). These protons align with a strong static magnetic field, the B0 field, which is parallel to the scanner bore. The protons precess at the Larmor frequency ω, which is equal to γB0, where γ is the gyromagnetic constant for a given nucleus. A radiofrequency (RF) pulse transmitted at a certain angle (the flip angle) at a frequency resonating with the frequency of proton precession (the Larmor frequency) can push the rotating net magnetization of the protons away from the B0 field to a desired angle. During this process, energy is transferred in a way conceptually similar to the build-up of energy that occurs when a child pushes another child on a swing.

Once the RF pulse is terminated, the magnetization decreases exponentially in a plane (the B1 field) perpendicular to the B0 field, governed by relaxation due to proton spin-spin interactions. This transverse relaxation rate is described by the time constant T2 (i.e., time duration until the signal has been reduced by 37% signal). Due to inter-tissue differences in spin-spin interactions, T2 differs between tissues, and this gives different image contrasts. At the same time as the transversal magnetization relaxes, the longitudinal magnetization recovers (increases) logarithmically, independent of the transversal magnetization relaxation. This recovery is described by the time constant T1, which is defined by the time when 63% of the signal is recovered. T1 is determined by spin-lattice interactions and is affected by the components in the tissue matrix. T2 is a theoretical value, and what is really observed after a given RF pulse is T2*, which is the effective transversal magnetization time constant ($T2^* \leq T2$). This differs from theoretical T2 due to local inhomogeneities in the B0 field caused by macroscopic tissue properties. At some point (echo time (TE)) after the RF pulse is transmitted, it is possible to measure the rotating transversal B1 field using a receive coil. The B1 field induces oscillating currents, which decreases over time and is measured as the tissue signals.

Slices are selected by gradient magnetic fields, which induce alignment changes in the protons inside the slice coverage, while keeping tissue outside of the slice insensitive to the RF pulse. Locating a signal in each volume element (voxel) within the slices in an image is enabled by the processes of phase encoding and frequency encoding. Fourier transformation of the signals recorded in the frequency space (k-

space) results in a two-dimensional array of values within a slice, which describes the location and intensity of every voxel.

MRI allows for several ways to record images of the brain, most of which fall beyond the scope of the present thesis. However, because echo planar imaging (EPI) with T2*-contrast weighting is widely used for blood oxygen level dependent (BOLD) fMRI, the governing principles of EPI are here presented: EPI makes use of 1) an RF pulse coupled with slice selection, 2) phase encoding, and 3) encoding of frequency (rapidly alternating directions) and phase. After every switch in the frequency encoding direction, the echo is measured at time TE. This makes it possible to fill k-space for an image slice with just a single RF pulse. The images are sensitive to changes in the BOLD signal, which is described in the following subsection.

BOLD fMRI

BOLD fMRI is a functional imaging technique capable of measuring changes in blood oxygenation levels and was first demonstrated in 1990 by Ogawa and colleagues (Ogawa *et al*, 1990a, 1990b). Subsequent early studies showed that MRI can measure BOLD signal changes in relevant cerebral regions after finger tapping (Bandettini *et al*, 1992), visual stimuli (Ogawa *et al*, 1992) and hand squeeze (Kwong *et al*, 1992). The oxygenation status of hemoglobin changes the magnetic field in such a way that oxyhemoglobin (diamagnetic) compared to deoxyhemoglobin (paramagnetic) induces less local magnetic field distortion, which affects the T2' (inhomogeneities in the magnetic field) and thus T2*. The BOLD hemodynamic response (HR) is a structured signal change after a stimulus (e.g., signal measured in visual cortex after visual stimulation) (Handwerker *et al*, 2012). Although the HR is similar between individuals, interindividual variability exists. The neurovascular coupling hypothesis describes the causal relation between neuronal activity and the related need for oxygen, which drives the HR and thus the BOLD signal (Raichle, 2015). The BOLD signal is thought to reflect integratory synaptic activity more so than spiking (Attwell and Laughlin, 2001), which is supported by within-scan recordings of local field potentials (LFP) (Logothetis *et al*, 2001; Schölvinck *et al*, 2010) and intracellular calcium measurement (Matsui *et al*, 2016; Schwalm *et al*, 2017).

Functional connectivity and cerebral networks

BOLD fMRI is not only capable of measuring changes in brain activation but can also provide information about functional connectivity (FC) between two or more regions in the brain. Bharat Biswal and colleagues were the first to demonstrate FC using BOLD fMRI (Biswal *et al*, 1995) and reported high correlations between the BOLD signal time series in sensorimotor cortex in the resting state (rs), i.e. resting state functional connectivity (RSFC). BOLD signal oscillations are slow (<0.1 Hz) (van den Heuvel and Hulshoff Pol, 2010). The functional coupling strength of VOIs can be estimated by computing the correlation coefficient for the time series. Highly correlated time series results in a higher correlation coefficient, which reflects higher FC.

Regions exhibiting similar BOLD signal time series can be construed as networks (Schaefer *et al*, 2018; Yeo *et al*, 2011). Commonly identified networks include the default mode network (DMN), (Greicius *et al*, 2003; Raichle *et al*, 2001; Shulman *et al*, 1997), fronto-parietal (control) network (FPN) (Shulman *et al*, 1997; Vincent *et al*, 2008), also referred to as the executive control network (ECN) (Menon and Uddin, 2010; Raichle, 2011; Seeley *et al*, 2007), dorsal and ventral attention networks (DAN and VAN) (Corbetta and Shulman, 2002; Fox *et al*, 2005, 2006), visual network (VN) (Smith *et al*, 2009), sensorimotor network (SMN) (Biswal *et al*, 1995), auditory network (AN) (Raichle, 2011) and a salience network (SAN) (Menon and Uddin, 2010; Raichle, 2011; Seeley *et al*, 2007).

Importantly, correlations between activation maps and functional network maps suggest that functional networks identified in the resting state contribute to rather specific functions (Margulies *et al*, 2016; Smith *et al*, 2009).

The role of unimodal networks (i.e., sensory and sensorimotor network) is more straightforward than networks “further downstream” from sensory input (Margulies *et al*, 2016; Smith *et al*, 2009). These more complex networks include heteromodal networks such as FPN/ECN, SAN, DAN and DMN. The DMN was initially identified as being more active during rest than task and hence named the “task-negative network” (Raichle *et al*, 2001; Shulman *et al*, 1997). The DMN has since been implicated in internal mentation, including memory, theory-of-mind and abstract brain function (Andrews-Hanna *et al*, 2014; Buckner and DiNicola, 2019; Carhart-Harris *et al*, 2012a; Qin and Northoff, 2011; Whitfield-Gabrieli and Ford, 2012). The ECN is more active during cognitive tasks,

leading to its initial inclusion in a “task-positive network” (Fox *et al*, 2005; Shulman *et al*, 1997; Vincent *et al*, 2008), which also included SAN and DAN. The DAN is hypothesized to constitute a top-down control system of visual attention is robustly activated visual search paradigms (Corbetta and Shulman, 2002). Although the DMN exhibits negative RSFC with DAN in the resting state (Fox *et al*, 2005), the coupling depends on cognitive state (Spreng *et al*, 2010).

Although functional networks display a large degree of within-network similarity of the BOLD signal time series, it is possible to further subdivide networks into subnetworks based on signal differences within each network (e.g., parcellate a DMN1 and a DMN2 from the total DMN) (Kernbach *et al*, 2018; Yeo *et al*, 2011). This indicates that larger networks consist of subnetworks, which may have functionally separate roles (Buckner and DiNicola, 2019).

More sophisticated analytical methods have been applied to BOLD fMRI data, yielding information about temporal variability in FC (dynamic functional connectivity) (Hutchison *et al*, 2013), directionality of FC (e.g., Granger causality analysis (Seth *et al*, 2015), dynamic causal modelling (Friston *et al*, 2003)), graph theory (Bullmore and Sporns, 2009) and brain states (Lord *et al*, 2019). Although these emerging methods provide complementary information to more traditional “static” FC assessments, they are not utilized in the analytical framework of the present thesis and further description is therefore considered superfluous.

Psilocybin

Psilocybin is the main tryptamine psychotropic substance contained in *Psilocybe* genus mushrooms, popularly known as magic mushrooms (Nichols, 2016). The ethnologist and banker R. Gordon Wasson described the hallucinogenic effects and ritual use of such mushrooms in Mexico (Wasson and Wasson, 1957). Wasson's mushroom species was mycologically characterized and cultivated by Roger Heim and named *Psilocybe mexicana* (Hofmann, 1959). This later led to the identification and synthesis of psilocybin by Albert Hofmann who also discovered LSD (Hofmann *et al*, 1958). Psilocybin belongs to a group of psychotropic compounds that all activate 5-HT receptors, including the 5-HT2AR. This group of drugs is often referred to as either psychedelics ("mind-manifesting"), hallucinogens (inducer of hallucinations) or entheogens ("releasing the divine from within") (Nichols, 2016). Because psilocybin plays a key role in the work included in the present thesis, an overview of its neuropsychopharmacology is presented in the following sections.

Psilocybin pharmacokinetics and product metabolism

Psilocybin is a prodrug of psilocin, which is the main psychoactive metabolite. This understanding stems from both non-human and human studies. Non-human studies showed that alkaline phosphatase turns psilocybin into psilocin by cleaving psilocybin's phosphoric ester group (Horita and Weber, 1961a, 1961b, 1962). Jejunal tissue took up psilocin but not psilocybin (Eivindvik et al. 1989), indicating that psilocin but not psilocybin is transferred into the blood from the gastrointestinal system. Kalberer and colleagues found in a study of 8 rats that 50% of orally applied [^{14}C]-labelled psilocin was absorbed and that psilocin was evenly distributed throughout the entire body (Kalberer *et al*, 1962). Thus, non-human studies suggest that psilocin is the active metabolite of psilocybin.

A similar line of research in humans also indicates that psilocin is the main psychoactive compound of psilocybin. Hasler and colleagues investigated the pharmacokinetic profile of oral (10-20 mg) and intravenous psilocybin (1 mg) in 8 subjects (**Table 1**) (Hasler *et al*, 1997), reporting almost immediate conversion of psilocybin to psilocin with psilocin plasma level (PPL) peaking within 2 minutes after injection. Likewise, Brown and colleagues administered high doses of oral psilocybin and reported that only psilocin and not psilocybin could be detected (Brown *et al*, 2017).

Human studies indicate that clearance of psilocin occurs renally, mainly as glucoronidated psilocin (Hasler *et al*, 1997, 2002). In a liquid chromatography/mass spectrometry study in one person, psilocin was 20% free and 80% glucoronidated five hours after intake (Kamata *et al*, 2006). Stich and Käferstein observed in two humans that after intake of psilocybin, psilocin was 35% free in serum and 13% free in urine, supporting a considerable contribution of glucoronidation of psilocin (Sticht and Käferstein, 2000).

An important aspect of psilocybin's pharmacokinetic profile is that there is substantial interindividual variability in terms of both peak psilocin concentration (C_{max}), time to C_{max} (t_{max}), and half-life $t_{1/2}$ (**Table 1**).

Dose (mg/kg)	c_{max} (mean ± SD, (range) μg/L)	c_{max} (median (25-75 percentile) μg/L)	t_{max} (mean ± SD (range) min)	$t_{1/2}$ (mean ± SD min)	Study
0.224	8.2 ± 2.4 (4.5-12.3)	NA	105 ± 37 (NA)	163.3 ± 63.5	Hasler et al 1997
0.2	11.34 ± 5.5 (6.0-16.4)	NA	81 ± 11 (70-90)	135 ± NA	Lindenblat et al 1998
0.3	NA	16 (14.5–17.2)	122 ± NA (60–124)		
0.45	NA	26 (22.7–35.1)	122±NA (78–180)	180 ± 66	Brown et al 2017
0.6	NA	37.6 (27.7–43.2)	123±NA (93–125)		

Table 1. Psilocybin pharmacokinetics studies. C_{max}: maximum psilocin blood concentration; t_{max}: time to reach C_{max}; $t_{1/2}$: half-life (time to reach 50% reduction in psilocin blood concentration). Oral administration.

Psilocin pharmacodynamics

Psilocin has a rich pharmacology, including partial agonist activity at other 5-HT targets such as 5-HT1AR, 5-HT2CRs (Blair *et al*, 2000; Kurrasch-Orbaugh *et al*, 2003; McKenna *et al*, 1990; Rickli *et al*, 2016) (**Table 2**), 5-HT2B/7Rs and the serotonin transporter (SERT) (Halberstadt and Geyer, 2011; Ray, 2010; Rickli *et al*, 2016).

5-HT1AR	5-HT2AR	5-HT2BR	5-HT2CR	Study
190 ± 40[a]	6.0 ±0.5[a]	410 ± 50[a]	NA	McKenna et al. 1990
49 ± 5.5[b]	25 ± 4.7[b]	NA	10 ± 1.4[b]	Blair et al. 2000
123 ± 20[c]	50 ± 10[c]	>20000[c]	94 ± 9[b]	Rickli et al. 2016

Table 2. Literature values of psilocin binding profile at 5-HT receptor subtypes. [a]IC50±SE (nM), 5HT1AR labelled with [^{3}H]8-OH-DPAT (rat cortex), 5HT2AR (rat cortex) labelled with [^{125}I]DOI, and 5HT2BR labelled with [^{3}H]ketanserin (bovine cortex) [b]K_i±SE (nM), labelling: [^{125}I]DOI 5HT2A/2C receptors and [3H]8-OH-DPAT (5HT1AR), [c]K_i±SD (nM), labelling: [^{3}H]8-hydroxy-2-(di-n-propylamine) (5-HT1AR), [^{3}H]ketanserin (5-HT2AR), [^{3}H]mesulgerine (5-HT2CR).

A sizeable body of literature suggests that it is stimulation of 5-HT2ARs that is primarily responsible for psychedelic effects of serotonergic psychedelic compounds, including psilocin (Glennon *et al*, 1984; González-Maeso *et al*, 2007; Halberstadt and Geyer, 2011; Kometer *et al*, 2012; Preller *et al*, 2017; Vollenweider *et al*, 1998).
Similar to other 5-HT2AR agonists, psilocin stimulation of 5-HT2ARs leads to activation of both $G_{q/11}$ and $G_{i/o}$ proteins and consequent protein kinase C (PKC) and PLA_2 activation (Halberstadt, 2015). Effects of psilocin on downstream-signaling in 5-HT2AR-expressing neurons in mice include increased expression of early genes (*erg-1, erg-2, c-fos, jun-B, period-1, gpcr-26, fra-1, N-10, I-κBα*). *I-κBα* was the only gene also increased after psilocin in a 5-HT2AR knock-out model, implying that 5-HT2AR is necessary for psilocin-induced changes in transcription of the mentioned genes except *I-κBα* (González-Maeso *et al*, 2007; González-Maeso and Sealfon, 2009).

A series of double-blind experiments assessing effects of psilocybin and ketanserin show that pretreatment with a 5-HT2AR antagonist almost completely prevents subsequent psychedelic effects after psilocybin intake (Carter *et al*, 2005, 2007; Kometer *et al*, 2012; Vollenweider *et al*, 1998), supporting that the 5-HT2AR is necessary for psychedelic effects. Interestingly, however, not all subjective effects were prevented since ketanserin intake potentiated the reduction in vigilance when administered together with psilocybin compared to psilocybin and ketanserin alone (Carter *et al*, 2005, 2007). A follow-up study by the same lab assessed moderating effects of the 5-HT1AR agonist buspirone on the psilocybin-induced psychedelic state and observed a blunting effect of buspirone on ASC scores (Pokorny *et al*, 2016). This is in line with the preclinical observation that stimulation of cortical 5-HT1ARs and 5-HT2ARs has opposite effects (Araneda and Andrade, 1991). These studies show that 5-HT2AR stimulation is key for psychedelic subjective effects of psilocybin in humans, and also that the engagement of non-5-HT2AR targets by psilocin or metabolites of psilocin likely contributes to subjective effects.

Few studies have examined the extent to which psilocin regulates 5-HT2AR levels. Five daily doses of psilocybin (1 mg/kg) reduced [^{3}H]-ketanserin binding with 38% in rat cortex, indicating down-regulation of 5-HT2AR, which is consistent with LSD-induced down-regulation of 5-HT2ARs in animal models (Buckholtz *et al*, 1985, 1988, 1990). Psilocin also reduced [^{3}H]-OH-DPAT with 16%, indicating down-regulation of 5-HT1AR (Buckholtz *et al*, 1990). In humans, a cross-sectional [^{18}F]Altanserin PET imaging study

compared users of psychedelics, including magic mushrooms, with healthy non-using control subjects. The study reported 9% lower neocortical 5-HT2AR levels in users of psychedelics, indicating that regular use of 5-HT2AR agonists may down-regulate 5-HT2ARs in humans (Erritzoe *et al*, 2011). Together these studies suggest that psilocin can regulate 5-HT2AR levels. It is possible that regulation of cerebral 5-HT2ARs constitutes a mechanism underlying potential long-term effects of psilocybin.

Somatic effects in humans

Somatic symptoms of psilocybin intake include mydriasis, changes in heart rate and/or blood pressure, hyperreflexia, drowsiness, yawning, paresthesia, dizziness, weakness, tremor, nausea, vomiting, blurred vision, dysmetria, and headache (Von Gnirss, 1958; Gouzoulis-Mayfrank *et al*, 1999b; Griffiths *et al*, 2006, 2011, 2016; Hasler *et al*, 2004; Isbell, 1959; Passie *et al*, 2002; Quetin, 1960; Studerus *et al*, 2011; Tyls *et al*, 2014; Wolbach *et al*, 1962). Cardiovascular changes are summarized in **Table 3**.

Study	Subjects	Dose (mg/kg)	Heart rate difference (bpm)	MAP difference (mmHg)
Gouzoulis-Mayfrank et al. 1999	N=8, healthy	0.2	11	16
Hasler et al. 2004	N=8, healthy	0.045	1	4
		0.12	-3	8
		0.22	7	7
Griffiths et al. 2006	N=30, healthy	0.43	10	14
Griffiths et al. 2011	N=18, healthy	0.071	3	8
		0.14	3	9
		0.29	6	9
		0.43	8	11
Griffiths et al. 2016	N=51, Cancer patients	0.31-0.43	7	14
Preller et al. 2016	N=21, healthy	0.2 mg/kg	5	11

Table 3. **Effects of oral psilocybin on the cardiovascular function.** MAP: mean arterial blood pressure; bpm: beats per minute. Values represent maximum difference of psilocybin compared to placebo, except Griffiths et al. 2006, here increases are relative to baseline. Griffiths et al. 2016 used 1 and 3 mg psilocybin as placebo comparison.

Psilocin does not seem to negatively affect cardiac electrophysiological measures. Hasler and colleagues did not observe effects of psilocybin (doses up to 0.3 mg/kg) on electrocardiogram-measures of cardiac electrophysiology (Hasler *et al*, 2004), and

Dahmane and coworkers reported a benign but statistically significant positive correlation between PPL and cardiac QT-interval (Dahmane *et al*, 2020).

Research on the effects of psilocybin on biomarkers of organ function and/or endocrinology is limited. Low to medium doses (up to 0.2 mg/kg) of psilocybin did not affect common biochemical parameters, including electrolytes, urea, creatinine, γ-glutamyltransferase (GGT), alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase and blood glucose (Hasler *et al*, 2004; Hollister, 1961; Tyls *et al*, 2014). Only in high doses (0.3 mg/kg) did psilocybin cause a brief and minor increase in GGT and ASAT (Hasler *et al*, 2004). Moderate doses of psilocybin (0.2 mg/kg p.o.) did not affect prolactin and cortisol (Gouzoulis-Mayfrank *et al*, 1999b), but medium to high doses (0.2 mg/kg and 0.3 mg/kg p.o.) caused transient increases 105 minutes after drug intake in thyroid-stimulating hormone (TSH), prolactin, adrenocorticotropic hormone (ACTH) and cortisol (Hasler *et al*, 2004).

In summary, human psilocybin studies indicate that psilocybin has benign somatic effects. This includes transient sympathomimetic effects, including blood pressure increases. This effect is likely only of potential harm in hypertensive patients or in the context of aneurisms.

Psychoactive effects

Psilocybin causes dose-dependent altered states of consciousness that may include stimulation of affect (e.g., euphoria, dysphoria, nervousness and anxiety); increased introspection; perceptual changes including synesthesia and illusions; hallucinations; changes in sense of body image; changes in sense of time and space; decreased attention; distorted thinking (e.g., magical thinking and delusions); depersonalization; and derealization (Griffiths *et al*, 2011; Nichols, 2016; Passie *et al*, 2002; Ruemmele and Gnirss, 1961; Vollenweider *et al*, 2007; Wolbach *et al*, 1962). Early studies claimed moderating effects of context (the environment ("setting") and psychological factors ("set"))) on the subjective experience (Hartogsohn, 2016; Hyde, 1960; Leary *et al*, 1963; Metzner *et al*, 1965; Unger, 1963), and this has been corroborated by more recent studies (Carhart-Harris *et al*, 2018b; Russ *et al*, 2019; Studerus *et al*, 2012).

Psychometric evaluation tools of subjective psilocybin experience include the revised Mystical Experiences Questionnaire (MEQ30), (Barrett *et al*, 2015; MacLean *et al*, 2012; Pahnke, 1969), Altered States of Consciousness Scale (ASC) (Dittrich *et al*,

2006; Studerus *et al*, 2011) and the Ego-Dissolution Inventory (EDI) (Nour *et al*, 2016). MEQ30 assesses four factors: mystical; positive mood; transcendence of time and space; and ineffability, and it is a tool for quantifying mystical type experiences (other labels include “transcendental”, “spiritual”, and “peak”) (Griffiths *et al*, 2006, 2011; Unger, 1963). ASC measures in its most recent version 11 dimensions of the psychedelic experience (Studerus *et al*, 2011). EDI measures perceptions of a reduced self, I or ego.

Long-term psilocybin effects

Intake of psilocybin has been associated with long-term beneficial effects, including increases in the personality trait Openness to Experience (Openness) in healthy individuals (Maclean *et al*, 2011) and in patients with major depression disorder (MDD) (Erritzoe *et al*, 2018). In the context of a five-day intensive mindfulness meditation retreat, psilocybin increased mindfulness measured at short-term follow-up. At long-term follow-up, the authors reported that psilocybin increased self-reported appreciation for life, self-acceptance and concern for others (Smigielski *et al*, 2019), which is in line with other psilocybin studies conducted in healthy human individuals (Griffiths *et al*, 2006, 2011).

The most clinically important effects of psilocybin include antidepressive and anxiolytic properties. Psilocybin led to significant beneficial effects in patients with MDD (Carhart-Harris *et al*, 2016a, 2018a; Roseman *et al*, 2017; Watts *et al*, 2017), cancer (Griffiths *et al*, 2016; Grob *et al*, 2011; Ross *et al*, 2016), obsessive-compulsive disorder (Moreno *et al*, 2006) and addiction to nicotine (Johnson *et al*, 2014, 2017) and alcohol (Bogenschutz *et al*, 2015). Although the reported effect sizes of these studies are large, sample sizes were generally small and few studies were conducted as double-blind placebo-controlled studies. Thus, although psilocybin shows promise as a novel transnosological therapeutic for a range of non-psychotic disorders, future studies are needed to define the clinical utility profile and better characterize side-effects.

Psilocybin neuroimaging studies

Human neuroimaging studies have yielded information about effects of psilocybin on brain function using electroencephalography (EEG), magnetoencephalography (MEG), fMRI, and PET imaging.

PET imaging studies have been conducted using as radioligands [^{18}F]fluorodeoxyglucose (FDG) (Gouzoulis-Mayfrank *et al*, 1999a; Vollenweider *et al*, 1997), [^{11}C]raclopride (Vollenweider *et al*, 1999) and [^{18}F]Altanserin (Quednow *et al*, 2010). The resting-state [^{18}F]FDG-PET study by Vollenweider and colleagues observed uniform cortical glucose metabolism increases (Vollenweider *et al*, 1997). Gouzoulis-Mayfrank and colleagues did not report effects of psilocybin on absolute regional metabolism changes, making it impossible to directly compare the findings with those of Vollenweider and colleagues (Gouzoulis-Mayfrank *et al*, 1999a). Instead, the authors calculated changes in regional glucose metabolism relative to the global average and reported increased metabolism in ACC and reduced in thalamus. Despite the interstudy discrepancies, the two studies indicate that psilocybin increases prefrontal glucose consumption, suggesting increased neuronal activity. The [^{11}C]raclopride study suggested increased striatal dopamine transmission after psilocybin (Vollenweider *et al*, 1999). The [^{18}F] Altanserin study, which was published as part of a book chapter, indicated measurable 5-HT2AR occupancy after psilocybin (Quednow *et al*, 2010). Although these PET-studies used small sample sizes and did not correct for multiple comparisons, they indicate that psilocybin increases prefrontal neuronal activity, striatal dopaminergic neurotransmission and binds to the 5-HT2AR.

Electrophysiological studies suggest substantial effects of psilocybin on brain function (Barnett *et al*, 2020; Bernasconi *et al*, 2014; Kometer *et al*, 2013, 2015; Muthukumaraswamy *et al*, 2013; Pallavicini *et al*, 2019; Schartner *et al*, 2017). The findings include 1) decreased oscillatory power in distributed brain regions, including core DMN regions such as the MPFC, PCC and parahippocampal gyrus (PHG) (Kometer *et al*, 2015; Muthukumaraswamy *et al*, 2013), 2) modulation of cortical responses to visual stimuli including faces (Bernasconi *et al*, 2014; Kometer *et al*, 2011, 2013), 3) increased signal diversity (Schartner *et al*, 2017), and 4) reduced FC in non-gamma bands (Pallavicini *et al*, 2019). Together these studies show that psilocybin modulates normal neuronal oscillatory patterns, including less well-ordered brain function.

Two arterial-spin-labelling (ASL) studies reported widespread reductions in absolute perfusion (Carhart-Harris *et al*, 2012a; Lewis *et al*, 2017). The latter study, however, also explored effects of normalizing perfusion to global signal and reported increased relative perfusion in some regions, including the ACC and bilateral insulae.

The extent to which such a normalization yields a more accurate picture of neuronal activity is unknown.

Resting state BOLD fMRI findings from the i.v. psilocybin studies show reduced DMN resting state functional connectivity (RSFC) (Carhart-Harris *et al*, 2012a); increased between-network RSFC (Roseman *et al*, 2014), including RSFC of DMN with the "task-positive network" (Carhart-Harris *et al*, 2013); increased global connectivity (Petri *et al*, 2014; Tagliazucchi *et al*, 2016); and decreased FC between medial temporal lobe (MTL) and higher cortical areas (Lebedev *et al*, 2015). In addition, studies applying more advanced analytical methods on the same data report an increased number of brain states (Atasoy *et al*, 2018; Tagliazucchi *et al*, 2014) and less expressed FPN brain states (Lord *et al*, 2019). A recent study investigating the temporal development of voxel-level whole-brain RSFC showed increases in sensory cortices and reductions in association cortices (Preller *et al*, 2020), contradicting the findings of Tagliazucchi (Tagliazucchi *et al*, 2016) who reported only increased global RSFC. Further studies are needed to more firmly establish psilocybin effects on global connectivity.

Main findings from fMRI task studies indicate that psilocybin reduces amygdala reactivity to aversive facial stimuli (Kraehenmann *et al*, 2014), moderates effective FC (i.e., directed FC) (Kraehenmann *et al*, 2016) and static FC during processing of facial stimuli (Grimm *et al*, 2018) and reduces dorsal ACC (dACC) activation in response to a social exclusion paradigm (Preller *et al*, 2016). Furthermore, psilocybin increased brain activation during recollection of autobiographical memories (Carhart-Harris *et al*, 2012b).

In summary, several psilocybin neuroimaging studies have assessed effects of psilocybin on various aspects of brain function compared to baseline or placebo. The current knowledge about psilocybin brain effects is based on relatively few experiments, all with small-to-moderate small sample sizes. Nevertheless, these studies suggest that psilocybin substantially alters several aspects of brain function, including the induction of a more entropic (more chaotic) brain function (Carhart-Harris and Friston, 2019).

Toxicology

Psilocybin's toxicity seems to be low. LD_{50} of psilocybin was 285 mg/kg in mice, 280 mg/kg in rats and 12.5 mg/kg in rabbits (Chemistry, 2001). A recent study in a sample of 250 mice found LD_{50} of psilocin to be 293 mg/kg with no lethal effects for doses up to 250 mg/kg (Zhuk *et al*, 2015). A single study examined mutagenic effects of psilocybin, reporting no effects of doses up to 16 mg/kg on micronuclei in nucleated bone marrow cells from mice (Wendt, 1977).

Agonist activity at 5-HT2BRs has been implicated in cardiac valvular pathology (Hutcheson *et al*, 2011). Since psilocin is an agonist at 5-HT2BRs, it is possible that exposure to psilocin increases the risk for valvular disease. Although the risk of cardiac valvulopathy is currently theoretical, it warrants further studies. Nevertheless, the physiological toxicity of psilocybin is in all likelihood very low, especially with a limited number of exposures.

Side-effects and participant safety in psilocybin studies

Very common self-reported acute and sub-acute (day after) side-effects of psilocybin include headache, fatigue, lack of energy, sleepiness and difficulties with concentration (Hendricks *et al*, 2015b; Studerus *et al*, 2011). Common side-effects include increased exhaustibility, rumination, decreased appetite, and musculoskeletal aches.

Participant safety in psychedelics studies also relies on preparation and on the guides/sitters. Due to the intoxication, participants may behave in a potentially dangerous manner to themselves or their surroundings. In a summary of participant safety based on 250 healthy participants, three incidences were highlighted as problematic: *"(1) a volunteer decided to stand up and engage in expressive movements; (2) a volunteer moved from the couch to the floor while vigorously moving legs and arms in an erratic fashion; and (3) a volunteer became confused and disoriented when in the restroom."* (Carbonaro *et al*, 2015).

Prolonged psychotic reactions (Johnson *et al*, 2008; Tyls *et al*, 2014) and hallucinogen persisting perceptual disorder (HPPD) have been reported in relation to ingestion of psychedelic drugs (Halpern and Pope, 2003; Strassman, 1984). These reactions are rare (Halpern and Pope, 2003; Johnson *et al*, 2008; Strassman, 1984) and did seemingly not occur in the approximately 360 healthy participants who have

received psilocybin in controlled studies conducted by research groups in Zürich and Boston (Carbonaro *et al*, 2015; Studerus *et al*, 2011).

Expert opinions on the danger of psychoactive compounds rate psilocybin as having a low harm potential compared to other drugs of abuse (van Amsterdam *et al*, 2010, 2015; Nutt *et al*, 2010). This is supported by epidemiological studies (Hendricks *et al*, 2015a, 2015b; Johansen and Krebs, 2015; Krebs and Johansen, 2013). However, two deaths partly ascribed to somatic effects after intake of magic mushrooms have been described in the literature (Amsterdam *et al*, 2011; Bück, 1961; Gerault, A.; Picart, 1996; Nichols, 2016), and several incidents involving jumping off of buildings under the influence of psychedelics have been reported (Nichols, 2016).

Although it is likely that side-effects in human psilocybin research is under-reported, the available literature suggests that psilocybin can be administered safely when interventions are done in a controlled environment with an experienced sitter/guide, and when screening procedures are enforced to exclude vulnerable individuals.

Motivation and aims

Study 1: Psychedelic effects of psilocybin are believed to arise when psilocin stimulates cerebral 5-HT2ARs (Vollenweider *et al*, 1998), but psilocin occupancy at 5-HT2ARs and relations with psychedelic effects in humans are unknown. Thus, in Study 1, we evaluated relations between plasma psilocin levels (PPL), 5-HT2AR occupancy measured using [^{11}C]Cimbi-36 PET, and subjective psychedelic effects, assessed with subjective drug intensity (SDI) ratings in eight healthy volunteers.

Study 2: Single doses of psilocybin can have long-lasting effects on well-being and behavior, including increases in personality trait Openness (Maclean *et al*, 2011; Unger, 1963). Anecdotal evidence indicates that psilocybin can increase attentiveness to self and surroundings (Watts *et al*, 2017), which is an essential part of mindfulness (i.e., attentive awareness) (Brown and Ryan, 2003; Jensen *et al*, 2016). However, long-term changes in mindfulness after psilocybin have not been quantified. Regulation of 5-HT2AR levels is a candidate mechanism by which psilocin could bring about long-term changes but regulation of cerebral 5-HT2ARs after a single psilocybin dose remains unexplored. Thus, in Study 2, we evaluated effects of a single dose of psilocybin on 5-HT2AR binding measured with [^{11}C]Cimbi-36 PET and on personality and mindfulness in 10 healthy psychedelic-naïve volunteers.

Study 3: Psilocin is the active metabolite of psilocybin, but relations between PPL, brain function and subjective effects are unknown. A recently published theory (Carhart-Harris and Friston, 2019) includes that an important neurobiological mechanism underlying psychoactive effects of psychedelics is a 5-HT2AR-mediated impairment of higher order networks, including the DMN, and an overall functionally desegregated brain. Thus, in Study 3, we evaluated associations of PPL and SDI with RSFC. We hypothesized that PPL and SDI would correlate negatively with DMN and across networks, indicating reduced network integrity. We also hypothesized that PPL and SDI would correlate positively with average between-network RSFC, indicating reduced network segregation. The study was conducted in 15 healthy volunteers.

Design

Study 1.

The design of Study 1 is depicted in **Fig. 4**. Eight healthy volunteers were included. Participants first underwent baseline MRI and PET scans. On a separate day, participants underwent a psilocybin intervention. Participants were blind to the dose they would receive but were aware of the possibility of strong psychoactive effects. Psilocybin was administered in the morning one hour prior to expected injection of [^{11}C]Cimbi-36, such that imaging was expected to coincide with PPL C_{max} (Hasler *et al*, 1997). A wide span of psilocybin doses (3-30 mg) were administered to yield a range of associated PPL and 5-HT2AR occupancy estimates. The first five participants underwent a second [^{11}C]Cimbi-36 PET occupancy scan later the same day. The three participants who received the highest dose (i.e., 0.3 mg/kg) underwent only one intervention PET scan (at expected peak levels).

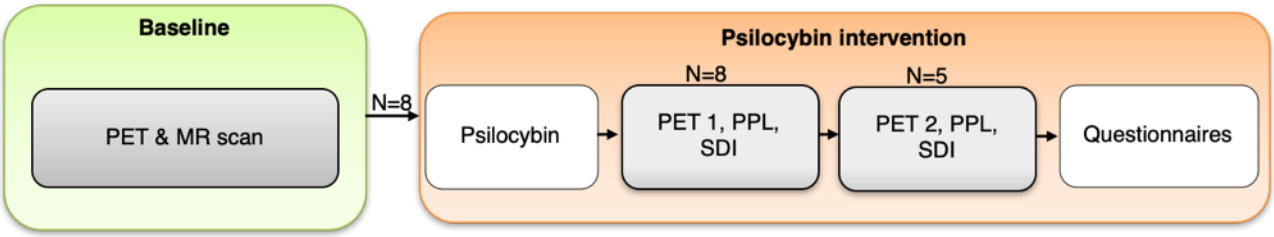

Fig. 4. Design of Study 1. PET: positron emission tomography; MR: magnetic resonance scan. PPL: plasma psilocin levels; SDI: subjective drug intensity. Questionnaires included Mystical Experiences Questionnaire, Altered States of Consciousness questionnaire and Ego-Dissolution Inventory.

Prior to psilocybin administration and at 20-minute intervals during PET scans, SDI was verbally rated, and a blood sample was collected for the purpose of PPL determination. At the end of the intervention day, participants completed questionnaires that measure aspects of the psychedelic experience (11-D ASC, MEQ30 and EDI). Participants in Study 1 participated as part of a larger single-blind study that also included a ketanserin intervention arm. Participants were aware of the possibility of receiving ketanserin and not psilocybin. Only data from the psilocybin arm is included in the present thesis.

In all three studies, participants were prepared for the experience by at least one of the psychologists who acted as one of two guides during psilocybin intervention. Participants also met with one or both of the guides the day after psilocybin to allow for integration of the experience.

Study 2.

The design of Study 2 is shown in **Fig. 5.** Ten healthy young adults naïve to psychedelic drugs were included. Baseline data collection included a baseline neuroimaging day ([^{11}C]Cimbi-36 PET and MRI) and completion of baseline questionnaires (mindfulness assessed with mindful attention awareness scale (MAAS) (Brown and Ryan, 2003) and NEO PI-R personality questionnaire (Costa, P.T., McCrae, 1992)). Participants underwent a psilocybin intervention day, each receiving a moderate-to-high dose of psilocybin (0.2 mg/kg (n=4), 0.3 mg/kg (n=6). The dose was increased from 0.2 mg/kg to 0.3 mg/kg after permission was obtained from the Danish Medicines Agency. Before the intervention, participants met with at least one of the two psychologists, who acted as guides during psilocybin interventions. A standardized list of music was played during the psilocybin sessions. MEQ30, ASC and EDI were completed at the end of the intervention day to assess aspects of the psychedelic experience. One week after psilocybin, participants underwent a rescan with [^{11}C]Cimbi-36 PET. Three months after psilocybin, participants completed MAAS and NEO PI-R again.

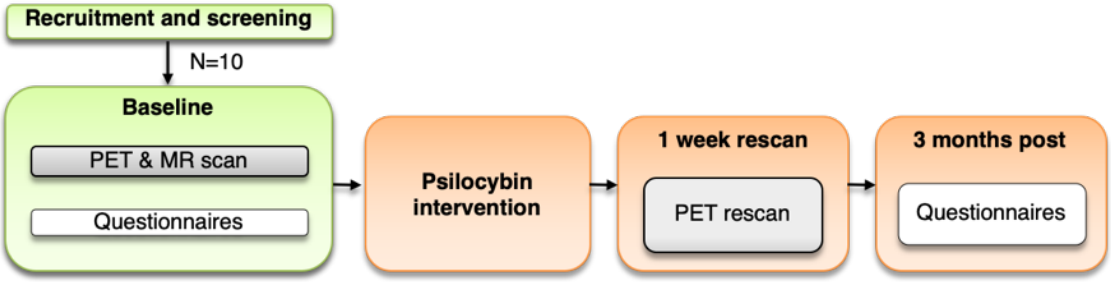

Fig. 5. Design of Study 2. PET: positron emission tomography; MR: magnetic resonance. Questionnaires included personality and mindfulness assessments.

Study 3.

The design of Study 3 is visualized in **Fig. 6**. A data triad consisting of rs BOLD fMRI, blood sample acquisition (for PPL determination) and SDI was acquired at pre-drug, and approximately 40, 80, 130, and 300 min after psilocybin administration (dose (0.2 mg/kg (n=4), 0.3 mg/kg (n=11)). The first participant underwent an additional three rounds of data acquisition; the subsequent data collection strategy was modified to provide participants with a break after the third post-drug scan.

Study 3 in the present PhD thesis include data derived from a larger single-blind study, and participants also underwent an MRI intervention day with the 5-HT2AR

antagonist ketanserin either three weeks before or after the psilocybin intervention. Only data concerning psilocybin interventions are included in the present thesis.

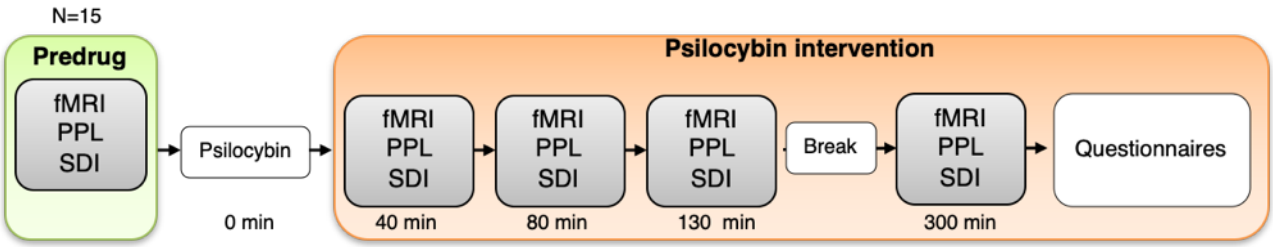

Fig. 6. Design of Study 3. fMRI: functional magnetic resonance imaging; PPL: plasma psilocin levels; SDI: subjective drug intensity. Questionnaires included 11-Dimension Altered States of Consciousness, Mystical Experiences Questionnaire and Ego-Dissolution Inventory.

Methods

Psychometric evaluations

Real-time assessment of SDI was assessed before and during the acute psilocybin experience ("How intense is it to you right now?", Likert-scale, 0 = "Not at all", 10 = "Very much"). Overall psychedelic experience was also characterized in all three studies, using retrospective questionnaires: the 94-item 11-D ASC (Dittrich *et al*, 2006; Studerus *et al*, 2010), the 30-item MEQ30 (Barrett *et al*, 2015), and the eight-item EDI (Nour *et al*, 2016). These questionnaires require the respondent to rate the extent to which certain phenomena were experienced during the acute psilocybin experience.

In Study 2, personality was measured using the 240-item personality trait questionnaire NEO PI-R (Costa, P.T., McCrae, 1992). NEO PI-R measures five personality factors: Openness, Conscientiousness, Extraversion, Agreeableness, and Neuroticism. The general degree of attentiveness (mindfulness) was measured using the 15-item MAAS (Brown and Ryan, 2003; Jensen *et al*, 2016).

Plasma psilocin concentration measurement

Blood samples were collected from a venous catheter in the antecubital vein. Blood samples were placed on ice and centrifuged, and the resulting plasma fraction was aliquoted and frozen. Plasma psilocin measurements included in Study 1 and 3 were carried out by the Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Science, University of Copenhagen, using ultra-performance liquid chromatography and tandem mass spectrometry on EDTA-stabilized plasma samples.

[^{11}C]Cimbi-36 PET data acquisition

Cerebral 5-HT2AR binding was measured in Study 1 and 2, using dynamic [^{11}C]Cimbi-36 PET imaging on a high-resolution research tomography (HRRT) PET-scanner (CTI/Siemens, Knoxville, USA). The in-plane resolution was approximately 8 mm^3. Participants were scanned for 120 minutes (45 frames: 6 × 10 s, 6 × 20 s, 6 × 60 s, 8 × 120 s, 19 × 300 s) after an i.v. injection of ≤ 600 MBq of [^{11}C]Cimbi-36. Subjects were instructed to lie still for the duration of the scan, and head motion was limited using head fixation cushioning.

Structural MRI acquisition

T1-weighted and T2-weighted MRI images were acquired for the purpose of PET image processing in Study 1 and 2 and for the purpose of functional image preprocessing in Study 3. Image acquisition was performed on a 3T Prisma scanner (Siemens, Erlangen, Germany) using a 64-channel head coil. T1-weighted images: inversion time = 900 ms, TE = 2.58 ms, TR = 1900ms, flip angle = 9°, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap. T2-weighted images: TE = 408 ms, TR = 3200 ms, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 208 slices and a slice thickness of 0.9 mm, no gap.

[^{11}C]Cimbi-36 PET data processing

PET images were motion-corrected, smoothed with a 10 mm Gaussian kernel and co-registered to structural MRI images. MRI images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Regions of interest were defined using Pvelab (Svarer *et al*, 2005). TACs were extracted from GM for kinetic modelling. Kinetic modelling was performed using SRTM (Lammertsma and Hume, 1996) with cerebellum as reference region (Ettrup *et al*, 2014). BP_{ND} in neocortex (a volume-weighted average of all cortical regions) was chosen as the primary outcome measure due to high cortical expression of 5-HT2ARs and close cortical interregional 5-HT2AR correlation (Erritzoe *et al*, 2009; Ettrup *et al*, 2014, 2016).

Resting state BOLD fMRI data acquisition

Resting state BOLD MRI data was acquired as part of the psilocybin intervention in Study 3 on the same 3T Siemens Prisma scanner as previously mentioned, using a T2*-weighted gradient echo sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90^{o}, in-plane matrix = 64 x 64 mm, in-plane resolution = 3.6 x 3.6 mm, 32 slices (thickness = 3.0 mm, gap = 0.75 mm). Three hundred brain volumes (10 min) were acquired for each BOLD fMRI data acquisition. Participants were instructed to lie still, have eyes closed, and allow thoughts to wander but not fall asleep. Each subject's head was fixed using foam cushions inside the head coil to reduce head motion.

BOLD fMRI data preprocessing

Preprocessing of the BOLD fMRI data was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). The preprocessing steps included 1) realignment of functional images to first functional image, 2) slice-timing correction, 3) unwarping, 4) co-registration of the first functional image to the T1-weighted structural image, 5) normalization of images into Montreal Neurological Institute (MNI) space, 6) smoothing (8 mm Gaussian kernel), and 7) reslicing into a final isotropic voxel size of 2 mm.

BOLD fMRI data denoising

Functional MRI data underwent a denoising procedure to clean the data from noise related to head motion, respiration and heart rate. This procedure was performed in the functional connectivity toolbox CONN version 17.c (https://web.conn-toolbox.org/)(Whitfield-Gabrieli and Nieto-Castanon, 2012). It included regression of motion (three translation and three rotation vectors describing framewise head motion and their 1st order derivatives), spike regression using ART (https://www.nitrc.org/projects/artifact_detect; flagging threshold: max motion 0.5 mm or global signal > 3 SD of timeseries average), and regression of physiological noise as contained in WM and CSF signal (the first five principal components and their 1st order derivatives from separate principal components analyses of WM and CSF time series (aCompCor, (Behzadi *et al*, 2007; Muschelli *et al*, 2014)). Only functional data sets with more than four minutes of unflagged images (as flagged by spike regression) were included in further analysis. Quality control functional connectivity (QC-FC) plots were inspected for all data sets as part of quality control (Ciric *et al*, 2017). QC-FC plots appeared normal for included (n = 68) but not for excluded (n = 6) datasets.

Estimation of resting state functional connectivity

Seven RSFC networks (36 ROIs) were defined using a 10 mm sphere at MNI coordinates previously described (Raichle, 2011). The networks were: DMN, DAN, ECN, SAN, AN, VN and SMN (See Appendix, Study 3, for ROI names and MNI coordinates). Fisher-transformed r-to-z value correlation coefficients were computed as a measure of coupling strength from denoised timeseries of percent signal change. RSFC within and between networks was computed as the average of RSFC for all relevant ROI pairs. Average within-network RSFC was calculated as mean RSFC for all

individual networks. Average between-network RSFC was calculated as the mean of all between-network RSFC estimates.

Data analysis

In Study 1, 5-HT2AR occupancy was calculated using equation (III). The relationship between occupancy, Occ_{max} and C_P was modelled using equation (IV).

In Study 2, changes in neocortical [^{11}C]Cimbi-36 binding from baseline to 1-week-rescan (1WRS) and changes in NEO PI-R personality and in mindfulness from baseline to 3-month-follow-up (3MFU) were assessed using paired t-tests. Openness was hypothesized to increase (Maclean *et al*, 2011), and a one-tailed paired t-test was used in this case. *Post-hoc* evaluation of changes in [^{11}C]Cimbi-36 with mindfulness and Openness was done using linear regression. The family-wise error rate (FWER) was controlled using Bonferroni-Holm (Holm, 1979); the statistical threshold was set to $p_{FWER} < 0.05$ for all tests with the exception of the hypothesized test for increased Openness, for which $p_{uncorrected} < 0.05$ was considered statistically significant.

Study 3 assessed relations between PPL, SDI and RSFC. The design made use of repeated measures, and associations were assessed using linear mixed effects modelling, allowing for variable y-intercepts but fixed slopes for each participant (Bates *et al*, 2015). We hypothesized that PPL and SDI would correlate negatively with DMN RSFC and with average within-network RSFC, indicating decreased network integrity, and positively with average between-network RSFC, indicating decreased network segregation (number of tests = 6). Associations with individual within- and between-network RSFC was evaluated separately for PPL and SDI (number of tests = 28). The statistical threshold was set to $p_{FWER} < 0.05$, controlling the family-wise error rate using Holm's method (Holm, 1979).

Statistical analyses were performed in R (version 3.3.1) with the exception of non-linear modelling in Study 1, which was performed in GraphPad Prism v.7.

Results

In the following chapter, main findings from the three studies are presented. Further results are contained within each paper in Appendix.

Study 1

Study 1 evaluated associations between PPL, 5-HT2AR occupancy and SDI in eight healthy participants (three females, mean age (SD): 33.0 (7.1) years) after an oral psilocybin intervention (3-30 mg). First, we saw that the time course of PPL and SDI followed a similar trajectory (**Fig. 7**), supporting that psilocin shapes subjective psychedelic experience.

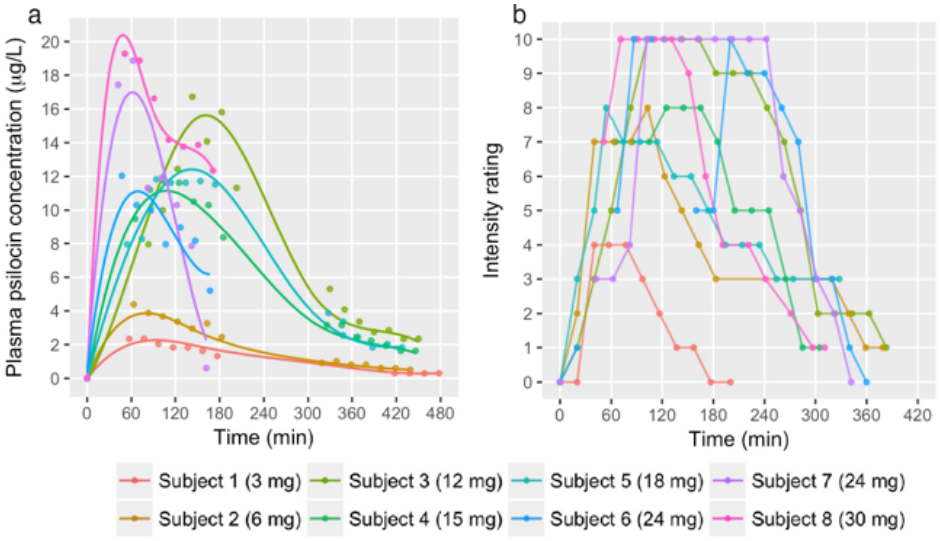

Fig. 7. Time course of plasma psilocin levels and subjective drug intensity ratings. (a) shows time course of plasma psilocin levels (measured at 20 min intervals during PET scans). Temporal development approximated using spline fits. (b) shows subjective intensity ratings over time.

Second, we found substantial 5-HT2AR occupancy following psilocybin intake (**Fig. 8**). PPL correlated positively with 5-HT2AR occupancy, an association well-described by the applied single-site binding model (EC_{50} [95% CI]:1.95 [1.16; 3.15] µg/L; Occ_{max} [95% CI]: 76.6 [67.3; 88.0]%) (**Fig. 9**).

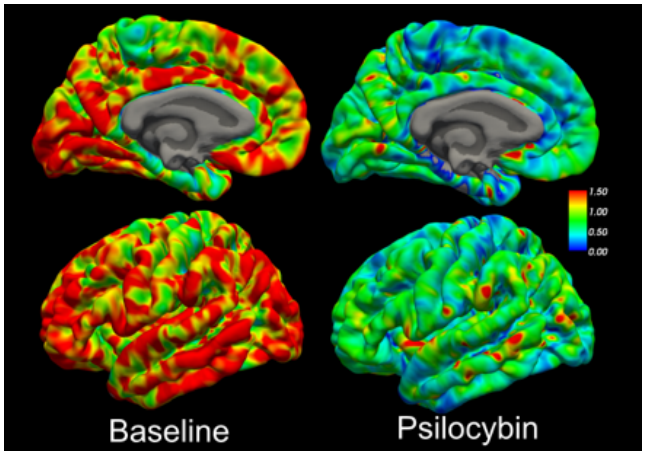

Fig. 8. 5-HT2AR occupancy after psilocybin. Surface visualization of [^{11}C]Cimbi-36 BP_{ND} of left hemisphere at baseline and at psilocybin intervention scan 1 (during the peak) for one subject. This subject displayed 72% occupancy.

Third, we observed that 5-HT2AR occupancy correlated positively with SDI, an association approximated using a second degree polynomial (Intensity = β1 * occupancy + β2 * occupancy^2; parameter estimates: β1[95% CI]: –0.02 [–0.13; 0.1], β2 [95% CI]: 0.002 [0.0006; 0.003]), (**Fig. 9**).

Fourth, SDI correlated positively with PPL, which was modelled using a non-linear model (the "Occ_{max} model") (**Fig. 9**): $Intensity_{max}$ [95% CI] was 10.8 [8.6; 14.7] and EC_{50} [95% CI] was 4.5 [2.1; 9.8] µg/L.

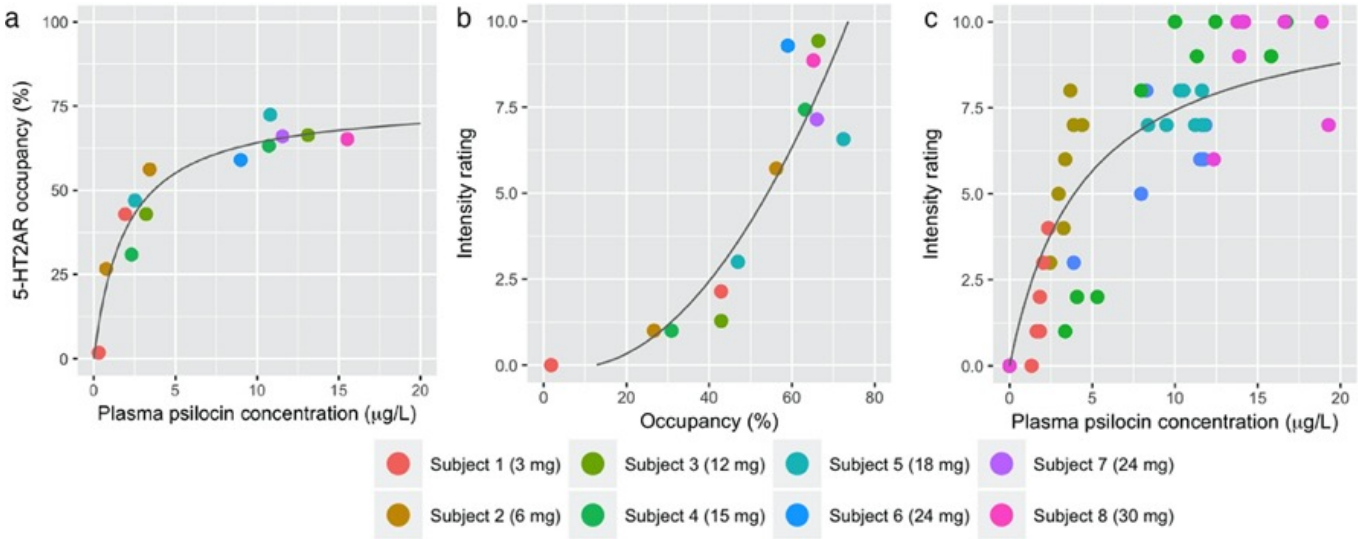

Fig. 9. Plasma psilocin level, subjective drug intensity and 5-HT2AR occupancy. (a) shows the association between average plasma psilocin concentration (PPL) and 5-HT2AR occupancy, (b) shows the association between 5-HT2AR occupancy and average subjective drug intensity (SDI), and (c) shows the association between individual measurements of PPL and SDI.

Study 2

In Study 2, we evaluated changes in neocortical [^{11}C]Cimbi-36 binding, personality and mindfulness in 10 healthy volunteers (four females, mean age (SD): 28.4 (3.4) years) after a single psilocybin intervention.

First, we found that neocortical [^{11}C]Cimbi-36 binding was unchanged across individuals (baseline mean BP_{ND} (SD): 1.15 (0.13)); 1WRS mean BP_{ND} (SD): 1.16 (0.14), p = 0.8, **Fig. 10**).

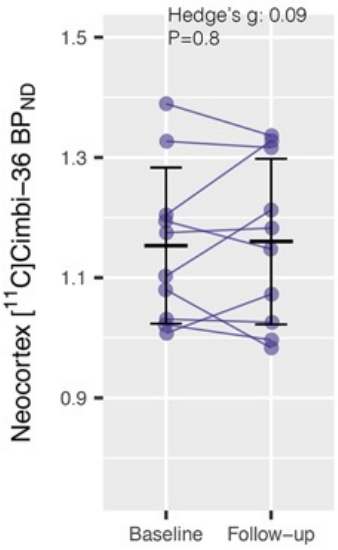

Fig. 10. Neocortex [^{11}C]Cimbi-36 BP_{ND} at baseline and 1-week-rescan.

Second, as hypothesized, personality trait Openness was increased at the 3MFU compared to baseline (baseline score mean (SD): 127.0 (16.6); 3MFU score mean (SD): 131.2 (17.2), p = 0.04, **Fig. 11**). Although not meeting the threshold for statistical significance, Neuroticism and Conscientiousness stood out by displaying effect sizes of -0.29 and 0.26, respectively.

Third, mindfulness was statistically significantly increased at the 3MFU compared to baseline (baseline score mean (SD): 4.2 (0.6); 3MFU score mean: 4.8 (0.6), p_{FWER} = 0.02) (**Fig. 11)**.

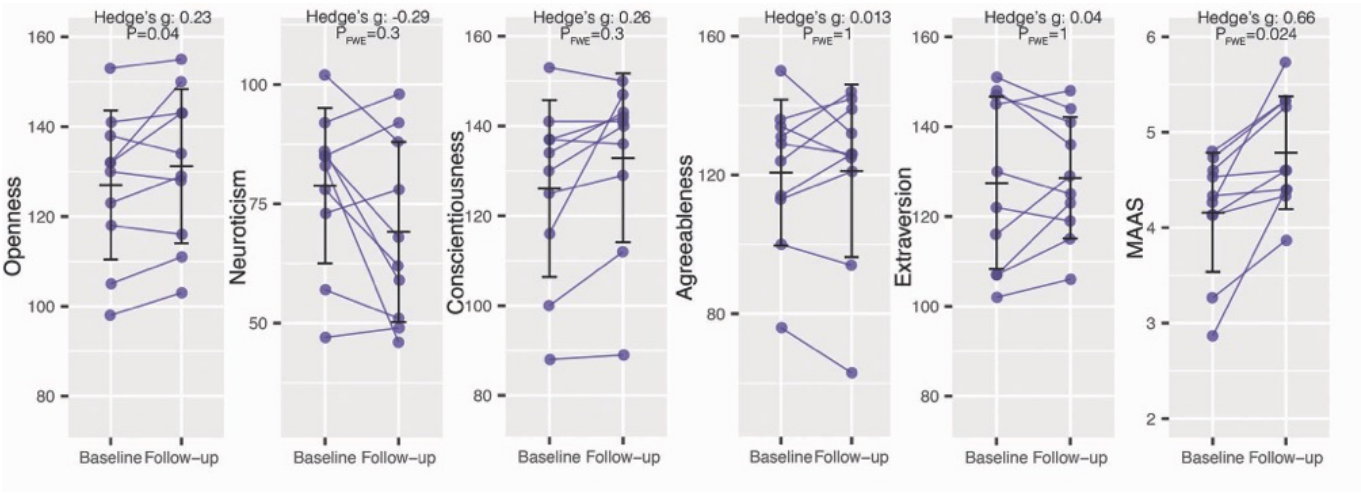

Fig. 11. Personality and mindfulness at baseline and at the three-months follow-up. NEO PI-R (Openness to experience, Neuroticism, Conscientiousness, Agreeableness, and Extraversion) and MAAS (mindfulness) measured at baseline and at 3-month follow-up.

Fourth, a *post-hoc* exploratory assessment found that individual changes in [^{11}C]Cimbi-36 BP_{ND} showed a statistically significant negative association with individual changes in MAAS (β [95%CI] = –5.0 [–9.0; –0.9], R^2 = 0.50, p_{FWER} = 0.046) (**Fig 12**) but not with changes in Openness (p_{FWER} = 0.3).

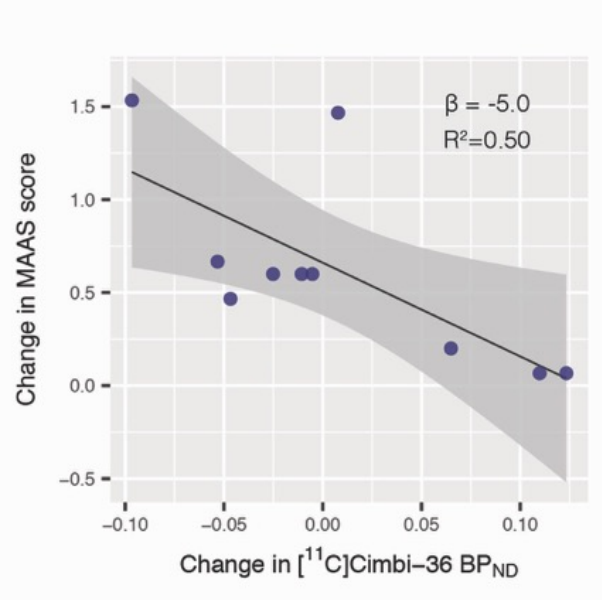

Fig 12. Changes in mindfulness correlates with changes in 5-HT2AR binding. MAAS: mindful attention awareness scale. MAAS was measured three months after drug; 5-HT2AR binding ([^{11}C]Cimbi-36 BP_{ND}) was measured one week after drug.

Study 3

Study 3 evaluated associations between PPL, SDI and RSFC in 15 healthy volunteers (six females, mean age (SD): 34.3 (9.8) years) throughout the psilocybin experience after peroral psilocybin.

First, we replicated findings from Study 1, showing that PPL and SDI displayed highly similar trajectories and SDI correlated positively (**Fig. 13**).

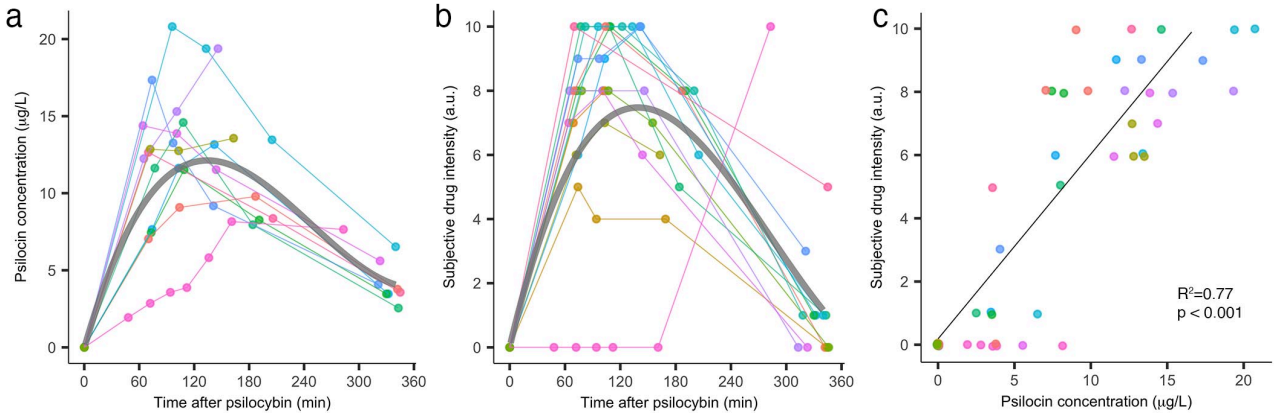

Fig. 13. Plasma psilocin levels and subjective drug intensity. Time course of plasma psilocin level (PPL) (a) and subject drug intensity (SDI) (b). PPL correlated positively with SDI (c). Grey line shows average time course approximated by spline fits for PPL (a) and SDI (b). a.u.: arbitratry units.

Second, we observed that PPL and SDI correlated negatively with RSFC in DMN and across networks, indicating reduced network integrity, and positively with average between-network RSFC, indicating reduced network segregation (**Fig. 14**).

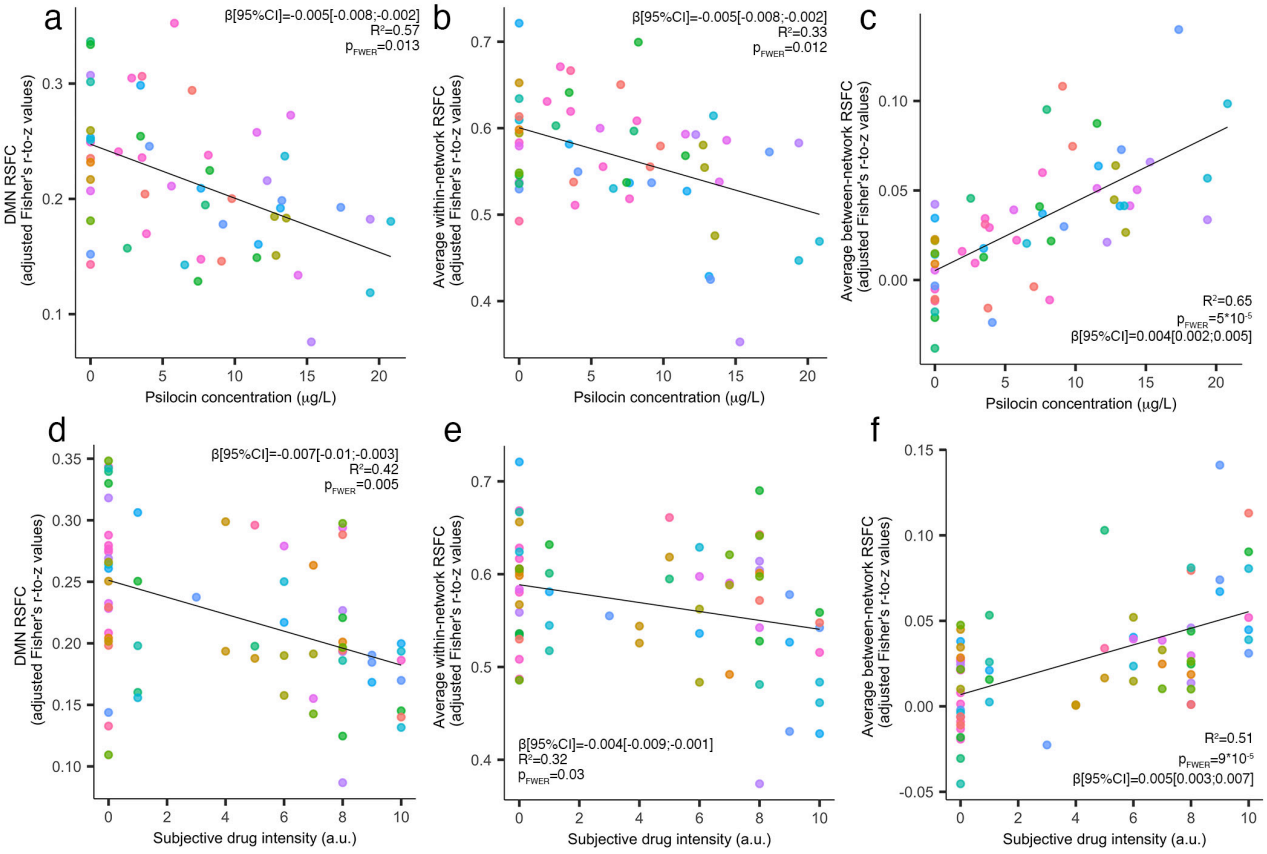

Fig. 14. Functional connectivity, psilocin and intensity. Plasma psilocin level (PPL) correlated negatively with average resting state functional connectivity (RSFC) in the default mode network (DMN) (a) and with average within-network RSFC (b), and positively with average between-network FC (c). Likewise, subjective drug intensity (SDI) correlated negatively with DMN RSFC (d) and average within-network RSFC FC (e). SDI correlated positively with average between-network FC (f). adjusted: partial correlation for RSFC estimate on SDI or PPL. a.u.: arbitratry units. Datapoints are color-coded by each subject.

Third, we evaluated PPL and SDI relations with individual network RSFC. We found that PPL correlated negatively with SAN RSFC (**Fig. 15**). Apart from the DMN, no other individual network exhibited statistically significant associations with either PPL or SDI. Further, for between-network RSFC, we found that PPL and SDI displayed positive correlations for DMN with ECN, DAN and SAN, and for DAN with AN and SMN. SDI also displayed a positive association with RSFC between ECN and SMN.

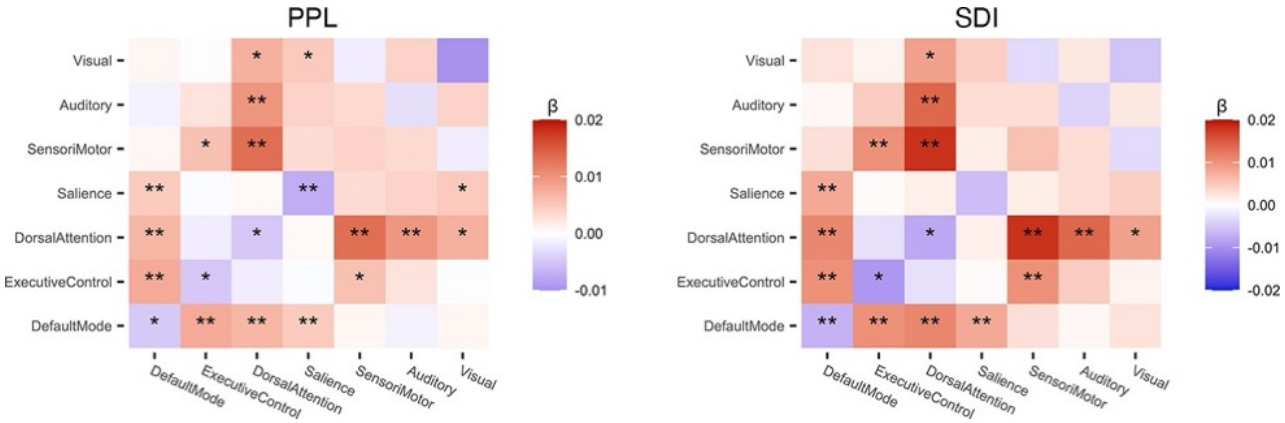

Fig. 15. Association of network functional connectivity with plasma psilocin level and subjective drug intensity. Heat map showing β-estimate (slope) for the association of network functional connectivity with plasma psilocin level (PPL) (left) and subjective drug intensity (SDI) (right). * $p_{unc}<0.05$, ** $p_{FWER}<0.05$. The diagonal represents within-network RSFC, and the off-diagonal represents between-network RSFC.

Fourth, current efforts to develop psilocybin as a medicine include giving a 25 mg psilocybin dose in the context of psychotherapy. A recent publication estimated that a 25 mg psilocybin dose is associated with psilocin C_{max} = 18.7 *μ*g/L (Dahmane *et al*, 2020). As a summary figure in this context, **Fig. 16** shows the average predicted time curve of 5-HT2AR occupancy, PPL, SDI and network RSFC and thus ties together main findings from Study 1 and 3.

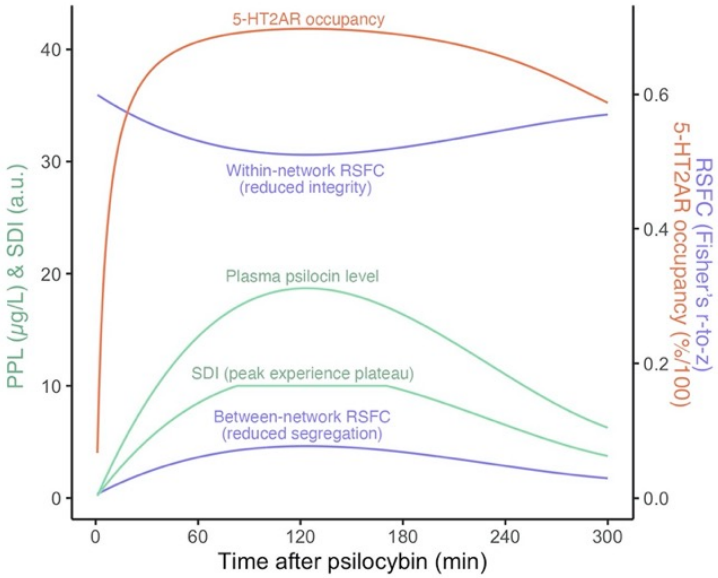

Fig. 16. Predicted response curve of 25 mg psilocybin dose. Predicted average time curves for a 25 mg psilocybin dose (Dahmane *et al*, 2020). PPL: plasma psilocin level; SDI: subjective drug intensity; RSFC: resting state functional connectivity. 5-HT2AR prediction based on model parameters from Study 1. RSFC and SDI prediction based on model parameters from Study 3. The peak experiential plateau corresponds to very intense effects (i.e., the maximum possible rating of 10). PPL curve defined by spline fits of Study 3 psilocin data scaled to C_{max} of 18.7 *μ*g/L.

Discussion

Study 1

We observed positive correlations between PPL, 5-HT2AR occupancy, and subjective effects. This supports that psilocin is a key determinant of psilocybin effects and that PPL can be used as an objective measure of 5-HT2AR occupancy and overall psychedelic effects. Although psilocin engages other serotonergic targets than the 5-HT2AR (McKenna *et al*, 1990; Rickli *et al*, 2016), our results are in line with 5-HT2AR agonism being a crucial mechanism for psychedelic effects of psilocin (Nichols, 2016).

However, some questions remain. First, we observed that the predicted maximum occupancy was 77%. Although this is similar to the predicted Occ_{max} of two other 5-HT2AR PET occupancy studies (Grunder *et al*, 1997; Nordstrom *et al*, 2008), it is unclear why predicted Occ_{max} is not 100%. Possible contributions to this may stem from real-world deviations from kinetic modelling assumptions and/or psilocin-induced increases in cortical 5-HT tonus, which could reduce occupancy and the predicted Occ_{max} (Jørgensen *et al*, 2016; Rickli *et al*, 2016; Sakashita *et al*, 2015). Potential future psilocin 5-HT2AR occupancy studies in animals or humans may benefit from collecting arterial blood samples, which would allow for full kinetic modelling, and from assessing changes in 5-HT levels after psilocybin. However, it is unlikely that the shape of the curve will change substantially.

Associations of SDI with occupancy and PPL appeared not to be linear. Whereas modelling of PPL and occupancy was informed by previous PET occupancy studies, the modelling of SDI relations with occupancy and PPL could not rely on a similar foundation. Thus, it is possible that other models more accurately describe these relations. Also, although a strength of the study was repeated observations for every subject, the sample size was small, and a larger number of observations would be helpful for more closely understanding the shape of SDI associations with occupancy and PPL.

The implemented SDI ratings has a maximum score of 10, corresponding to “very intense” effects. A ceiling effect was present at higher PPL for several subjects (**Fig. 7**). Had the scale been constructed in another way (e.g., 0-100 or substituting the upper classifier as “extreme” instead of “very”), it is probable that a ceiling effect would be less

evident. Nevertheless, within the range of PPL recorded in the present study, the 5-TH2AR occupancy, PPL and SDI relations seemed to perform well and were closely positively correlated, supporting that psilocin binds with high affinity to 5-HT2ARs *in vivo* and that PPL stimulation of 5-HT2ARs is important for subjective effects of psilocybin.

Study 2

Interestingly, we found a long-term increase in mindfulness after psilocybin. At baseline, mindfulness was comparable with normative data (Jensen *et al*, 2016) but was robustly increased at 3MFU. Mindfulness is negatively correlated with psychological distress (Jensen *et al*, 2016) and is a prominent part of mindfulness-based stress reduction (MBSR) (Kabat-Zinn, 1996), which is effective for reducing anxiety and depressive symptoms (Fjorback *et al*, 2011; Forman *et al*, 2007). Thus, it is possible that psilocybin-induced increases in mindfulness constitute an independent mechanism by which psilocybin therapy may promote mental health.

We observed that Openness was increased three months after psilocybin. Openness has previously been identified as a personality trait sensitive to psilocybin (Erritzoe *et al*, 2018; Maclean *et al*, 2011). Thus, our finding confirms that Openness is amenable to psilocybin intervention. Further studies are needed to understand neurobiological mechanisms of personality change in the context of psilocybin.

On a group basis, [^{11}C]Cimbi-36 binding was unchanged across individuals at the 1WRS, which does not support that cerebral 5-HT2AR density changes. This is noteworthy because Study 1 found pronounced 5-HT2AR occupancy, and because a previous human [^{18}F]Altanserin PET study observed decreased 5-HT2AR binding in psychedelics users compared to non-users (Erritzoe *et al*, 2011). However, individual changes in [^{11}C]Cimbi-36 binding correlated negatively with changes in mindfulness. This implicates changes in 5-HT2AR levels, 5-HT tonus or, more speculatively, neuroplastic brain changes in subsequent mindfulness increases. This interesting finding, however, should be regarded as preliminary and more studies are needed to establish the extent to which neurobiological changes (e.g., 5-HT2AR levels or 5-HT tonus changes) play a role in mindfulness changes after psilocybin.

Some limitations deserve mention. We did not employ a placebo-control condition in the present study, and it is likely that factors other than psilocybin could contribute to changes in Openness, mindfulness or, less likely, changes in [^{11}C]Cimbi-36 binding.

PET scans were conducted one week after psilocybin, and personality and mindfulness assessments were conducted three months after psilocybin. This discrepancy between the time of data collection makes direct interpretations challenging.

Thus, Study 2 confirmed that Openness is a personality trait sensitive to psilocybin and found that mindfulness was increased long-term, which warrants a larger study employing a placebo-control condition. Although 5-HT2AR binding appeared stable at the group-level, changes in 5-HT2AR binding correlated with changes in MAAS, indicating that changes in 5-HT2AR levels or 5-HT tonus may play a role in subsequent mindfulness increase.

Study 3

Study 3 found, as hypothesized, that PPL and SDI correlated negatively with RSFC in DMN and across networks. We interpret this finding as reduced network integrity, presumably marking impairment of normal network function. We also observed that PPL and SDI correlated positively with average between-network RSFC, which we interpret as reduced network segregation. These results support previous psilocybin studies, which indicate impaired network function (Carhart-Harris *et al*, 2012a; Muthukumaraswamy *et al*, 2013) and increased between-network communication (Carhart-Harris *et al*, 2013; Roseman *et al*, 2014), and support proposed neurobiological mechanisms underlying the psychedelic state (Carhart-Harris and Friston, 2019). Our findings implicate the expression of network integrity and segregation in psychedelic experiences and consciousness in general.

Interestingly, we observed that PPL and SDI associations with between-network RSFC presented in a pattern similar to the repeating spatial motif of networks observed in the brain (Buckner and DiNicola, 2019; Margulies *et al*, 2016; Yeo *et al*, 2011). In this spatial motif, the DMN is situated at one end, with ECN/DAN/SAN in the middle, and unimodal sensory/motor networks in the other end. Our findings suggest that it is primarily adjacent networks that desegregate as a function of PPL, driving the overall network desegregation after psilocybin.

We saw that as a function of PPL and SDI, RSFC was increased for the DAN with DMN and sensory networks. The DAN is implicated in externally directed attention (Corbetta and Shulman, 2002) and DMN is involved in mental functions related to internally directed attention (Buckner and DiNicola, 2019). The two networks display

anticorrelations in the resting state (Chai *et al*, 2012). It is possible that DMN-DAN desegregation is involved in psilocybin-induced unitive experiences, which includes a perceived merging of the "self" with the external world (Griffiths *et al*, 2006; Nour *et al*, 2016). Future studies are needed to more closely understand neurobiological effects of psychedelic experiences, including unitive experiences.

As mentioned, the temporal trajectories of PPL and SDI were highly similar in Study 3, as was the case in Study 1. However, one subject (**Fig. 13**, color-coded in pink) displayed a disjunction of PPL and SDI time courses. Pharmacokinetics was very slow for this subject, as true C_{max} occurred 180-240 min after drug. Although this subject exhibited signs of intoxication in his interactions with staff already after one hour, he rated SDI zero. It is probable that the sluggish rise in PPL (and consequently slow onset of brain function changes) made the transition from normal consciousness to psychedelic consciousness less clear to this subject than it was for the other subjects for whom the rise in PPL occurred more rapidly. Nevertheless, SDI and PPL correlated overall very well, supporting that PPL critically shapes psychedelic experience.

Study 3 did not employ a placebo condition. It is clear that factors other than the pharmacological action of psilocin (e.g., music, psychological factors and interaction with guides) shape subjective experiences (Carhart-Harris *et al*, 2018b; Griffiths *et al*, 2016; Haijen *et al*, 2018). In other words, study designs including a placebo-control condition would be helpful in identifying placebo effects and the true pharmacological effect of psilocybin. Nevertheless, the salient experience of psilocybin unblind participants rather quickly, and it is unclear what constitutes the best placebo (e.g., active vs inactive) in the context of psychedelics studies. Importantly, however, Study 1 and Study 3 correlated PPL with SDI, 5-HT2AR occupancy and RSFC, and these variables were measured throughout the psilocybin experience. It is difficult to imagine a placebo effect mimicking a similar time course and magnitude as PPL.

Arguably, Study 3 constitutes the most clinically relevant neuroimaging assessment of psilocybin brain effects to date. It captures the entire acute psilocybin experience (5-6 hours) after clinically relevant doses of peroral psilocybin. The direct linking of psilocin engagement of cerebral targets and subjective experience (indexed by PPL and SDI) with changes in the cerebral functional network architecture provides a clear picture of overall psilocybin effects throughout the acute psilocybin experience.

Perspectives and conclusion

The present PhD thesis is based on PET and fMRI with the aim to better understand neurobiological effects of 5-HT2AR modulation induced by psilocin. The studies illuminate aspects of psilocybin's neuropsychopharmacology and are especially timely given the emergence of psilocybin as a novel therapeutic for several brain disorders.

An important finding is that PPL correlates closely with 5-HT2AR occupancy, brain function and subjective experience. This shows that PPL is a key determinant of psilocybin effects, and suggests that PPL can be used as an objective marker of effects of psilocybin. It is probable that some interindividual variability in clinical outcomes after psilocybin therapy (Carhart-Harris *et al*, 2016a; Griffiths *et al*, 2016; Ross *et al*, 2016) can be explained by interindividual variability in PPL (Brown *et al*, 2017; Hasler *et al*, 1997; Lindenblatt *et al*, 1998). Thus, a clear suggestion informed by our studies is that clinical trials could benefit from determining PPL.

As described in the Background section, a large body of literature suggests that 5-HT2AR stimulation is crucial for effects of psychedelics, including psilocin. Our findings support that stimulation of cerebral 5-HT2ARs is important for psychedelic effects of psilocin. However, psilocin engages other neuroreceptors than the 5-HT2AR, including 5-HT1ARs (McKenna *et al*, 1990). Stimulation of 5-HT1ARs has opposite effects of 5-HT2AR stimulation on neuronal membrane potential (Araneda and Andrade, 1991), and it would be interesting to better understand the extent to which psilocin engagement of 5-HT1ARs contribute to psilocin effects on brain function and subjective experience. The role of 5-HT1AR in psilocin effects could be elucidated by carrying out a study similar to Study 1, but using a 5-HT1AR radioligand instead of [^{11}C]Cimbi-36. Also, information about the contribution of different neuroreceptors could be gained by pretreatment with or concurrent administration of selective receptor antagonists. For instance, the importance of the 5-HT2AR has been examined using the 5-HT2A/2CR antagonist ketanserin (Kometer *et al*, 2012; Vollenweider *et al*, 1998), but this could be further improved upon by using a more selective 5-HT2AR antagonist such as volinanserin, and the importance of the 5-HT1AR could be elucidated by using pindolol. In a similar vein, it would be interesting to compare effects of a more selective 5-HT2AR agonist with those of psilocin. Given the ongoing emergence of psychedelics as

medicines, it is possible that more selective 5-HT2AR agonist compounds will become available for use in humans, which would allow for such a study.

Study 2 showed that mindfulness was increased long-term after psilocybin. This is an interesting finding, deserving of additional scientific scrutiny. It would be interesting to evaluate whether this effect can be reproduced in a double-blind placebo-controlled experiment. This could be done in combination with a traditional mindfulness intervention in a 2-by-2 factorial design. It is possible that both psilocybin and the mindfulness intervention independently increase mindfulness, and that an interaction effect can be observed of combined psilocybin and mindfulness intervention (boosting the mindfulness effects compared to each of the conditions alone). Given that psilocybin and MBSR both can have beneficial effects on anxiety and depressive symptoms in cancer patients (Griffiths *et al*, 2016; Ross *et al*, 2016; Zhang *et al*, 2015), it would be interesting to conduct the experiment in a population in which effects on psychiatric symptoms could be evaluated also. Such a population could be patients with work-related stress, cancer patients or patients with depression or anxiety disorder.

Study 3 showed that functional network integrity and segregation was reduced as a function of PPL and mapped similarly onto SDI ratings, implicating reduced expression in psychedelic experience. Conversely, these findings suggest that high within-network connectivity and low between-network connectivity is important for maintaining normal consciousness.

We applied more traditionally oriented static FC analyses in Study 3, but many other methods have been developed. Since it is likely that applying complementary analytical methods may lead to additional insights, it would be interesting to consider their application. Additional analytical frameworks could include graph theoretical assessments of functional brain integration and segregation (Bullmore and Sporns, 2009), effective connectivity methods, assessment of the directionality of FC (Friston *et al*, 2003; Seth *et al*, 2015) or assessment of changes in brain states (Lord *et al*, 2019). It would also be relevant to consider other parcellations of brain (Kernbach *et al*, 2018; Schaefer *et al*, 2018; Shen *et al*, 2013; Yeo *et al*, 2011).

Serotonergic targets of psilocin (e.g., 5-HT2AR and 5-HT1AR) are not evenly distributed throughout the brain but display substantial interregional variability (Beliveau *et al*, 2017). Interestingly, the DMN contains high levels of 5-HT2ARs, supporting that psilocin stimulation of 5-HT2ARs contributes to reduced DMN integrity. Future psilocin

studies could benefit from including spatial molecular information (e.g., 5-HT2AR and 5-HT1AR brain maps) in the analytical framework.

Although SDI is an easily obtainable and useful measure of the overall psychedelic experience, it does not enable an understanding of more specific dimensions of psychedelic experience (e.g., ego-dissolution or visual imagery). Previous psychedelic neuroimaging studies have made use of more elaborate real-time ratings of subjective experience (Carhart-Harris *et al*, 2012a, 2016b; Lebedev *et al*, 2015; Tagliazucchi *et al*, 2016), showing that this approach is feasible and can yield knowledge about neural correlates of psychedelic experience. Although not included in the present thesis, additional real-time ratings were collected in Study 3, and it will be interesting to see future studies make use of this data, since this may provide additional insights into brain mechanisms of individual dimensions of psychedelic experience.

Together, Study 1 and 3 exemplify how valuable information can be gained about a drug's neuropsychopharmacology by combining PET occupancy assessment, plasma drug measurement, functional brain imaging and characterization of subjective experience. An interesting—but long-term—perspective would be to apply these methods in the context of pharmacological challenges with selective agonists and antagonists. This could yield a library of functional brain changes associated with both magnitude-specific and receptor-specific modulation. Coupled with information about differences in brain function between healthy individuals and patients, it is possible that such a library could guide drug development.

In conclusion, the present PhD thesis provides novel information about psilocybin's neuropsychopharmacology, including long-term increased mindfulness, and shows that psilocin engages a large fraction of 5-HT2ARs after psychoactive doses of psilocybin. It demonstrates that psilocin critically shapes effects of psilocybin on brain function and subjective experience and implicates the expression of functional network integrity and segregation as important for both psychedelic experience and normal consciousness.

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Appendix

Study 1

Madsen, M. K., Fisher P.M., Burmester D., Dyssegaard A., Stenbæk D.S., Kristiansen S., Johansen S.S., Lehel S., Linnet K., Svarer C., Erritzoe D., Ozenne B., Knudsen G.M. (2019). Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. Neuropsychopharmacology 44, 1328–1334.

GRADUATE SCHOOL OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

PHD-THESIS
DECLARATION OF CO-AUTHORSHIP

The declaration is for PhD students and must be completed for each conjointly authored article. Please note that if a manuscript or published paper has ten or less co-authors, all co-authors must sign the declaration of co-authorship. If it has more than ten co-authors, declarations of co-authorship from the corresponding author(s), the senior author and the principal supervisor (if relevant) are a minimum requirement.

1. Declaration by	
Name of PhD student	Martin Korsbak Madsen
E-mail	martin@nru.dk
Name of principal supervisor	Gitte Moos Knudsen
Title of the PhD thesis	Neurobiological effects of 5-HT2AR modulation

2. The declaration applies to the following article	
Title of article	Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels
Article status	
Published ☒ Date: Jan 26 2019	Accepted for publication ☐ Date:
Manuscript submitted ☐ Date:	Manuscript not submitted ☐
If the article is published or accepted for publication, please state the name of journal, year, volume, page and DOI (if you have the information).	Neuropsychopharmacol. 33, 71–80 (2020). 10.1038/s41386-019-0324-9

3. The PhD student's contribution to the article ***(please use the scale A-F as benchmark)*** Benchmark scale of the PhD-student's contribution to the article **A**. Has essentially done all the work (> 90 %) **B**. Has done most of the work (60-90 %) **C**. Has contributed considerably (30-60 %) **D.** Has contributed (10-30 %) **E.** No or little contribution (<10 %) **F.** Not relevant	**A, B, C, D, E, F**
1. Formulation/identification of the scientific problem	E
2. Development of the key methods	E
3. Planning of the experiments and methodology design and development	D
4. Conducting the experimental work/clinical studies/data collection/obtaining access to data	B
5. Conducting the analysis of data	B
6. Interpretation of the results	B
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	A
Provide a short description of the PhD student´s specific contribution to the article.[i] Recruited, included participants, conducted PET experiments in collaboration with co-workers, performed data processing and statistical analyses under supervision, wrote the first draft, circulated to co-authors and finalized the manuscript according to discussions with collaborators.	

4. Material from another thesis / dissertation[ii]	
Does the article contain work which has also formed part of another thesis, e.g. master's thesis, PhD thesis or doctoral dissertation (the PhD student's or another person's)?	Yes: ☐ No: ☒
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5. Signatures of the co-authors[iii]

	Date	Name	Title	Signature
1.	May 4 2020	Gitte Moos Knudsen (senior & corresponding author)	Professor	
2.				
3.				
4.				
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6. Signature of the principal supervisor

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: 25.5.2020
Principal supervisor:

7. Signature of the PhD student

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: May 25 2020
PhD student: Martin Korsbak Madsen

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ARTICLE

Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels

Martin K. Madsen[1,2], Patrick M. Fisher[1], Daniel Burmester[1,2], Agnete Dyssegaard[1], Dea S. Stenbæk[1], Sara Kristiansen[1], Sys S. Johansen[3], Sczabolz Lehel[4], Kristian Linnet[3], Claus Svarer[1], David Erritzoe[5], Brice Ozenne[1,6] and Gitte M. Knudsen[1,2]

The main psychedelic component of magic mushrooms is psilocybin, which shows promise as a treatment for depression and other mental disorders. Psychedelic effects are believed to emerge through stimulation of serotonin 2A receptors (5-HT2ARs) by psilocybin's active metabolite, psilocin. We here report for the first time the relationship between intensity of psychedelic effects, cerebral 5-HT2AR occupancy and plasma levels of psilocin in humans. Eight healthy volunteers underwent positron emission tomography (PET) scans with the 5-HT2AR agonist radioligand [^{11}C]Cimbi-36: one at baseline and one or two additional scans on the same day after a single oral intake of psilocybin (3–30 mg). 5-HT2AR occupancy was calculated as the percent change in cerebral 5-HT2AR binding relative to baseline. Subjective psychedelic intensity and plasma psilocin levels were measured during the scans. Relations between subjective intensity, 5-HT2AR occupancy, and plasma psilocin levels were modeled using non-linear regression. Psilocybin intake resulted in dose-related 5-HT2AR occupancies up to 72%; plasma psilocin levels and 5-HT2AR occupancy conformed to a single-site binding model. Subjective intensity was correlated with both 5-HT2AR occupancy and psilocin levels as well as questionnaire scores. We report for the first time that intake of psilocybin leads to significant 5-HT2AR occupancy in the human brain, and that both psilocin plasma levels and 5-HT2AR occupancy are closely associated with subjective intensity ratings, strongly supporting that stimulation of 5-HT2AR is a key determinant for the psychedelic experience. Important for clinical studies, psilocin time-concentration curves varied but psilocin levels were closely associated with psychedelic experience.

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INTRODUCTION

Psilocybin is a classic serotonergic psychedelic drug and is the primary psychoactive compound in magic mushrooms [1]. Its effects are in many ways similar to those of LSD and mescaline [2]. Recent clinical trials have shown that psilocybin may be an effective treatment for neuropsychiatric disorders, including treatment-resistant major depressive disorder (MDD) [3], cancer-related anxiety and depression [4, 5], and for addiction to nicotine [6] and alcohol [7]. Thus, psilocybin is an emerging and promising drug for a range of mental disorders where existing drugs have shown shortcomings.

Preclinical findings [8], human blocking studies [9, 10] and preliminary data from a PET study [11] strongly suggest that serotonergic psychedelics exert their psychoactive effects through the serotonin 2A receptor (5-HT2AR). However, 5-HT2AR target engagement of psilocybin's active metabolite, psilocin, as well as the pharmacodynamics, i.e., the relation between plasma psilocin levels and 5-HT2AR occupancy, still remain to be established. Importantly, the relationship between the subjective psychedelic experience, plasma psilocin levels and 5-HT2AR occupancy in the human brain is currently unknown.

Positron emission tomography (PET) is an imaging technique capable of quantifying receptor binding in vivo [12, 13]. Coupled with drug administration and appropriate radiotracer selection, PET-studies can provide valuable knowledge about relationships between drug levels, drug target occupancy, and associations with clinical response or side-effects [14]. In the present study we took advantage of the recent development of a 5-HT2R agonist radioligand, [^{11}C]Cimbi-36 [15, 16], to elucidate the direct role of 5-HT2ARs in psilocybin's psychedelic effects in humans. Here, we for the first time describe the relationships between subjective psychedelic effects, 5-HT2AR occupancy and psilocin plasma concentrations.

METHODS AND MATERIALS

Participants

Eight healthy participants (three females, mean age ± SD 33.0 ± 7.1 years) were recruited from a database of individuals interested in participating in a human neuroimaging study investigating psilocybin. After providing written informed consent, participants underwent a screening procedure including screening for present or previous psychiatric disorders using Mini-International

[1]Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Copenhagen 2100, Denmark; [2]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark; [3]Department of Forensic Medicine, Section of Forensic Chemistry, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark; [4]PET and Cyclotron Unit, Copenhagen University Hospital Rigshospitalet, Copenhagen 2100, Denmark; [5]Department of Medicine, Psychedelic Research Group, Neuropsychopharmacology Unit, Centre for Psychiatry, Division of Brain Sciences, Imperial College London, London, UK and [6]Department of Public Health, Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark
Correspondence: Gitte M. Knudsen (gmk@nru.dk)

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Neuropsychiatric Interview, Danish translation version 6.0.0 [17], neurological illness or significant somatic illness. Participants were healthy, see Supplementary data for complete exclusion criteria and individual participant descriptive data. History of serotonergic psychedelic drug use was noted for the five subjects with such experience (number of times used: 1 [0–55] (median [range]), time since last intake: 42 [6–156] months; Supplementary data, Table 1). Participants were thoroughly informed about the study prior to inclusion, including effects of psilocybin, potential side-effects, and risks. On the day of information and screening (prior to intervention day), all participants attended a preparatory meeting with at least one of the psychologists present on intervention days to familiarize with the study setting and establish a rapport. The study was approved by the ethics committee for the capital region of Copenhagen (journal identifier: H-16028698, amendments: 56023, 56967, 57974, 59673, 60437, 62255) and Danish Medicines Agency (EudraCT identifier: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295).

Procedures

Participants underwent a physical exam, including ECG, blood screening for pathology, and a screening for psychopathology. Participants completed baseline [^{11}C]Cimbi-36 PET (PET 0) and MR imaging prior to the psilocybin intervention day (mean ± SD: 49 ± 12 days). A screening procedure for amphetamines, opioids, benzodiazepines, barbiturates, tetrahydrocannabinol, cocaine, ketamine, phencyclidine, and gamma hydroxybutyrate was done using a urine test (Rapid Response, BTNX Inc., Markham, Canada). Participants were asked to be well-rested, refrain from alcohol the day before neuroimaging, have only a light breakfast and abstain from caffeine on study days. On the intervention day and before psilocybin administration, participants were informed again about potential psilocybin effects and safety precautions, as suggested previously [18]. Two psychologists providing interpersonal support were present on intervention days. During all PET scans (including baseline), a standardized list of music was played on a stereo system in the PET room. The playlist was adapted from one kindly provided by Prof. Roland Griffiths, Johns Hopkins Medicine.

Psilocybin interventions

On the intervention day, participants ingested between 3 and 30 mg psilocybin (3 mg capsules) approximately one hour prior (mean ± SD: 58 min ± 13) to the first [^{11}C]Cimbi-36 post-drug PET scan (PET 1). Subjects 1–5 underwent a second post-drug PET scan (PET 2) later the same day (344 min ± 41 after psilocybin ingestion), while subjects 6, 7, and 8 underwent only PET 1 on the intervention day. Participants were blind to the dose of psilocybin they were given. Each scan lasted 120 min, descriptive data pertaining to PET scans are available in supplementary data (Supplementary Table 2). For assessment of plasma psilocin levels, venous blood samples were taken simultaneously with the [^{11}C]Cimbi-36 injection and at 20-min intervals throughout each scan session. Subjective psychedelic intensity ratings (0–10 Likert scale, 0 = not intense at all, 10 = very intense) were assessed at 20-min intervals throughout the day until effects had waned. Between the two intervention scans, participants listened to music in the scanner room with staff support as appropriate. This three-scan protocol enabled the determination of 5-HT2AR occupancy during high and low plasma psilocin levels in five individuals. At the end of the intervention day (mean ± SD: 468 ± 80 min after psilocybin), participants filled out questionnaires capturing aspects of psychedelic experiences: 11-dimension altered states of consciousness questionnaire (11D-ASC) [19, 20], the 30-item mystical experiences questionnaire (MEQ30) [21] and the ego-dissolution inventory (EDI) [22]. All questionnaires were administered in Danish, having been translated and back-translated to English by native Danish, English, and bilingual speakers.

Psilocin plasma concentrations

Plasma psilocin concentrations were determined using ultra performance liquid chromatography and tandem mass spectrometry. Analysis was performed in units of µg/kg, although data are here presented in units of µg/L. For detailed description of analysis, see supplementary data.

Magnetic resonance imaging

High resolution 3D T1-weighted and T2-weighted images were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) using a 64-channel head coil for the purpose of PET-image coregistration and segmentation (T1-weighted images: inversion time = 900 ms, echo time = 2.58 ms, repetition time = 1900ms, flip angle = 9°, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap; T2-weighted images: echo time = 408 ms, repetition time = 3200 ms, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 208 slices and a slice thickness of 0.9 mm, no gap).

[^{11}C]Cimbi-36 PET data acquisition, processing, and kinetic modeling

Acquisition and processing of [^{11}C]Cimbi-36 PET data has been described previously [15, 16], a similar pipeline was used here. PET images were acquired for 120-min on a high-resolution research tomography PET-scanner (CTI/Siemens, Knoxville, USA) after a bolus injection of [^{11}C]Cimbi-36 (Supplementary data, Table 2). Regions of interest were defined using Pvelab, a fully automated regional delineation procedure, and regional time-activity curves were extracted for kinetic modeling [15, 23].

Kinetic modeling was performed using the simplified reference tissue model (SRTM) [13, 15] with neocortex (a volume-weighted average of all cortical regions) chosen a priori as the region of interest due to the high expression of 5-HT2ARs and the consequent beneficial signal-to-noise ratio within this region [24]. Cerebellum was chosen as the reference region [15]. Non-displaceable binding potential (BP_{ND}) was the primary outcome measure [12].

[^{11}C]Cimbi-36 metabolism

Analysis of [^{11}C]Cimbi-36 radiometabolites was described in recent publications by our lab [15, 25]. We did not observe effects of the psilocybin intervention on [^{11}C]Cimbi-36 radiometabolism or protein binding (see Supplementary data for details).

Data analysis

Within-scan plasma psilocin area under curve ($psilocin_{AUC}$) was calculated from psilocin plasma concentration time curves (Fig. 1), using the trapezoid method in GraphPad Prism (version 7.01, GraphPad Software, Inc., CA, USA) and normalized by 120 min (duration of blood sampling and PET scan) to yield a mean psilocin concentration, which was used for statistical analyses and figures (Table 1).

Neocortical [^{11}C]Cimbi-36 BP_{ND} was plotted against mean psilocin concentration and the relationship modeled using the following equation:

$$\text{Occupancy} = \frac{Occ_{max} * C_P}{EC_{50} + C_P},$$

where Occ_{max} denotes the predicted highest attainable occupancy, C_P is plasma psilocin concentration and EC_{50} is the plasma psilocin concentration at 50% Occ_{max} [26] Modeling and curve fitting was performed in GraphPad Prism.

Subject 1 psilocin concentrations were below limit of quantification (LOQ, 0.5 µg/kg) but above limit of detection (LOD, 0.1 µg/kg) during all second scan time points. We evaluated psilocin-occupancy relations considering LOQ and LOD. Model parameters were similar (Occ_{max} = 75.5% vs. 77.9%,

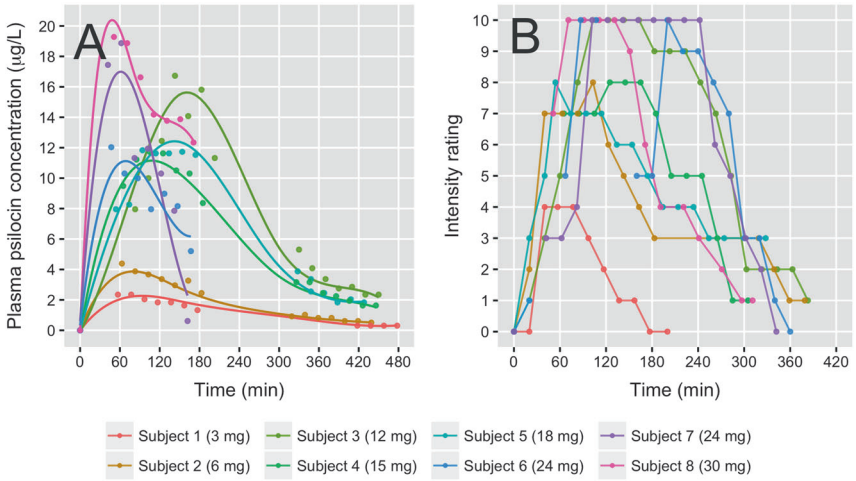

Fig. 1 Psilocin and intensity rating time course. **a** Plasma psilocin levels. Individual data points are measured plasma psilocin concentrations, fitted with spline fits. **b** Time course of subjective intensity ratings. Time = 0 indicates time of psilocybin ingestion

Table 1. Descriptive data related to psilocybin interventions and corresponding 5-HT2AR occupancy estimates

ID	Dose (mg)	Weight-adjusted dose (mg/kg)	C_{max} (µg/L)	Mean psilocin PET 1 (µg/L)	Mean psilocin PET 2 (µg/L)	Occupancy PET 1 (%)	Occupancy PET 2 (%)
Subject 1	3	0.05	2.3	1.9	<LOQ*	42.9	1.8
Subject 2	6	0.07	4.4	3.5	0.7	56.2	26.7
Subject 3	12	0.14	16.7	12.6	3.4	66.4	42.9
Subject 4	15	0.2	11.7	10.5	2.3	63.2	30.9
Subject 5	18	0.2	11.8	10.6	2.6	72.4	47.0
Subject 6	24	0.27	12.0	9.0	NA	60	NA
Subject 7	24	0.3	18.9	11.5	NA	66	NA
Subject 8	30	0.3	19.3	15.6	NA	65.2	NA

*Below level of quantification

EC_{50} = 1.81 µg/L vs. 2.12 µg/L, respectively). Due to the minor difference in outcomes, we set plasma psilocin concentrations for all time points to the mean value (0.3 µg/kg).

We calculated the EC_{50} [27] corresponding to PET 1 and PET 2 for each participant (mean EC_{50} ± SD: PET 1 = 4.5 ± 1.9 µg/L, PET 2 = 6.2 ± 6.0 µg/L). The determined EC_{50} did not differ between the two intervention scans (paired *t*-test, mean difference = −1.7, 95%CI [−10.2, 6.7], $p = 0.6$).

All statistical tests apart from non-linear modeling were performed in the statistical software package R (version 3.3.1).

We chose to assess associations between occupancy, plasma psilocin levels and subjective intensity ratings because the latter single, compound measure of drug-intensity was acquired simultaneously with PET 1 and PET 2, which was not the case with the MEQ-30, 11D-ASC and EDI questionnaires. Intensity ratings have previously been used in psychedelics research [28]. The questionnaires were not obtained until the end of the last scan session as we did not want to induce suggestive experiences by such a detailed questionnaire. Further, we believed that the intensity ratings would (1) be less sensitive to non-pharmacological modulators of psilocybin-induced altered states of consciousness (i.e., the context in which the drug is administrated [29]), (2) be feasible to administer during scans, and (3) yield a better temporal resolution. Intensity rating was stopped before the end of PET 2 for all participants (n = 5). Thus, for the purpose of calculating mean within-PET 2 intensity, participants were asked if intensity had changed during PET 2 compared to the last recorded rating. All participants responded that intensity had not changed during PET 2, and thus the last recorded score was extrapolated and used to calculate mean PET 2 intensity. For the purpose of modeling the association between occupancy and intensity, a quadratic function was used Intensity = $\beta1 * \text{occupancy} + \beta2 * \text{occupancy}^2$), and for the purpose of modeling the association between psilocin levels and intensity, a non-linear stimulus-response function similar to the occupancy model was used: Intensity = $\frac{\text{Intensity}_{max} * C_P}{EC_{50} + C_P}$. 95% Wald-type confidence intervals were computed for $\beta1$ and $\beta2$ using quantiles of the Student's *t*-distribution.

Post-hoc linear regression analyses of the association between mean PET 1 intensity ratings and three questionnaire responses (MEQ30, 11-D ASC, EDI) were performed. Our main hypothesis was that the outcome of the questionnaires would correlate with intensity ratings during PET 1. For these analyses, we report the unadjusted ($p_{unc.}$) and Bonferroni-adjusted (p_{FWE}) *p*-values. Further exploratory post hoc linear regression analyses are available in Supplementary data. The coefficient of determination (R^2) is reported as a measure of data variance explained by the respective model.

Voxel-level [^{11}C]Cimbi-36 BP_{ND} maps were estimated using the PETSurfer tool within Freesurfer [30] as described previously [24] and used for visualization purposes only.

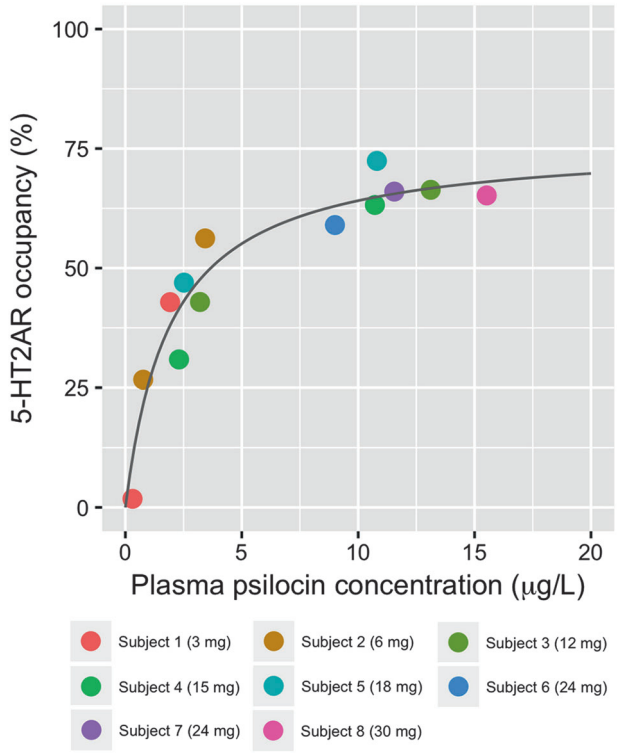

Fig. 2 Relationship between mean within-scan plasma psilocin levels and neocortical 5-HT2AR occupancy. Estimated EC_{50} [95% CI]: 1.95 [1.16; 3.15] µg/L and Occ_{max} [95% CI]: 76.6 [67.3; 88.0]%

RESULTS

Psilocin occupancy at neocortical 5-HT2ARs

Psilocybin intake was in all PET scans associated with considerable dose-related 5-HT2AR occupancies (PET 1 range 43–72%). Occupancies at PET 2 were also substantial (range 27–47%) with the exception of Subject 1 for which occupancy was 2% (Table 1; Fig. 2).

Psilocin levels and receptor occupancy relations

We found a high inter-individual variability in the dose response curves (e.g., maximum concentration (C_{max}) median [range]: 11.9 [2.3–19.3] µg/L) Fig. 1). The relation between plasma psilocin levels and neocortex 5-HT2AR occupancy conformed well to the non-linear regression model. Occ_{max} [95% CI] determined from this model was 76.6 [67.3; 88.0]%, EC_{50} [95% CI] was 1.95 [1.17; 3.15] µg/L, and R^2 was 0.92 (Fig. 3).

Subjective intensity ratings correlate with occupancy and psilocin levels

Subjective intensity ratings had a qualitatively similar time course compared to plasma psilocin levels (Fig. 1). We found a positive nonlinear association between mean within-scan intensity ratings and psilocin levels. $Intensity_{max}$ [95% CI] was 10.8 [8.6; 14.7] and EC_{50} [95% CI] was 4.5 [2.1; 9.8] µg/L, and R^2 was 0.35 (Fig. 4). We also observed a positive association between intensity ratings and occupancy that was well described by a quadratic relationship (β1 [95% CI]: −0.02 [−0.13; 0.1], β2 [95% CI]: 0.002 [0.0006; 0.003], R^2: 0.81, Fig. 4).

Psychedelic questionnaire responses

As expected, psilocybin had profound effects on the mental state of the participants (MEQ30 total score median [range]: 2.9 [1.6–4.5], 11D-ASC global score (sum of all dimensions) median [range]: 428.1 [35.1–772.1], EDI median [range]: 52 [4.0–97.9]) (see Figs. S1–2 and Table S3 for detailed responses). Post hoc linear regressions showed positive associations between mean PET 1 intensity ratings and total MEQ30 score (β-estimate [95% CI]: 0.34 [0.044; 0.64], $p_{unc.} = 0.03$, $p_{FWE} = 0.09$, R^2: 0.57), global 11-D ASC score (β-estimate [95% CI]: 76.4 [27.8; 125], $p_{unc.} = 0.008$, $p_{FWE} = 0.024$, R^2: 0.71) and EDI score (β-estimate [95% CI]: 11.1 [2.23; 20], $p_{unc.} = 0.02$, $p_{FWE} = 0.06$, R^2: 0.61). For further information, see Figure S3.

DISCUSSION

We here show that psilocybin ingestion of between 3 and 30 mg is associated with dose-dependent occupancy of cerebral 5-HT2ARs. Further, plasma psilocin concentration and 5-HT2AR occupancy are positively associated and the relationship conforms with a single-site binding model. Lastly, subjective intensity ratings are positively correlated with both neocortical 5-HT2AR occupancy and plasma psilocin levels, strongly supporting that stimulation of cerebral 5-HT2ARs is paramount for the psychedelic effects of psilocybin.

Similar to previous 5-HT2AR PET-imaging occupancy studies with other 5-HT2AR drugs [31, 32], we found that the single-site binding model provided a good fit of the relation between drug blood levels and 5-HT2AR occupancy, and predicted maximum occupancies were similar. Here, it is important to emphasize that the occupancies detected with an agonist radioligand (such as [^{11}C]Cimbi-36) may differ from that of antagonist radioligands because an agonist radioligand may bind preferentially to receptors in the high-affinity state [33, 34]. Thus, given that high-affinity receptors are believed to be most important for neurotransmission, an agonist radioligand may yield a more relevant estimate of receptor levels.

We found the EC_{50} of psilocin to be 1.95 µg/L. This corresponds to 10 nM, which is in the same range of K_i values from in vitro studies (rat cortex) performed with another 5-HT2AR agonist, [^{125}I] DOI: 6 nM [35] or 25 nM [36].

The post hoc linear regressions showed positive associations between mean PET 1 intensity ratings and MEQ30, global 11-D ASC score, and EDI score, and intensity ratings correlated also with both occupancy and with psilocin levels (Fig. 4). Thus, although the participants scored their overall intensity of the psychedelic experience based on a number of different components (e.g., imagery, changes in perception, stimulation of mood, feeling of enhanced meaning, somatic sensations, etc.), and probably also as a function of previous drug experience and psychological make-up ("set"), including personal coping style, our results show that intensity ratings constitute a meaningful global measure of psychedelic experience that is feasible to obtain with high temporal resolution.

Previous studies in humans reported that antagonists at 5-HT2A and 2C receptors can prevent perceptual effects after subsequent ingestion of psilocybin [9, 10]. Our data show that psilocin plasma levels correlate with occupancy (Fig. 3), that psilocin levels and occupancy correlate with intensity (Fig. 4), and that intensity correlates with scores of MEQ30, 11D-ASC and EDI. Thus, our findings strongly support that 5-HT2AR stimulation is central for psychedelic experiences in humans, and adding our findings to the existing literature, the evidence is by now strong that the 5-HT2AR is indeed the critical molecular mediator of psychedelic effects of psilocybin.

Our model can in future studies assist to estimate psilocin brain 5-HT2AR receptor occupancy without the use of PET-imaging, by determining plasma psilocin levels. For example, Brown and colleagues recently reported that ingestion of 25 mg psilocybin results in a mean C_{max} of about 15 ng/mL [37]. Assuming analysis methods of similar quality, similar stability of psilocin samples and a plasma density of 1.02 g/mL [38], this plasma psilocin level corresponds to 69% occupancy. There is considerable

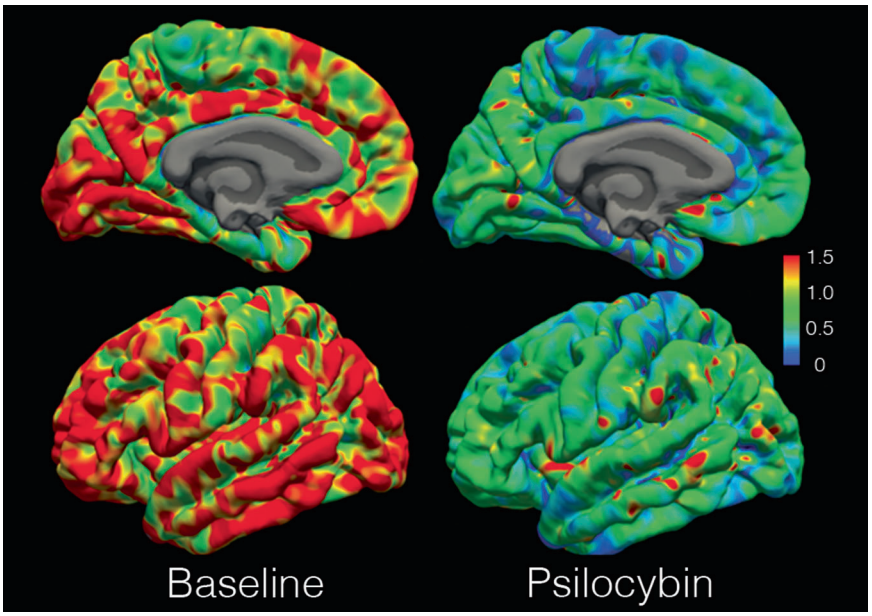

Fig. 3 Psilocybin occupancy of 5-HT2AR. [^{11}C]Cimbi-36 BP_{ND} map of the cortical surface of the left hemisphere of Subject 5 at baseline and at the first post-psilocybin intervention scan. Color bar in units BP_{ND}

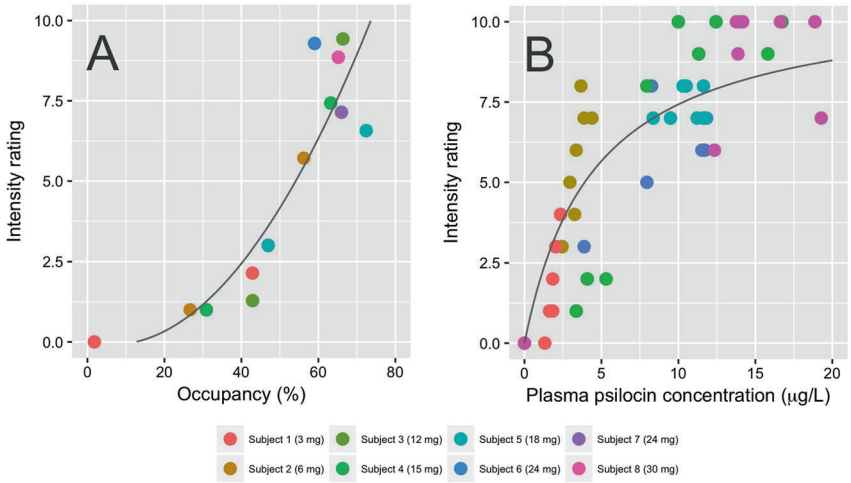

Fig. 4 Subjective intensity of the psychedelic experience at the time of the PET scan, neocortical 5-HT2AR occupancy and plasma psilocin concentration. **a** Relationship between intensity ratings and neocortical 5-HT2AR occupancy. The fitted line was obtained using a quadratic function. **b** Relationship between intensity and psilocin concentration, fitted to a single site receptor binding model

inter-individual variability in psilocybin pharmacokinetics [37, 39, 40]. Consistent with this, C_{max} for Subject 3 (12 mg, 0.14 mg/kg) was higher than C_{max} values for Subjects 4, 5, and 6 (15, 18, and 24 mg, respectively; 0.2, 0.2, and 0.3 mg/kg). Importantly, our data convincingly demonstrate that plasma psilocin levels correlate closely with the overall psychedelic experience, and it is possible that future clinical trials may benefit from relating psilocin levels and/or estimated occupancies to clinical effects, rather than absolute doses.

Recently, it has been argued that psychedelic "microdosing", entailing a dose small enough to avoid noticeable perceptual effects [41], comes with benefits such as enhanced creativity, social interaction and mood. Although a dose range of 0.5–2 mg psilocybin has been suggested as a psilocybin microdose (Dr. James Fadiman, Institute of Transpersonal Psychology, personal communication), there are currently no data available to identify such a cut-off. Subject 1 received 3 mg (0.05 mg/kg), had noticeable perceptual effects and an occupancy of 43%. This indicates that a smaller dose/lower occupancy would be needed for microdosing studies. Based on our data, a dose range of 0.5–2 mg is a reasonable suggestion for potential psilocybin microdose studies.

A few limitations of the study should be noted. When fitted to a single-site binding model without constraining $Occ_{max} = 100\%$, we found $Occ_{max} = 77\%$. Possible explanations for this include violations of kinetic modeling assumptions [13, 42], rapid internalization of 5-HT2AR or psilocybin-associated lowering of brain 5-HT levels. Although weaker than for 5-HT2AR, psilocin has also affinity to 5-HT 2B, 5-HT 2C, and 5-HT 1A receptors [36, 43]; the affinity for the serotonin transporter (SERT) is about 100 times lower [43]. A net decrease in cerebral 5-HT levels due to psilocin agonist activity at 5-HT1A autoreceptors could lead to an underestimation of occupancy due to decreased competition at 5-HT2ARs during intervention scans [44]. In vitro studies reported that 5-HT2AR stimulation led to 5-HT2AR internalization [45–48]. We cannot exclude that [^{11}C]- Cimbi-36, being an agonist

radioligand, has different affinity to internalized 5-HT2AR, leading to an underestimation of occupancy. We did not observe a difference between EC_{50} values of PET 1 and 2, suggesting that if internalization occurred, it occurred either very rapidly (within a few minutes) or very slowly (days after). For Subject 1 who received only 3 mg, occupancy was 43% at PET 1 and 2% at PET 2, speaking against 5-HT2AR internalization. Nevertheless, it would be interesting to investigate long-term effects of a single psilocybin dose on cerebral 5-HT2AR levels, as a potential molecular mediator of the long-term effects on personality and mood [3–5, 49]. Such a study is currently ongoing in our lab.

We did not observe statistically significant median head motion during PET 1 or PET 2 compared to baseline scans (Supplementary Methods and Materials). Participants 7 and 8 exhibited maximum motion of up to 35 and 20 mm during PET 1, respectively. Although this could affect the kinetic modeling, model fits were acceptable and comparable to baseline scans. Our conclusions are based on only eight participants, but five were investigated three times which generated two occupancy measures for each of these participants. The majority of male participants, that participants were recruited as specifically interested in a neuroimaging study investigating psilocybin, and the narrow age range decreases generalizability of our findings to the extent there are sex-dependent or age-dependent differences in psilocybin [50] or radioligand kinetics and differences in psilocin levels, occupancy or intensity ratings as a function of propensity to seek study participation in a psychedelics research study. PET-environment was positively correlated with anxiety during a psilocybin intervention [51] and we cannot exclude that the PET-environment influenced the psychedelic experience [29], making experiences less comparable to therapeutic or naturalistic settings. Yet, our participants experienced anxiety only to a very limited extent (11-D ASC anxiety subscale (median [range]: 4.25 [0; 17.3]). The study was not placebo-controlled and it is possible that this may have ultimately affected intensity ratings. Also, we cannot rule out that metabolites of psilocin or expectation-induced changes in 5-HT levels could affect the occupancy estimates, although we are unaware of evidence suggesting this.

In summary, we find that in humans, psychedelic effects of psilocybin are closely correlated with psilocin stimulation of the 5-HT2AR, and our data allows for an objective assessment of psilocybin effects on 5-HT2AR in future studies, by measuring plasma psilocin levels.

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ADDITIONAL INFORMATION

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Competing interests: The authors declare no competing interests.

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Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels

Supplementary information.

Martin Korsbak Madsen[a,b], Patrick MacDonald Fisher[a], Daniel Burmester[a,b], Agnete Dyssegaard[a], Dea Siggaard Stenbæk[a], Sara Kristiansen[a], Sys Stybe Johansen[d], Sczabolz Lehel[c], Kristian Linnet[d], Claus Svarer[a], David Erritzoe[e], Brice Ozenne[a,f], Gitte M. Knudsen[a,b,*]

[a]Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark;
[b]Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[c]PET and Cyclotron Unit, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark
[d]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[e]Psychedelic Research Group, Neuropsychopharmacology Unit, Centre for Psychiatry, Division of Brain Sciences, Department of Medicine, Imperial College London, London, United Kingdom.
[f]Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

Gitte M. Knudsen
E-mail: gmk@nru.dk

Exclusion criteria.

Exclusion criteria included: 1) personal or immediate family history of psychiatric disorder including substance misuse disorder, 2) present or previous neurological condition/disease, significant somatic condition/disease or intake of drugs suspected to influence test results; 3) non-fluent Danish language skills; 4) vision or hearing impairment; 5) learning disability; 6) pregnancy; 7) breastfeeding; 8) MRI contraindications; 9) alcohol or drug abuse 10) allergy to test drugs; 11) significant exposure to radiation within the past year (e.g., medical imaging investigations); 12) intake of QT-prolonging medication or electrocardiogram (ECG) results indicative of heart disease, 13) history of significant adverse response to a hallucinogenic drug. 14) use of hallucinogenic drugs less than 6 months prior to inclusion; 15) blood donation less than 3 months before project participation; 16) bodyweight less than 50 kg; 17) low plasma ferritin levels (< 12 µg/L).

Individual participant characteristics.

	Sex	Age (years)	Bodyweight (kg)	Past psychedelic drug use*
Subject 1	Female	33	57	Psilocybin: 2 (10) LSD: 1 (13)
Subject 2	Male	32	86	Psilocybin: 2 (2)
Subject 3	Male	31	83	Ayahuasca: 1 (3.5)

Subject 4	Male	29	75	Ayahuasca: 1 (3.5)
Subject 5	Male	30	88	None
Subject 6	Female	28	89	None
Subject 7	Female	50	79	None
Subject 8	Male	32	97	Psilocybin: 25 (0.5) LSD: 30 (1)

Table S1: Subject characteristics. *Drug: number of times used (number of years between last intake and study participation).

[^{11}C]Cimbi-36 PET-imaging data.

	Baseline BP_{ND}	PET 1 BP_{ND}	PET 2 BP_{ND}	Baseline scan	PET 1		PET 2	
				Dose (MBq)	Mass (μg)	Dose (MBq)	Mass (μg)	Dose (MBq)	Mass (μg)
Subject 1	1.02	0.59	1.00	454.9	0.9	546.7	0.48	586.5	0.70
Subject 2	1.58	0.69	1.16	580. 9	1.18	526.0	0.62	530.0	0.49
Subject 3	1.16	0.39	0.66	585.9	1.02	452.2	1.54	570.95	0.83
Subject 4	1.16	0.43	0.80	391.4	1.23	177.9	1.17	189.2	0.74
Subject 5	1.35	0.37	0.72	596.4	0.6	590.99	1.0	593.5	1.19
Subject 6	1.19	0.49	NA	279.4	1.45	439.8	1.46	NA	NA
Subject 7	1.052	0.36	NA	513.0	0.53	524.8	1.24	NA	NA
Subject 8	1.36	0.47	NA	550.5	0.38	508.5	0.61	NA	NA

Table S2. Non-displaceable binding potentials (BP_{ND}) in neocortex, injected radioactivity dose and mass dose of Cimbi-36.

Psychometric evaluation of psilocybin effects

In order to assess the participants' more detailed subjective experience of psilocybin intake, we administered questionnaires immediately after the last [^{11}C]Cimbi-36 PET-scan or when participants felt normal (app. 5-8 hours after psilocybin administration). In order to avoid to induce suggestive responses, we deliberately chose not to administer these questionnaires until the end of the study day. Thus, we cannot exclude that the questionnaire scores could be influenced by recall bias. The questionnaires were the 11-dimension altered states of consciousness **(Fig. S1)** (1, 2), the revised mystical experiences questionnaires **(Fig. S2)** (MEQ-30) (3), and the ego dissolution inventory **(Table S3)** (4).

The outcomes of all three questionnaires were congruent with staff members subjective evaluation of the participants' mental states, specifically that apart from subject 1, all participants experienced profound effects of psilocybin.

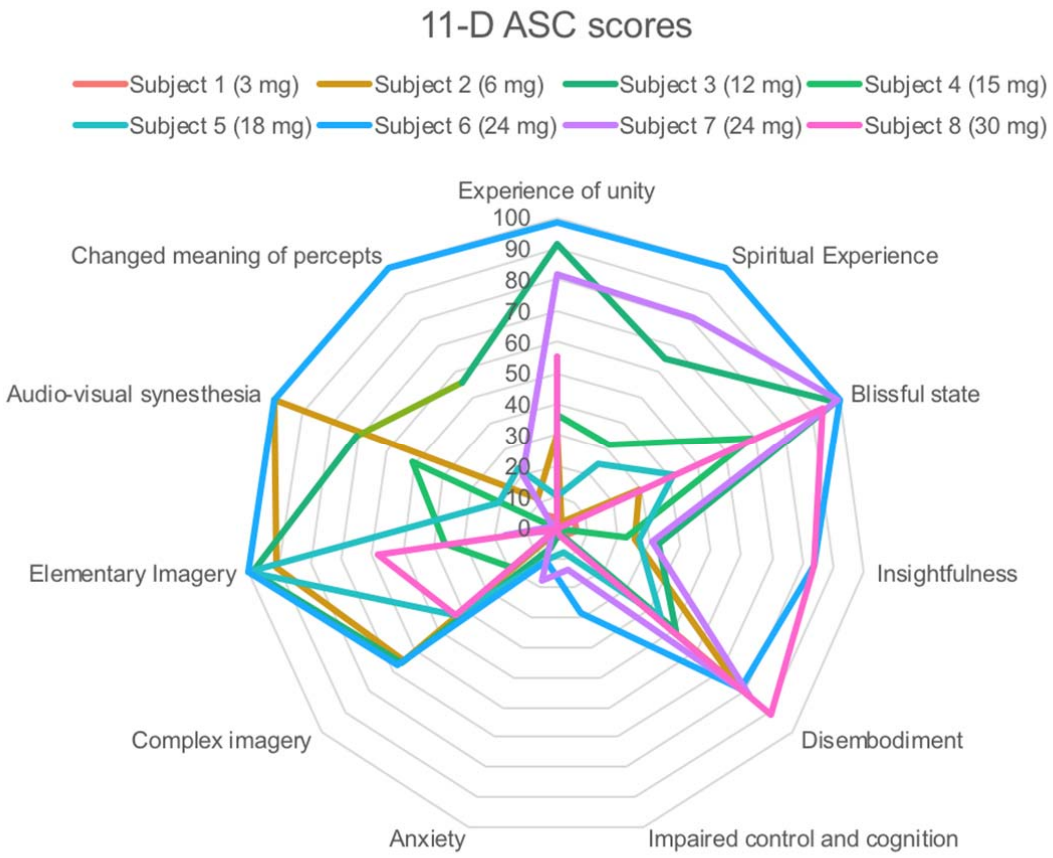

Fig. S1. 11-dimensional altered states of consciousness scale questionnaire results, maximum score is 100.

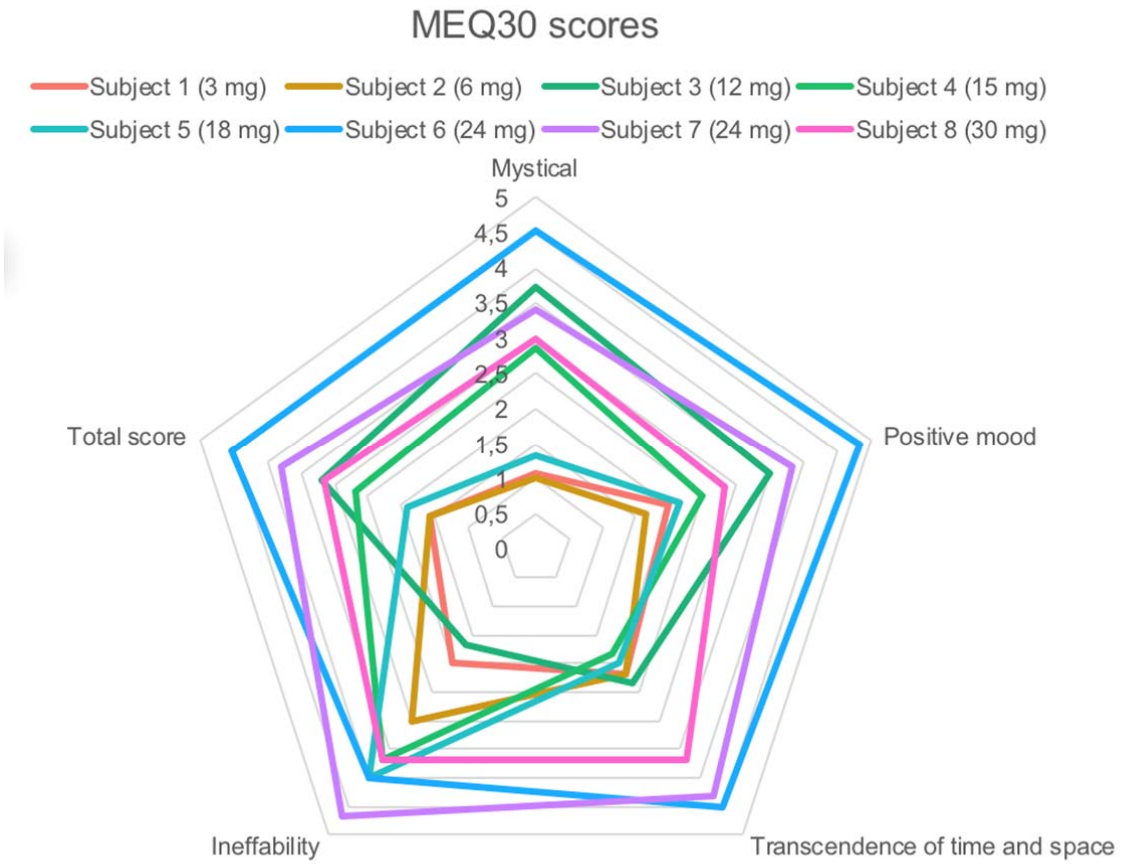

Fig. S2. Results of revised mystical experiences questionnaires (MEQ-30), maximum score is 5. Mean total score ± SD: 2.8 ± 1.1

ID	EDI score
Subject 1 (3 mg)	4
Subject 2 (6 mg)	38.5
Subject 3 (12 mg)	65.5
Subject 4 (15 mg)	37.375
Subject 5 (18 mg)	18.375
Subject 6 (24 mg)	80.625
Subject 7 (24 mg)	86.75
Subject 8 (30 mg)	97.875

Table S3. Results of ego dissolution inventory (EDI), maximum score is 100. Mean score ± SD: 53.6 ± 34.1

Correlations between results of MEQ30, 11-D ASC, EDI, C_{max}, mean psilocin levels, and occupancy.

Exploratory linear regressions were performed to evaluate associations of questionnaire responses of MEQ30, 11-D ASC, EDI with intensity ratings from PET 1, dose, adjusted dose, C_{max}, mean psilocin levels (n=8). Total MEQ-score, global ASC-score (i.e., the sum of all ASC dimensions) and EDI scores were selected *a priori* as variables of interest. After controlling Type I error rate, using Bonferroni-correction, no p-values were < 0.05.

Dependent variable	**β**	**95% CI lower bound**	**95% CI upper bound**	**SE**	**R^2**	**$p_{unc.}$**
MEQ30 total	0.34	0.0444	0.636	0.121	0.569	0.0306
ASC Global	76.4	27.8	125	19.9	0.711	0.00853
EDI	11.1	2.23	20	3.63	0.61	0.0221
MEQ30 Mystical	0.453	0.131	0.775	0.132	0.663	0.0138
MEQ30 Positive mood	0.298	-0.0335	0.63	0.136	0.446	0.0701
MEQ30 Transcendence of time & space	0.208	-0.203	0.619	0.168	0.204	0.262
MEQ30 Ineffability	0.126	-0.289	0.54	0.169	0.084	0.486
ASC Unity	12.2	2.83	21.5	3.81	0.629	0.0188
ASC Spiritual experience	9.25	-4.19	22.7	5.49	0.321	0.143
ASC Blissful state	14	7.41	20.5	2.68	0.819	0.00199
ASC Insightfulness	8.56	0.209	16.9	3.41	0.512	0.046
ASC Disembodiment	7.82	-4.8	20.4	5.16	0.277	0.18
ASC Impairment of	1.31	-2.44	5.07	1.53	0.109	0.425
ASC Anxiety	0.592	-1.99	3.17	1.05	0.0499	0.595
ASC Complex imagery	6.46	-3.02	15.9	3.88	0.317	0.146
ASC Elementary Imagey	9.66	-4.12	23.4	5.63	0.329	0.137
ASC Synaesthesia	6.28	-10.7	23.2	6.93	0.12	0.4
ASC Changed meaning of percepts	7.6	-4.73	19.9	5.04	0.275	0.182

Table S4. Statistical outcome of linear regression. Independent variable: mean PET-1 subjective intensity.

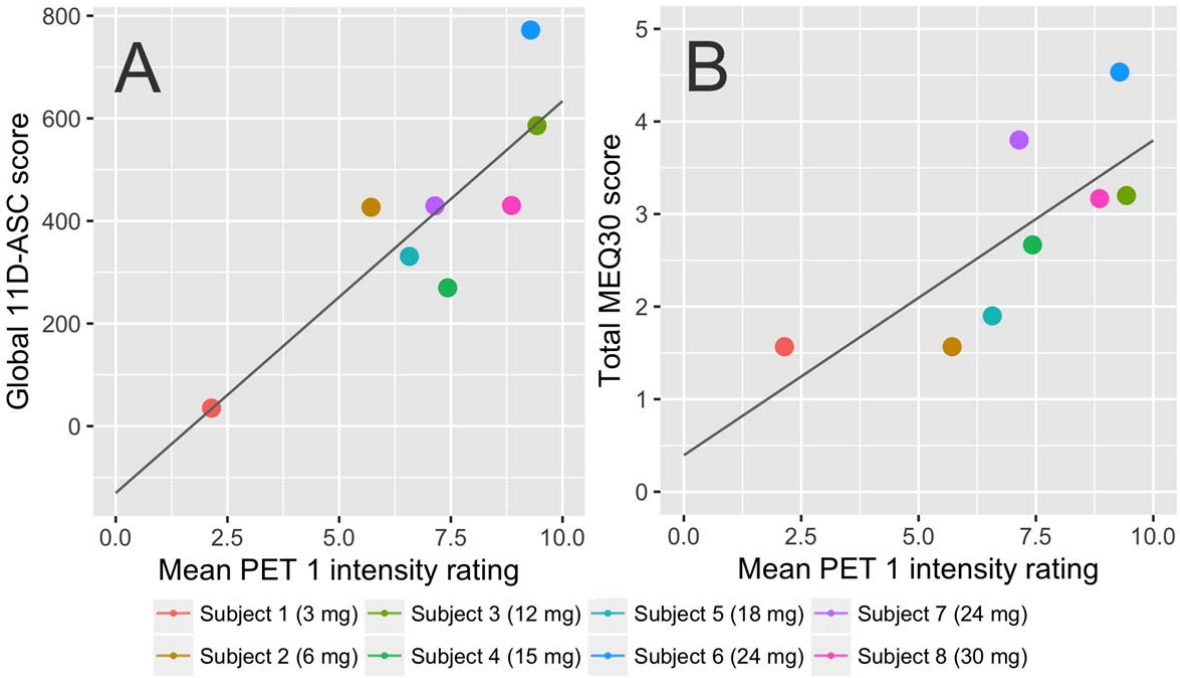

Fig. S3. Intensity and psychedelic questionnaire scores. *A)* Relationship between mean PET 1 intensity ratings and global 11-D ASC score, R^2 = 0.71, β [95% CI] = 76.4 [27.8;125.0]. *B)* Relationship between mean PET 1 intensity ratings and total score of MEQ30, R^2 = 0.57, β [95% CI] = 0.34 [0.04;0.64]. Global 11-D ASC and MEQ30 scores were both obtained after the completion of PET 2.

Dependent variable	β	95% CI lower bound	95% CI upper bound	SE	R^2	$p_{unc.}$
MEQ30 total	0.0105	-0.112	0.133	0.0502	0.00725	0.841
ASC Global	1.56	-23.2	26.3	10.1	0.00393	0.883
EDI	1.44	-2.17	5.06	1.48	0.137	0.366
MEQ30 Mystical	0.0189	-0.132	0.17	0.0617	0.0154	0.77
MEQ30 Positive mood	-0.0106	-0.132	0.111	0.0497	0.0075	0.838
MEQ30 Transcendence of time & space	0.00202	-0.124	0.128	0.0516	0.000257	0.97
MEQ30 Ineffability	0.028	-0.0873	0.143	0.0471	0.0556	0.574
ASC Unity	-0.0902	-4.28	4.1	1.71	0.000462	0.96
ASC Spiritual experience	-1.42	-5.66	2.81	1.73	0.101	0.443
ASC Blissful state	1.46	-2.51	5.42	1.62	0.119	0.403
ASC Insightfulness	1.63	-1.21	4.47	1.16	0.247	0.21
ASC Disembodiment	0.911	-3.05	4.87	1.62	0.0501	0.594
ASC Impairment of	-0.312	-1.36	0.731	0.426	0.0818	0.492
ASC Anxiety	-0.22	-0.911	0.471	0.282	0.092	0.465
ASC Complex imagery	0.44	-2.67	3.55	1.27	0.0196	0.741
ASC Elementary Imagey	0.889	-3.63	5.41	1.85	0.0372	0.647
ASC Synaesthesia	-1.74	-6.38	2.9	1.9	0.123	0.394
ASC Changed meaning of percepts	-1.16	-4.96	2.63	1.55	0.086	0.481

Table S5. Statistical outcome of linear regression. Independent variable: dose (mg psilocybin).

Dependent variable	β	95% CI lower bound	95% CI upper bound	SE	R^2	$p_{unc.}$
MEQ30 total	8.61	1.74	15.5	2.81	0.611	0.022
ASC Global	1080	-847	3010	788	0.239	0.219
EDI	272	56.8	488	88.1	0.614	0.0213
MEQ30 Mystical	9.31	-0.563	19.2	4.04	0.47	0.0605
MEQ30 Positive mood	7.17	-1.03	15.4	3.35	0.432	0.0763
MEQ30 Transcendence of time & space	8.46	1.04	15.9	3.04	0.564	0.0316
MEQ30 Ineffability	8.27	1.66	14.9	2.7	0.61	0.0222
ASC Unity	211	-97.2	520	126	0.319	0.145
ASC Spiritual experience	199	-147	544	141	0.249	0.209
ASC Blissful state	302	75.2	528	92.5	0.639	0.0173
ASC Insightfulness	205	-3.83	413	85.2	0.49	0.0531
ASC Disembodiment	191	-117	499	126	0.278	0.18
ASC Impairment of	50.2	-33	133	34	0.266	0.19
ASC Anxiety	30.4	-26.7	87.5	23.3	0.22	0.24
ASC Complex imagery	-17.2	-297	263	114	0.00375	0.885
ASC Elementary Imagey	12.1	-399	423	168	0.000858	0.945
ASC Synaesthesia	-110	-538	318	175	0.0614	0.554
ASC Changed meaning of percepts	80.1	-265	425	141	0.0511	0.59

Table S6. Statistical outcome of linear regression. Independent variable: adjusted dose (mg psilocybin per kg bodyweight).

Dependent variable	β	95% CI lower bound	95% CI upper bound	SE	R^2	$p_{unc.}$
MEQ30 total	0.123	-0.00334	0.249	0.0516	0.486	0.0546
ASC Global	17.4	-13.4	48.2	12.6	0.242	0.215
EDI	4.52	1.28	7.75	1.32	0.66	0.0142
MEQ30 Mystical	0.153	-0.0019	0.307	0.0631	0.493	0.0521
MEQ30 Positive mood	0.101	-0.041	0.243	0.0581	0.335	0.132
MEQ30 Transcendence of time & space	0.0966	-0.0554	0.249	0.0621	0.287	0.171
MEQ30 Ineffability	0.0708	-0.0831	0.225	0.0629	0.174	0.303
ASC Unity	3.97	-0.501	8.45	1.83	0.44	0.0728
ASC Spiritual experience	2.9	-2.77	8.58	2.32	0.207	0.257
ASC Blissful state	5.37	2.63	8.12	1.12	0.793	0.00303
ASC Insightfulness	2.49	-1.46	6.45	1.62	0.284	0.174
ASC Disembodiment	2.86	-2.18	7.91	2.06	0.243	0.215
ASC Impairment of	0.261	-1.27	1.79	0.626	0.0282	0.691
ASC Anxiety	0.411	-0.539	1.36	0.388	0.158	0.33
ASC Complex imagery	0.171	-4.31	4.65	1.83	0.00145	0.929
ASC Elementary Imagey	0.722	-5.81	7.26	2.67	0.012	0.796
ASC Synaesthesia	-1.86	-8.68	4.95	2.79	0.0694	0.528
ASC Changed meaning of percepts	0.896	-4.7	6.49	2.29	0.025	0.709

Table S7. Statistical outcome of linear regression. Independent variable: Cmax.

Dependent variable	β	95% CI lower bound	95% CI upper bound	SE	R^2	$p_{unc.}$
MEQ30 total	0.137	-0.0559	0.33	0.0789	0.335	0.133
ASC Global	20.8	-22	63.6	17.5	0.191	0.28
EDI	5.16	-0.232	10.6	2.21	0.478	0.0577
MEQ30 Mystical	0.183	-0.0438	0.41	0.0928	0.394	0.0957
MEQ30 Positive mood	0.105	-0.104	0.315	0.0856	0.201	0.266
MEQ30 Transcendence of time & space	0.0826	-0.145	0.31	0.093	0.116	0.409
MEQ30 Ineffability	0.0795	-0.134	0.293	0.0872	0.122	0.397
ASC Unity	4.24	-2.6	11.1	2.79	0.277	0.18
ASC Spiritual experience	2.68	-5.47	10.8	3.33	0.0973	0.452
ASC Blissful state	6.62	1.94	11.3	1.91	0.666	0.0135
ASC Insightfulness	3.39	-1.91	8.68	2.16	0.29	0.169
ASC Disembodiment	2.78	-4.52	10.1	2.98	0.126	0.388
ASC Impairment of	0.0992	-1.99	2.19	0.853	0.00225	0.911
ASC Anxiety	0.287	-1.07	1.65	0.556	0.0426	0.624
ASC Complex imagery	0.793	-5.18	6.77	2.44	0.0173	0.756
ASC Elementary Imagey	1.97	-6.65	10.6	3.52	0.0495	0.596
ASC Synaesthesia	-2.2	-11.4	7.05	3.78	0.0534	0.582
ASC Changed meaning of percepts	0.808	-6.76	8.38	3.09	0.0112	0.803

Table S8. Statistical outcome of linear regression. Independent variable: normalized $psilocin_{AUC}$ (mean psilocin concentration)

Plasma psilocin concentrations.

Blood samples were drawn from an intravenous access in the antecubital vein and collected in EDTA vials, placed on ice, centrifuged, and plasma was aliquoted and stored at -20°C. Psilocin was obtained from Lipomed (Arlesheim, Switzerland) and Cerilliant (Round Rock, TX, USA) for calibrator and control batches, respectively, while the deuterated internal standard (IS) psilocin-d10 was from Cerilliant. Acetonitrile, methanol HPLC grade and water were obtained from Fishers Scientific (Loughborough, UK). Ascorbic acid was obtained from VWR (Hassrode, Belgium).

Stock solution (1000 mg/l) of psilocin and IS were prepared in acetonitrile and stored in amber ampoules at -20°C until use. Working standard solutions from 0.5 µg/l to 1000 µg/l were freshly prepared in 50% methanol in water for each analysis, and the IS-solution was 100 µg/l in 50% methanol. For preparation of calibration standards and quality controls blank plasma was preserved with 1% fluoride and stored at -20°C. Two quality controls (QC) were prepared at low (5.0 µg/kg) and high (50 µg/kg) levels and stored at -80°C. These two along with a freshly spiked blank plasma sample at 2.5 µg/kg were analyzed in each run.

Protein precipitation was performed on a fully automated Tecan Freedom EVO 200 robotic platform (Tecan group Ltd, Männedorf, Switzerland) that included all pipetting, centrifugation, and evaporation steps. Each plasma sample (100 mg) was transferred to a 96-well 2.0 ml deep-well plate and 20 µl IS-solution was added to each well, followed by precipitation with 700 µl acetonitrile and shaking. The samples were centrifuged at 1000 g for 10 min, and the supernatant was evaporated to dryness under a stream of nitrogen at 35°C. Afterwards, the samples were reconstituted in 100 µl mixture of 12.5% methanol:12.5% acetonitrile:75% 0.05% formic acid in

water, shaken and centrifuged again. Finally, the supernatant was transferred to a 96-well plate and 1 µl was injected into the chromatographic system.

Chromatographic separation was performed on a HSS T3 column (100 x 2.1 mm, 1.8um, Waters, Milford, MA, USA) using an ACQUITY Ultra Performance Liquid Chromatography system (UPLC) from Waters. The mobile phase was composed of solvent A: 1 mM ammoniumformate in 0.1% formic acid in water and B: 0.1% formic acid in 1:1 mixture of acetonitrile:methanol. The column was maintained at 45°C with a flow 0.4 ml/min, and a gradient elution was applied from 2% to 100% B within 3.2 min with a total analysis time of 4.5 min. Detection was done by tandem mass spectrometry using an ACQUITY TQS from Waters. Ionization was achieved by electrospray in positive mode, and the source temperature was set at 150°C and desolvation temperature at 600°C. Two transitions were used for psilocin, m/z 205 -> 58 and 205 -> 160, with a cone voltage of 20 V and collision energy at 14 and 18 eV, respectively. For the IS the transition was m/z 215 -> 164 with cone 20 V and collision energy of 18 eV. Argon was used as collision gas at 0.45 Pa, and desolvation and cone gasflow were fixed at 1000 L/hr and 150 L/hr, respectively. Data were acquired and processed with MassLynx 4.2 software (Waters).
Quantification was performed by an eight-point linear calibration curve (0.1, 0.5, 1.0, 5.0, 25, 50, 100, 200 µg/kg) with weighting 1/x. Limits of detection (LOD) and quantification (LOQ) were 0.1 and 0.5 µg/kg, respectively, while the upper limit of quantification was 200 µg/kg. The overall process efficiency was found to be 63% based on an obtained extraction efficiency of 81% and matrix effect of 25% that the stable isotope labelled IS adjusted for. QC plasma samples were measured in each series with a RSD of 5% and an accuracy of 78% for the low level and less than 5% and an accuracy 89% for the high level. Similar performance was obtained with QC´s preserved with 1 mM ascorbic acid demonstrating that stored plasma QC´s at -80°C without ascorbic acid were stable for at least 6 months. The freshly spiked plasma QC at 2.5 µg/kg analyzed in each series had a RSD of 13% and an accuracy of 88% (n = 11). For final presentation of results, units of concentration (µg/kg) were converted into µg/L using a conversion factor or 1.02 kg per liter plasma.

[^{11}C]Cimbi-36 kinetic modelling.

We selected neocortex as our region of interest due to the high cortical expression of 5-HT2AR, high correlation in 5-HT2AR across regions (5), and large volume and consequent beneficial signal-to-noise ratio (SNR). In agreement with previous studies, cerebellum was chosen as reference region (6, 7). The outcome measure of the SRTM is non-displaceable binding potential (BP_{ND}), which is "the ratio at equilibrium of specifically bound radioligand to that of non-displaceable radioligand in tissue" (8) and is proportional to the number of receptors available for binding in the tissue:

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

Here, f_{ND} is the free radioligand fraction in the non-displaceable compartment and is assumed to be equal in the reference region (cerebellum) and in the tissue of interest (neocortex). B_{avail} is the number of available receptors, and K_D is the dissociation constant (K_{off}/K_{on}) (8).

[^{11}C]Cimbi-36 metabolism and psilocybin intervention

Venous blood samples were collected 5, 10, 20, 40, 60, and 120 minutes after tracer injection and plasma phase (2 mL) was subsequently analyzed using a column-switching high-performance liquid chromatography (HPLC) system to determine the fractions of unchanged tracer and radiometabolites as previously described (6, 9).[^{11}C]Cimbi-36 was metabolized rapidly after

administration **(S11)** and two fractions of radiolabelled metabolites were identified in the radiochromatogram (M1: polar and M2: less polar). Small polar metabolites (M1) may penetrate the blood-brain-barrier and decrease the SNR. To evaluate potential effects of psilocybin intervention on metabolism of [^{11}C]Cimbi-36, plasma radiometabolism profiles were plotted and compared to baseline. The free fraction of [^{11}C]Cimbi-36 in human plasma was estimated during baseline and the first intervention scan.

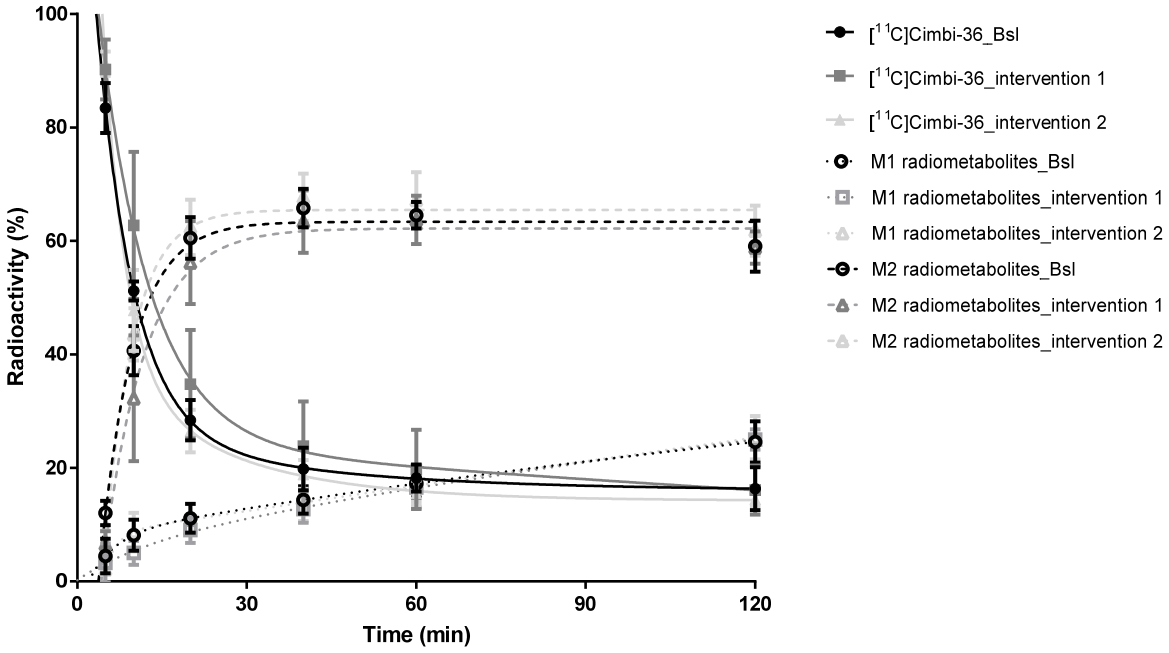

Fig. S4. Parent compound ([11C-Cimbi-36]) and M1 and M2 components at baseline (Bsl) and intervention 1 and 2 as a function of time after injection.

The time course of fractions of intact radioligand and radiometabolites were fitted to a non-linear regression curve using a two-phase model. Data are shown as mean ± SD. No significant differences were found when the average fraction of intact [^{11}C]Cimbi-36 during baseline (Bsl) was compared to the two interventions scan ($p= 0.76$ and $p=0.98$ for Bsl vs Intervention 1 and Bsl vs Intervention 2, respectively; unpaired t-test of mean values). Also, the levels of M1 radiometabolites were similar across scans. The rate of [^{11}C]Cimbi-36 metabolism was much slower for one subject, who was omitted from the present analysis (including the subject did not affect results, $p= 0.77$ and $p=0.77$ for Bsl vs Intervention 1 and Bsl vs Intervention 2, respectively). The free fraction of [^{11}C]Cimbi-36 in plasma at equilibrium was 3.6±0.5% at baseline and 2.9±0.4% at first intervention scan, and no significant difference was found between scans ($p=0.30$; unpaired t-test of mean values). All plots and statistical tests were performed using GraphPad Prism (version 7.03, GraphPad Software Inc., CA, USA).
Taken together, these observations indicate that psilocybin administration did not affect the rate of [^{11}C]Cimbi-36 metabolism in plasma, making differences in radioligand metabolism unlikely to confound the PET quantification.

Motion assessment

We assessed effects of psilocybin on motion (mean median max motion ± SD, baseline: 3.4 ± 0.8 mm; PET1: 3.0 ± 1.5 mm, PET2: 2.6 ± 0.6 mm) using a paired t-test on median maximum motion data from baseline and intervention scans. There was not statistically significant effect of psilocybin on motion: Baseline vs PET 1: p=0.6, Baseline vs PET2: p=0.13. PET1 vs PET2: p = 0.11.

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Study 2

Madsen, M. K., Fisher P.M., Stenbæk D.S., Kristiansen S., Burmester D., Lehel S., Páleníček, T., Kuchař M., Svarer C., Ozenne B., Knudsen G.M. A single psilocybin dose is associated with long-term increased mindfulness, preceded by a proportional change in neocortical 5-HT2A receptor binding (2020). *Eur. Neuropsychopharmacol.* **33**, 71–80.

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UNIVERSITY OF COPENHAGEN

PHD-THESIS
DECLARATION OF CO-AUTHORSHIP

The declaration is for PhD students and must be completed for each conjointly authored article. Please note that if a manuscript or published paper has ten or less co-authors, all co-authors must sign the declaration of co-authorship. If it has more than ten co-authors, declarations of co-authorship from the corresponding author(s), the senior author and the principal supervisor (if relevant) are a minimum requirement.

1. Declaration by	
Name of PhD student	Martin Korsbak Madsen
E-mail	martin@nru.dk
Name of principal supervisor	Gitte Moos Knudsen
Title of the PhD thesis	Neurobiological effects of 5-HT2AR modulation

2. The declaration applies to the following article	
Title of article	A single psilocybin dose is associated with long-term increased mindfulness, preceded by a proportional change in neocortical 5-HT2A receptor binding
Article status	
Published ☒ Date: 4 March 2020	Accepted for publication ☐ Date:
Manuscript submitted ☐ Date:	Manuscript not submitted ☐
If the article is published or accepted for publication, please state the name of journal, year, volume, page and DOI (if you have the information).	Eur. Neuropsychopharmacol. 33, 71–80 (2020). j.euroneuro.2020.02.001

3. The PhD student's contribution to the article ***(please use the scale A-F as benchmark)*** Benchmark scale of the PhD-student's contribution to the article **A**. Has essentially done all the work (> 90 %) **B**. Has done most of the work (60-90 %) **C**. Has contributed considerably (30-60 %) **D.** Has contributed (10-30 %) **E.** No or little contribution (<10 %) **F.** Not relevant	**A, B, C, D, E, F**
1. Formulation/identification of the scientific problem	E
2. Development of the key methods	E
3. Planning of the experiments and methodology design and development	D
4. Conducting the experimental work/clinical studies/data collection/obtaining access to data	C
5. Conducting the analysis of data	B
6. Interpretation of the results	B
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	A
Provide a short description of the PhD student´s specific contribution to the article.[i] Recruited, included participants, conducted PET experiments and psilocybin interventions in collaboration with co-workers, performed data processing and statistical analyses under supervision, wrote the first draft, circulated to co-authors and finalized the manuscript according to discussions with collaborators.	

4. Material from another thesis / dissertation[ii]	
Does the article contain work which has also formed part of another thesis, e.g. master's thesis, PhD thesis or doctoral dissertation (the PhD student's or another person's)?	Yes: ☐ No: ☒
If yes, please state name of the author and title of thesis / dissertation.	
If the article is part of another author's academic degree, please describe the PhD student´s and the author´s contributions to the article so that the individual contributions are clearly distinguishable from one another.	

5. Signatures of the co-authors[iii]

	Date	Name	Title	Signature
1.	May 4 2020	Gitte Moos Knudsen (senior & corresponding author)	Professor	
2.				
3.				
4.				
5.				
6.				
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10.				

6. Signature of the principal supervisor

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: 25.5.2020
Principal supervisor:

7. Signature of the PhD student

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: May 25 2020
PhD student: Martin Korsbak Madsen

Please learn more about responsible conduct of research on the Faculty of Health and Medical Sciences' website.

[i] This can be supplemented with an additional letter if needed.
[ii] Please see Ministerial Order on the PhD Programme at the Universities and Certain Higher Artistic Educational Institutions (PhD Order) § 12 (4):
"Any articles included in the thesis may be written in cooperation with others, provided that each of the co-authors submits a written declaration stating the PhD student's or the author's contribution to the work."
[iii] If more signatures are needed please add an extra sheet.

European Neuropsychopharmacology (2020) 33, 71–80

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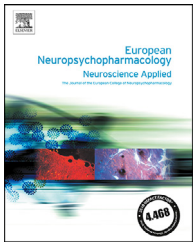

A single psilocybin dose is associated with long-term increased mindfulness, preceded by a proportional change in neocortical 5-HT2A receptor binding

Martin Korsbak Madsen[a,b], Patrick MacDonald Fisher[a], Dea Siggaard Stenbæk[a], Sara Kristiansen[a], Daniel Burmester[a,b], Szabolcs Lehel[c], Tomas Páleníček[d], Martin Kuchař[d,e], Claus Svarer[a], Brice Ozenne[a,f], Gitte M. Knudsen[a,b,*]

[a] *Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark*
[b] *Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark*
[c] *PET and Cyclotron Unit, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark*
[d] *National Institute of Mental Health, 250 67 Klecany, Czech Republic*
[e] *Forensic Laboratory of Biologically Active Substances, Department of Chemistry of Natural Compounds, University of Chemistry and Technology, 166 28 Prague, Czech Republic*
[f] *Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark*

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KEYWORDS
Psilocybin;
Personality;
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PET;
Mindfulness

Abstract
A single dose of the serotonin 2A receptor (5-HT2AR) agonist psilocybin can have long-lasting beneficial effects on mood, personality, and potentially on mindfulness, but underlying mechanisms are unknown. Here, we for the first time conduct a study that assesses psilocybin effects on cerebral 5-HT2AR binding with [^{11}C]Cimbi-36 positron emission tomography (PET) imaging and on personality and mindfulness. Ten healthy and psychedelic-naïve volunteers underwent PET neuroimaging of 5-HT2AR at baseline (BL) and one week (1W) after a single oral dose of psilocybin (0.2–0.3 mg/kg). Personality (NEO PI-R) and mindfulness (MAAS) questionnaires were completed at BL and at three-months follow-up (3M). Paired t-tests revealed statistically significant increases in personality Openness ($p_{uncorrected} = 0.04$, mean change [95%CI]: 4.2[0.4;∞]),

* Corresponding author at: Copenhagen University Hospital Rigshospitalet, Neurobiology Research Unit, NRU 6931, Rigshospitalet, Blegdamsvej 9, Copenhagen O DK-2100, Denmark.
E-mail address: gmk@nru.dk (G.M. Knudsen).

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which was hypothesized *a priori* to increase, and mindfulness ($p_{FWER} = 0.02$, mean change [95%CI]: 0.5 [0.2;0.7]). Although 5-HT2AR binding at 1W versus BL was similar across individuals ($p_{uncorrected} = 0.8$, mean change [95%CI]: 0.007 [−0.04;0.06]), a *post hoc* linear regression analysis showed that change in mindfulness and 5-HT2AR correlated negatively (β [95%CI] = −5.0 [−9.0; −0.9], p_{FWER}= 0.046). In conclusion, we confirm that psilocybin intake is associated with long-term increases in Openness and - as a novel finding - mindfulness, which may be a key element of psilocybin therapy. Cerebral 5-HT2AR binding did not change across individuals but the negative association between changes in 5-HT2AR binding and mindfulness suggests that individual change in 5-HT2AR levels after psilocybin is variable and represents a potential mechanism influencing long-term effects of psilocybin on mindfulness.

1. Introduction

Psilocybin is a serotonergic psychedelic and the main psychoactive constituent of *Psilocybe* mushrooms (i.e., "magic mushrooms") (Hofmann et al., 1958; Wolbach et al., 1962). Psilocybin's active metabolite, psilocin, stimulates cerebral serotonin 2A receptors (5-HT2AR) (Vollenweider et al., 1998; González-Maeso et al., 2007; Madsen et al., 2019), which gives rise to dose-dependent psychedelic effects lasting approximately six hours (Griffiths et al., 2006, 2011). Acute subjective effects include an altered state of consciousness, closed-eyes imagery, changes in perception, mood and affect, a weakened sense of self or ego (Griffiths et al., 2006; Studerus et al., 2011; Nour et al., 2016), and less rumination (Watts et al., 2017). The experience is often perceived as highly meaningful and of spiritual value (Griffiths et al., 2006, 2011; Studerus et al., 2011).

A growing body of evidence indicates that a single dose of psilocybin can positively affect well-being and reduce clinical symptoms of depression and anxiety and that these effects can be long lasting. For example, clinical trials in patients with cancer (Grob et al., 2011; Griffiths et al., 2016; Ross et al., 2016) and treatment-resistant depression (Carhart-Harris et al., 2016, 2018) suggest improved effects on mood, lasting several months after a single psychoactive dose of psilocybin. Similar long-lasting positive effects on mood and behavior have been observed in healthy individuals (Griffiths et al., 2006, 2008, 2011, 2018; Studerus et al., 2011). In patients with depression, the personality trait Openness to experience (Openness) was increased and Neuroticism decreased, three months after psilocybin (Erritzoe et al., 2018). Similarly, healthy volunteers showed increased Openness 14 months after psilocybin (Maclean et al., 2011).

Mindfulness is positively associated with income and socioeconomic status but negatively associated with psychological distress and mental health scores (Jensen et al., 2016, 2019). Mindfulness signifies "presence of mind" or "attentiveness to the present" (Bodhi, 2000) and is purposely cultivated within Buddhism and as part of mental health interventions such as Mindfulness-Based Stress Reduction (MBSR) (Kabat-Zinn, 1996) and Acceptance and Commitment Therapy (ACT) (Hayes et al., 2006). MBSR and ACT interventions are viable treatment options in anxiety and depression (Forman et al., 2007; Fjorback et al., 2011), suggesting that other mindfulness-improving interventions may also be related to clinical symptom improvement. Reports from healthy volunteers (Griffiths et al., 2011) and patients with depression (Watts et al., 2017) indicate that mindfulness may increase after psilocybin (e.g., "not stuck on thoughts—more in the moment now" (Watts et al., 2017)). A recent study showed that a psilocybin intervention during a five-day mindfulness retreat increased trait mindfulness measured immediately after the retreat (Smigielski et al., 2019). Studies of ayahuasca (containing the serotonergic psychedelic compound *N*,*N*-dimethyltryptamine) also indicate that psychedelics may enhance mindfulness-related capabilities (Soler et al., 2016; Sampedro et al., 2017). However, the extent to which psilocybin modulates mindfulness long-term has not been formally evaluated.

In recent years, several neuroimaging studies have evaluated acute effects of psilocybin on brain function, showing distinct effects on functional connectivity (Carhart-Harris et al., 2012; Roseman et al., 2014), perfusion (Carhart-Harris et al., 2012; Lewis et al., 2017), glucose metabolism (Vollenweider et al., 1997; Gouzoulis-Mayfrank et al., 1999), dopamine transmission (Vollenweider et al., 1999) and threat-related brain function (Kraehenmann et al., 2014). Although this work has greatly enhanced our understanding of the neural mechanisms underlying acute psilocybin effects, knowledge about long-term effects and related neurobiological mechanisms is lacking.

Stimulation of cerebral 5-HT2ARs accounts for the acute effects of psilocybin (Vollenweider et al., 1998; González-Maeso et al., 2007; Kometer et al., 2013; Madsen et al., 2019). Individual differences in the 5-HT2AR levels are linked to cortico-limbic brain function (Fisher et al., 2009, 2011) and Neuroticism (Frokjaer et al., 2008; Erritzoe et al., 2009), but not Openness (Stenbaek et al., 2019). Preclinical studies show that 5-HT2AR agonists, such as psilocybin, can internalize (Berry et al., 1996; Karaki et al., 2014) and down-regulate 5-HT2ARs (Buckholtz et al., 1990). A cross-sectional human [^{18}F]-Altanserin PET study supports these findings, reporting that regular users of 5-HT2AR agonist psychoactive drugs (e.g., magic mushrooms, LSD and MDMA) had lower 5-HT2AR levels than non-using controls (Erritzoe et al., 2011). Thus, several lines of research suggest that psilocybin-induced changes in cerebral 5-HT2AR levels is a candidate molecular mechanism underlying long-lasting effects of psilocybin.

In the present study, we for the first time conducted a molecular neuroimaging study evaluating potential mechanisms underlying long-term effects of psilocybin. Specifically, healthy individuals underwent [^{11}C]Cimbi-36 PET imaging at baseline and one week after a single oral psychoactive dose of psilocybin to evaluate effects on cerebral 5-HT2AR levels. Next, we investigated effects of psilocybin on personality and mindfulness three months after psilocybin intake and associations with preceding changes in cerebral 5-HT2AR.

2. Experimental procedures

2.1. Participants

Ten healthy volunteers without prior intake of psychedelic drugs (mean age (SD) [range]: 28.4 (3.4) [24.2–35.0], four females) were recruited from a list of participants that expressed interest in participating in a psilocybin brain scanning study. After obtaining informed consent, participants underwent screening for somatic illness, including a medical examination, an ECG, blood screening for somatic disease, and screening for psychiatric disorders using Mini-International Neuropsychiatric Interview, Danish translation version 6.0.0 (Sheehan et al., 1998). Exclusion criteria were: (1) present or previous primary psychiatric disease (DSM axis 1 or WHO ICD-10 diagnostic classifications) or in first-degree relatives; (2) present or previous neurological condition/disease, significant somatic condition/disease; (3) intake of drugs suspected to influence test results; (4) non-fluent Danish language skills; (5) vision or hearing impairment; (6) previous or present learning disability; (7) pregnancy; (8) breastfeeding; (9) magnetic resonance imaging (MRI) contraindications; (10) alcohol or drug abuse; (11) allergy to test drugs; (12) significant exposure to radiation within the past year (e.g., medical imaging investigations); (13) intake of QT-prolonging medication or electrocardiogram (ECG) results indicative of heart disease, (14) blood donation less than 3 months before project participation; (15) bodyweight less than 50 kg; (16) low plasma ferritin levels (< 12 μg/L). The study was approved by the Danish Medicines Agency (EudraCT ID: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295); and by the ethics committee for the capital region of Copenhagen (journal ID: H-16028698, with amendments). The study was preregistered at ClinicalTrials.gov (identifier: NCT03289949).

2.2. Procedures

Prior to inclusion, participants were informed about the study, including safety precautions and potential effects and side-effects of psilocybin. Before the psilocybin session, all participants met one of the two staff members present on the psilocybin intervention day. A urine test was used to screen for common drugs of abuse (Rapid Response, BTNX Inc., Markham, Canada) on baseline imaging days. At baseline, participants filled out questionnaires before undergoing neuroimaging with [^{11}C]Cimbi-36 PET and MRI. On a separate day, psilocybin sessions were conducted by two staff members familiar with effects of psilocybin, safety precautions and interpersonal support methods (number of days between baseline PET imaging and psilocybin session mean (SD) [range]: 21.7 (13.3) [1–50]). Psilocybin capsules were taken with a glass of water (dose: 0.2 mg/kg ($n = 4$) and 0.3 mg/kg ($n = 6$)). Participants listened to a standardized music playlist, adapted from one kindly provided by Prof. Roland Griffiths, Johns Hopkins Medicine. Music was played using a stereo system. One week after psilocybin, participants underwent neuroimaging with [^{11}C]Cimbi-36 PET and MRI (number of days between psilocybin session and PET rescan: mean (SD) [range] = 7.1 (0.9) [6–8] days).

2.3. Psychometric evaluations

At the end of psilocybin session days, participants completed the 11-dimension Altered States of Consciousness questionnaire (11D-ASC) (Dittrich et al., 2006; Studerus et al., 2010), the revised Mystical Experiences Questionnaire (MEQ30) (Barrett et al., 2015), and the Ego-Dissolution Inventory (EDI) (Nour et al., 2016) (median [range]: 6.4 [5.9–7.4] hours after psilocybin intake). Participants also completed the personality questionnaire NEO PI-R (Costa, P.T., Jr, McCrae, 1992; Skovdahl-Hansen et al., 2004; McCrae and Costa, 2006) and the mindfulness questionnaire mindful attention awareness scale (MAAS) (Brown and Ryan, 2003; Jensen et al., 2016) at baseline and three-months follow-up (number of days between psilocybin sessions and follow-up questionnaires: mean (SD) [range] = 97.8 (11.9) [79–120 days]. NEO PI-R measures the five personality factors Openness, Conscientiousness, Extraversion, Agreeableness, and Neuroticism. The Persistent Effects Questionnaire (PEQ), which measures changes that are subjectively perceived to be due to the psilocybin experience, was administered at three-months follow-up (Griffiths et al., 2006, 2011).

2.4. [^{11}C]Cimbi-36 PET acquisition

Full description of [^{11}C]Cimbi-36 PET imaging acquisition has been published previously (Ettrup et al., 2014, 2016; Madsen et al., 2019). Arterial blood samples were not collected in the present study. Briefly, PET data was acquired for 120 min after a bolus injection of [^{11}C]Cimbi-36 on a high-resolution research tomograph (HRRT) PET-scanner (CTI/Siemens, Knoxville, USA). The injected dose was similar at baseline and rescan PET imaging (paired t-test: mean difference [95% CI]: 10.6 [−15.7;36.8] MBq, $p = 0.4$, Table 1).

2.5. [^{11}C]Cimbi-36 PET image processing and kinetic modeling

Regional time-activity curves were extracted from 42 cortical and subcortical regions for kinetic modeling based on the automated regional delineation procedure in Pvelab, as described previously (Svarer et al., 2005). Our *a priori* region of interest was neocortex, which is a volume-weighted average of all cortical regions, and cerebellum was used as reference region. The simplified reference tissue model (SRTM) was used for kinetic modeling (Lammertsma et al., 1996; Ettrup et al., 2014). Non-displaceable

Table 1 Main PET imaging results at baseline and one week after psilocybin. Statistical test: two-tailed paired t-test.

	Baseline (mean (SD))	Rescan (mean (SD))	Change (mean (SD))	$P_{uncorrected}$
[^{11}C]Cimbi-36 Neocortex BP_{ND}	1.15 (0.13)	1.16 (0.14)	0.007 (0.07)	0.8
Injected dose	519.8 (44.0) MBq	509.2 (45.4) MBq	10.6 (36.7) MBq	0.4

binding potential (BP_{ND}) is the outcome of the SRTM and is defined as "the ratio at equilibrium of specifically bound radioligand to that of non-displaceable radioligand in tissue" (Innis et al., 2007). BP_{ND} is proportional to the number of 5-HT2ARs available for binding, assuming stable levels of the endogenous ligand (5-HT).

2.6. Magnetic resonance imaging

MRI data was acquired using a 64-channel head coil on a 3T Prisma scanner (Siemens, Erlangen, Germany). High resolution 3D T1- and T2-weighted images were acquired for the purpose of PET-image processing as recently described (Madsen et al., 2019): T1-weighted images: inversion time = 900 ms, echo time = 2.58 ms, repetition time = 1900 ms, flip angle = 9°, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap; T2-weighted images: echo time = 408 ms, repetition time = 3200 ms, in-plane matrix = 256 × 256, in-plane resolution = 0.9 × 0.9 mm, 208 slices and a slice thickness of 0.9 mm, no gap.

2.7. Data analysis

Neocortex was selected *a priori* as region of interest in the main statistical evaluation of changes in [^{11}C]Cimbi-36 BP_{ND} following psilocybin due to the high expression of 5-HT2ARs in cortex (Beliveau et al., 2017). Change was assessed using a paired *t*-test. To explore possible localized changes in [^{11}C]Cimbi-36 BP_{ND}, *post hoc* exploratory paired t-tests were performed on the 14 bilateral cortical regions (orbitofrontal cortex, medial inferior frontal gyrus, anterior cingulate cortex, insula, superior temporal gyrus, parietal cortex, medial inferior temporal gyrus, superior frontal gyrus, occipital cortex, sensorimotor cortex, posterior cingulate cortex, dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and entorhinal cortex).

Vertex-level [^{11}C]Cimbi-36 BP_{ND} maps were estimated using the PetSurfer tool within FreeSurfer (Greve et al., 2014; Beliveau et al., 2017). We performed paired t-tests on BP_{ND} estimates at each vertex across the left and right hemisphere surfaces.

Because the personality trait Openness has been reported to increase after psilocybin (Maclean et al., 2011; Erritzoe et al., 2018), we tested an *a priori* hypothesis that Openness would be increased at the three-month follow-up relative to baseline, using a one-tailed paired *t*-test. Two-tailed paired t-tests were performed on the remaining four NEO PI-R personality traits (Conscientiousness, Extraversion, Agreeableness and Neuroticism) and mindfulness (MAAS scores).

Post hoc linear regressions were performed, evaluating associations between change in [^{11}C]Cimbi-36 BP_{ND} and change in MAAS and Openness.

The statistical threshold for assessing change in total neocortex BP_{ND} and Openness was $p < 0.05$, uncorrected, whereas the family-wise error rate for all other statistical tests (regional and whole-brain BP_{ND} analyses, remaining personality factors and mindfulness (five tests), and *post hoc* linear regression of (two tests) was controlled using Bonferroni-Holm correction (Holm, 1979), statistical threshold: $p_{FWER} < 0.05$).

Hedge's g is provided as an expression of effect size (Hedges, 1981).

All statistical analyses were conducted in the statistical software R (version 3.3.1).

3. Results

3.1. Acute effects of psilocybin

Psilocybin induced an altered state of consciousness: ASC (global ASC mean (SD) [95% CI]: 600.9 (147.6) [495.3;706.5]), MEQ30 (total score mean (SD) [95% CI]: 3.9 (0.8) [3.3;4.5]), EDI (mean (SD) [95% CI]: 58.2 (30.0) [36.7;79.7]) (Fig. 1). Eight of the ten participants had a "complete mystical experience", defined as a score of ≥60% of possible maximum on all four MEQ30 factors (ineffability, positive mood, transcendence of time and space, and mystical experience) (Barrett et al., 2015).

3.2. [^{11}C] Cimbi-36 PET results

We did not observe evidence for a difference between neocortical [^{11}C]Cimbi-36 BP_{ND} at baseline and at rescan ($p_{uncorrected} = 0.8$, mean change [95%CI]: 0.007 [−0.04;0.06], (Table 1, Fig. 2)), Hedge's g [95% CI]: 0.09 [−0.8;1]. *Post hoc* exploratory analyses did not reveal evidence for psilocybin

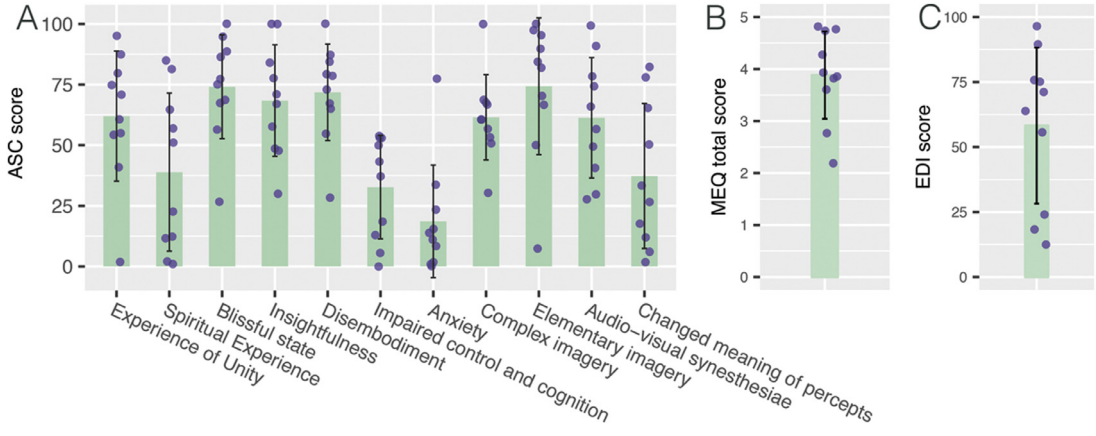

Fig. 1 Acute effects of psilocybin. (A) 11-dimensions Altered States of Consciousness questionnaire. (B) Mystical experiences questionnaire. (C) Ego-dissolution inventory. Colored circles: individual values, column: mean, error bars: SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

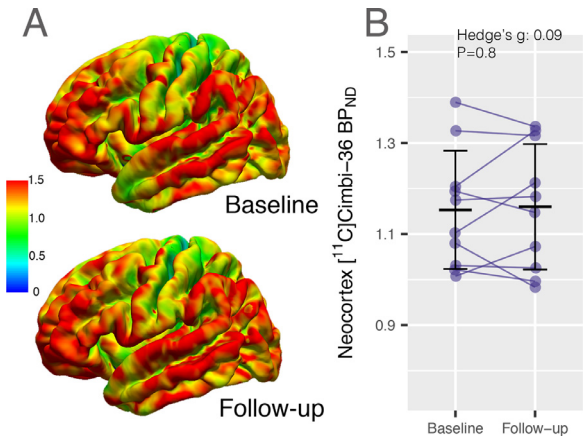

Fig. 2 Effects of psilocybin on [^{11}C]Cimbi-36 BP_{ND}. (A) Mean vertex-level cortical [^{11}C]Cimbi-36 BP_{ND}, left hemisphere, at baseline and one-week follow-up. (B) Neocortical [^{11}C]Cimbi-36 BP_{ND} at baseline and one-week follow-up. Colored points: individual values; middle horizontal black line: mean; error bar: SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects across the group in any of the 14 bilateral cortical regions ($p_{uncorrected} > 0.11$). Similarly, the vertex-based analysis did not identify subregions showing statistically significant changes in [^{11}C]Cimbi-36 BP_{ND} (Fig. 2).

3.3. Long term effects of psilocybin on personality and mindful attentive awareness

We found that personality trait Openness was statistically significantly increased from baseline to three-month follow-up ($p_{uncorrected} = 0.04$, mean change [95%CI]: 4.2[0.4;∞], Table 2, Fig. 3). Mindfulness (MAAS) was also statistically significantly increased at follow-up ($p_{FWER} = 0.023$, mean change [95%CI]: 0.6 [0.3;1.0], Fig. 3). PEQ responses showed more self-rated positive changes than negative changes, Table 3.

3.4. *Post hoc* linear regression analyses

Post hoc exploratory linear regression analyses revealed a statistically significant negative correlation between changes in [^{11}C]Cimbi-36 BP_{ND} and MAAS scores (β [95%CI] $= -5.0$ [-9.0; -0.9], $R^2 = 0.50$, $p_{FWER} = 0.046$)

Table 2 Personality traits before and 3 months after a single dose of psilocybin. P-values are derived from paired t-tests. Bold designates statistically significant differences. Statistical tests: two-tailed paired *t*-test; correction for multiple comparisons: Bonferroni-Holm (Openness: one-tailed paired *t*-test, not subjected to correction for multiple comparisons).

	Baseline Mean(SD)	Follow-up Mean(SD)	Change Mean(SD)	$P_{uncorrected}$	P_{FWER}	Hedge's g
NEO PI-R						
Openness	127.0(16.6)	131.2(17.2)	4.2(6.6)	0.04	0.04	0.23
Conscientiousness	126.1(19.7)	132.9(18.8)	6.8(9.9)	0.06	0.3	0.26
Extraversion	127.5(19.2)	128.6(13.6)	1.1(9.2)	0.7	1	0.04
Agreeableness	120.7(21.0)	121.2(24.9)	0.5(11.1)	0.9	1	0.013
Neuroticism	78.8(16.3)	69.1(18.9)	−9.7(15.1)	0.07	0.3	−0.29
MAAS	4.2(0.6)	4.8(0.6)	0.6(0.5)	0.004	0.02	0.66

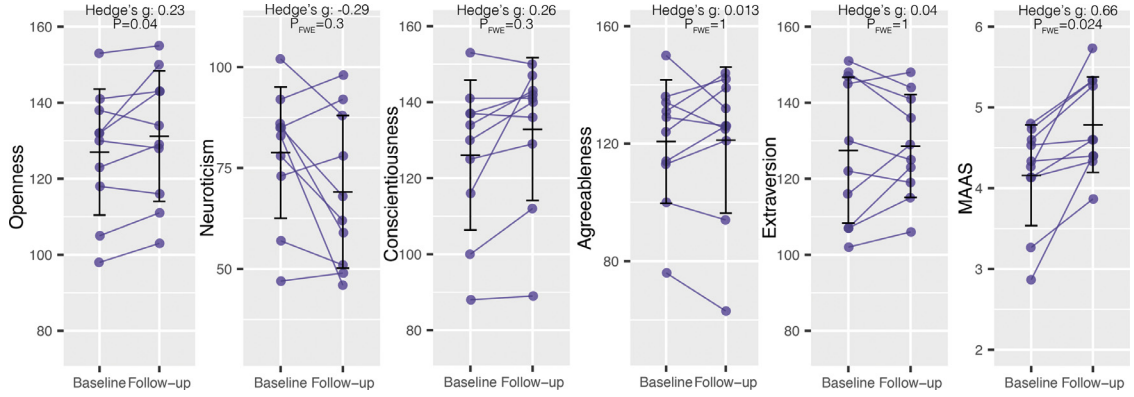

Fig. 3 Personality and mindful attention awareness at baseline and three-month follow-up. Colored points: individual values; middle horizontal black line: mean; error bar: SD. Openness analysis was *a priori* hypothesized to increase and was not subjected to correction for multiple comparisons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3 Outcome of Persisting Effects Questionnaire completed three months after the psilocybin session. All scores have a range of 0–100.

	Mean (SD)	Median [range]
Positive attitudes about life	36 (10)	37 [4.6;71]
Negative attitudes about life	3.4 (5.3)	0 [0;12]
Positive attitudes about self	25 (21)	25 [0;69]
Negative attitudes about self	2.7 (3.7)	0 [0;9.1]
Positive mood changes	23 (22)	18 [0;78]
Negative mood changes	1.1 (3.5)	0 [0;11]
Altruistic/positive social effects	16 (21)	7.8 [0;64]
Antisocial/negative social effects	0.67 (1.5)	0 [0;4.4]
Positive behavior changes	34 (34)	30 [0;80]
Negative behavior changes	0 (0)	0 [0;0]
Increased spirituality	22 (24)	12 [0;70]
Decreased spirituality	0.67 (1.6)	0 [0;4.8]

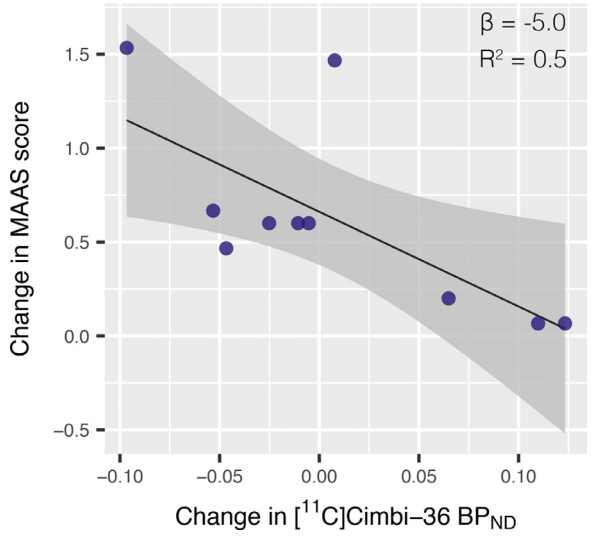

Fig. 4 Relation between changes in mindfulness and [^{11}C]Cimbi-36 BP_{ND} . Colored points: individual data points; shading: 95% CI for regression line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4) but not for Openness (β [95%CI] = −33.1 [−101.9; 37.3], R^2=0.13, p_{FWER}=0.3).

4. Discussion

In the present study, we for the first time evaluated long-term brain changes after a single dose of psilocybin. We report an increase in mindfulness at least three months following a single oral psilocybin dose in psychedelic-naïve individuals. As expected, and consistent with previous studies, we observed improvements in mood, behavior and trait Openness. These intriguingly persistent changes were not preceded by a consistent change in neocortical 5-HT2AR binding across individuals, one week after exposure. However, *post hoc* analysis showed that change in 5-HT2AR binding after one week correlated negatively with change in mindfulness three months after psilocybin. This observation suggests that the psilocybin effect on 5-HT2AR binding is variable and that individual-specific 5-HT2AR regulation may influence long-term improvements in mindfulness.

In line with previous studies (Maclean et al., 2011; Erritzoe et al., 2018), we observed that the personality trait Openness was statistically significantly increased after psilocybin. PEQ scores indicated that the psilocybin session enhanced mood, spirituality and outlook on life and self. This replicates previous findings in healthy volunteers that psilocybin can elicit long-lasting positive changes in behavior and mood (Griffiths et al., 2011).

We observed that mindfulness was increased least three-months following psilocybin. This is in line with a recent study demonstrating that psilocybin, relative to placebo, potentiated an increase in mindfulness from intensive mindfulness training, as reported one day after the retreat (Smigielski et al., 2019). Notably, the change we observed in our sample occurred in the absence of explicit mindfulness training. We reanalyzed our data, including data from eight additional individuals who previously participated in a psilocybin PET study (Madsen et al., 2019). Although two individuals received a low dose (0.05 and 0.07 mg/kg), the analysis in this larger sample confirmed an increase in mindfulness following psilocybin (mean change [95%CI]: 0.5 [0.2;0.7], Hedge's g: 0.78). The effect size found in our study is comparable to that of MBSR (Shapiro et al., 2011). MAAS measures the general degree of attentiveness to the present moment (example question: "I find myself doing things without paying attention") (Brown and Ryan, 2003; Jensen et al., 2016). MAAS scores are highly stable over time, considered a psychological trait (Brown and Ryan, 2003; Jensen et al., 2016), and correlate negatively with stress and mood disturbance (Carlson and Brown, 2005; Jensen et al., 2012). Increases in MAAS following mindfulness training (e.g., MBSR) were associated with improvements in mood and affect in healthy individuals and patients (Brown and Ryan, 2003). Considering MBSR effects on clinical symptoms, it is possible that increases in mindfulness constitutes a key element of psilocybin therapy.

Our study has several strengths: first, we employed a within-subject design, which increases the power to detect changes. Further, all volunteers in the present study were naïve to psychedelic drugs, which is crucial because previous 5-HT2AR stimulation could have moderated psilocybin effects, e.g., induced receptor desensitization (Gray and Roth, 2001) or affected personality structure (Maclean et al., 2011; Erritzoe et al., 2018), which would make interpretation more challenging. Finally, [^{11}C]Cimbi-36 binding in neocortex has an excellent test-retest variability of 3.8% (Ettrup et al., 2016), and is a 5-HT2AR agonist (Ettrup et al., 2014; Finnema et al., 2014), which means that [^{11}C]Cimbi-36 presumably measures the same pool of receptors that are stimulated by psilocin.

According to the ternary complex model (Leff et al., 1997; Kenakin, 2002), [C^{11}]Cimbi-36, being an agonist, binds with higher affinity to membrane-bound receptors (active state) as compared to internalized receptors (inactive state), which is supported by some preclinical studies (Titeler et al., 1987; Egan et al., 2000). Consequently,

a reduction in membrane-bound receptors should theoretically be detectable as a reduction in BP_{ND}, assuming equal concentrations of the endogenous ligand (5-HT) at baseline and rescan. Conversely, if 5-HT levels change, effects on [^{11}C]Cimbi-36 BP_{ND} would be opposite due to competition between the endogenous ligand and radioligand (i.e., increased 5-HT would lead to a decrease in [^{11}C]Cimbi-36 BP_{ND}) (Jørgensen et al., 2016). Thus, our observation that [^{11}C]Cimbi-36 BP_{ND} is unchanged at the group level could be due to counterbalancing effects of 5-HT2AR down-regulation and increased 5-HT levels in the brain or vice versa. However, in vitro studies show that following agonist-induced internalization (Berry et al., 1996; Bhattacharyya et al., 2002), most receptors recycle back to the cell membrane within 2.5 h (Bhattacharyya et al., 2002). Also, we recently showed that psilocin K_D values for early post-drug PET-scan (one hour after drug intake) vs late post-drug PET-scans (five-six hours after drug intake) were similar (Madsen et al., 2019). These results speak against substantial and sustained reductions in membrane bound 5-HT2ARs after one dose of psilocybin that might have confounded our study.

We did not observe a direction-specific change in [^{11}C]Cimbi-36 BP_{ND} across individuals but we did observe a negative correlation between change in [^{11}C]Cimbi-36 BP_{ND} at one-week and change in mindfulness at three-months. This suggests that psilocybin may indeed affect [^{11}C]Cimbi-36 BP_{ND} levels, but that this effect varies between individuals and possibly shapes the long-term outcome on mindfulness. Further studies are warranted to elucidate possible variable 5-HT/5-HT2AR regulation in response to psilocybin and possible relations between mindfulness, psilocybin intake, and the 5-HT system, including 5-HT2ARs. To gain information about potential contributions of 5-HT to [^{11}C]Cimbi-36 BP_{ND}, such studies could leverage the 5-HT4R PET radioligand [^{11}C]SB207145 as a biomarker for endogenous serotonergic tonus (Haahr et al., 2014).

Our study is not without limitations. Based on normative material (Ettrup et al., 2014), our study was designed to detect a 15% change in BP_{ND} in 10 participants ($\alpha = 0.05$, power $= 0.8$), and a larger sample size would have improved our ability to detect more subtle changes. Our study would have benefitted from employing a placebo control group (Griffiths et al., 2016; Ross et al., 2016) since other factors than psilocybin could have contributed to the observed effects. Considering the time discrepancy between follow-up PET scans (one week after psilocybin) and personality measurements (three months after psilocybin), we cannot exclude that [^{11}C]Cimbi-36 BP_{ND} levels could have changed between PET rescan and personality re-assessment. The timing of the PET-rescan was based on the assumption that 5-HT2AR levels would have stabilized one week after stimulation and that plasma psilocin concentrations would be zero (time between drug and rescan: 50–90 half-lives). Questionnaire re-assessment could have been performed one week after psilocybin. Although this would have avoided temporal discrepancy between PET and questionnaire data collection, we abstained from doing this since short-lasting "after-glow" effects possibly would have inflated measured effects.

In conclusion, we replicate the previous finding of long-lasting increases in trait Openness following psilocybin administration measured at three-months follow-up. We report a significant increase in mindfulness at three-months follow-up, which may constitute an important part of psilocybin therapy. Although on a group level, [^{11}C]Cimbi-36 BP_{ND} levels did not differ significantly before and one week after psilocybin, our finding of a negative correlation between individual change in 5-HT2AR and mindfulness suggests a potential mechanism through which psilocybin exerts long-term effects on mindfulness.

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Contributors

GMK and PMF designed the study, wrote the protocol and contributed to data analysis. MKM wrote the protocol, and contributed with data collection and analysis, and wrote the first draft of the manuscript. DB assisted with data collection and analysis. DSS and SK assisted with psilocybin interventions. SL produced the radiotracer. CS assisted with processing of PET data. BO contributed with data analysis. TP and MK produced the study drug. All authors contributed substantially to interpretation and discussion of study results, critical review of the submitted manuscript, and approve of the final version.

Conflict of interest

GMK: H. Lundbeck A/S (research collaboration), Novo Nordisk/Novozymes/Chr. Hansen (stock holder), Janssen Pharmaceutica NV (research collaboration), Sage Therapeutics (Advisory Board). GMK is currently the president of the European College of Neuropsychopharmacology. All other authors declare no conflicts of interest.

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Study 3

Madsen, M.K., Stenbæk D.S., Arvidsson A., Armand S., Marstrand-Jørgensen M., Johansen S.S., Linnet K., Ozenne B., Knudsen G.M. Fisher P.M. Integrity and segregation of macroscale cerebral functional networks correlate with plasma psilocin level and psychedelic experience. Manuscript

GRADUATE SCHOOL OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

PHD-THESIS
DECLARATION OF CO-AUTHORSHIP

The declaration is for PhD students and must be completed for each conjointly authored article. Please note that if a manuscript or published paper has ten or less co-authors, all co-authors must sign the declaration of co-authorship. If it has more than ten co-authors, declarations of co-authorship from the corresponding author(s), the senior author and the principal supervisor (if relevant) are a minimum requirement.

1. Declaration by	
Name of PhD student	Martin Korsbak Madsen
E-mail	martin@nru.dk
Name of principal supervisor	Prof. Gitte Moos Knudsen
Title of the PhD thesis	Neurobiological effects of 5-HT2AR modulation

2. The declaration applies to the following article	
Title of article	Integrity and segregation of macroscale cerebral functional networks correlate with plasma psilocin level and psychedelic experience
Article status	
Published ☐ Date:	Accepted for publication ☐ Date:
Manuscript submitted ☐ Date:	Manuscript not submitted ☒
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3. The PhD student's contribution to the article ***(please use the scale A-F as benchmark)*** Benchmark scale of the PhD-student's contribution to the article **A**. Has essentially done all the work (> 90 %) **B**. Has done most of the work (60-90 %) **C**. Has contributed considerably (30-60 %) **D.** Has contributed (10-30 %) **E.** No or little contribution (<10 %) **F.** Not relevant	**A, B, C, D, E, F**
1. Formulation/identification of the scientific problem	D
2. Development of the key methods	F
3. Planning of the experiments and methodology design and development	B
4. Conducting the experimental work/clinical studies/data collection/obtaining access to data	B
5. Conducting the analysis of data	B
6. Interpretation of the results	A
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	F
Provide a short description of the PhD student´s specific contribution to the article.[i] The PhD student has recruited and included test subjects, performed psilocybin interventions, incl. fMRI acquisition, blood sampling & questionnaires data collection; analysis and interpretation of data, and writing the first draft of the manuscript.	

4. Material from another thesis / dissertation[3]	
Does the article contain work which has also formed part of another thesis, e.g. master's thesis, PhD thesis or doctoral dissertation (the PhD student's or another person's)?	Yes: ☐ No: ☒
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1.	25/5-2020	SVS STYRE JOHANSEN	Ass. Prof.	
2.	25/5-20	KRISTIAN LINNET		
3.	25/5	Brice Ozenne		
4.	25/5 - 2020	Albin Arvidsson		
5.	25/5	Patrick Fisher		
6.	25/5 - 2020	Maja Rou Mostrand-Jørgensen		
7.	26/5-20	Sophia Armand		
8.	27/5 2020	PER SIGGAARD STENBÆK		
9.	27/5 2020	Gitte Moos Knudsen	Prof.	
10.				

6. Signature of the principal supervisor
I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge. Date: 27/5 2020 Principal supervisor: Gitte Moos Knudsen

7. Signature of the PhD student
I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge. Date: May 25 2020 PhD student: Martin K. Madsen

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Integrity and segregation of macroscale cerebral functional networks correlate with plasma psilocin level and psychedelic experience

Martin Korsbak Madsen[a,b], Dea Siggaard Stenbæk[a], Albin Arvidsson[a], Sophia Armand[a], Maja Marstrand Jørgensen[a], Sys S. Johansen[c], Kristian Linnet[c], Brice Ozenne[a,d], Gitte M. Knudsen[a,b], Patrick MacDonald Fisher[a*]

[a]Neurobiology Research Unit and NeuroPharm, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark;
[b]Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[c]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[d]Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

*Corresponding Author:
Patrick MacDonald Fisher, PhD
Copenhagen University Hospital Rigshospitalet
Neurobiology Research Unit
NRU 6931, Rigshospitalet
Blegdamsvej 9
Copenhagen O DK-2100
DENMARK
Phone: +45 3545 6714
E-mail: patrick.fisher@nru.dk

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Abstract

Consciousness-altering effects of psychedelics such as psilocin are suggested to occur because of increased entropy, reduced functional connectivity (FC) within networks (reduced integrity) and increased FC between networks (reduced segregation). Here we investigate effects of a psychoactive dose of psilocybin on temporal trajectories and interrelations of plasma psilocin levels (PPL), subjective drug intensity ratings (SDI) and FC as measured with fMRI. We show that PPL and SDI correlate negatively with both network integrity (including default mode network) and segregation. We also find evidence that as a function of PPL and SDI, the dorsal attention network desegregate with other networks. These findings demonstrate that psilocin critically determines the time course and magnitude of changes in the cerebral functional architecture after psilocybin and, importantly, implicate the expression of network integrity and segregation as crucial for psychedelic experience and for the brain's ability to maintain consciousness states conducive to everyday life.

Introduction

Current neuroscientific beliefs informed by functional neuroimaging studies hold that multiple macroscale cerebral functional networks shape behavior and phenomenal experience (i.e., subjective experience of contents in consciousness) through concerted activity [1–3]. These functional networks are reliably identified in the resting state (rs) using blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) [4–6], which is an imaging technique capable of measuring functional connectivity (FC) [7]. An axiomatic feature of these functional networks is high FC between nodes belonging to the same-network ("network integrity") and low FC between nodes belonging to different networks ("network segregation") [5]. Reduced network integrity and segregation has recently been proposed to underlie psychedelic experience after intake of psychedelic compounds[8] but neuroimaging experiments evaluating these relations are sparse [9,10].

The psychedelic compound psilocybin potently induces an altered state of consciousness and is emerging as a promising novel therapeutic, showing long-lasting beneficial effects with fast onset after a single dose both in healthy individuals [11–14] and in patients with depression, anxiety and addiction [15–21]. We recently showed that plasma level of psilocybin's active metabolite psilocin predict both cerebral 5-HT2AR occupancy and psychedelic experience [23], highlighting psilocin as a crucial pharmacological mediator of acute psilocybin effects. Thus, given psilocybin's transformative potential as a transnosological treatment modality, it is important to understand psilocin plays in psilocybin effects on brain function, psychedelic phenomenology and clinical outcomes.

Acute psilocybin effects have been studied in two BOLD fMRI resting state functional connectivity (RSFC) experiments: 2 mg i.v. psilocybin in fifteen subjects [24], and 0.2 mg/kg oral psilocybin in 23 subjects [25]. Important findings from the first experiment include reduced RSFC within the DMN [24], largely increased between-network RSFC [26], and increased global RSFC [10]. The

second experiment assessed BOLD fMRI scans acquired during the ascent phase and reported a reduction in global brain connectivity (GBC) in associative regions and increased GBC in sensory regions. Although these studies contribute substantially to the understanding of psilocybin effects on brain function, neither assessed relations between plasma psilocin level (PPL) and brain function nor investigated peak and descent phases after oral psilocybin. This knowledge is needed for a more complete understanding of psilocybin's neuropsychopharmacology and may ultimately lead to optimized treatment regimens.

In the present study, we sought to elucidate the extent to which network integrity and segregation map on to PPL and phenomenal experience alterations induced by clinically relevant doses of oral psilocybin in healthy participants with no or limited experience with psychedelics. To accomplish this, we collected simultaneous data of BOLD fMRI images, subjective drug intensity (SDI) ratings (i.e., real-time self-report psychedelic phenomenal experience changes in relation to psilocybin [23]) and PPL (via blood samples) at multiple time points during the psilocybin psychedelic experience (5-6 hours). This scan strategy made it possible to map brain function, phenomenal experience and PPL throughout psilocybin's three pharmacological and experiential phases of ascent, peak and descent [13,23,28–31].

We hypothesized that throughout all phases, PPL and SDI would correlate negatively with DMN and average within-network RSFC, reflecting reduced network integrity, and positively with average-between network RSFC, reflecting reduced network segregation. To more closely identify potential network-specific effects, we also evaluated relations of RSFC within and between individual networks with PPL and SDI. Lastly, we evaluated voxel-level associations between PPL and SDI with: A) local correlation (LCOR)[32], reflecting local changes in integrity, and B) global correlation (GCOR)[33], reflecting changes in global segregation.

Results

Fifteen healthy individuals (mean age ± SD 34.3 ± 9.8 years, six females) participated in the study. All participants were thoroughly prepared and supported before, during and after psilocybin by two psychologists, acting as dedicated guides. The volunteers underwent a psilocybin fMRI intervention during which 10-min BOLD fMRI scans were acquired prior to the administration of a moderate-to-high dose of psilocybin (0.2-0.3 mg/kg) and at approximately 40, 80, 130 and 300 mins after administration (see **Fig 1**., **Methods** and **SI** for further information about the study design and participants). Immediately after every 10-min rs-fMRI acquisition, each participant verbally rated SDI ("How intense is it to you right now?", 1-10 Likert scale: 0 = "not at all intense", 10 = "very intense") and a venous blood sample was collected for determination of PPL [23]. At the end of the intervention day, participants completed self-report questionnaires to assess the overall psychedelic experience.

Plasma psilocin level and subjective effects

In accordance with our previous findings [23], PPL and SDI exhibited highly similar temporal trajectories and correlated closely ($R^2 = 0.77$, $p < 0.001$), confirming that PPL is a key determinant of subjective experience after psilocybin (**Fig 2**) [23].

Retrospective assessment of the psychedelic experience showed that the chosen dose of psilocybin induced a profound altered state of consciousness, assessed with the 11-Dimension Altered States of Consciousness questionnaire (11D-ASC) [34,35], the revised Mystical Experiences Questionnaire (MEQ30) [36], and the Ego-Dissolution Inventory (EDI) [37] (**SI**). Area under curve (AUC) for SDI correlated positively with average 11D-ASC, total MEQ and EDI scores (**SI**), replicating our previous observation that SDI is sensitive to typical psilocybin-induced subjective experience.[23]

Network integrity and segregation decrease as a function of PPL and SDI

Seven networks were analyzed, comprising regions defined by 10 mm spheres about coordinates previously reported [5] (36 regions-of-interest (ROIs), see **SI** for coordinates). Within- and between-network RSFC was estimated by averaging FC estimates between all pairs of relevant regions.

As hypothesized, both PPL and SDI were statistically significantly negatively correlated with DMN (PPL: β[95% CI] = -0.005[-0.008;-0.002], p_{FWER} = 0.013, R^2 = 0.57; SDI: β[95% CI] = -0.007[-0.01;-0.003], R^2 = 0.42, p_{FWER} = 0.005) and average within-network RSFC (PPL: β[95% CI] = -0.005[-0.008;-0.002], p_{FWER} = 0.012, R^2 = 0.33; SDI: [95% CI] = -0.004[-0.009;-0.001], R^2 = 0.32, p_{FWER} = 0.03), reflecting reduced network integrity, and positively correlated with average between-network RSFC (PPL: β[95% CI] = 0.004[0.002;0.005], p_{FWER} < 0.001, R^2 = 0.65; SDI: [95% CI] = 0.005[0.003;0.007], R^2 = 0.51, p_{FWER} < 0.001), reflecting reduced network segregation (**Fig 3**).

PPL and SDI associations with individual network integrity and segregation

To better resolve the effects of PPL and SDI on the functional network architecture, we evaluated associations of PPL and SDI with RSFC of individual networks and between network pairs (**Fig 4**). Integrity for salience network (SAN) was statistically significantly negatively correlated with PPL, the only individual network other than DMN. Between-network analyses showed that as a function of PPL and SDI, segregation was reduced for 1) DMN with SAN, executive control network (ECN, corresponding to the frontoparietal control network [6,38]) and dorsal attention network (DAN), 2) ECN with sensorimotor network (SMN), 3) DAN with sensory networks (see **SI** for parameter estimates and statistics).

Local correlation associations with PPL and SDI

LCOR measures FC between each voxel and its local neighborhood voxels. The LCOR analysis showed that PPL correlated negatively with LCOR in several regions belonging to DMN (medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC) and precuneus), ECN (bilateral anterior

prefrontal cortex (APFC), and visual cortex (occipital pole and lateral occipital cortex) (**Fig 5, SI**). SDI was statistically significantly negatively correlated with LCOR in PCC/precuneus, APFC, visual cortex, and in bilateral temporal regions and a region at the left intraparietal sulcus.

Global correlation associations with PPL and SDI.

GCOR measures average FC between every voxel and the brain and provides a measure of whole-brain connectivity (WBC) similar to previous psychedelic neuroimaging studies [10,25]. PPL and SDI showed comparable statistically significant positive associations with GCOR in areas belonging to DAN and ECN (bilateral superior frontal gyri, bilateral intraparietal sulci, and dorsomedial cingulate gyrus) and SMN (supplementary motor area (SMA)) (**Fig 7**, **SI**). SDI also exhibited a statistically significant positive association with GCOR in thalamus.

Discussion

In the present study, we evaluated how RSFC measures of functional network integrity and segregation map on to blood level of psilocin and the psychedelic experience measured with SDI. As hypothesized, PPL correlated negatively with functional network integrity of the DMN and across all networks. Similar associations were observed for SDI, implicating functional network integrity in phenomenal experience. PPL and SDI correlated negatively with LCOR in central DMN regions (MPFC and PCC), APFC of the ECN, and visual cortex, suggesting that psilocin impairs local network integrity. These findings are consistent with previous studies showing reduced DMN integrity with fMRI [24] and for other networks also with MEG [39] after i.v. psilocybin. Our results thus demonstrate that psilocin shapes the integrity of DMN and across networks and implicates these neuropharmacological dynamics in the transformation of consciousness from the normal to the psychedelic state.

Also consistent with our hypotheses, overall network segregation decreased as a function of PPL and SDI, showing that functional networks increasingly synchronize as psilocin engages

cerebral targets (**Fig 7**). This is noteworthy because average between-network FC is approximately zero in the unstimulated state (pre-drug) (**Fig 3**). This finding of reduced network segregation is consistent with previous observations of a functionally more connected brain in the psychedelic state [8,26]. Interestingly, however, effects of PPL and SDI on individual network integrity and segregation were observed for some networks and not others (**Fig 4**). Specifically, effects at the individual network level matched the repeating spatial motif of functional macroscale networks previously described in the literature [40,41]. In this motif, the DMN is situated at one end, juxtaposed to heteromodal networks (ECN, DAN or SAN). These heteromodal networks are, in turn, situated next to unimodal networks such as auditory or visual networks, which reside at the other end. We found that as function of PPL and SDI, segregation is reduced in a similar pattern for 1) DMN with DAN, ECN and SAN, and for 2) DAN and ECN with unimodal sensory networks. These findings show that although global network segregation is reduced, this effect is more strongly driven by desegregation of adjacent functional networks.

The GCOR analysis showed increased WBC as a function of PPL and SDI for frontoparietal and midline regions within the DAN and ECN [5,6]. Thus, the spatially unconstrained GCOR analysis and the spatially constrained networks analysis converge, showing that ECN and DAN increasingly link together sensory networks with DMN as PPL rises and psychedelic phenomenology intensifies. The DMN is believed to be responsible for internal mentation, i.e., cognition dependent on constructed representations with minimal external stimuli [40,42–44], and the DAN is considered critical for externally oriented attention [45,46]. Interestingly, subjective psychedelic experience includes unitive experiences and the perceived breakdown of borders between self and the external world [47]. We propose that this "flowing together" of normally segregated functional streams related to internally directed (DMN) and externally directed (DAN) attention contributes to the psychedelic

unitive experience, which may be therapeutically important for good clinical outcomes of psilocybin therapy [22].

Interestingly, the GCOR analysis also showed that thalamic GCOR correlated positively with SDI. This is compatible with the thalamic gating theory of psychedelic drug action, which posits that thalamic filtering of external and internal information to the cortex is impaired by psilocin, resulting in information overload [48]. Our observation of SDI-dependent increase in thalamic GCOR indicates that impaired thalamic gating contributes to psychedelic experiences in the context of psilocybin.

Two other fMRI studies used WBC analyses, which yield measures similar to the GCOR analysis [10,25]. The first study showed increased WBC for frontoparietal networks, and also SAN and DMN, but not DAN, after i.v. psilocybin [10]. The second study reported only wide-spread decreased WBC when global-signal regression (GSR) was not used but increased WBC in sensory regions and reduced in associative and subcortical regions when GSR was used [25]. Although Tagliazucchi and colleagues in the former study did not identify DAN as exhibiting increased whole-brain connectivity after psilocybin, our findings agree that other frontoparietal regions increase WBC after psilocybin. The WBC study by Preller and colleagues in the latter study are neither in line with our findings nor the findings of Tagliazucchi and colleagues. It is possible that interstudy discrepancies are influenced by differences related to psilocin pharmacokinetics (i.v. vs oral formulation), BOLD signal denoising procedures (e.g., use of GSR [49]), head motion or the analytical method. Although future studies are warranted to more firmly establish psilocybin effects on voxel-level GBC, an important strength of our study resides in the direct linking of WBC with psilocin-induced changes in neurotransmission and in phenomenal experience (as indexed by PPL and SDI).

The recently proposed "relaxed beliefs under psychedelics (REBUS)" theory hypothesizes that a core mechanism of 5-HT2AR agonist psychedelic compounds, including psilocybin, is impairment of the function of DMN and other high order networks (e.g., ECN), resulting in a less ordered and more desegregated functional architecture [8]. We show that psilocin dose-dependently reduces network integrity and desegregation, both at the global and individual network level (**Fig 4**). Importantly, our findings are thus compatible with neurobiological mechanisms proposed in the REBUS hypothesis.

We replicate our previous findings that PPL and SDI are tightly coupled (**Fig 2**) and also that SDI AUC correlates positively with global ASC questionnaire score [23], which we here also extend to ego-dissolution (EDI) and mystical type experiences (MEQ30) (**SI**). Together these findings show that SDI is tightly coupled to PPL and measures appraised integrated perceptual changes related to psychedelic phenomenology.

Consistent with previous pharmacokinetics studies employing similar peroral doses (0.2-0.3 mg/kg) [28,30,31], we observed considerable interindividual variability in terms of maximum PPL (C_{max}) and time to reach C_{max} (t_{max})(**SI**). Considering this pharmacological variability and the strong correlations of PPL with both phenomenal experience and FC changes, we believe it is very likely that some of the interindividual differences in subjective effects and clinical response, which sometimes has been ascribed to psychological factors, can be explained by interindividual differences in PPL [13,15–17]. Thus, our results clearly indicate that future psilocybin studies would benefit from measuring PPL, as an objective measure of psilocybin effects.

Psilocybin is currently being evaluated in clinical trials for major depression disorder. The intervention strategy includes a 25 mg psilocybin dose in the context of psychosocial support and possibly psychotherapy[50]. Extrapolating our findings in this context, we provide a visualization of the predicted average time course of PPL, SDI and network integrity and segregation after a 25 mg

dose (**Fig 7**). This visualization shows the temporal development of PPL, network function, and SDI over the course of the acute psilocybin experience and ties together important aspects of psilocybin's neuropsychopharmacology.

Our study is not without limitations. A placebo condition could have helped identify effects on RSFC and SDI not tied to psilocybin (i.e., placebo effects). Nevertheless, our study evaluated gradients of RSFC, PPL and SDI, and it is difficult to imagine how placebo could mimic the tight associations with PPL with a similar time course and magnitude. Although head motion during rs-fMRI scans correlated positively with PPL (**SI**), quality control functional connectivity (QC-FC) plots of included datasets indicated that physiological noise was successfully removed in included but not excluded datasets, and *post hoc* analyses indicate that motion did not confound our main results (see **Methods** and **SI** for more information). Lastly, our analysis strategy incorporated data points collected throughout the acute psilocybin effects into a linear model, and we cannot exclude that some neural pathways display non-linear associations with PPL and SDI in this context (i.e., a different association strength "coming down" vs "going up").

In conclusion, the present study evaluated real-time measures of PPL, alterations in phenomenal experience and FC measures of network integrity and segregation, covering ascent, peak and descent phases of clinically relevant psilocybin doses in individuals with no or limited experience with psychedelic drugs. These findings convincingly demonstrate that psilocin critically determines the time course and magnitude of changes in the cerebral functional architecture after psilocybin and implicate the expression of network integrity and segregation as important for consciousness.

Methods

Participants. Fifteen healthy individuals (mean age ± SD 34.3 ± 9.8 years, six females) were recruited from a list of volunteers who signed up to participate in a neuroimaging experiment

investigating psilocybin. The data are part of a broader neuroimaging study; only results pertaining to psilocybin interventions are presented here. Prior to obtaining written informed consent, participants were informed about the study, side-effects and risks. After written informed consent forms were obtained, participants underwent a screening process, including a screening for neurological illness or significant somatic illness, and a screening interview for present or previous psychiatric disorders using Mini-International Neuropsychiatric Interview, Danish translation version 6.0.0 [51]. Exclusion criteria were: 1) present or previous primary psychiatric disease (DSM axis 1 or WHO ICD-10 diagnostic classifications) or in first-degree relatives; 2) present or previous neurological condition/disease, significant somatic condition/disease; 3) intake of drugs suspected to influence test results; 4) non-fluent Danish language skills; 5) vision or hearing impairment; 6) previous or present learning disability; 7) pregnancy; 8) breastfeeding; 9) MRI contraindications; 10) alcohol or drug abuse; 11) allergy to test drugs; 12) significant exposure to radiation within the past year; 13) intake of QT-prolonging medication or electrocardiogram (ECG) results indicative of heart disease, 14) blood donation less than 3 months before project participation; 15) bodyweight less than 50 kg; 16) low plasma ferritin levels (< 12 μg/L). Self-reported history of hallucinogenic drug use was obtained and is presented in **SI**. Before the intervention day, participants were further prepared by the psychologist that would be primary guide. Prior to fMRI data acquisition, a urine sample was obtained from each participant and subjected to a dip-stick test for common drugs of abuse (Rapid Response, BTNX Inc., Markham, Canada). The study was approved by the ethics committee for the capital region of Copenhagen (journal identifier: H-16028698, amendments: 56023, 56967, 57974, 59673, 60437, 62255) and Danish Medicines Agency (EudraCT identifier: 2016-004000-61, amendments: 2017014166, 2017082837, 2018023295).

Psilocybin interventions. Participants underwent MRI data acquisition (T1-weighed structural image, and BOLD fMRI data) prior to psilocybin administration. Oral psilocybin (0.2 mg/kg (n=4),

0.3 mg/kg (n=11), relative dose mean ± SD = 0.26 ± 0.04 mg/kg, absolute dose mean ± SD = 19 ± 3.5 mg) was administered shortly after the pre-drug scan with a glass of water. Participants were blind to dose but were prepared for eventual experience of strong psychoactive effects. Post-drug rs-fMRI scan acquisition was performed approximately 40, 80, 130 and 300 minutes after psilocybin, aimed at capturing FC associated with a range of plasma psilocin levels and associated experiential phases (i.e., ascent, peak, and descent phases) (**Fig 1**). For the first subject, seven rounds of post-drug rs-fMRI data acquisitions were obtained. Although this subject did not report substantial distress due to the number of acquired scans, the acquisition plan was adjusted for all following participants to provide participants a break from fMRI scan acquisition after the third post-drug rs scan. Immediately after each round of the rs fMRI data acquisition, participants verbally rated the subjectively perceived intensity of the drug experience (SDI) of the experience ("How intense is it to you right now?", 1-10 Likert scale: 0 = "not at all intense", 10 = "very intense". Following the SDI rating, a blood sample was obtained from an intravenous catheter in the antecubital vein to quantify plasma psilocin level. Post-drug rs fMRI data from the 300 min acquisition was missing for two subjects (one due to scanner problems, the other due to the participant feeling nauseous).

Two psychologists provided psychosocial support throughout the intervention day. At least one of the two psychologists met with participants the day after the intervention to allow for integration of the experience. After psilocybin administration, participants were free to listen to music in a room adjacent to the MRI scanner room, i.e., from the time of psilocybin administration to the first post-drug acquisition and between the 130 and 300 min rs-fMRI acquisitions (i.e., during the break). No music was played in the context of rs-fMRI acquisition. After the 300-min scan session and when SDI was rated 1 or less, participants filled out three questionnaires measuring aspects of the psychedelic experience: 11D-ASC [34,35], MEQ30 [36] and the EDI [37].

Psilocin plasma concentrations. Plasma psilocin analyses were performed using ultra performance liquid chromatography and tandem mass spectrometry as previously described [23].

MRI acquisition. MRI data was obtained on a 3T Siemens Prisma scanner (Siemens, Erlangen, Germany), using a 64-channel head coil. BOLD fMRI data was obtained using a T2*-weighted gradient echo-planar imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90°, in-plane matrix = 64x64 mm, in-plane resolution=3.6x3.6 mm, 32 slices (thickness = 3.0 mm, gap = 0.75 mm). Three hundred volumes were acquired for each BOLD fMRI data acquisition. A high-resolution, T1-weighted 3D structural image was acquired at the pre-drug scan (inversion time = 900 ms, TE = 2.58 ms, TR = 1900 ms, flip angle = 9°, in-plane matrix = 256x256, resolution = 0.9x0.9 mm, 224 slices; slice thickness = 0.9 mm, no gap).

fMRI data preprocessing. Preprocessing of the BOLD fMRI data was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). The preprocessing steps included 1) realignment of functional images to first functional image, 2) co-registration of the T1-weighted structural image, 3) slice-timing correction, 4) unwarping, 5) normalization of images into Montreal Neurological Institute (MNI) space, 6) smoothing (8 mm Gaussian kernel), and 7) reslicing into a final voxel size of 2 x 2 x 2 mm.

fMRI data denoising. Previous neuroimaging studies report increased head motion after psychedelic drug intake [9,24,52], and head motion is potentially problematic as it may affect analytical results [53]. Drug-associated changes in respiratory or cardiac activity may likewise impact image quality and hence analytical results [54]. Thus, it is important to clean the data from motion and physiological noise. In the present study, head motion was limited by firmly fixing each subject's head inside the head coil using foam pads. Post-acquisition denoising performed in CONN [55], included: regression of noise sources at the level of data from each scan by including in the general linear model: 1) six motion parameters (three translation, three rotation) and their 1st order

derivatives, 2) the first five principal components and their 1st order derivatives from separate principal components analyses of white matter and cerebrospinal fluid time series (aCompCor, [54,56]), and 3) a regressor denoting images for which maximum voxel displacement exceeded 0.5 mm or for which global signal exceeded three standard deviations above the time series mean (spike regression) using ARTifact Detection Tools ((ART), https://www.nitrc.org/projects/artifact_detect). Both spike regression [53,57,58] and aCompCor [53,54,56] improves BOLD fMRI data quality and, compared to global signal regression (GSR), has the benefit of not including gray matter signal, which can remove true neuronal signal [49,59,60]. The residuals represent the BOLD signal unexplained by the noise sources, i.e., the BOLD signal cleaned from noise. Functional datasets with more than 120 censored images were excluded from further analysis as previously suggested [53,58]. This procedure resulted in the exclusion of six datasets from the analysis, resulting in a total of 68 included datasets.

Histograms of FC for a 1000 region adjacency matrix (QC-FC plots) computed before and after denoising were inspected as part of the denoising assessment [61]. The QC-FC plots for the omitted scans indicated that these scans indeed were tainted, even after denoising, supporting the exclusion of these datasets. The remaining QC-FC plots for the denoised BOLD data were unremarkable and suggested successful denoising.

Functional connectivity estimation

Pairwise interregional analysis. The network ROIs employed in the present analysis were defined by a 10 mm sphere at MNI coordinates previously reported [5] (see **SI** for ROIs and coordinates used to delineate the networks). Estimation of FC was performed in CONN version 17.c (https://web.conn-toolbox.org/). The denoised BOLD signal time series for each ROI (percent of mean signal) was included in linear regression models, and the Fisher-transformed r-to-z correlation coefficients were calculated for all possible region pairs.

Local correlation (LCOR) analysis. LCOR is a measure of local FC [32,33]. It is calculated for each voxel by computing a weighted-average of Fisher-transformed r-to-z-value correlation coefficients between each voxel time series and the time series of neighboring voxels, where the weighting is defined by an 8 mm full-width at half-maximum Gaussian kernel.

Global correlation (GCOR) analysis. GCOR is implemented in CONN and is a FC method that gives a voxel-wise measure of GCOR [33]. Each whole brain time-series was represented as a 64-component single value decomposition. GCOR was calculated as the average of Fisher-transformed r-to-z-value correlation coefficients for each individual voxel and the 64-component time-series.

Data analysis

Network analysis. Within-network FC was computed as the average of Fisher-transformed r-to-z values between regions within a given network. Average within-network RSFC was calculated as the average of all individual within-network RSFCs. Between-network RSFC was calculated as the average of all possible connections between regions in a pair of networks. Average between-network FC was calculated as the average of pairwise between-network RSFC estimates.

Linear mixed effects model regression analysis was used to model the association between FC and PPL and SDI, respectively [62], and p-values were calculated using Satterthwaite's method for approximating degrees of freedom [63]. The family-wise error rate for all statistical tests was controlled using the Bonferroni-Holm method [64], with a threshold for statistical significance of $p_{FWER} < 0.05$. First, statistical testing for the associations of PPL and SDI with average within-network, average between-network and DMN RSFC was conducted (number of tests = 6). Second, PPL and SDI associations were evaluated separately with all within- and between-network RSFC estimates (number of tests = 28). Third, associations of PPL and SDI with ROI-to-ROI RSFC were performed separately for PPL and for SDI (number of tests = 630), and was thresholded using false-discovery-rate-corrected p-values ($q_{FDR} < 0.05$) [65].

Statistical analysis of GCOR and LCOR. Whole-brain GCOR and LCOR maps were included in separate linear mixed-effects models with PPL or SDI as independent variables, respectively. Based on the smoothness of the residuals, cluster sizes unlikely to occur by change ($p < 0.05$) at a voxel-level threshold of $p < 0.001$[66] was estimated, using 3dFWHMx and 3dClustSim in AFNI version 16.2.01 (https://afni.nimh.nih.gov/). The cluster size threshold was calculated to be 560 voxels.

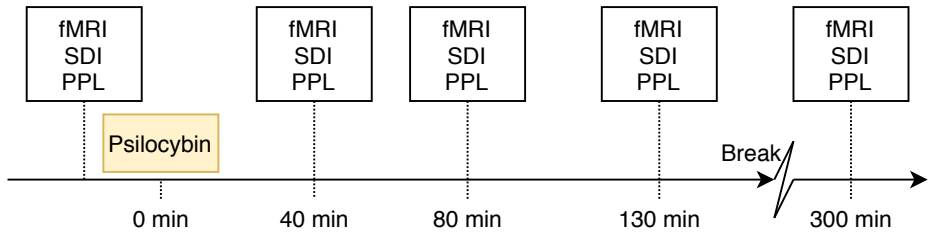

Fig. 1. Study design. fMRI: functional magnetic resonance imaging, SDI: subjective drug intensity rating, PPL: plasma psilocin levels. After the third post-drug scan acquisition, participants had a break from fMRI acquisition before the fourth post-drug scan acquisition. Post-drug time designates anticipated beginning of scan.

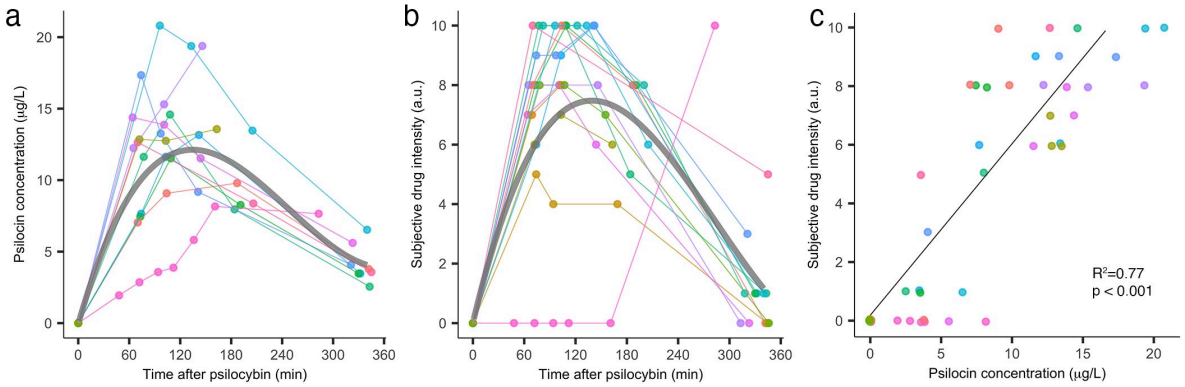

Fig. 2. Plasma psilocin levels and subjective drug intensity. Time course of plasma psilocin level (PPL) (a), and subjective drug intensity (SDI) (b). (c) Correlation between PPL and SDI. a.u.,

arbitrary units. Average time course of PPL and SDI approximated by spline fits is visualized in (a) and (b) in grey. Datapoints are color-coded for each subject.

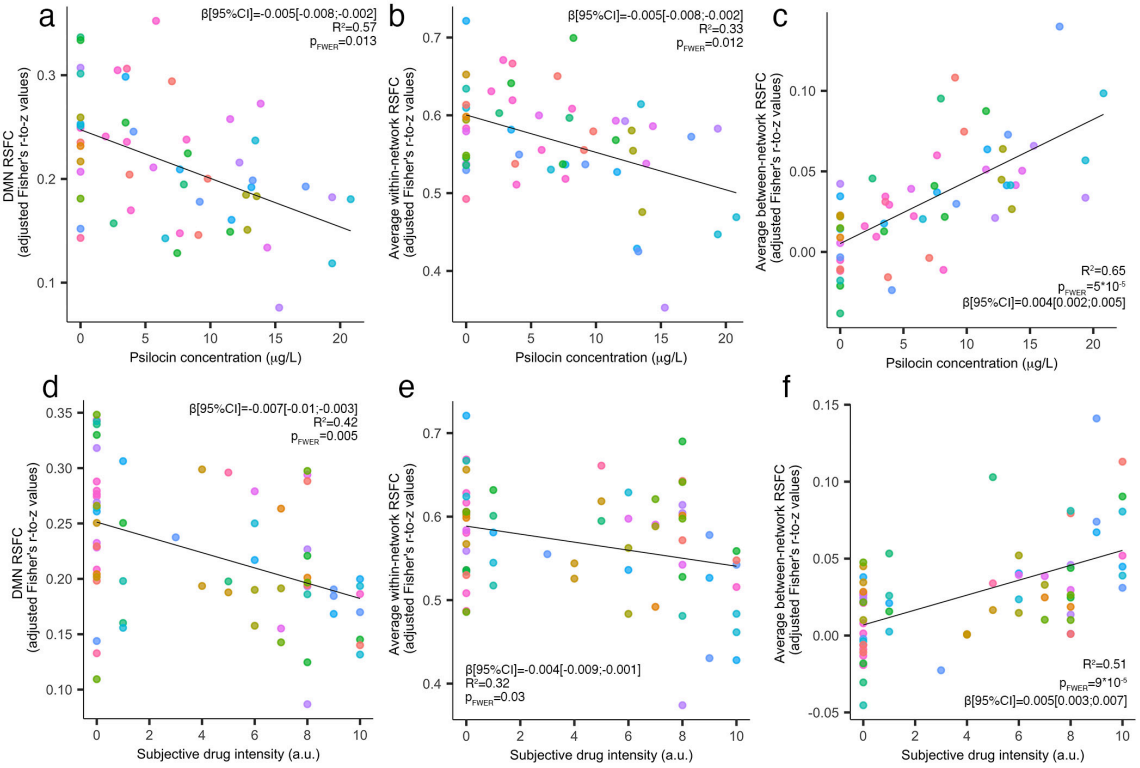

Fig 3. Network connectivity, plasma psilocin level and subjective drug intensity. Plasma psilocin level (PPL) was negatively associated with default mode network (DMN) resting state functional connectivity RSFC (a) and average within-network RSFC (b), and positively associated with average between-network RSFC (c). Similar associations were observed for SDI (d, e, f). a.u., arbitrary units. adjusted: partial correlation for RSFC estimate on SDI or PPL.

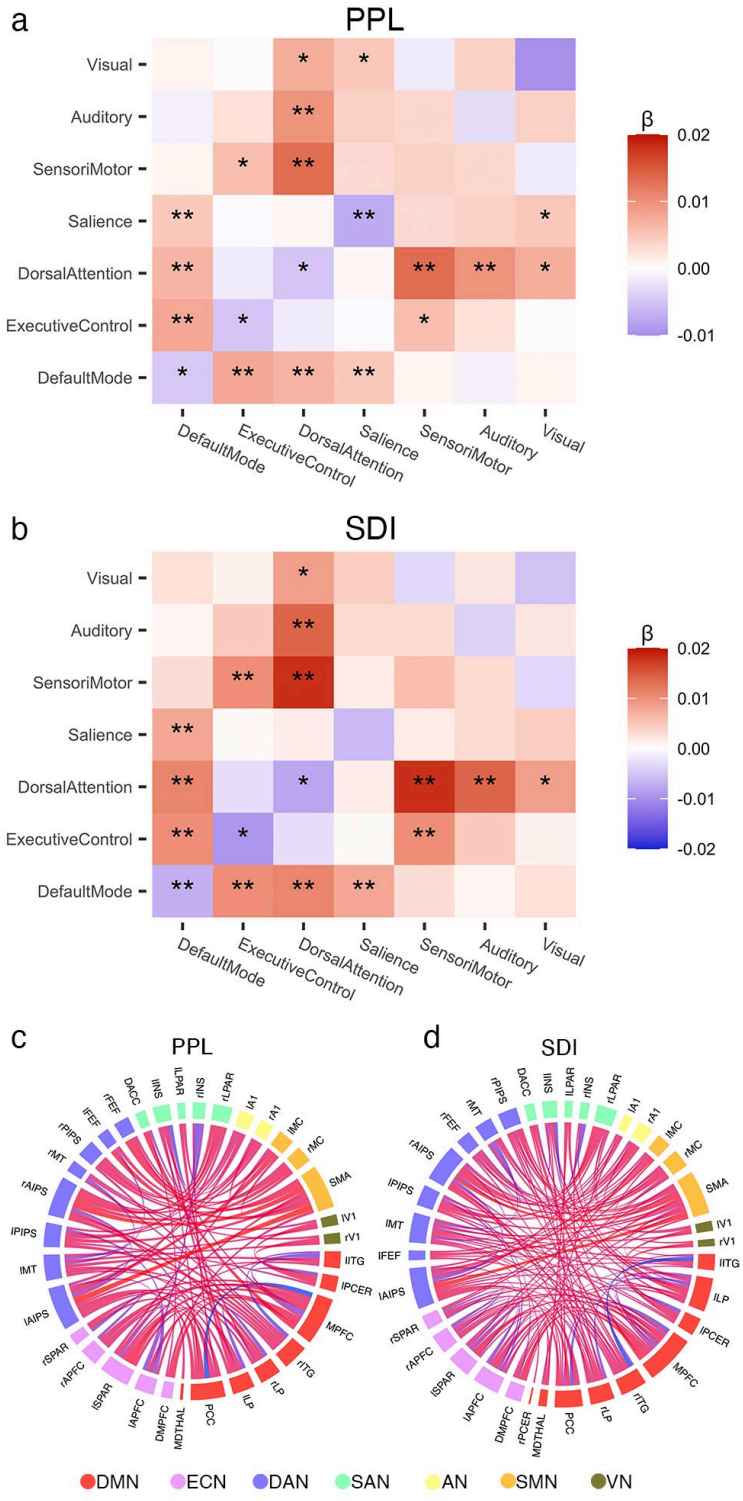

Fig. 4. Results of individual networks analysis. Plasma psilocin level (PPL) (a) and subjective drug intensity (SDI) (b) displayed similar negative associations with within-network resting state functional connectivity (RSFC) and positive associations with between-network RSFC for

individual networks. * $p_{unc} < 0.05$, ** $p_{FWER} < 0.05$. A graphical representation is shown of the slope estimates (β) for the correlation of region-to-region RSFC with PPL (88 connections) (a) and with SDI (138 connections) (b), respectively, thresholded at $q_{FDR} < 0.05$. Red links signify a positive association; blue signifies a negative association. Line thickness is proportional to β estimate within the same circle.

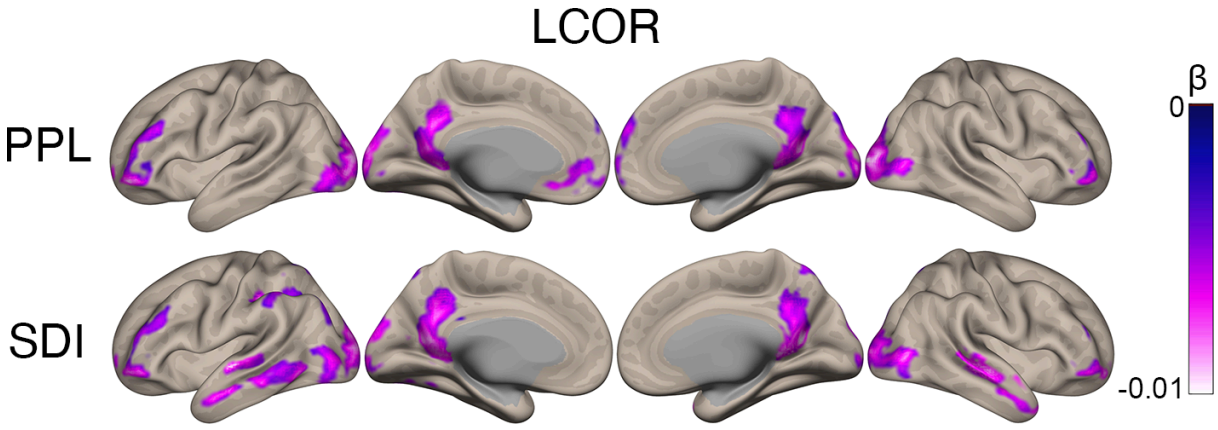

Fig 5. Results of the local correlation (LCOR) analysis. Surface projection of areas for which LCOR is significantly correlated with plasma psilocin levels (PPL, top row) and subjective drug intensity (SDI, bottom row). Associations were observed in visual cortex; in regions within default mode network hubs of medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC) and temporal cortex; and in bilateral anterior prefrontal cortex (APFC), which is part of the ECN. Color bars designates slope estimates (β) for association. The cluster-level threshold for statistical significance was 560 voxels.

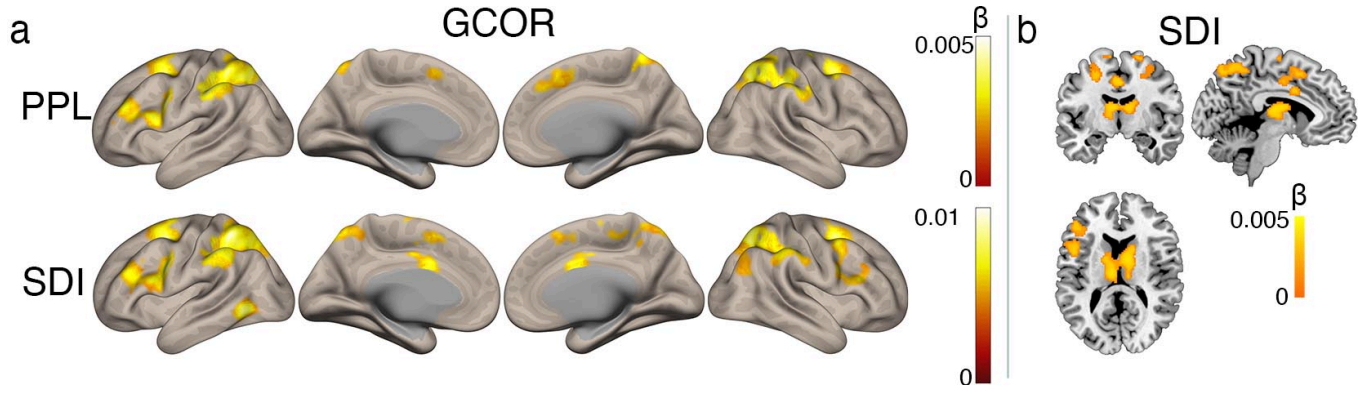

Fig 6. Results of the global correlation (GCOR) analysis. Surface projection of clusters for which GCOR correlated positively with subjective drug intensity (SDI) (a) and plasma psilocin

level (PPL). Only positive associations were observed. Clusters correspond primarily to dorsal attention network (DAN) regions and to a lesser extent executive control network (ECN). SDI correlated statistically significantly positively with GCOR in thalamus (b). Color bars designates slope estimates (β) for association of LCOR with SDI and PPL. The cluster-level threshold for statistical significance was 560 voxels.

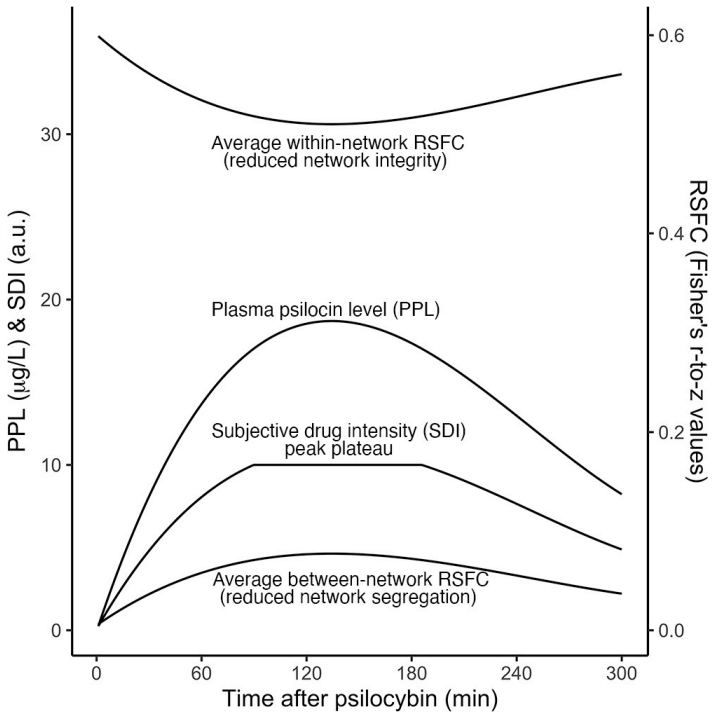

Fig 7. Predicted response profile for a 25 mg clinical psilocybin dose. The figure shows a predicted response profile for a 25 mg clinical dose of psilocybin, constructed by spline fits of psilocin time course data, scaled to C_{max} of 18.7 μg/L [50]. Resting state functional connectivity (RSFC) and subjective drug intensity (SDI) predictions reflect average predicted values from model estimates of PPL relations. a.u., arbitrary units.

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Author contributions

PMF and GMK designed the study, wrote the protocol and contributed to data analysis, and interpretation. MKM designed the study, wrote the protocol, contributed with data collection,

analysis, interpretation and wrote the first draft of the manuscript. MMJ and AA assisted with data collection. DSS conceptualized the main psychological outcome of the study (SDI), and set up, translated and implemented the applied psychological and contributed with guiding at psilocybin interventions, participant preparation and integration. SA contributed with guiding at psilocybin interventions, and participant preparation and integration. KL and SSJ determined plasma psilocin levels. BO contributed with data analysis and interpretation. All authors contributed substantially to interpretation and discussion of the study results, critical review of the submitted manuscript, approve of the final version and agree to be both personally accountable for own contributions and willing to participate in resolving any question that may arise regarding the present paper.

Competing interests

GMK has received honoraria as a speaker for Janssen Pharmaceutica NV and as expert consultant for Sage Therapeutics and Sanos. All other authors declare no conflicts of interest.

Data availability

Data is available upon reasonable request as permitted by Danish and European Union law.

Code availability

The present results were produced using freely available software, and customized code was applied only in the context of calling functions from the existing software packages. Code is available upon request.

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SUPPLEMENTARY INFORMATION

Integrity and segregation of macroscale cerebral functional networks correlate with plasma psilocin level and psychedelic experience

Martin Korsbak Madsen[a,b], Dea Siggaard Stenbæk[a], Albin Arvidsson[a], Sophia Armand[a], Maja Marstrand Jørgensen[a], Sys S. Johansen[c], Kristian Linnet[c], Brice Ozenne[a,d], Gitte M. Knudsen[a,b], Patrick MacDonald Fisher[a*]

[a]Neurobiology Research Unit and NeuroPharm, Copenhagen University Hospital Rigshospitalet, 2100 Copenhagen, Denmark;
[b]Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[c]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen, Denmark
[d]Section of Biostatistics, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

*Corresponding Author:
Patrick MacDonald Fisher, PhD
Copenhagen University Hospital Rigshospitalet
Neurobiology Research Unit
NRU 6931, Rigshospitalet
Blegdamsvej 9
Copenhagen O DK-2100
DENMARK
Phone: +45 3545 6714
E-mail: patrick.fisher@nru.dk

Short title: Functional connectivity & psilocybin

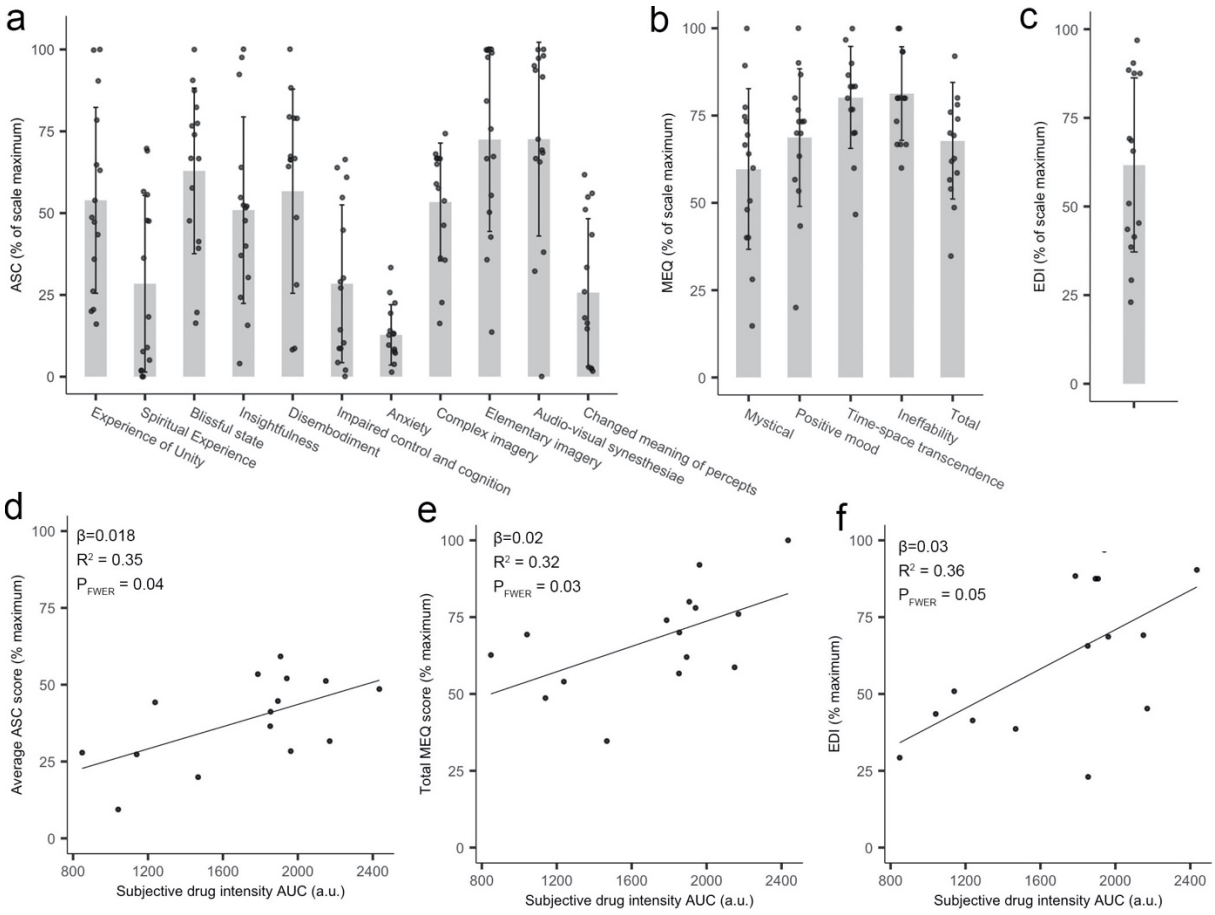
Number of tables: 5
Number of figures: 2

Subject	Life time	Preceding year
Subject 1	No use	No use
Subject 2	No use	No use
Subject 3	No use	No use
Subject 4	No use	No use
Subject 5	No use	No use
Subject 6	No use	No use
Subject 7	*Psilocybin: 1*	No use
Subject 8	Ketamine: 1 *Psilocybin: 1*	No use
Subject 9	*Psilocybin: 1*	No use
Subject 10	*Psilocybin: 1*	No use
Subject 11	*Psilocybin: 1* *Ayahuasca: 1*	No use
Subject 12	*Psilocybin: 1*	No use
Subject 13	No use	No use
Subject 14	*Psilocybin: 9*	No use
Subject 15	*Psilocybin: 1*	No use

SI Table 1. Participant history of hallucinogenic drug use. Self-reported history of use of hallucinogens. Serotonergic psychedelics italicized.

Dose (mg/kg)	n	C_{max} median;mean [range] (μg/L)	t_{max} median; mean [range] (min)
0.2	4	13.8; 13.3 [8.2-17.3]	108; 110 [64-161]
0.3	7	13.6; 14.6 [9.8-20.9]	109; 125 [70;187]

SI Table 2. Plasma psilocin levels. C_{max}: maximum plasma psilocin concentration. t_{max}: time to C_{max}. Plasma psilocin levels available for first 11 subjects only.



SI Fig 1. Retrospective psychedelic questionnaire results and relation to subjective drug intensity. Self-report responses for three questionnaires, measuring aspects of the psychedelic experience: (a) 11-Dimension Altered States of Consciousness (ASC), (b) Mystical Experiences Questionnaire (MEQ), (c) Ego-Dissolution Inventory (EDI). Correlation between the subjective drug intensity (SDI) area under curve (AUC) and ASC (d), MEQ30 (e) and EDI (f). SDI AUC correlates positively with all three questionnaire outcomes, supporting that SDI is a useful real-time single-readout of subjective psychedelic effects. a.u., arbitrary units.

Network	Region	MNI[a] coordinate		
		X	Y	Z
Default mode network	Posterior cingulate cortex / precuneus	0	-51	27
	Medial prefrontal cortex	-1	54	27
	Left lateral parietal cortex	-46	-66	30
	Right lateral parietal cortex	49	-63	33
	Left inferior temporal gyrus	-61	-24	-9
	Right inferior temporal gryus	58	-24	-9
	Medial dorsal thalamus	0	-12	9
	Right posterior cerebellum	-25	-81	-33
	Left posterior cerebellum	25	-81	33
Dorsal attention network	Left frontal eye field	-29	-9	54
	Right frontal eye field	29	-9	54
	Left posterior intraparietal sulcus	-26	-66	48
	Right posterior intraparietal sulcus	26	-66	48
	Left anterior intraparietal sulcus	-44	-39	45
	Right anterior intraparietal sulcus	41	-39	45
	Left medial temporal	-50	-66	-6
	Right medial temporal	53	-63	-6
Executive control network	Dorsal medial prefrontal cortex	0	24	46
	Left anterior prefrontal cortex	-44	45	0
	Right anterior prefrontal cortex	44	45	0
	Left superior parietal cortex	-50	-51	45
	Right superior parietal cortex	50	-51	45
Salience network	Dorsal anterior cingulate	0	21	36
	Left anterior prefrontal cortex	-35	45	30
	Right anterior prefrontal cortex	32	45	30
	Left insula	-41	3	6
	Right insula	41	3	6
	Left lateral parietal cortex	-62	-45	30
	Right lateral parietal cortex	62	-45	-40
Sensorimotor network	Left motor cortex	-39	-26	41
	Right motor cortex	38	-26	48
	Supplementary motor area	0	-21	48
Visual network	Left V1	-7	83	2
	Right V1	7	83	2
Auditory network	Left A1	-62	-30	12
	Right A1	59	-27	15

SI Table 3. Region-of-interest-based networks delineation. The table shows the center of mass coordinate for each 10 mm sphere used for delineation of each network, as proposed by Raichle(Raichle, 2011). [a]Montreal Neurological Institute coordinates.

Network	Intercept	95% CI of intercept	ß-estimate	95% CI of ß	p_{unc}	p_{FWER}
AN	0.82	[0.70;0.93]	-0.0029	[-0.013;0.0067]	0.6	1
DMN	0.25	[0.21;0.30]	-0.0047	[-0.0078;-0.0016]	0.004	0.1
DAN	0.44	[0.39;0.48]	-0.0050	[-0.0091;-0.00058]	0.02	0.4
ECN	0.43	[0.36;0.51]	-0.0050	[-0.0096;-0.00051]	0.04	0.6
SAN	0.42	[0.37;0.47]	-0.0073	[-0.012;-0.0031]	0.001	0.03
SMN	0.44	[0.35;0.53]	0.0041	[-0.0049;0.011]	0.3	1
VN	1.40	[1.27;1.53]	-0.0050	[-0.022;0.0021]	0.1	1
DMN-AN	-0.089	[-0.15;-0.026]	-0.0013	[-0.0062;0.0036]	0.6	1
DMN-DAN	-0.15	[-0.19;-0.11]	0.0067	[0.0032;0.0099]	0.0002	0.004
DMN-ECN	-0.0069	[-0.058;0.043]	0.0078	[0.0046;0.011]	$1.6*10^{-5}$	0.0004
DMN-SAN	-0.095	[-0.13;-0.061]	0.0048	[0.0021;0.0075]	0.001	0.03
DMN-SMN	-0.038	[-0.074;-0.0017]	0.00078	[-0.0029;0.0046]	0.7	1
DMN-VN	-0.0044	[-0.058;0.048]	0.00090	[-0.0038;0.0055]	0.7	1
DAN-AN	0.087	[0.019;0.15]	0.0097	[0.0036;0.016]	0.002	0.04
DAN-ECN	0.079	[0.023;0.14]	-0.0017	[-0.0061;0.0026]	0.5	1
DAN-SAN	0.046	[0.00087;0.090]	0.00061	[-0.0031;0.0042]	0.7	1
DAN-SMN	0.087	[0.030;0.14]	0.013	[0.0073;0.019]	$7*10^{-6}$	0.0002
DAN-VN	0.0060	[-0.055;0.068]	0.0073	[0.0020;0.013]	0.01	0.2
ECN-AN	-0.089	[-0.14;-0.038]	0.0026	[-0.0020;0.0073]	0.3	1
ECN-SAN	0.010	[0.051;0.15]	-0.00038	[-0.0041;0.0033]	0.8	1
ECN-SMN	-0.10	[-0.14;-0.063]	0.0058	[0.0016;0.0098]	0.008	0.2
ECN-VN	-0.052	[-0.11;0.0083]	0.00024	[-0.0050;0.0056]	0.9	1
SAN-AN	0.20	[0.13;0.26]	0.0040	[-0.0017;0.0093]	0.1	1
SAN-SMN	0.011	[-0.040;0.058]	0.0033	[-0.0012;0.0077]	0.1	1
SAN-VN	-0.090	[-0.14;-0.043]	0.005	[0.00032;0.0098]	0.04	0.7
SMN-AN	0.17	[0.010;0.24]	0.0035	[-0.0044;0.010]	0.3	1
SMN-VN	0.047	[-0.035;0.13]	-0.0017	[-0.0091;0.0055]	0.7	1
VN-AN	0.027	[-0.075;0.13]	0.0041	[-0.0035;0.012]	0.3	1

SI Table 4. Networks RSFC and plasma psilocin levels. Result of linear mixed-effects model analysis of the association between plasma psilocin levels and functional connectivity within and between networks (networks separated by "-"). AN: auditory network; DMN: default mode network; DAN: dorsal attention network; ECN: executive control network; SAN: salience network; SMN: sensorimotor network; VN: visual network. FC: functional connectivity. The intercept represents the model-estimated FC at pre-drug and thus gives a measure of FC in the unstimulated state.

Network	Intercept	95% CI of intercept	ß-estimate	95%CI of ß	p_{unc}	p_{FWER}
AN	0.79	[0.68;0.91]	-0.0039	[-0.016;0.0083]	0.5	1
DMN	0.25	[0.22;0.28]	-0.0069	[-0.011;-0.0029]	0.001	0.03
DAN	0.45	[0.41;0.48]	-0.0078	[-0.014;-0.0018]	0.01	0.2
ECN	0.42	[0.35;0.48]	-0.0093	[-0.016;-0.0026]	0.008	0.17
SAN	0.39	[0.34;0.44]	-0.0060	[-0.012;0.00024]	0.06	1
SMN	0.44	[0.36;0.53]	0.0058	[-0.0041;0.015]	0.2	1
VN	1.40	[1.25;1.51]	-0.0053	[-0.025;0.014]	0.6	1
DMN-AN	-0.010	[-0.16;-0.041]	0.00073	[-0.0053;0.0068]	0.8	1
DMN-DAN	-0.16	[-0.20;-0.12]	0.011	[0.0061;0.016]	$3.9*10^{-5}$	0.001
DMN-ECN	0.0067	[-0.035;0.048]	0.010	[0.0059;0.014]	$1.4*10^{-5}$	0.0004
DMN-SAN	-0.095	[-0.14;-0.054]	0.0080	[0.0046;0.011]	$1.4*10^{-5}$	0.0004
DMN-SMN	-0.041	[-0.074;-0.0084]	0.0030	[-0.0019;0.008]	0.2	1
DMN-VN	-0.014	[-0.065;0.037]	0.0027	[-0.0041;0.0093]	0.4	1
DAN-AN	0.086	[0.024;0.15]	0.014	[0.0065;0.0213]	0.0004	0.009
DAN-ECN	0.075	[0.026;0.12]	-0.0029	[-0.0084;0.0028]	0.3	1
DAN-SAN	0.041	[-0.00010;0.083]	0.0016	[-0.0032;0.0063]	0.5	1
DAN-SMN	0.10	[0.049;0.14]	0.018	[0.011;0.025]	$2.5*10^{-6}$	$6.9*10^{-5}$
DAN-VN	0.0096	[-0.051;0.071]	0.0090	[0.00041;0.017]	0.04	0.9
ECN-AN	-0.081	[-0.13;-0.035]	0.0047	[-0.0017;0.011]	0.2	1
ECN-SAN	0.092	[0.046;0.140]	0.00042	[-0.0043;0.0051]	0.9	1
ECN-SMN	-0.10	[-0.13;-0.072]	0.010	[0.0050;0.015]	0.0003	0.006
ECN-VN	-0.055	[-0.11;-0.0011]	0.0011	[-0.0061;0.0083]	0.8	1
SAN-AN	0.21	[0.16;0.27]	0.0031	[-0.0044;0.010]	0.4	1
SAN-SMN	0.022	[-0.019;0.063]	0.0017	[-0.0041;0.0076]	0.6	1
SAN-VN	-0.072	[-0.11;-0.030]	0.0044	[-0.0021;0.011]	0.2	1
SMN-AN	0.17	[0.12;0.23]	0.0031	[-0.0052;0.011]	0.5	1
SMN-VN	0.028	[-0.045;0.10]	-0.0033	[-0.013;0.0069]	0.5	1
VN-AN	0.014	[-0.070;0.10]	0.0022	[-0.0081;0.013]	0.7	1

SI Table 5. Networks RSFC and subjective drug intensity. Result of linear mixed-effects model analysis of the association between subjective drug intensity and functional connectivity within and between networks (networks separated by "-"). AN: auditory network; DMN: default mode network; DAN: dorsal attention network; ECN: executive control network; SAN: salience network; SMN: sensorimotor network; VN: visual network. FC: functional connectivity. The intercept represents the model-estimated FC at pre-drug and thus gives a measure of FC in the unstimulated state.

Effects of head motion on main study outcomes

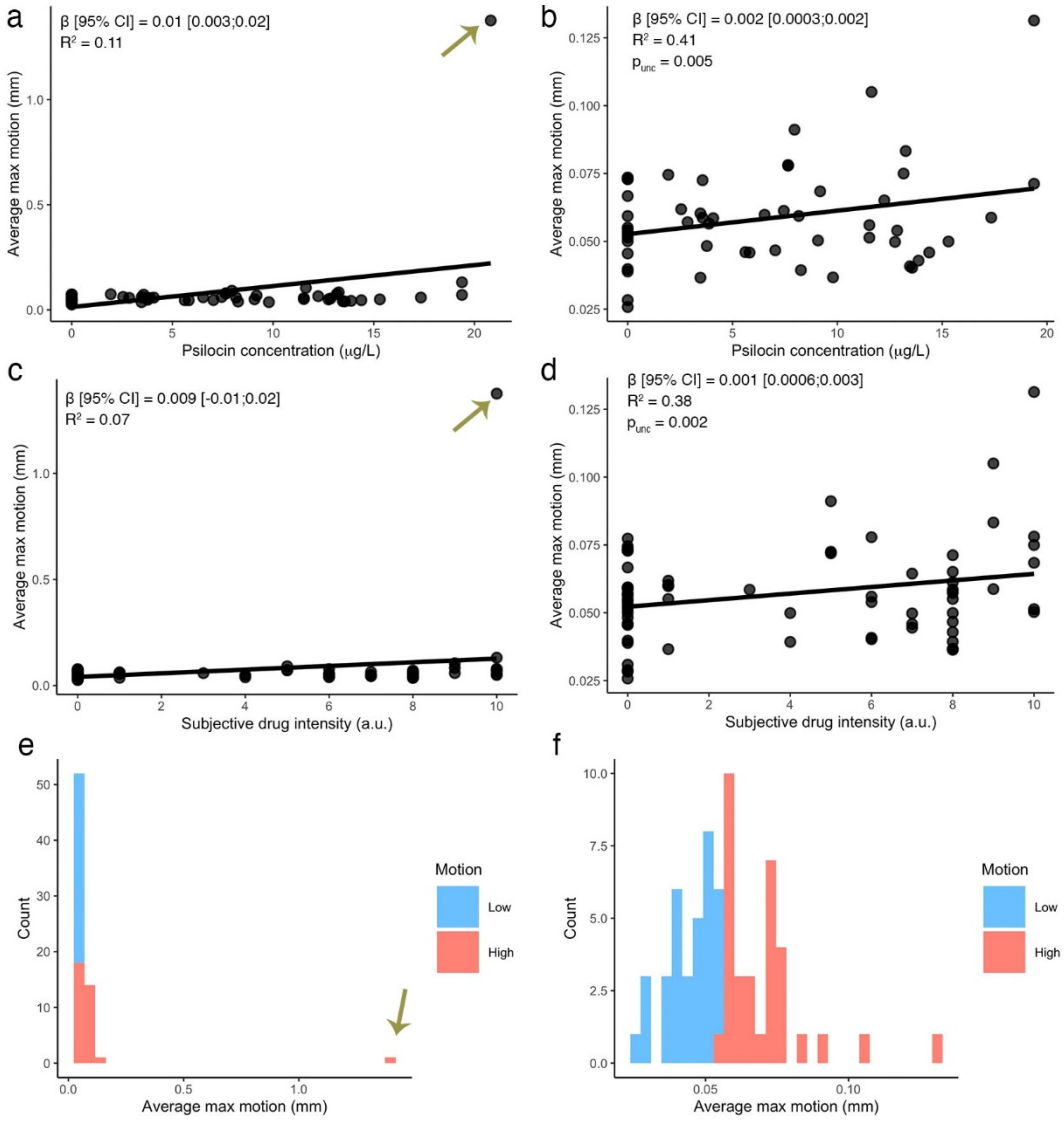
The implemented denoising procedure (regression of motion parameters, aCompCor, and spike regression, see **Methods** for further description) removes noise associated with physiological sources (respiration and pulse) and head motion from the BOLD signal (Behzadi et al., 2007). Although the BOLD signal is cleaned from noise at the level of each scan, it is still possible that residual motion artifacts could influence the results. To investigate this, we evaluated the extent to which head motion correlated with PPL and SDI and whether motion influenced our main conclusions (DMN RSFC, average within-network RSFC, and average between-network RSFC).

First, PPL and SDI correlated with the average maximum head motion (i.e., the average of the greatest framewise voxel displacement observed both when including and excluding one outlier observation (**SI Fig 2**). This shows that PPL dose-dependently increases head-motion.

Second, theoretically it would be possible to adjust for head motion at the group-level analysis. However, since motion is cleaned from the BOLD signal during denoising, and since head motion correlate with PPL and SDI, it is likely that effects of PPL and SDI are erroneously removed if adding head motion as regressor in the group level analysis.

Thus, to evaluate whether head motion may have affected the main results, we compared main study outcomes for high vs low motion scans (median-split), when adjusting for PPL or SDI, using linear mixed effects modelling. This assumes that the effect of PPL and SDI on RSFC is the same within each group. The analysis showed that main study outcomes (DMN RSFC, average within-network RSFC, and average between-network RSFC) did not differ for high vs low motion scans for the ($p_{FWER} > 0.8$ (six tests)), when

adjusting for PPL or SDI. Although the inability to reject the null hypothesis does not provide evidence that there is no difference between high and low motion groups, nor that there is no effect of head motion on the BOLD signal, it nevertheless indicates that potential residual motion-related noise in the signal is likely of small magnitude and consequently less likely to confound our findings.



SI Fig 2. Head motion, plasma psilocin levels and subjective drug intensity. Average head motion for each scan correlated positively with plasma psilocin levels (PPL) (a) and subjective drug intensity (SDI) (d) also when outlier (indicated by arrow) was removed (c) and (d). A histogram of average head motion is shown with outlier (e) and without outlier (f), color coded by median-split into high (red) or low motion (blue) scans, which were used to assess effect of head motion on main results.

Cluster	Regions	Size (voxels)	Coordinate of peak voxel
1			
Negative association	Left middle occipital	3697	(20,-98,6)
	Right middle occipital		
	Right superior occipital		
	Left superior occipital		
	Left calcarine		
	Right calcarine		
	Left inferior occipital		
	Right inferior occipital		
	Left fusiform		
	Right middle temporal		
2			
Negative association	Right superior medial frontal	808	(-6,38,-2)
	Left superior medial frontal		
	Left medial frontal, orbital part		
	Left anterior cingulate		
	Right medial frontal, orbital part		
3			
Negative association	Left middle frontal	1468	(-36,54,-8)
	Left inferior frontal, triangular part		
	Left middle frontal, orbital part		
	Left inferior frontal, orbital part		
	Left superior frontal, orbital part		
4			
Negative association	Right middle frontal	871	(44,50,-8)
	Right middle frontalm orbital part		
	Right inferior frontal, orbital part		
	Right inferior frontal, triangular part		
5			
Negative association	Right precuneus	2500	(15,-52,14)
	Left precuneus		
	Left calcarine		
	Right calcarine		
	Right posterior cingulate		
	Left posterior cingulate		
	Left middle cingulate		
	Right middle cingulate		
	Right lingual		
	Left lingual		

SI Table 6. Results of analysis of LCOR and plasma psilocin levels. The table shows clusters within which there was a statistically significant correlation between local correlation (LCOR) and plasma psilocin levels. Coordinate in Montreal Neurological Institute (MNI) space. Cluster size threshold was 560 voxels. Only negative associations were observed.

Cluster	Regions	Size (voxels)	Peak voxel coordinate
1			
Negative association	Right middel temporal	1045	(52,-22,-8)
	Right middle temporal pole		
	Right superior temporal		
2			
Negative association	Left middel temporal	3495	(-64,-14,-6)
	Left middle occipital		
	Left inferior temporal		
	Left superior occipital		
	Left inferior occipital		
	Left cuneus		
	Left calcarine		
3			
Negative association	Right middle frontal	1034	(26,60,-8)
	Right middle frontal, orbital part		
	Right inferior frontal, orbital part		
4			
Negative association	Left middle frontal	1391	(-36,54,-8)
	Left middle frontoorbital		
	Left inferior frontal triangular part		
	Left inferior frontal orbital part		
5			
Negative association	Right middle occipital	1380	(48,-74,2)
	Right superior occipital		
	Right inferior occipital		
	Right middle temporal		
	Right inferior temporal		
6			
Negative association	Right precuneus	3077	(16,-52,14)
	Left precuneus		
	Left posterior cingulate		
	Right posterior cingulate		
	Left middle cingulate		
	Right middle cingulate		
	Right calcarine		
	Left calcarine		
7			
Negative association	Left inferior parietal	1973	(-38,-32,38)
	Left superior parietal		
	Left middle occipital		
	Right precuneus		
	Left precuneus		
	Right superior parietal		

SI Table 7. Results of analysis of LCOR and subjective drug intensity. The table shows clusters within which there was a statistically significant correlation between local correlation (LCOR) and subjective drug intensity (SDI). Coordinate in Montreal Neurological Institute (MNI) space. Cluster size threshold was 560 voxels. Only negative associations were observed.

Cluster	Regions	Size (voxels)	Peak voxel coordinate
1			
Positive association	Left inferior frontal, triangular part	1462	(-52,6,34)
	Left inferior frontal, opercular part		
	Left middle frontal		
2			
Positive association	Left middle frontal	1680	(-26,2,54)
	Left superior frontal		
	Right middle cingulate		
	Left sup. motor area		
	Left middle cingulate		
	Right sup. motor area		
3			
Positive association	Left inferior parietal	2978	(-42,-38,44)
	Left superior parietal		
	Left supramarginal		
	Left post central		
	Left precuneus		
4			
Positive association	Right superior parietal	2988	(38,-36,42)
	Right post central		
	Right inferior parietal		
	Right supramarginal		
	Right angular		
5			
Positive association	Right superior frontal	936	(30,0,60)
	Right middle frontal		
	Right precentral		

SI Table 8. Results of analysis of GCOR and plasma psilocin levels. The table shows clusters within which there was a statistically significant correlation between global correlation (GCOR) and plasma psilocin levels (PPL). Coordinate in Montreal Neurological Institute (MNI) space. Only positive associations were observed. Cluster size threshold was 560 voxels.

Cluster	Regions	Size (voxels)	Peak voxel coordinate
1			
Positive association	Left inferior temporal	563	(-52,-60,-6)
	Left middle temporal		
	Left inferior occipital		
	Left middle occipital		
2			
Positive association	Left thalamus	791	(10,-2,14)
	Right thalamus		
	Left caudate		
	Right caudate		
3			
Positive association	Left inferior frontal, triangular part	1794	(-50,6,30)
	Left precentral		
	Left inferior frontal, opercular part		
	Left middle frontal		
4			
Positive association	Right middle frontal	1977	(24,4,50)
	Right inferior frontal, opercular part		
	Right superior frontal		
	Right precentral		
	Right inferior frontal, triangular part		
5			
Positive association	Left inferior parietal	7862	(-42,-46,56)
	Right inferior parietal		
	Left superior parietal		
	Right superior parietal		
	Left precuneus		
	Right precuneus		
	Left supramarginal		
	Right superior occupital		
	Left superior occipital		
	Right middle occipital		
	Left middle occipital		
	Left post central		
	Right post central		
	Right middle cingulum		
	Left middle cingulum		
6			
Positive association	Left sup. motor area	778	(2,6,30)
	Left middle cingulate		
	Right sup. motor area		
	Right middle cingulate		
	Left anterior cingulate		
	Right anteriate cingulate		
7			
Positive association	Left superior frontal	929	(-26,4,50)
	Left middle frontal		

SI Table 9. Results of analysis of GCOR and subjective drug intensity. The table shows clusters within which there was a statistically significant correlation between global correlation (GCOR) and subjective drug intensity (SDI). Coordinate in Montreal Neurological Institute (MNI) space. Only positive associations were observed. Cluster size threshold was 560 voxels.