

ACUTE & LONG-TERM NEURAL, BEHAVIOURAL & PSYCHOLOGICAL EFFECTS OF PSILOCYBIN & 5-HT_{2A}-MEDIATED VASOACTIVITY

A Comprehensive Approach Combining Pulse Oximetry & Behavioural Pharmacology in Mice with Diverse Behavioural & Psychological Assessments & Multimodal Neuroimaging in Humans

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THE CENTRE FOR
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This thesis is submitted for the degree of Doctor of Philosophy (Ph.D)
Supervised by Prof Thomas Knöpfel, Prof David Nutt and Dr Robin Carhart-Harris

Dedicated to my wonderful parents, Karen and Mark.

“Family: where life begins and love never ends.”

*“If I have seen further it is by standing
on the shoulders of Giants.”*

– Isaac Newton (1675)

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As I come to write this (final) section of my thesis, it suddenly dawns on me that this sometimes challenging but extremely rewarding journey that I've been on is actually coming to fruition. It's hard to fathom the mixed range of emotions that I currently feel. Although completing this work is a massive load off my mind and has brought me a great sense of relief, achievement and excitement for what's next to come, I also feel somewhat reluctant to leave my PhD days behind me. Perhaps bittersweet best describes my conflicting emotions – or to borrow a term from the culinary world, *agrodolce* (meaning both sweet and sour). Yet as I move forward and progress in my professional and personal life, this text will live on forever, way beyond my time on this earth. It's comforting to know that this writing is *timeless* even if my research was bound within the constraints of time. And at this irony I have to chuckle, because I could have written this text forever if I wasn't bound by the strict deadline!

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“Great things are done when men and mountains meet.”

— William Blake

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Statement of Publications

Parts of this thesis have been published. Data presented in Chapter 3 (STUDY 1) has been published in *Frontiers in Psychology* (Lyons and Carhart-Harris, 2018b). Portions of data presented in Chapter 4 (STUDY 2) have been published as a video presentation from *Horizons Conference 2019*. Portions of data presented in Chapter 5 (STUDY 3) have been published: sections 5.5.1 – 5.5.3 have been published in *Frontiers in Pharmacology* (Buchborn et al., 2018) and sections 5.5.4 – 5.5.8 have been published in the *Journal of Psychopharmacology* (Buchborn et al., 2020).

Lyons T and Carhart-Harris RL (2018b) More Realistic Forecasting of Future Life Events After Psilocybin for Treatment-Resistant Depression. *Frontiers in Psychology* 9(1721)¹.

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Statement of Originality

I declare that all work contained within this thesis is my own, unless otherwise stated, and conforms to the rules and guidelines set out by Imperial College London. All referenced work has been cited to the best of my knowledge. A statement of personal and team contributions is presented in the methods sections corresponding to each study presented in this thesis.

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Abbreviations

25CN-NBOH	N-(2-Hydroxybenzyl)-2,5-Dimethoxy-4-Cyanophenylethylamine
2C series	2,5-dimethoxy, 4-substituted phenethylamines
2C-B	2,5-dimethoxy-4-bromophenethylamine
5-HT	Serotonin
5-HT2AR	Serotonin 2A Receptor
5-MeO-DMT	5-methoxy-N,N-dimethyltryptamine
5D-ASC	5-Dimensional Altered States of Consciousness Questionnaire
11D-ASC	11-Dimensional Altered States of Consciousness Questionnaire
ACC	Anterior Cingulate Cortex
AF	Advanced Fluorescence
AFNI	Analysis of Functional Neuroimages
ANTS	Advanced Normalisation Tools
APZ	Altered States of Consciousness Questionnaire
ASL	Arterial Spin Labelling
AUA	Auditory Alterations
AUD	Auditory Network
BDI	Beck Depression Inventory
bEAQ	Brief Experiential Avoidance Questionnaire
BMR	Bayesian Model Reduction
BOLD	Blood-Oxygen-Level Dependent
CBF	Cerebral Blood Flow
CBS	Central Biomedical Services
CCD	Charged-Coupled Device
CEQ	Challenging Experiences Questionnaire
ChIVSFP	Chimeric Butterfly Voltage-Sensitive Fluorescent Protein
CNS	Connectedness to Nature Scale
CSF	Cerebrospinal Fluid
DAN	Dorsal Attention Network
DED	Dread of Ego Dissolution
dIPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DMT	N,N-Dimethyltryptamine
DOI	2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-iodo-/methyl-amphetamine
EBI	Emotional Breakthrough Inventory
ECG	Electrocardiogram
EDI	Ego Dissolution Inventory

EEG	Electroencephalography
EPI	Echo-Planar Images
EPSC	Excitatory Postsynaptic Current
FD	Frame-wise Displacement
FDA	Food and Drug Administration
FEAT	fMRI Expert Analysis Tool
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FPN	Frontoparietal Network
FRET	Fluorescence Resonance Energy Transfer
FS	Flourishing Scale
FSL	FMRIB Software Library
FWHM	Full Width at Half-Maximum
GCP	Good Clinical Practice
GCPR	G Protein-Coupled Receptor
GDI	Gains from Dream Interpretation
GECI	Genetically Encoded Calcium Indicators
GEVI	Genetically Encoded Voltage Indicators
GLM	General Linear Model
GMP	Good Manufacturing Practice
GPI	Gains from Psychedelic Integration
GQ-6	Gratitude Questionnaire
GRAPPA	Generalised Autocalibrating Partial Parallel Acquisition
HRF	Hemodynamic Response Function
HTR	Head Twitch Response
IC	Independent Component
ICA	Independent Component Analysis
ICRF	NIH Research/Wellcome Trust Imperial Clinical Research Facility
IDED	Intra-Dimensional/Extra-Dimensional
IFG	Inferior Frontal Gyrus
IGD	Internet Gaming Disorder
IPSC	Inhibitory Postsynaptic Current
IRI	Interpersonal Reactivity Index
K_i	Inhibitory constant
LAS	Leica Application Suite
IFPN	Left Frontoparietal Network
LMM	Linear Mixed-effects Model
LOT-R	Life Orientation Test-Revised
LPFC	Lateral Prefrontal Cortex
IPoCG	Left Postcentral Gyrus

LSA	Lysergic acid amide
LSD	Lysergic acid diethylamide
LZc	Lempel-Ziv Complexity
MDD	Major Depressive Disorder
MEG	Magnetoencephalography
MEQ	Mystical Experience Questionnaire
MFG	Middle Frontal Gyrus
MFL	Medial Frontal Lobe
MINI	Mini-International Neuropsychiatric Interview
MIS	Mastery-Insight Scale
MLQ	Meaning in Life Questionnaire
MNI	Montreal Neurological Institute and Hospital coordinate system
MODTAS	Modified Tellegen Absorption Scale
mPFC	Medial Prefrontal Cortex
MPRAGE	Magnetisation Prepared Rapid Gradient Echo
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTL	Medial Temporal Lobe
NBOMe	N-methoxybenzyl
NDE	Near-Death Experience
NHS	National Health Service
OBN	Oceanic Boundlessness
OCD	Obsessive-Compulsive Disorder
OTX2	Orthodenticle Homeobox 2
PAR	Parietal Cortex Network
PCA	Principal Component Analysis
pCAG	Cytomegalovirus promoter
PCC	Posterior Cingulate Cortex
PCR	Polymerase Chain Reaction
PE	Parameter Estimate
PET	Positron-Emission Tomography
PFC	Prefrontal Cortex
PH	Parahippocampus
PI	Parallel Imaging
PIL	Personal Licence (Home Office)
PIS	Psychological Insight Scale
PIS-BC	PIS Behavioural Change subscale
PIS-PI	PIS Psychological Insight subscale
POFLE	Prediction of Future Life Events
POMS	Profile of Mood States

POP	Posterior Opercular Network
PPL	Project Licence (Home Office)
PSI	Psychotomimetic States Inventory
RCT	Randomised Controlled Trial
REBUS	RElaxed Beliefs Under pSychedelics
REM	Repetitive Eye Movement
RF	Radio Frequency
rFPN	Right Frontoparietal Network
ROI	Region of Interest
RSES-S	Short Rosenberg Self-Esteem Scale
rsFC	Resting-State Functional Connectivity
RSN	Resting State Network
SAL	Salience Network
SCBCS	Santa Clara Brief Compassion Scale
sCMOS	Scientific Complementary Metal Oxide Semiconductor
SCS	Social Connectedness Scale
SFG	Superior Frontal Gyrus
sgACC	Subgenual Anterior Cingulate Cortex
sgPFC	Subgenual Prefrontal Cortex
SMN	Sensorimotor Network
SSS	Short Suggestibility Scale
SRIS	Self-Reflection and Insight Scale
SRIS-ESR	SRIS Engagement in Self-Reflection subscale
SRIS-IN	SRIS Insight subscale
SRIS-NSR	SRIS Need for Self-Reflection subscale
STAI	State-Trait Anxiety Inventory
STAR	Scale to Assess Therapeutic Relationships
TE	Echo Time
tetO	Tetracycline resistance protein
TFCE	Threshold Free Cluster Enhancement
THH	Tetrahydroharmine
TR	Repetition Time
TRD	Treatment-Resistant Depression
TRE	Tetracycline Response Element
VAS	Visual Analogue Scale
VIR	Vigilance Reduction
VisL	Lateral Visual Network
VisM	Medial Visual Network
VisO	Occipital Pole Network
vmPFC	Ventromedial Prefrontal Cortex

VRS	Visionary Restructuralisation
WEMWBS	Warwick-Edinburgh Mental Wellbeing Scale
WHO	World Health Organisation
YR	Yellow-Red

Abstract

Background: Our understanding of psychedelic drug action has rapidly developed over recent years. Yet despite impressive progress, fundamental questions remain. Determining post-psychedelic long-term brain effects and neurobiological mechanisms underlying positive psychological change would hold significant value – not just for the field of psychedelic science, but for the wider fields of neuroscience and mental health. Moreover, serotonin 2A receptors – the key site of action for psychedelic drugs – are widely expressed across the vascular system in addition to their expression in the brain. This complicates our understanding of the neurophysiological correlates of the human psychedelic brain state which largely stems from indirect measures of neuronal activity by extraction of blood-flow related parameters. **Aim:** To address the knowledge gaps and further our understanding, this thesis investigated (i) post-psilocybin changes in pathological beliefs in patients with treatment-resistant depression (TRD), (ii) acute and long-term effects of psilocybin in healthy humans, and (iii) psychedelic vasoactivity in mice. **Methods:** STUDY 1 investigated changes in pessimism in TRD patients versus controls via an objective measure of cognitive biases. STUDY 2 measured the acute and long-term effects of psilocybin in healthy humans via multimodal neuroimaging and a broad repertoire of psychological and behavioural assessments. STUDY 3 characterised the behavioural and vascular effects of 25CN-NBOH in mice via behavioural pharmacology with the head twitch response (HTR) as a behavioural read-out and pulse oximetry, respectively. **Results:** STUDY 1: Psilocybin treatment remediated pessimism in TRD patients one week post-dosing. STUDY 2: Brain signal complexity was related to insightfulness experienced during the peak intensity of a high-dose psilocybin experience. Psychological insight modulated positive long-term outcomes and persistent brain effects correlated with positive long-term psychological changes four weeks after a high-dose psilocybin experience. STUDY 3: 25CN-NBOH induced a (i) dose- and time-dependent, ketanserin-sensitive increase in HTRs, which was subject to short-interval and subchronic tolerance, and (ii) temperature-dependant increase in heart rate, decrease in breath rate, and increase in arterial blood flow. **Conclusions:** This thesis revealed post-psilocybin treatment changes in pathological beliefs in TRD patients. In healthy participants, the present findings identify insight as a mediator of long-term positive outcomes, provide a potential neural correlate of acute insight during a high-dose psilocybin experience, and show persistent neural changes that correlate with well-being outcomes long after that experience. The vasoactivity intrinsic to psychedelic drug action revealed here in mice provides considerations for blood-flow based neuroimaging techniques to measure acute psychedelic brain states. Further, using optogenetics to image cortical activities in awake behaving mice might take our mechanistic understanding of the underlying processes from basic receptor pharmacology to systemic circuit dynamics. In conclusion, this thesis presents novel results that considerably broaden the field's knowledge, provide inspiration for future work and potentially impact wider society and mental health care.

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1. FOREWORD

“Why if [clinical LSD projects] were worthwhile six months ago, why aren’t they worthwhile now? ... Why didn’t you just let them continue? ... We keep going around and around... If I could get a flat answer about that I would be happy. Is there a misunderstanding about my question?”

... Perhaps to some extent we have lost sight of the fact that LSD can be very, very helpful in our society if used properly”

– Senator Robert F. Kennedy (1966)

1.1. WHAT ARE PSYCHEDELICS?

Psychedelics are mind-altering substances that have been used by a variety of cultures for thousands of years. The serotonin 2A receptor (5-HT_{2A}R) is widely accepted as the key site for psychedelic drug action. Through their serotonergic mechanism of action (i.e., agonism at the 5-HT_{2A}R), psychedelics produce profound effects on consciousness and alter perception, cognition and mood in a characteristic way. These powerful mind-altering qualities provide the basis for commonly reported reasons for psychedelic use, which include recreational, spiritual, experimental and therapeutic purposes. The most well-known psychedelics are: (i) psilocybin, a naturally occurring compound found in over 200 species of mushrooms; (ii) lysergic acid diethylamide (LSD), a semi-synthetic compound derived from ergot; (iii) mescaline, a naturally occurring compound found in some cacti species; and (iv) dimethyltryptamine (DMT), a naturally occurring compound found in many plants and animals and the major ingredient in the Amazonian brew ayahuasca (Figure 1-1). Despite differences in their chemical structure, the various chemical classes of psychedelics all act as 5-HT_{2A}R agonists, induce cross-tolerance and produce remarkably similar experiences in humans (Abramson and Rolo, 1965; Abramson et al., 1958; Balestrieri and Fontanari, 1959; Hollister et al., 1969; Isbell et al., 1961; Wolbach et al., 1962b). As well as being the key site of action for psychedelic drugs, the 5-HT_{2A}R is considered an important drug target with potential implications in the treatment of various psychiatric conditions (Carhart-Harris et al., 2016a; Carhart-Harris et al., 2017b; Carhart-Harris and Goodwin, 2017; Carhart-Harris and Nutt, 2017; Ross et al., 2016).



Figure 1-1. Illustrations of psychedelic botanicals. (A) Psilocybin mushrooms (*Agaricus semiglobatus*). (B) Ergot growing on rye. Illustrations from *Medical Botany* (1836) by John Stephenson and James Morss Churchill. (C) Peyote cactus (*Lophophora williamsii*). Illustration from *The Botanical Magazine* (1847; plate 4296) by William Curtis. (D) Ayahuasca vine (*Banisteriopsis caapi*). Illustration from *A Golden Guide to Hallucinogenic Plants* (1977) by Richard Evans Schultes & Elmer W. Smith. Copyright information: (A-B) Universal Creative Commons Public Domain (CC0 1.0). (C-D) Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

Psychedelics have been defined in many ways throughout history, reflecting the different attitudes and intentions with which these compounds have been approached. I will refer to these substances as psychedelics throughout this thesis. However, as psychedelics have been given numerous different

names since their discovery, a brief discussion of the most popular and commonly used terms seems relevant.

Psychedelics were defined as *psychotomimetic* compounds when they were introduced to psychiatry in the 1950s, a term suggesting that they mimic the symptoms of psychosis. The notion that psychedelics can model psychosis has since been discredited, resulting in the rejection of this term by the World Health Organisation (WHO, 1958). Psychedelics later reverted back to their previous name, *hallucinogens*, when it was realised that they poorly resemble the psychotic state. However, the term hallucinogen is quite broad and encompasses many other types of compounds, such as deliriants and dissociatives. Further, it erroneously implies that psychedelics produce true hallucinations. As it is now accepted that these substances do not produce true hallucinations or have effects that resemble psychosis, terms like these (i.e. psychotomimetics, hallucinogens) are misleading and therefore inappropriate.

The term *psychedelic* was coined following a battle of rhymes between the British psychiatrist, Humphry Osmond, and the author, Aldous Huxley. Osmond was among the first people to research psychedelic compounds before they were made illegal in 1966. In 1953, Osmond introduced Huxley to the cacti-derived psychedelic, mescaline, which inspired Huxley's seminal *The Doors of Perception* (1954). Following Huxley's psychedelic experience, the relationship between Osmond and Huxley continued and they wrote a series of letters to each other. Both Huxley and Osmond were dissatisfied with the names given to these compounds (such as psychotomimetics and hallucinogens for reasons outlined above), so they wrote rhymes to each other in an attempt to find a more representative term.

In a letter from Huxley to Osmond in 1956, Huxley wrote the following:

"To make this trivial world sublime, take half a gram of phanerothyme."

Osmond responded to Huxley with his own rhyme:

"To fathom hell or soar angelic, just take a pinch of psychedelic."

Both Huxley and Osmond borrowed ancient Greek words to give their new terms meaning. Huxley borrowed *phanein* (to reveal) and *thymós* (spirit) to produce the term *phanerothyme* (translating to "spirit-revealing"), but it was Osmond's term *psychedelic* derived from *psychē* (mind) and *dēloun* (to make visible), translating to "mind-manifesting", that was adopted in 1957. The term psychedelic therefore signifies that these compounds can bring a wide spectrum of latent psychological content into awareness. The fact that this content can be both strongly positive or negative in emotional valence is indicated in Osmond's rhyme: *"to fathom hell or soar angelic"*.

The central use of psychedelics in many historical and present-day religious practices has led to yet another term for psychedelic compounds – *entheogens*. The term entheogen (translating to "divine-revealer") was coined by a group of ethnobotanists and scholars of mythology in 1979 and is derived

from the ancient Greek words *éntheos* (God within) and *genésthai* (to come into being). By placing these substances within a sacred context, this term emphasises that psychedelics are part of a visionary healing tradition that is as old as humanity itself. However, the use of the term entheogen is largely limited to mystical traditions due to the controversial attachment of ideas of divinity to the psychedelic state.

In summary, the terms attached to these substances reflect the attitudes, intentions and understanding of their value and use. For example, use of the term ‘hallucinogen’ may not reflect a recognition of the therapeutic and beneficial applications for these substances. On the other hand, use of the term ‘entheogen’ reflects the view that these substances have an inherent sacredness. Despite gaining more popularity as time progresses, the term ‘psychedelic’ remains to be adopted by some members of the scientific community. Perhaps this is because some members of scientific and medical community have not yet accepted that psychedelics can have beneficial effects, and the problem is perpetuated by the relative lack of support for research on their potential therapeutic use from government funding agencies.

1.2. WHY STUDY PSYCHEDELICS?

My motivation to study in this field stems from my desire to further our understanding of psychedelic drug action, which ultimately teaches us about the inner workings of our minds. Humans from various different cultures have used psychedelics for millennia for their mind-altering effects and therapeutic value. Psychedelics have a profound ability to alter our consciousness, and by investigating how they do so, we subsequently gain a better understanding of ourselves. Based on ideas surrounding the neural substrates of consciousness and the recognition that psychedelics target the most important of these, it should be no surprise that psychedelics can be powerful tools to study how the brain works. This notion is beautifully illustrated in a quote from the Czech psychiatrist, Dr Stanislav Grof:

“The potential significance of LSD and other psychedelics for psychiatry and psychology was comparable to the value the microscope has for biology or the telescope has for astronomy.”

My motivation also stems from the need to fill large knowledge gaps borne out of the relative dearth of research in this field (Figure 1-2). The lack of psychedelic research was not because of disinterest, but instead is a consequence of political forces and the legislative status of these drugs. Subsequently, the banning of psychedelics brought with it yet another misleading way to define these drugs: *“dangerous drugs of abuse”*. Although it brings me great sadness that we missed out on so many valuable years of research, it also provides an exciting opportunity to address this issue and be part of the growing ‘psychedelic renaissance’. Who knows what advances in the understanding of brain function, brain diseases, and psychedelic-based treatments would currently be available if psychedelic research had continued unhindered. Fortunately, interest in psychedelic compounds and their effects has reappeared

and is most certainly growing. There is a plethora of new studies and therefore a rapidly growing body of psychedelic knowledge appearing in the scientific literature. On top of this, articles that positively present psychedelics are even appearing in the popular media and the stigma associated with these drugs that was caused by their banning is beginning to dissipate. These are fascinating times with much still to learn, and I am extremely grateful to be part of it.

“Adversity causes some men to break, and others to break records.”

- William A Ward

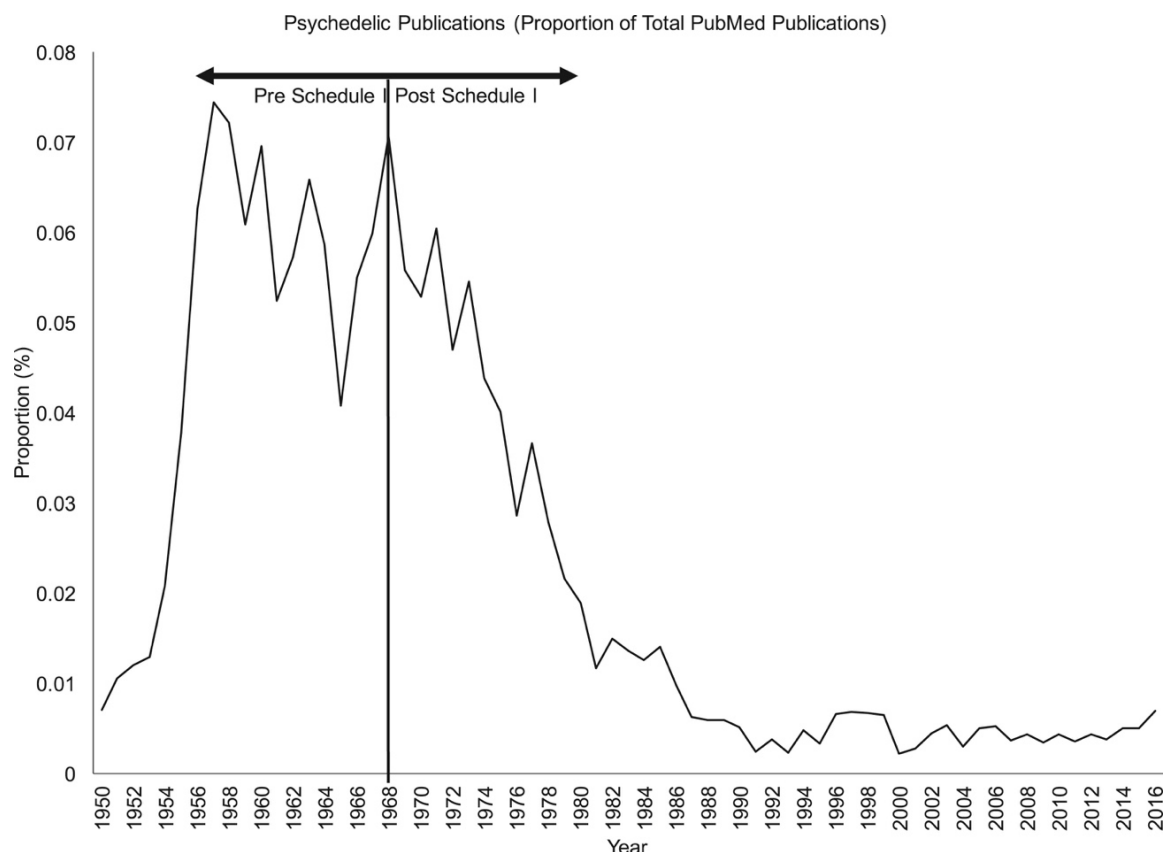


Figure 1-2. The effect of Schedule I on psychedelic drug research. Number of PubMed publications in which a classical psychedelic drug is found in the title expressed as a proportion of all PubMed publications by year, from 1950 to 2016. Reproduced from Rucker et al. (2018). Copyright information: Creative Commons Attribution 4.0 International (CC BY 4.0).

2. INTRODUCTION

“As shortcuts to spiritual and transcendent experiences, psychedelics played an important role in human evolution and galvanized pre-historic ritualistic cultures. In modern times, banning psychedelic drugs has proven to be counterproductive... Despite their classification and the legal hurdles around working with Schedule I substances... psychedelics have undergone something of a renaissance among researchers, and for good reason.”

– Alex M. Vikoulov

2.1. A BRIEF HISTORY

2.1.1. Prehistoric and historical use

Psychedelics may be the oldest class of psychopharmacological agents known to man. Archeological evidence reveals that humans have been using psychedelic drugs since prehistoric times (Winkelman, 2019). The earliest fossil remains of the San Pedro cactus (containing mescaline) date back to between 8600 and 5600 B.C. (Lynch, 1980) and evidence suggests peyote cacti were used by Native North Americans around 5700 years ago (Bruhn et al., 2002). Chemical analyses of material found in smoking pipes dated to approximately 2000 B.C. revealed that *Anadenanthera* seeds (containing DMT) have been ingested for more than 4000 years (Torres, 1995; Torres, 1998). Furthermore, small sculptures called "mushroom stones" (Figure 2-1 A) have been found in Mesoamerica and suggest that *Psilocybe* mushrooms were used approximately 3500 years ago (De Borhegyi, 1963; Schultes, 1998). Likewise, magic mushrooms are depicted in the 7000 year old *Selva Pascuala* mural paintings in Spain and hint that psilocybin was also used in prehistoric Europe (Akers et al., 2011). It is clear that psychedelic compounds have been consumed for thousands of years, and their consumption continues today.

It has been suggested that psychedelics have profoundly impacted philosophy and religiosity throughout history and played a vital role in the origins of many religions around the world (Winkelman, 2019). The ritualistic use of psychedelics within religious contexts in the past is widely studied by a growing number of philosophers, religious scholars, theologians and anthropologists. Biblical stories describe Moses guiding the Israelites to find "Manna", "the bread of god" (Exodus 16:15), which appeared as circular disks in the morning dew of the fields and made god visible to the human eye after ingesting it (Merkur, 2000). Biblical stories such as these provide support for the arguments put forth in the notorious and highly criticised book, *The Sacred Mushroom and the Cross*, wherein Allegro argues that Jesus was a mythological creation of early Christians under the influence of psychoactive mushrooms (Allegro et al., 2009). In ancient India, many Vedic hymns were written in praise of a psychedelic called Soma, which supposedly elevated the consumer to become god-like (Wasson, 1971). Psychedelic compounds were also widely used in Maya, Aztec, Olmec and Zapotec cultures of Mesoamerica (Carod-Artal, 2015). For example, psilocybin mushrooms were used by the Aztecs in a variety of religious ceremonies and were known as *teonanacatl*, meaning "God's flesh" (Ott and Bigwood, 1978; Schultes and Hofmann, 1979). Furthermore, Persephone and Demeter from ancient Greek mythology have been depicted sharing mushrooms and are associated with the Eleusian Mysteries, a then highly popular cult (Figure 2-1 B). For the cult, an annual secret religious ceremony took place for more than 2000 years whereby attenders drank a psychedelic brew called Kykeon and partook in an all-night celebration (Wasson et al., 2008).



Figure 2-1. Historical evidence of psychedelic use. (A) Mushroom stones discovered in Maya ruins of Kaminaljuyu in Guatemala. Kaminaljuyu is a Pre-Columbian site of the Maya civilisation that was primarily occupied from 1500 B.C. to 1200 A.D. The mushroom stones were used between approximately 1000 B.C. to 500 A.D. to prepare mixtures of psychedelic mushrooms for ceremonial use associated with a mushroom stone cult. Photographer: unknown. (B) Persephone and Demeter holding mushrooms and a food wallet, an implement of the Mysteries used to hide secret edible objects. From the Pharsalos bas-relief (470-460 B.C.), now in the Louvre Museum. Excavated in 1863 by Heuzey and Daumet in Pharsalos, Thessaly. Photographer: Marie-Lan Nguyen. (A-B) Copyright information: Universal Creative Commons Public Domain (CC0 1.0).

2.1.2. *Psychedelic use in modern day traditional societies*

Traditional societies around the world continue to use psychedelic compounds. In many societies, the natives have integrated psychedelics into their social, religious and medicinal practices and view psychedelic drug use as beneficial for their society (Winkelman, 2010). Because of this, psychedelic plants are regarded as sacred and treated with a great deal of respect. Further to the use of psychedelics as sacraments, psychedelics are also directly worshipped as vegetal incarnations of the deities in many cultures (Stuart, 2002). Because psychedelics are vision-producing and consciousness-altering drugs that feature in shamanic or religious rites, they have often been referred to as “plants of the gods” (Schultes and Hofmann, 1979).

“The feature that distinguishes the psychedelic agents from other classes of drug is their capacity reliably to induce states of altered perception, thought, and feeling that are not experienced otherwise except in dreams or at times of religious exaltation.”

– Jaffe (1990)

2.1.3. *Psychedelic use in Western science and clinical therapy*

Mainstream science didn’t take an interest in psychedelic compounds until the early 20th century. This interest partly stemmed from the birth of ethnopharmacology, with the work of Richard Evans Schultes providing a significant contribution. The extensive work of Schultes brought the use of psychedelic

plants in traditional societies to the attention of the Western scientific community (Schultes and Hofmann, 1980; Schultes and Hofmann, 1979). Interest in psychedelics from mainstream science also partly stemmed from Albert Hofmann's discovery of LSD whilst working in Sandoz Laboratories in Switzerland (Hofmann, 1980). In 1938, Hofmann synthesised a range of ergot derivatives in search for a novel respiratory and circulatory stimulant, one of which turned out to be LSD (Hofmann, 1980). The project was set aside until 1943 when Hofmann decided to resume it, during which he accidentally intoxicated himself.

"I suddenly became strangely inebriated. The external world became changed as in a dream. Objects appeared to gain in relief; they assumed unusual dimensions; and colors became more glowing. Even self-perception and the sense of time were changed. When the eyes were closed, colored pictures flashed past in a quickly changing kaleidoscope. After a few hours, the not unpleasant inebriation, which had been experienced whilst I was fully conscious, disappeared. what had caused this condition?"

– Hofmann (1943): Laboratory notes

A few days after his accidental experience, Hofmann intentionally ingested LSD to determine if it was indeed responsible for the effects he had experienced a few days earlier. Hofmann reported that he woke up the next day with a remarkable mental clarity (Hofmann, 1980) and it was his positive experience the following day that provided inspiration for the many therapeutic investigations that soon followed. Sandoz Laboratories were keen to find a new use for LSD, and based on his experience, Hofmann believed that it could play an important role in psychiatry. When *in vivo* tests found LSD to be non-toxic, the compound was made freely available to psychiatrists for use in their research. In the 1940s and 50s, LSD revolutionised the field of psychiatry with more than 40,000 people receiving psychedelic therapy (Grinspoon and Bakalar, 1979).

"A sensation of well-being and renewed life flowed through me. The world was as if newly created. All my senses vibrated in a condition of highest sensitivity."

– Hofmann (1980)

When LSD was first introduced to Western medicine, psychiatrists initially believed that it could be a useful tool to mimic psychoses. This led many doctors to self-administer LSD in the belief that it provided a glimpse into the world of their psychotic patients and also led to use of the term *psychotomimetics* (see Chapter 1: FOREWORD) to describe psychedelic compounds (see Passie et al., 2008: for a review). It was the suggestion that LSD could be used as a psychotomimetic that first caught the attention of Osmond, the psychiatrist that later coined the term *psychedelic* through his correspondence with Huxley. Yet throughout the 1950s and 60s, LSD was also used as an adjunct to psychotherapy in order to catalyse the treatment of various mental health issues (Passie et al., 2008). Two new therapy-centred paradigms began to emerge from the initial psychotomimetic model: *psychedelic therapy* and *psycholytic therapy* (Passie, 1997).

In collaboration with Abram Hoffer, Osmond pioneered the development of psychedelic therapy in Canada during the early 1950s. Hoffer and Osmond investigated the use of LSD with psychotherapy to treat alcoholism and obtained abstinence rates of 50 – 90% (Abramson, 1967). Although research standards of that time were substantially less rigorous than they are today, these results far surpassed the outcomes achieved using other treatments for alcoholism, before and since (Moos and Moos, 2006; Nathan, 1986). Whereas psychedelic therapy tended to use high (400 – 1500 µg) doses of LSD, psycholytic therapy typically used low (30 – 200 µg) doses (Grof, 1980; Sandison, 1954; Sandison, 1966; Sandison et al., 1954; Sandison and Whitelaw, 1957). Psycholytic therapy was first developed by Ronald Sandison at Powick Mental Hospital in England. Over the course of the early 1950s, Sandison began using LSD as part of his psychotherapy program by giving it to patients that appeared stuck in traditional psychotherapy (Sandison, 1954; Sandison, 1966; Sandison et al., 1954; Sandison and Whitelaw, 1957). Using LSD-assisted psychotherapy, Sandison achieved a therapeutic response in 36 treatment-resistant patients (Sandison et al., 1954). In 1959, Sandison introduced the term psycholytic ('mind-loosening') to describe the action of LSD (Sandison, 1959). The development of these two therapeutic models demonstrated that LSD poorly resembled the psychotic state and the psychotomimetic paradigm began to lose support. By 1965, several dozen books, numerous international conferences and more than 2000 published papers in over 40,000 subjects showed that LSD could be used to treat addictions and a range of mood disorders, such as anxiety and obsessive-compulsive disorder (Grinspoon and Bakalar, 1979). Hofmann's vision of LSD as a "medicine for the soul" (Smith, 2006) appeared to be coming to fruition.

At around the same time as these medical studies, the creative potential of LSD and other psychedelics was explored by members of the elite society that were able to obtain such compounds. It wasn't long before the general public became aware of the mind-altering potential of psychedelics and recreational use became popular in the 1960s. This rise in recreational use was partly helped by Timothy Leary, a Harvard University psychologist with an anti-establishment attitude (Greenfield, 2006). Leary preached about the benefits of widespread psychedelic drug use and helped leak these drugs to the general public after losing his position at Harvard (Greenfield, 2006). Leary is remembered for straying away from what he called the 'mainstream game' and is regarded as one of the major influencers of the 60s counter-culture movement.

2.1.4. Prohibition

The movement of psychedelic drugs away from the clinical environment and into the world at large had a huge impact on Western culture. Psychedelic use massively influenced art, literature and music and has contributed to the acceptance of different philosophies, religions and lifestyles as well as the development of a high-tech information society (Grunenberg et al., 2005). But the mass use of psychedelics within mainstream culture also brought with it many problems. By 1966, millions of people had engaged in non-clinical recreational psychedelic drug use. When used without undue care paid to

the 'set and setting'², some users had negative experiences and reports of the dangers of psychedelics began to emerge (Hartogsohn, 2017). Casualties, which were few and far between when psychedelics were used in the clinical environment, began to appear and there was a surge of negative publicity surrounding psychedelic drugs in the press. Psychedelics were banned in 1966 despite the protestations of those therapists who were using these drugs safely and effectively with their patients. The consequences of the ban together with the increasing number of media scare stories, despite many stories being erroneous, forced many medical professionals to distance themselves from psychedelic research (Bonson, 2018). Despite the ground-breaking findings on the therapeutic potential of these drugs, psychedelic research was brought to a halt because of socio-political forces. Yet the new legislation didn't curb recreational psychedelic drug use, which continued to grow among the public (Lee and Shlain, 1985).

The scientific community are not solely responsible for the spread of psychedelic drugs to the general public. Ironically, government military agencies in America and Europe also played a major role in the development of psychedelic drug culture during the 1950s and 60s. Through the now well-known CIA project, MK-ULTRA, the US government hoped to develop LSD as a truth drug for the military (Senate, 1977; Valentine, 2017). The MK-ULTRA experiments involved secretly spiking unsuspecting members of the public with LSD before observing their behaviour as they interacted with stooges (Senate, 1977; Valentine, 2017), and far exceeded any unethical practices attributed to the scientific and medical community during this period (Stevens, 1998).

"Drugs needn't kill people. Prohibition does."

– Ben Sessa (2018)

2.1.5. Psychedelic renaissance

After a long hiatus, today's society and scientific community has witnessed a resurgence of interest in psychedelics in the last 25 years, especially in this past decade (see Sessa, 2012; and Sessa, 2018: for an overview). Prohibition ultimately resulted in a halt to a wealth of knowledge that could have been gained during this lengthy lull in research. Yet, despite the legal restrictions that make psychedelic research extremely difficult, many prominent researchers believe that it's still worthwhile and that these drugs should not be overlooked as potential medicines (Horgan, 2005). Most of the safety concerns that contributed to prohibition were later shown to be unfounded and fallacious (Byock, 2018; Davies, 2017). Moreover, despite the methodological flaws, many early studies showed promising results and great potential (see Carhart-Harris and Goodwin, 2017; Rucker et al., 2018).

² *Set and setting* is a term used to refer to various internal and external contextual factors that shape one's response to a psychedelic drug. The 'set' typically refers to the participant's mindset, mood, traits and expectations. The 'setting' typically refers to external environmental factors, such as interpersonal relationships with others present and comfortable surroundings.

Remarkable advances in neuroscience technologies now enable a much better understanding of psychedelic drug action and therapeutic potential than was previously possible. Over this past few years, a more accepting cultural and socio-political climate has enabled an explosion of psychedelic research and medical acceptance is closer than ever. For instance, psychedelics possess great clinical potential in the treatment of addiction and depression, which are both serious global problems (Carhart-Harris and Goodwin, 2017). Depression affects 350 million people, accounts for over half of all suicides and is the leading cause of disability worldwide (James et al., 2018). Similarly, alcoholism is now the leading cause of disability for men in Europe with the prevalence of addiction rapidly on the rise (James et al., 2018). Psychedelics are also therapeutically useful for reducing anxiety in terminally ill cancer patients and could offer a much more humane alternative to sedatives and opiates (Gasser et al., 2014; Griffiths et al., 2016; Ross et al., 2016). As psychedelics help us to understand the neurochemistry of the brain and show promise in a wide range of disorders, further investigation is warranted and should continue despite being under difficult conditions. Fortunately, psychedelic research is currently undergoing a period of rapid growth and development and we are now beginning to unveil the neural mechanisms by which psychedelics produce their profound effects (e.g. Carhart-Harris et al., 2012a; Palhano-Fontes et al., 2015; Vollenweider et al., 1997a; Carhart-Harris et al., 2016c; Komater et al., 2015; Muthukumaraswamy et al., 2013; Puig et al., 2010; Wood et al., 2012; Schenberg et al., 2015; Riba et al., 2004; Riba et al., 2002; Tagliazucchi et al., 2014; Lebedev et al., 2016; Lebedev et al., 2015; Roseman et al., 2014) and open new avenues of treatment for mental and physical conditions (e.g. Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a; Osorio Fde et al., 2015; Grob et al., 2011; Griffiths et al., 2016; Gasser et al., 2014; Ross et al., 2016; see Carhart-Harris and Goodwin, 2017; Rucker et al., 2018; Schenberg, 2018). These are exciting times with much still to learn.

Psychedelic drugs are also capturing people's imagination and permeating popular culture on a scale not seen since the 1960s (Pollan, 2019; Hanks, 2009; Bayne and Carter, 2018; Keshavan and Sudarshan, 2017). Recent years have witnessed an upsurge in psychedelic resources, advocacy groups and multidisciplinary scientific conferences solely dedicated to the field of psychedelics. This has been helped by social media and the infrastructure it provides for advocacy groups and information, a re-established psychedelic community, the growth in society's awareness of the limitations of the war on drugs and a wealth of evidence from robust psychedelic experiments (Morris, 2008; Hari, 2015; Dunne, 2018). Society has changed in its general perception of pharmacology and understanding of different forms of drug use and misuse, whereby we are now observing a shift in the attitudes of mainstream society about illegal substances and their potential as therapies (Morris, 2008). Providing the public with objective information about drugs and reforming the current state of affairs on drug policy is already in progress. For example, the legalisation of cannabis in the US has resulted in the treatment of a variety of disorders (Carliner et al., 2017) and in 2019, Denver and Oakland became the first and second cities in the US to decriminalise psilocybin, respectively (Marlan, 2019). Psilocybin also inches closer to becoming an approved treatment for major depressive disorder (MDD) after being designated as a "breakthrough therapy" by the U.S. Food and Drug Administration (FDA; Muensterman et al.,

2020). Hopefully the UK will follow suit, as the current drug laws in the UK hinder scientific research into these compounds.

2.2. PHARMACOLOGY OF PSYCHEDELICS

Serotonin (5-hydroxytryptamine; 5-HT) was first discovered in the 1930s (Erspamer and Vialli, 1937), but its presence in the mammalian brain and role in neurotransmission wasn't realised until 1953 (Twarog, 1954; Twarog and Page, 1953). Chemists were quick to recognise the tryptamine template in the structure of LSD and it was initially hypothesised that the potent psychedelic effects might derive from the blockade of 5-HT receptors in the brain (Gaddum, 1953; Gaddum and Hameed, 1954; Woolley and Shaw, 1954). Yet, further studies showed that this theory was incorrect (Cerletti and Rothlin, 1955; Rothlin, 1957; Votava et al., 1958) and it was instead suggested that LSD might behave as a direct agonist at 5-HT receptors in the brain (Anden et al., 1968). Further studies found that various psychedelic compounds either increased serotonin levels in the brain and/or decreased the turnover of serotonin (Anden et al., 1971; Leonard, 1973; Randic and Padjen, 1971), thus providing support for the agonism hypothesis.

The serotonin 2A receptor (5-HT_{2A}R) is a member of the 5-HT₂ seven-transmembrane receptor family and is expressed in various cells and tissues across the mammalian body, with highest expression levels in the brain (see GCPR database; Regard et al., 2008). 5-HT_{2A}Rs are specifically enriched in the cerebral cortex, particularly on the apical dendrites of pyramidal cells in layer 5 (Weber and Andrade, 2010). By enhancing cortical glutamate release, 5-HT_{2A}Rs may modulate cognitive processes, such as working memory and attention (Williams et al., 2002; Mirjana et al., 2004). The 5-HT_{2A}R is considered as an important drug target, with potential implications of both agonists and antagonists in the treatment of various psychiatric conditions, including depression and anxiety (Carhart-Harris et al., 2016a; Carhart-Harris et al., 2017b; Carhart-Harris and Goodwin, 2017; Carhart-Harris and Nutt, 2017; Ross et al., 2016).

The 5-HT_{2A}R is widely accepted as the key site for psychedelic drug action due to the vast body of compelling evidence to support this hypothesis. Specifically, 5-HT_{2A}R of layer 5 pyramidal cells are thought to be a key mediator of psychedelic activity (Vollenweider and Kometer, 2010; Muthukumaraswamy et al., 2013; Nichols, 2016). The strong positive correlation between the affinity of psychedelic drugs for the 5-HT_{2A}R and psychedelic potency (Glennon et al., 1984) suggests that the stimulation of this receptor subtype is important in their mechanism of action. Psychedelics are also devoid of activity in 5-HT_{2A}R knock-out mice and selective 5-HT_{2A}R antagonists ablate the effects of psychedelics in animals and humans (Vollenweider et al., 1998; Glennon et al., 1984; Colpaert et al., 1985; Gresch et al., 2005; Fiorella et al., 1995; Smith et al., 1995). The rapid development of tolerance to psychedelics also correlates with the downregulation of the 5-HT_{2A}R. Furthermore, despite differences in their chemical structure, the various chemical classes of psychedelics all act as 5-HT_{2A}R

agonists, induce cross-tolerance and produce remarkably similar experiences in humans (Abramson and Rolo, 1965; Abramson et al., 1958; Balestrieri and Fontanari, 1959; Hollister et al., 1969; Isbell et al., 1961; Wolbach et al., 1962b).

There are two major chemical classes of psychedelic drugs: (i) the indoleamines and (ii) the phenethylamines. The indoleamines can be further classified into two subsets: (i) the tryptamines, which possess considerable conformational flexibility, and (ii) the ergolines, which are relatively rigid tryptamine analogues (

Figure 2-2). In contrast with the high selectivity of phenethylamines for the 5-HT₂ family of receptors (Table 2-2), indoleamines are relatively non-selective at 5-HT sites (Table 2-1; Pierce and Peroutka, 1989; Titeler et al., 1988; McKenna et al., 1990).

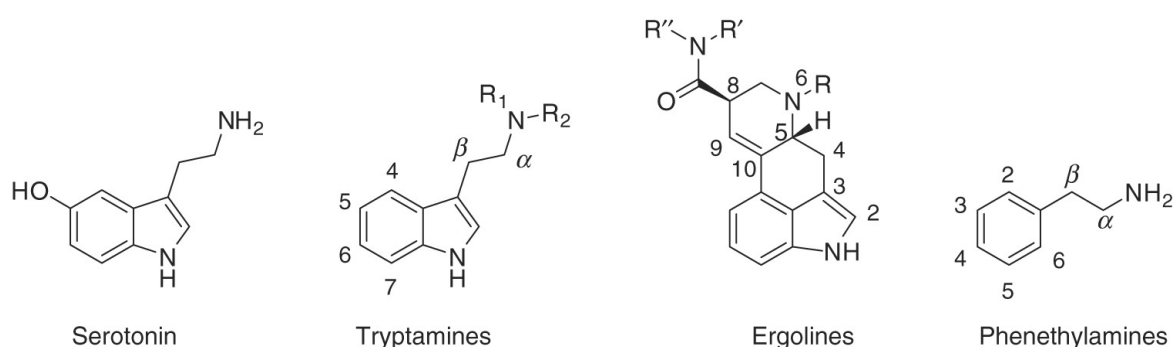


Figure 2-2. Chemical structures. The structure of the neurotransmitter serotonin and the three basic chemotypes of serotonin 5-HT_{2A} agonists. Reproduced with permission from Nichols (2012). Copyright information: © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.2.1. Indoleamines

Tryptamines are a family of compounds structurally derived from tryptamine, which consist of an indole ring attached to an amino group through an ethyl side chain. Psychedelics classified as tryptamines are the chemotypes that bear the most resemblance to the neurotransmitter serotonin and therefore have significant activity at 5-HT receptors (Nichols, 2012). However, many of the tryptamines also bind to non-5-HT receptors and have a relatively lower affinity for the 5-HT_{2A}R than the ergolines and phenethylamines (see

Table 2-1 and Table 2-2). Examples of psychedelics that belong in the tryptamine family include DMT, 5-MeO-DMT, bufotenin, psilocybin and psilocin.

DMT (N,N-dimethyltryptamine) is a derivative of tryptamine with two methyl groups attached to the amine nitrogen atom. DMT, the major ingredient in the Amazonian Brew ayahuasca, is a naturally-

occurring compound that is found in many plants and animals. 5-MeO-DMT (5-methoxy-N,N-dimethyltryptamine) and bufotenin (5-hydroxy-N,N-dimethyltryptamine) are naturally occurring psychedelics found in a variety of plants and *Bufo Alvarius* toads. 5-MeO-DMT and bufotenin both begin with the structure of DMT, but 5-MeO-DMT has the addition of a methoxy group at the 5-position whereas bufotenin has the addition of a hydroxy group at the 5-position.

Psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine) has the structure of DMT but with a phosphoryloxy group at the 4-position. Psilocybin is primarily a prodrug that is dephosphorylated by alkaline phosphatase to active metabolite psilocin. Psilocin (4-hydroxy-N,N-dimethyltryptamine) is structurally very similar to bufotenin, but the hydroxy group is at the 4-position with psilocin and not the 5-position as it is with bufotenin. In contrast to the tryptamines discussed in the previous paragraph, this small change ensures that psilocin, like psilocybin, is orally active. Psilocybin and psilocin are naturally-occurring psychedelics found in a variety of mushrooms.

Ergolines are derived from alkaloids in the ergot fungus, of the genus *Claviceps*. Relative to the tryptamines, ergolines have a more complex structure based around an indole system and tetracyclic ring scaffold (Nichols, 2012). As such, ergolines are elaborated, conformationally restrained tryptamines. Psychedelics classified as ergolines include LSA (lysergic acid amide) and LSD (lysergic acid diethylamide). LSA, a naturally-occurring psychedelic found in *Rivea corymbosa* seeds, is an ergoline alkaloid that lacks hydrogens at positions 9 and 10 and also has a methyl group attached to the piperidine nitrogen. LSA is derived from a hydride of an ergoline. LSD is a semi-synthetic ergoline alkaloid arising from formal condensation of lysergic acid with diethylamine and is derived from a lysergamide. It is important to note that the complex ring system of the ergolines contains the phenethylamine backbone in addition to the tryptamine backbone.

Table 2-1. Binding affinities (K_i , nM; <3000 nM) of indoleamines. Data was collated from the PDSP K_i database[†] (Roth et al., 2000).

Drug	Receptor	K_i (nM)	Reference
Psilocin	5-HT1A	49	Blair et al. (2000)
	5-HT1B	219.6	PDSP certified data
	5-HT1D	36.4	PDSP certified data
	5-HT1E	52.2	PDSP certified data
	5-HT2A	25	Blair et al. (2000)
	5-HT2B	4.6	PDSP certified data
	5-HT2C	10	Blair et al. (2000)
	5-HT5	83.7	PDSP certified data
	5-HT6	57	PDSP certified data
	5-HT7	3.5	PDSP certified data
	Histamine H1	304.6	PDSP certified data
Psilocybin	5-HT1E	194.8	PDSP certified data
	5-HT2A	13	McKenna et al. (1990)

	5-HT2B	98.7	PDSP certified data
	5-HT5	6,181	PDSP certified data
	5-HT6	413.5	PDSP certified data
	5-HT7	597.9	PDSP certified data
DMT	5-HT1A	119.51	Pierce and Peroutka (1989)
	5-HT1D	270	Peroutka et al. (1989)
	5-HT2A	230.27	Pierce and Peroutka (1989)
	5-HT2B	550	McKenna et al. (1990)
	5-HT6	68	Kohen et al. (1996)
5-MeO-DMT	5-HT1B	351	Offord et al. (1988)
	5-HT2A	207	Sadzot et al. (1989)
	5-HT2B	1,300	McKenna et al. (1990)
	5-HT2C	100	Hoyer et al. (1989)
Bufotenin	5-HT1A	5	Peroutka (1986)
	5-HT1B	52	Peroutka (1986)
	5-HT1D	3.71	Peroutka (1985)
	5-HT1F	20.4	Waeber et al. (1989)
	5-HT2A	15	Egan et al. (2000)
	5-HT2C	70.8	Engel et al. (1986)
	5-HT3	34	Milburn and Peroutka (1989)
	5-HT5	1,000	Plassat et al. (1992)
	5-HT6	4.46	Boess et al. (1997)
	5-HT7	19.95	Tsou et al. (1994)
LSD	5-HT1A	1.1	Nichols et al. (2002)
	5-HT1B	3.9	Nichols et al. (2002)
	5-HT1D	14	Peroutka et al. (1989)
	5-HT1E	93	Nichols et al. (2002)
	5-HT2A	0.75	Knight et al. (2004)
	5-HT2B	3.7153	Wacker et al. (2013)
	5-HT2C	1.096	Knight et al. (2004)
	5-HT4L	1,000	Gerald et al. (1995)
	5-HT5A	9	Nichols et al. (2002)
	5-HT5B	3.23	Boess and Martin (1994)
	5-HT6	2.29	Hirst et al. (2003)
	5-HT7	6.6	Nichols et al. (2002)
	Adrenergic a	220	U'Prichard et al. (1977)
	Adrenergic b1	140	Nichols et al. (2002)
	Adrenergic b2	740	Nichols et al. (2002)
	Dopamine D1	180	Nichols et al. (2002)
	Dopamine D2	120	Nichols et al. (2002)
	Dopamine D3	27	Nichols et al. (2002)
	Dopamine D4	56	Nichols et al. (2002)
	Dopamine D5	340	Nichols et al. (2002)
	Histamine H1	1540	Nichols et al. (2002)

[†]Note: the apparent affinity estimated from displacement experiments depends on the intrinsic efficacy of the radioligand used to label the binding site. Comparisons of affinities for various psychedelics across multiple sites of action must be regarded as tentative, particularly

when distinct receptor populations have been labelled with ligands of varying efficacy.

2.2.2. Phenethylamines

The chemical backbone for the phenethylamines is based upon phenethylamine, a prevalent structure found in a range of endogenous compounds including the neurotransmitters serotonin, dopamine and norepinephrine (Nichols, 2012). The phenethylamine structure contains a phenyl ring attached to an amino group through an ethyl side chain. Phenethylamines may be chemically modified along this phenyl ring, side chain, or amino group and they have a relatively higher affinity for the 5-HT_{2A}R than the tryptamines. Examples of phenethylamine psychedelics include mescaline, the 2C series (2,5-dimethoxy, 4-substituted phenethylamines), DOI (2,5-Dimethoxy-4-iodoamphetamine) and 25CN-NBOH (N-(2-Hydroxybenzyl)-2,5-Dimethoxy-4-Cyanophenylethylamine).

Mescaline (3,4,5-trimethoxyphenethylamine) is a naturally-occurring psychedelic alkaloid found in some species of cacti, including the peyote cactus (*Lophophora williamsii*) and the San Pedro cactus (*Echinopsis pachanoi*). No other naturally-occurring phenethylamine psychedelics have yet been discovered. Mescaline is phenethylamine substituted at positions 3, 4 and 5 by methoxy groups and is the lead compound that has served as the starting point for many structure-activity relationship (SAR) studies. These SAR investigations have resulted in the design of relatively selective and potent compounds (compared to mescaline, the reference standard) to probe psychedelic mechanisms of action.

Shulgin first synthesised the 2C compounds in the 1970s and he coined the term 2C to represent the two carbon homologues of mescaline. The 2C series of compounds lack the alpha-methyl group and contain methoxy groups attached to the 2- and 5-position carbons of the phenyl ring. There are many compounds in the 2C series, but 2C-B (2,5-dimethoxy-4-bromophenethylamine) is one of the oldest and best-known members of the 2C family. In addition to the methoxy groups at positions 2 and 5, 2C-B has a bromo substituent at the 4-position. On the other hand, DOI (2,5-dimethoxy-4-iodoamphetamine) is structurally very similar to 2C-B in that it has two methoxy substituents at the 2- and 5- position, but instead has an iodo substituent at the 4-position.

25CN-NBOH (N-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenethylamine) is a N-benzylphenethylamine with the addition of a hydroxy group at the 2-position of the N-benzyl structure and a cyano group at the 4-position of the phenethylamine part of the molecule. 25CN-NBOH is a very novel compound and these structural variations in both the phenethylamine and N-benzyl parts of the molecule have rendered it the most selective 5-HT_{2A}R agonist yet discovered.

Table 2-2. Binding affinities (K_i , nM) of phenethylamines at 5-HT₂ sites. Data was collated from the PDSP K_i database[†] (Roth et al., 2000).

Drug	Receptor	K_i (nM)	Reference
Mescaline	5-HT _{2A}	150	McKenna et al. (1990)
	5-HT _{2A}	0.001	McLean et al. (2006)
2C-B	5-HT _{2B}	0.034	Glennon et al. (1992)
	5-HT _{2C}	0.036	Glennon et al. (1992)
DOI	5-HT _{2A}	0.65	Wainscott et al. (1996)
	5-HT _{2B}	20	Nelson et al. (1999)
	5-HT _{2C}	2.36	Wainscott et al. (1996)
25CN-NBOH	5-HT _{2A}	2.2	Halberstadt et al. (2016)
	5-HT _{2B}	58	Halberstadt et al. (2016)
	5-HT _{2C}	49.8	Halberstadt et al. (2016)

[†]Note: the apparent affinity estimated from displacement experiments depends on the intrinsic efficacy of the radioligand used to label the binding site. Comparisons of affinities for various psychedelics across multiple sites of action must be regarded as tentative, particularly when distinct receptor populations have been labelled with ligands of varying efficacy.

“...the phenomena induced by LSD (and probably by any similar drug) cannot be predicted or understood in purely pharmacological terms; the personality of the drug taker plays an enormous and critical role in determining how much effect there will be and of what particular type.”

- Barr et al (1972)

2.3. SAFETY OF PSYCHEDELICS

As mentioned previously (see 2.1.4 Prohibition), the media have historically portrayed psychedelics as extremely dangerous drugs of abuse and psychedelic compounds have remained illegal since the ban in 1966. Psychedelics are classified under the current legislation as Schedule 1 Class A drugs, which are the most restrictively controlled drugs considered to be the most harmful with a high potential for abuse and no therapeutic value. Yet, analyses have shown that there is no relation between the harms of a range of psychoactive drugs and their current legal status in the United Kingdom (Nutt et al., 2010). The decisions made about which drugs should be controlled under the legislation seem to be unclear and inconsistent, and may have been made for political rather than health purposes (Nutt et al., 2010). In fact, the legislation has greatly hindered research into the therapeutic potential of such compounds, including psychedelic drugs (Nutt et al., 2013). Evidence suggests that these compounds are safe and have therapeutic potential when used in a controlled environment (see Carhart-Harris and Goodwin, 2017: for a review), which is in contrast to what their legislative status suggests.

Psilocybin and related psychedelic compounds possess an extremely low level of physiological toxicity and no known risk of mortality. Nevertheless, although classic psychedelics have not directly caused deaths, users may experience impaired judgement while under the influence of these compounds (Nutt et al., 2010). This is a particular concern when psychedelics are used recreationally and not in a controlled environment. Notably, acute adverse reactions can occur in any context, but are generally well-managed in a controlled setting with interpersonal support and do not persist when the drug effects have worn off (Johnson et al., 2008). Contrary to previous sensational media reports, there is no evidence that there is any risk of prolonged psychotic reactions to psilocybin in individuals without a personal or family history of psychotic illness. Moreover, no incidents of prolonged emotional or perceptual disturbances have been reported in modern clinical studies involving the administration of psilocybin or related psychedelics to humans. Several thousand participants received psychedelics in clinical research during the 1950s and 60s without serious concerns and significant adverse reactions caused by psychedelic use are rare. In the few cases in which long-term adverse effects have occurred from repeated use, they were regarded as subtle or nonsignificant (Halpern and Pope, 1999; Strassman, 1984; Johnson et al., 2008; Nichols, 2004; Nichols, 2016). There is no animal or human data to suggest that psilocybin and related psychedelics, even in very high doses, are neurotoxic, and there is no evidence of these drugs inducing compulsive drug seeking behaviours in animals or dependency in humans (Hintzen and Passie, 2010). To this end, attempts to train animals to self-administer psychedelics, an animal model that can predict the potential for abuse, have been unsuccessful (O'Brien, 2001). In recreational users, psychedelic drug-use is associated with lower rates of suicidality and psychological distress (Hendricks et al., 2015) and is not associated with increased mental health problems (Krebs and Johansen, 2013) or decreased neuropsychological performance (Halpern et al., 2005).

Many psychedelics have been safely administered in contemporary research settings in both healthy and patient populations, including participants with no previous psychedelic experience (e.g. Griffiths et al., 2008; Griffiths et al., 2011; Griffiths et al., 2006; Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a). In controlled studies, the risk of adverse reactions to psychedelics is low (Halpern and Pope, 1999; Halpern and Pope, 2003) and harm assessments show them to be among the least harmful drugs, with psilocybin containing mushrooms consistently ranked as the least harmful (Figure 2-2; Nutt et al., 2010). Psilocybin is considered significantly less harmful than some legal and licensed prescription medications (Morgan et al., 2010; Nutt et al., 2010). Moreover, our team has safely administered psychedelic drugs in well over 100 acute administration sessions for multiple neuroimaging studies without any serious adverse events (e.g. Carhart-Harris et al., 2016a; Carhart-Harris et al., 2012a; Carhart-Harris et al., 2013; Carhart-Harris et al., 2012b; Carhart-Harris et al., 2011; Muthukumaraswamy et al., 2013; Petri et al., 2014; Tagliazucchi et al., 2014; Turton et al., 2014; Terhune et al., 2016; Carhart-Harris et al., 2015a; Kaelen et al., 2016; Kaelen et al., 2015; Tagliazucchi et al., 2016; Lebedev et al., 2016; Lebedev et al., 2015; Roseman et al., 2016).

Despite the physiologic safety of psychedelics and their positive effects on mental health (e.g. Hendricks et al., 2015; Krebs and Johansen, 2013; Carhart-Harris and Goodwin, 2017), it is important to consider their potential to induce negative effects. Through their serotonergic mechanism of action, very high doses of psychedelic compounds can induce acute problems in vascular functioning, such as high blood pressure (Nagatomo et al., 2004). Moreover, psilocybin has been found to induce transient and delayed headache, whereby the occurrence, duration and severity are dose-dependent (Johnson et al., 2012). The most common adverse reaction to psychedelics is acute anxiety and dysphoria (Johnson et al., 2008; Elsey, 2017; Strassman, 1984); however, in healthy participants, evidence suggests that these experiences are short-lived (Hintzen and Passie, 2010) and there is some evidence that over-coming challenging psychedelic experiences can have therapeutic benefits (Carbonaro et al., 2016; Barrett et al., 2016). Persistent sensory changes (known as ‘hallucinogen persisting perceptual disorder’, HPPD) have previously been observed following psychedelic use. Although the prevalence of HPPD is unknown, the incidence is believed to be rare (i.e. ~3%) and primarily associated with LSD use (Halpern and Pope, 2003; Halpern et al., 2018; Hermle et al., 2015). HPPD phenomena usually occur in the visual domain and have been found to be effectively treated with benzodiazepines, although HPPD usually subsides with abstinence from psychedelic drugs (Halpern and Pope, 2003).

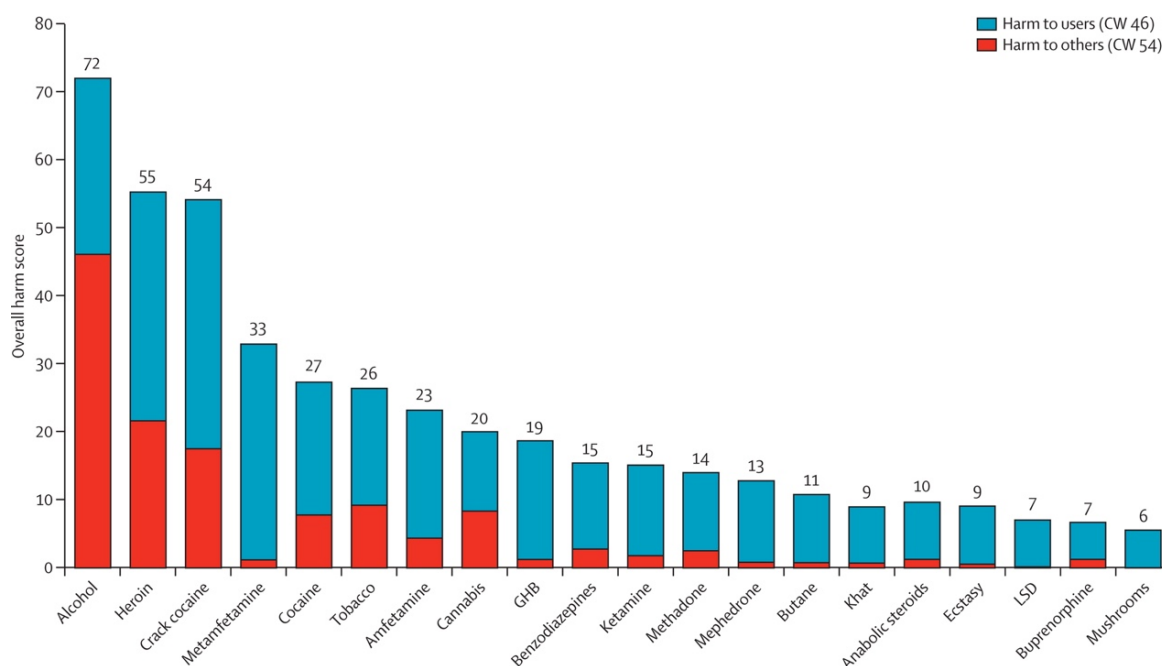


Figure 2-3. Drugs ordered by their overall harm scores. Separate contributions to the overall scores of harms to users and harm to others. Reproduced with permission from Nutt et al. (2010). Copyright information: © 2010 Elsevier Ltd. All rights reserved.

2.3.1. Novel NBOMe compounds: deadly exception

The previous section showed that classic psychedelics are among the safest drugs that act on the central nervous system. Unfortunately, this is not the case for the NBOMe (*N*-methoxybenzyl)

compounds, which are novel and highly potent synthetic phenethylamines. The NBOMe compounds – which have recently become popular as recreational drugs – have proven to be toxic and users have experienced a variety of adverse events, such as agitation, tachycardia, hypertension, seizures and fatal overdoses (Wadowski et al., 2019; Suzuki et al., 2015; Gee et al., 2016). Further, NBOMe compounds have recently been shown to induce neurotoxicity and rhabdomyolysis (Kawahara et al., 2017; Xu et al., 2019). Much interest has been generated to determine why the NBOMe compounds, unlike other psychedelics, cause such devastating consequences. There has never been a recorded overdose with classical psychedelics, such as LSD, psilocybin or mescaline. On the other hand, the extremely potent and synthetic NBOMe compounds thicken the blood and thin the blood vessels (Suzuki et al., 2015), which is the main catalyst behind a range of other issues, such as ventricular fibrillation leading to heart attack, renal failure and stroke. Thus, it appears that NBOMe toxicity may be primarily caused by the drugs' effect on the cardiovascular system (Suzuki et al., 2015).

2.4. PSYCHOLOGICAL EFFECTS

2.4.1. *Predictability of acute effects*

In contrast to other classes of drugs, where the action is usually predictable, the effects of psychedelics are heavily dependent on the expectations of the user (“set”) and the environment (“setting”) in which the use takes place (Carhart-Harris et al., 2018; Haijen et al., 2018). For example, expectations and environments that encourage spirituality increase the probability of psychedelics producing such an effect. Conversely, unstructured or recreational use can have unpredictable effects. To complicate matters further, an individual may respond differently to repeated administrations of the same drug at the same dose (see Nichols, 2016).

The disruption of sensory and cognitive functions is generally dose-dependent (e.g. Hasler et al., 2004). However, when considering the effects produced by different doses of psychedelic compounds, it is not as simple as low doses producing the same effects as large doses at a reduced intensity. In fact, lower doses of psychedelics can elicit quantitatively and qualitatively different responses (Nichols, 2004). Psychedelics can produce a profoundly altered state of consciousness in which the user may have a “peak” or “mystical” experience” (Barrett and Griffiths, 2017). Although peak experiences occur more often with high doses, they can also occur unpredictably and independently of the dose. This unpredictability of drug effects can make psychedelic research challenging and is also a factor in the occurrence of adverse reactions during recreational use (Else, 2017; Johnson et al., 2008).

“I consider LSD to be a powerful unspecific amplifier or catalyst of biochemical and physiological processes in the brain”

- Grof (1975).

2.4.2. Altered states of consciousness and the phenomenon of “peak” experiences

Through their serotonergic mechanism of action, psychedelics produce profound effects on consciousness during the acute phase of administration. The Altered States of Consciousness Questionnaire (APZ; Dittrich, 1998) and APZ variants, such as the five- and eleven- dimensional altered states of consciousness questionnaire (5D-ASC, Dittrich et al., 2010; 11D-ASC, Studerus et al., 2010), are rating scales that are widely used to assess the acute subjective effects of psychedelics. These instruments are designed to measure altered states of consciousness independent of their aetiology (Dittrich, 1998). Both indoleamines and phenethylamines produce profound increases on three core dimensions of the 5D-ASC: (i) *Oceanic Boundlessness*, which measures a pleasant state of depersonalisation and derealisation; (ii) *Anxious Ego Dissolution*, which measures dysphoric effects such as ego disintegration, delusions, loss of self-control and anxiety; and (iii) *Visionary Restructuralisation*, which measures elementary and complex visual imagery (Dittrich et al., 2010). Using the further parcellated version of the 5D-ASC (i.e. 11D-ASC; Studerus et al., 2010), psychedelics have been shown to increase personal awareness, insightfulness, feelings of pure unity, ineffability and a transcendence of space and time (e.g. MacLean et al., 2011; Kometer et al., 2015; Griffiths et al., 2006; Griffiths et al., 2011; Griffiths et al., 2008; Carhart-Harris et al., 2016b; Carhart-Harris et al., 2012a; Barrett et al., 2015).

Dimensions within the 5D- and 11D-ASC and the Mystical Experience Questionnaire (MEQ) are often used to measure psychedelic “peak experiences”. Peak experiences elicited by psychedelic drugs are often referred to as “mystical experiences”³ (Pahnke, 1963; Griffiths et al., 2006). However, the term “mystical” may be viewed as problematic due to metaphysical or supernatural connotations that may obstruct its acceptance by the scientific mainstream, thereby potentially hindering scientific progress in this area. To overcome this issue, one might argue that the (secular) term “peak experience” – which is synonymous with the mystical-type experience – is more appropriate for modern science.

Psychedelic peak experiences are often accompanied by emotions of wonder, bliss and awe and are generally characterised by the following dimensions of “mystical experience” described by Stace (1960): (i) a sense of unity with all that exists; (ii) a transcendence of space and time; (iii) a deeply felt positive mood; (iv) a sense of sacredness; (v) a sense of the experience of truth and reality at a fundamental level (noetic quality); (vi) paradoxicality; (vii) difficulty explaining the experience in words (ineffability); (viii) transiency; and (ix) persisting positive changes in different domains, including attitudes and behaviour towards the self, others, life and the experience itself. These peak experiences are often associated with an absence or resolution of inner conflict and a sense of serenity, confidence and meaningful purpose in one’s life (Maslow, 1959; Maslow, 1964; Stace, 1960).

³ The term “mystical-type experience” is based on Walter Stace’s work on mysticism (Stace, 1960), and is synonymous with the term “peak experience” derived from the work of Abraham Maslow (Maslow, 1959, 1964). Note: use of the term “mystical” in this thesis refers only to the phenomenology of the experience, devoid of supernatural or metaphysical associations.

"I am not looking at the world, not confronting it; I am knowing it by a continuous process of transforming it into myself, so that everything around me, the whole globe of space, no longer feels away from me but in the middle."

- Watts (1962)

Ego-dissolution, which refers to a temporary dissolution of the boundaries between the self (i.e. the "me") and other (i.e. the "not me"), is a commonly reported effect elicited by psychedelic compounds (Nour et al., 2016). The loss of the sense of self is an important feature of peak experiences occasioned by psychedelics. As mentioned previously, psychedelic peak experiences are characterised by feelings of unity with all that exists. This unitive experience is therefore explicitly related to disturbed ego-boundaries between the self (i.e. the "me") and the other/object (i.e. the "not me"). Early researchers suggested that the disturbance of ego-functioning may represent an important mechanism through which psychedelics produce their therapeutic effects (Grof, 1980). More recently, it has been argued that the ego-dissolving quality of psychedelics represents an exciting opportunity to study the brain mechanisms responsible for creating a coherent sense of self (Carhart-Harris and Friston, 2010; Lebedev et al., 2015).

"I took my [mescaline] pill at eleven ... I spent several minutes – or was it several centuries? – not merely gazing at those bamboo legs, but actually being them – or rather being myself in them; or, to be still more accurate (for "I" was not involved in the case, nor in a certain sense were "they") being my Not-self in the Not-self which was the chair."

- Huxley (1956)

Psychedelic peak experiences have been reported as among the most meaningful events experienced in one's life (Griffiths et al., 2018) and evidence suggests that they may mediate some of the enduring positive psychological effects observed following psychedelic use (see Barrett and Griffiths, 2017: for a review). For instance, the persistent feeling of elevated and energetic mood is termed the psychedelic 'afterglow' and has been shown to last for as long as a few weeks (Majic et al., 2015). Long-term positive outcomes associated with psychedelic peak experiences have also been demonstrated in patient populations, suggesting these experiences mediated therapeutic efficacy (Roseman et al., 2017). As such, psychedelic peak experiences have been likened to "*quantum change experiences*" (Griffiths et al., 2018), which are described as sudden and often profoundly meaningful experiences that are said to result in dramatic and enduring behavioural, emotional and cognitive changes (Miller, 2004).

It has been suggested that the intensity of challenging psychedelic experiences may predict enduring positive outcomes, whereas the duration of struggle, on the other hand, is predictive of negative outcomes (Carbonaro et al., 2016). Relatedly, proponents of psychoanalysis argue that one must overcome psychological resistance in order to achieve emotional breakthrough⁴, resolve psychological

⁴ The phenomenon of emotional breakthrough is defined as the overcoming of challenging emotions/memories and thereby experiencing emotional release.

struggle and have peak experience phenomena (Grof, 1980; Fischer, 2015; Eisner, 1958; Crockett et al., 1963). This notion is supported by patient references to emotional breakthroughs in post-psychedelic treatment reports (Watts et al., 2017; Gasser et al., 2014; Belser et al., 2017). However, measures such as the ASC, MEQ and Challenging Experience Questionnaire (CEQ; Barrett et al., 2016) are unable to sufficiently detect struggle resolution. This need for more sensitive measures led to the recent development of the Emotional Breakthrough Inventory (EBI; Roseman et al., 2019), a new scale specifically designed to measure emotional breakthrough following the resolution of psychological struggle. Using this new scale, emotional breakthrough has since been determined as an essential component of psychedelic-assisted therapy (Roseman et al., 2019), thereby supporting use of the EBI alongside measures of peak and challenging experiences.

Taken together, it is clear that emotional breakthroughs and peak and challenging experiences may represent mechanisms through which psychedelics promote their long-term effects. Nonetheless, other factors that are as yet unmeasured and therefore unaccounted for may also significantly contribute to the mediation of enduring post-psychedelic outcomes. For instance, my research has demonstrated the importance of psychological insight and the quality of post-psychedelic integration work; key factors focused on in a later chapter of this thesis (see Chapter 4, STUDY 2). For now though, having outlined the predictive potential of acute psychedelic experience phenomena in determining long-term outcomes, it is necessary to explain what some of these outcomes are.

2.4.3. Enduring effects

Psychedelics have been shown to induce positive psychological effects that are sustained long-term. Remarkably, a long-term follow-up of Pahnke's "Good Friday Experiment" (Pahnke, 1963) found that enduring positive effects may persist for many years, albeit with substantial study limitations (Doblin, 1991). As I mentioned in the previous section (see section 2.4.2), evidence suggests that peak experiences may mediate some of the enduring positive psychological effects observed following psychedelic use (see Barrett and Griffiths, 2017: for a review). It is particularly notable that positive long-term changes have been observed following a single exposure to a psychedelic compound. For instance, a single high dose of psilocybin in healthy participants was found to increase well-being, life satisfaction and trait openness 14 months after administration, whereby these outcomes were mediated by peak experience phenomena (Griffiths et al., 2008; MacLean et al., 2011).

Enduring positive effects on mental health have also been demonstrated in patient populations. In a recent study by our group, psilocybin was found to decrease depressive symptoms in patients with treatment-resistant depression (TRD) for up to six months post-dosing (Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a). Moreover, the occurrence and intensity of the psilocybin-induced peak experience was associated with positive long-term clinical outcomes observed in this TRD sample (Roseman et al., 2018b). Consistent with this, a single dose of psilocybin was found to reduce depression scores in patient anxiety related to terminal illness six months after treatment (Grob et al.,

2011; Griffiths et al., 2016). Ayahuasca has also demonstrated antidepressant potential, with a single dose resulting in a reduction of depressive symptoms for up to three weeks (Osorio Fde et al., 2015).

In addition to their antidepressant potential, psychedelics have shown promise in disorders of addiction. For instance, a single high dose of psilocybin was found to stop cigarette smoking in 80% of individuals six months after psilocybin-assisted smoking-cessation treatment (Garcia-Romeu et al., 2014; Johnson et al., 2014), and improve drinking behaviours in alcohol-dependent patients nine months after psilocybin-assisted motivational therapy (Bogenschutz et al., 2015). These positive long-term outcomes following psychedelic therapy for addiction have also been associated with the occurrence of peak experiences (Garcia-Romeu et al., 2014; Bogenschutz et al., 2015), suggesting these experiences may mediate therapeutic efficacy.

Taken together, it's apparent that psychedelic compounds have the capacity to promote positive changes that sustain long-term. The predictive value of psychedelic peak experiences in promoting such changes is well-established, but a growing awareness and understanding of other phenomenological features of the psychedelic experience encourage further research into additional psychological mechanisms at play. Moreover, science is currently gaining an understanding of the acute and post-acute brain effects of psychedelics, but the long-term brain effects and neurobiological mechanisms underlying long-term psychological outcomes are yet to be elucidated. My research addresses this knowledge gap in a later chapter of this thesis (see Chapter 4, STUDY 2), but in the meantime, the brain effects of psychedelics uncovered thus far is the topic of the next section (see section 2.5).

2.5. BRAIN EFFECTS

2.5.1. *Acute and post-acute effects*

Through the use of direct depth and scalp electroencephalography⁵ (EEG) to record spontaneous brain activity, early research provided important insights into the possible brain mechanisms through which psychedelics might produce their subjective effects. Early work found that psychedelics decreased the power and amplitude of spontaneous brain activity in animals (Bradley and Elkes, 1953; Delay et al., 1952; Evarts, 1957; Macadar et al., 1970; Rinaldi and Himwich, 1955) and humans (Bradley et al., 1953; Rinkel et al., 1952). These reductions in oscillatory power often occurred in the alpha range and were accompanied by a simultaneous increase in the alpha frequency (Bradley et al., 1953; Rinkel et al., 1952). Animal experiments also demonstrated psychedelic-induced disorganised activity within the hippocampus (Macadar et al., 1970) and a lack of psychedelic-induced effects in chimpanzees with no temporal lobes (Baldwin et al., 1959). Consistent with this, human experiments also demonstrated

⁵ An electroencephalogram (EEG) is an electrophysiological monitoring method to record electrical activity of the brain.

psychedelic-induced disorganised activity within the hippocampus, which was found to be associated with visual hallucinations and emotionally charged memories (Monroe et al., 1957). Moreover, human studies also demonstrated a lack of psychedelic-induced changes in the amplitude and frequency of cortical activity following temporal lobectomy in epileptic patients (Serafetinides, 1965). As such, these early studies led researchers to hypothesise that the temporal lobe may play an important role in producing the subjective effects of psychedelics.

Modern work has since corroborated and extended the findings of early studies by demonstrating decreased oscillatory power across different frequency bands after the administration of various psychedelic compounds (Carhart-Harris et al., 2016c; Kometer et al., 2015; Muthukumaraswamy et al., 2013; Puig et al., 2010; Wood et al., 2012; Schenberg et al., 2015; Riba et al., 2004; Riba et al., 2002), particularly in the alpha frequency range (Muthukumaraswamy et al., 2013). These decreases appeared localised in the posterior cingulate cortex (PCC), and the magnitude of the decreases in alpha activity correlated positively with ratings for ego-dissolution (Carhart-Harris et al., 2016c; Muthukumaraswamy et al., 2013). Furthermore, psychedelic-induced decreases in oscillatory power have been shown to extend into parahippocampal regions, which form part of the medial temporal lobe (MTL) system (Kometer et al., 2015).

PET studies have shown increased blood supply and glucose metabolism in cortical and subcortical regions after psilocybin, including the anterior cingulate cortex (ACC), insula, thalamus, MTL and lateral temporal lobes (LTL; Vollenweider et al., 1997a). In particular, increased glucose metabolism in the cingulate cortex, MTL and medial frontal lobes (MFL) correlated with the occurrence of visual disturbances and ego dissolution (Vollenweider et al., 1997a). Similarly, increased blood perfusion following ayahuasca has been demonstrated in the anterior insula, ACC, amygdala and parahippocampal gyrus (Riba et al., 2006). However, fMRI studies do not corroborate the PET findings. Instead, fMRI studies have observed decreased BOLD signal and cerebral blood flow (CBF) in cortical and subcortical regions, which appeared in high-level association regions and correlated with the subjective effects (Carhart-Harris et al., 2012a; Palhano-Fontes et al., 2015). It is unclear whether timing differences play a role in this controversy, as PET measures are averaged over a longer period of time relative to fMRI. The alteration in neurovascular coupling reported by Spain et al. (2015) might also help explain the discrepancy between fMRI and PET findings. Nonetheless, in an attempt to bridge this paradox, it has been suggested that the reduced BOLD activity reflects the desynchronisation of oscillatory rhythms while the PET metabolic increases correspond with the increased excitability of layer 5 pyramidal neurons (Halberstadt, 2015).

Investigations of intra-network functional integration have provided insights into the psychedelic-induced effects on the brain. The medial prefrontal cortex (mPFC), PCC and MTL are structurally well-connected and functionally well-coupled under normal resting-state conditions (Raichle et al., 2001; Greicius et al., 2003). These regions represent important high-level association areas, acting as central nodes in the Default Mode Network (DMN) that receive and integrate information from many dispersed and functionally specialised brain regions. Significant decoupling between the mPFC and PCC has

been observed following psilocybin (Carhart-Harris et al., 2012a), ayahuasca (Palhano-Fontes et al., 2015) and LSD (Carhart-Harris et al., 2016c). Psychedelics have also been shown to decouple the MTL from the parietal lobes (Lebedev et al., 2015) and the parahippocampus from the DMN (Carhart-Harris et al., 2016c). Psilocybin has also been shown to induce increased BOLD signal variability, especially within the hippocampus and ACC (Tagliazucchi et al., 2014). Thus, it has been suggested that the psychedelic-induced decreases in intra-network functional connectivity might represent a key mechanism via which psychedelics disorganise or disintegrate high-level brain network integrity to produce their profound subjective effects (Carhart-Harris et al., 2014; Carhart-Harris, 2018). Furthermore, research has demonstrated that psilocybin and LSD produce significant increases in brain signal complexity (entropy), suggesting that brain activity becomes more disorganised and unpredictable (Lebedev et al., 2016; Lebedev et al., 2015).

Further insights have emerged from studying inter-network connectivity – the coupling between different brain networks. The DMN and the Task Positive Network usually display anti-correlated or orthogonal activity under normal resting-state conditions but are shown to become positively coupled under psilocybin (Carhart-Harris et al., 2013) and ayahuasca (Palhano-Fontes et al., 2015). Marked increases in the coupling between a number of networks that are usually strongly segregated have been reported under psilocybin (Roseman et al., 2014), particularly for networks that abundantly express 5-HT_{2A}Rs (Erritzoe et al., 2009). Similarly, significant increases in coupling between normally segregated brain networks have been observed under LSD, accompanied by simultaneous disintegrated intra-network activity (Carhart-Harris et al., 2016c). Furthermore, an increase in global functional connectivity coinciding a decrease in network integrity, mediated by high-level association areas, has been observed following LSD administration (Tagliazucchi et al., 2016). Notably, this increase in global integration in cortical association regions selectively correlated with subjective ratings of ego-dissolution (Tagliazucchi et al., 2016).

Taken together, the evidence presented above suggests that psychedelics desynchronise and disorganise neuronal activity via 5-HT_{2A}R activation, particularly within high-level brain networks. Additionally, the above evidence indicates that psychedelics facilitate increased coupling between brain regions and networks that are usually functionally segregated. This “collapse” of the usual hierarchy, and the resulting decrease in the brain’s modularity, may represent important mechanisms through which psychedelics induce their profound subjective effects.

2.5.2. Enduring effects

The long-term brain effects of psychedelics are not as well studied nor understood. Recent work suggests that brain changes may persist for up to one week following a psychedelic experience, however, results have not been replicated and the direction of findings can be challenging to interpret. For instance, increased amygdala responsiveness to emotional face stimuli was found one day after psilocybin treatment for depression (Roseman et al., 2018a), yet decreased amygdala responsiveness

was found one week post-psilocybin in healthy participants (Barrett et al., 2020). It's possible that this discrepancy is merely a function of the different populations studied (i.e. depressed patients versus healthy volunteers). Alternatively, the increase in amygdala responsiveness found post-acutely (Roseman et al., 2018a) might reflect a transient alteration in serotonergic signalling due to 5-HT_{2A}R internalisation – to this end, if long-term scanning was completed in the depression study, amygdala responsiveness may have also subsequently decreased one week post-dosing in the depressed patients, as in the healthy volunteers study by Barrett et al. (2020). It is clear that much work is needed to determine the long-term effects of psychedelics on brain function in different populations, with a particular focus on brain scanning at different post-treatment phases.

Reduced cortical thickness has been observed in the PCC of experienced ayahuasca users, with the magnitude of changes correlating with the extent of previous psychedelic use (Bouso et al., 2015). Cortical thinning was also observed in the middle frontal gyrus (MFG), inferior frontal gyrus (IFG), precuneus and superior frontal gyrus (SFG), whereas cortical thickening was observed in the precentral gyrus and the ACC (Bouso et al., 2015). Notably, agonism at the 5-HT_{2A}R dose-dependently increases the expression of brain-derived neurotrophic factor (Gewirtz et al., 2002) and stimulates the expression of immediate early genes that encode transcription factors associated with synaptic plasticity and cognition (Gonzalez-Maeso et al., 2007). It therefore seems plausible that long-term adaptation through changes in gene expression may partially account for the enduring psychological effects observed following psychedelic use (e.g. see Nichols and Sanders-Bush, 2002). Taken together, it is clear that further research is required to elucidate, better understand and untangle the enduring brain effects of psychedelic compounds. To this end, unveiling the long-term brain effects of psilocybin is a key focus of a later chapter in this thesis (see Chapter 4, STUDY 2).

2.6. REBUS and the anarchic brain: a model of psychedelic drug action

Given that interest in psychedelics is rapidly growing and a wealth of knowledge on their action is currently accumulating, a compelling model to account for the brain mechanisms of psychedelics has recently been developed. To this end, the relaxed beliefs under psychedelics (REBUS) and the anarchic brain (Carhart-Harris and Friston, 2019) model attempts to account for both the acute and long-term effects of psychedelics. The REBUS model was developed from the unification of two closely related formulations of brain function: (i) the free-energy principle (Friston, 2010) and (ii) the entropic brain hypothesis (Carhart-Harris, 2018).

The entropic brain hypothesis (Carhart-Harris, 2018; Carhart-Harris et al., 2014) proposes that the subjective quality of any given conscious state and the 'richness' of its content can be indexed by the magnitude of entropy⁶ in a given parameter of spontaneous brain activity. Normal waking

⁶ Entropy in the information theoretic sense, where there is maximum uncertainty about the system due to a lack of order on which to base any predictions. "Uncertainty" is a synonym for entropy, and disorder and uncertainty are effectively equivalents.

consciousness is proposed to arise within a critical zone at which the entropy of brain activity is neither too ordered nor disordered, whereas psychedelic compounds have been shown to increase brain entropy within the acute psychedelic drug state. The entropic brain suggests that this psychedelic-induced increase in criticality towards greater conscious content, flexibility of mind and emotional lability results in increased sensitivity to perturbation via intrinsic (i.e. “set”) and extrinsic (i.e. “setting”) sources. However, the entropic brain also suggests that uncertainty and information-content are closely related and that entropic-brain-states, and thus acute psychedelic states, are experienced as content-rich but unpredictable and uncertain (Carhart-Harris, 2018; Carhart-Harris et al., 2014).

The free-energy principle⁷ attempts to explain how biological systems, particularly the brain, maintain order by minimising the entropy of sensory states (Friston, 2010; Friston, 2005; Friston et al., 2006; Friston, 2012). The free-energy principle proposes the following arguments. Biological systems act on and selectively sample the environment to avoid phase transitions that may lead to an irreversible alteration in their structure. Ensemble density is encoded by the state of the system, and this density is used to construct a free energy for any system that is in exchange with its environment. The free-energy principle suggests that any adaptive change in the brain will minimise free-energy, which is a function of the ensemble density encoded by the organism's configuration and the sensory data to which it is exposed. Free energy minimisation occurs through action-dependent changes in sensory input and the ensemble density implied by internal changes. This minimisation results in perceptual inference about the world, encoding of perceptual context, perceptual learning about the causal structure of the environment and, finally, a principled exchange with, or sampling of, that environment. The free energy induces a generative model that involves the system's structure. Identifying this model enables one to predict how a system will change if it conforms to the free energy principle. Therefore, the brain may be regarded as a hierarchical dynamic system in which neural activity encodes the conditional modes of environmental states and its connectivity encodes the causal context in which these states evolve (Friston, 2010; Friston, 2005; Friston et al., 2006; Friston, 2012).

In brief, the entropic brain measures the uncertainty of neuronal fluctuations across time (Carhart-Harris, 2018; Carhart-Harris et al., 2014), whereas free-energy measures the uncertainty of beliefs encoded by neuronal fluctuations (Friston, 2010; Friston, 2005; Friston et al., 2006; Friston, 2012). The entropic brain hypothesis and the free-energy principle both relate to hierarchical predictive coding (Figure 2-4). Hierarchical predictive coding posits that the brain generates mental models that predict upcoming sensory inputs. These predictive models are called “priors” (i.e. “prior beliefs.”) and are layered in a hierarchy. The higher levels send predictions down the hierarchy and the lower levels report sensory information upwards. When the model's top-down predictions don't match the bottom-up sensory input, the model either updates its priors based on the new sensory information or ignores this information and maintains its priors.

⁷ The free-energy principle is closely related to hierarchical predictive coding and the Bayesian Brain. Simply put, free energy can be thought of as a measure of surprise. It is therefore closely related to information theoretic entropy, which is a measure of uncertainty or average surprise.

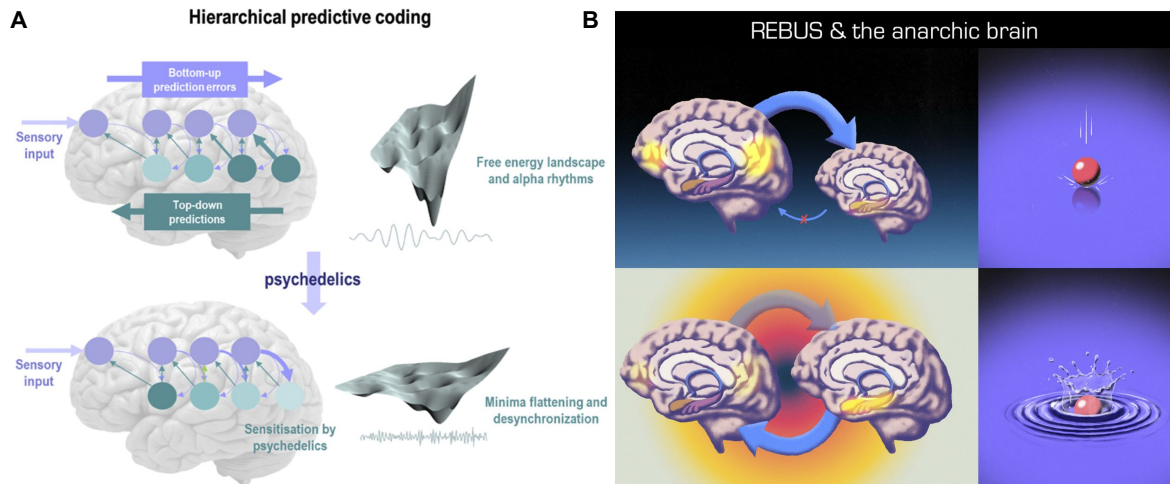


Figure 2-4. Hierarchical predictive coding and REBUS and the anarchic brain. (A) Hierarchical predictive coding. Schematic illustration of the proposed effects of psychedelics on hierarchical predictive coding, a predominant process theory for Bayesian inference and variational free-energy minimisation in the brain. Under these computational architectures, sensory input arrives at the sensory epithelia and is compared with descending predictions. The ensuing prediction error (blue circles; e.g., neuronal populations of superficial pyramidal cells) is then passed forward into hierarchies, to update expectations at higher levels (blue arrows). These posterior expectations (teal circles; e.g., deep pyramidal cells) then generate predictions of the representations in lower levels, via descending predictions (teal arrows). The recurrent neuronal message passing (i.e., neuronal dynamics) tries to minimise the amplitude of prediction errors at each and every level of the hierarchy, thereby furnishing the best explanation for sensory input at multiple levels of hierarchal abstraction. Crucially, this process depends upon the precision (ascribed importance or salience) afforded to the ascending prediction errors (surprise) and the precision (felt confidence) of posterior beliefs. The basic idea of the REBUS model is that psychedelics act preferentially via stimulating 5-HT_{2A}Rs on deep pyramidal cells within the visual cortex as well as at higher levels of the cortical hierarchy. Deep-layer pyramidal neurons are thought to encode posterior expectations, priors, or beliefs. The resulting disinhibition or sensitisation of these units lightens to precision of higher-level expectations so that (by implication of the model) they are more sensitive to ascending prediction errors (surprise/ascending information), as indicated by the thick blue arrow in the lower panel. Computationally, this process corresponds to reducing the precision of higher-level prior beliefs and an implicit reduction in the curvature of the free-energy landscape that contains neuronal dynamics. Effectively, this can be thought of as a flattening of local minima, enabling neuronal dynamics to escape their basins of attraction and — when in flat minima — express long-range correlations and desynchronised activity. This schematic uses the format in (Bastos et al., 2012), to which the reader is referred for details. **(B) REBUS and the anarchic brain.** These schematics depict: on the top row, brain organisation in psychopathologies such as depression in which high-level priors (e.g., instantiated by the DMN) are overweighted (thick top-down arrow), causing a suppression of and insensitivity to bottom-up signalling (e.g., stemming from the limbic system). In this figure, we show compromised bottom-up signalling via a thin arrow with a red cross over its centre. The graphic on the top right depicts a pathologically rigid or frozen system, insensitive to perturbation, represented in this figure as a heavy ball dropped on a solid surface having a minimal effect on the system, i.e., the ball lands with an uneventful thud. The bottom row depicts brain organisation under a psychedelic. In this figure, the top-down arrow has been made translucent to reflect a deweightening or relaxation of high-level priors or beliefs (this component of the model is referred to by the acronym REBUS). The effect of this deweightening is to enable bottom-up information intrinsic to the system, to travel up the hierarchy with greater latitude and compass. We refer to this component of the model as the anarchic brain. That the two brains on the bottom row

are on the same level and of the same size is intended to reflect a generalised decrease in hierarchical constraints under the psychedelic. The graphic on the bottom right represents a phenomenon known as critical slowing, i.e., systems at criticality display maximal sensitivity to perturbation. In this figure, one can see ripples appearing after a heavy ball is dropped into a liquid surface, reflecting how, in this particular system, and unlike its frozen counterpart above, there will be a slow recovery to the same perturbation. Illustrations by Pedro Oliveira, courtesy of Favo Studio. Both Figures reproduced from Carhart-Harris and Friston (2019). Copyright information: Creative Commons Attribution 4.0 International (CC BY 4.0).

REBUS⁸ places psychedelics in a predictive coding framework to provide a unified model of psychedelic drug action that may explain the acute and enduring effects occasioned by psychedelic drugs. The REBUS model (Carhart-Harris and Friston, 2019) theorises that psychedelics induce an acute state of heightened brain entropy via their action on 5-HT_{2A}Rs (Carhart-Harris, 2018), and that this reflects a relaxation of precision weighting of priors (REBUS) that coincides with the increased weight of bottom-up signalling (anarchic brain). This allows bottom-up information to have more influence on our conscious experience, and also on the configuration of the hierarchy overall. Both components of the REBUS model (i.e. (i) REBUS and (ii) anarchic brain) are regarded as mutually dependent because relaxed priors naturally imply increased prediction error (Carhart-Harris and Friston, 2019). For instance, REBUS suggests that psychedelics disrupt functioning at the level of the system that encodes the precision of priors, beliefs, or assumptions. This includes alterations in the sensitivity of pyramidal neurons, the power of low frequency oscillations and the integrity of large-scale neural networks. As 5-HT_{2A}Rs are densely expressed in the visual cortex, a low dose of a psychedelic drug may primarily induce perceptual effects within the visual domain. However, high doses may produce more profound subjective effects due to the disrupted functioning of higher levels in the hierarchy, which might explain phenomena such as ego dissolution and enduring alterations of high-level priors, perspectives, or beliefs.

2.7. PRIMARY RESEARCH OBJECTIVES OF THIS THESIS

This introduction chapter provided a brief historical overview of psychedelic use from prehistorical times to the present day. The safety and pharmacology of psychedelics was also discussed, as well as the psychological and brain effects that characterise these compounds. As well as introducing the wealth of knowledge that has accumulated over the years, this introduction highlighted significant knowledge gaps that need to be addressed. Accordingly, further quantifying the mechanisms underlying the profound and persisting effects observed following psychedelic use demands attention. Likewise, science is currently gaining an understanding of the acute and post-acute brain effects of psychedelics,

⁸ REBUS is an acronym for “RElaxed Beliefs Under psychedelics.” As the anarchic brain follows naturally from the liberation of bottom-up signalling, which is logically implied by relaxed precision on priors, both components of the REBUS model (i.e. (i) REBUS and (ii) anarchic brain) are regarded as mutually dependent. Therefore, REBUS is used as an acronym for both components of the model.

yet the paradoxical effects obtained using different (blood-flow based) neuroimaging methods thwarts our confidence when trying to interpret such data and deserves experimental scrutiny. Consequently, learning how psychedelics – through their serotonergic mechanism of action – effect the brain's blood supply is essential to disentangling mechanisms involved in the psychedelic brain state. Moreover, the long-term brain effects and neurobiological mechanisms underlying long-term psychedelic outcomes are yet to be elucidated.

Broadening the scope of psychedelic phenomena under investigation as well as unveiling the long-term brain effects of psychedelics and determining the neural correlates of their long-term outcomes will help fill this knowledge gap and would hold significant value. Similarly, elucidating the 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action will help further our understanding and interpretation of data obtained in humans using measures such as BOLD-fMRI. As such, somewhat filling these knowledge gaps to contribute to the field's understanding of psychedelic activity is the ultimate aim of my work outlined here. To this end, this thesis outlines three separate studies. The specific hypotheses, methods, results and interpretations pertaining to each of these studies are discussed in more detail in their corresponding chapters, but a brief overview of what's to come is outlined here:

- **CHAPTER 3: STUDY 1 |**
Can psilocybin therapy remediate negative cognitive biases in severe depression?

Objective: to investigate the presence of a negative cognitive bias in patients with treatment-resistant depression and the ability of psilocybin therapy to alleviate this.

- **CHAPTER 4: STUDY 2 |**
What are the acute and long term effects of psilocybin in healthy participants?

Objective: to investigate the acute and long term effects of psilocybin on the brain, behaviour and psychology in healthy participants, with a key focus on psychological insight.

- **CHAPTER 5: STUDY 3 |**
Can we untangle neuro- and haemodynamic mechanisms in the psychedelic brain state?

Objective: to investigate (i) the suitability of the novel and most 5-HT_{2A}-selective psychedelic, 25CN-NBOH, in mice for prospective *in vivo* optical imaging studies; and (ii) 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic (25CN-NBOH) drug action.

3. STUDY 1 |

Can psilocybin therapy remediate negative cognitive biases in severe depression?

“If the doors of perception were cleansed every thing would appear to man as it is, Infinite. For man has closed himself up, till he sees all things thro' narrow chinks of his cavern.”

– William Blake

3.1. FOREWORD

The previous chapter finished with a proposed model of psychedelic action – i.e., the relaxed beliefs under psychedelics and the anarchic brain model (REBUS; Section 2.6, page 22). REBUS places psychedelics in a predictive coding framework to provide a unified model of psychedelic drug action that may explain the acute and enduring effects occasioned by psychedelic drugs (Carhart-Harris and Friston, 2019). In brief, the REBUS model theorises that psychedelics induce an acute state of heightened brain entropy via their action on 5-HT_{2A}Rs (Carhart-Harris, 2018), and that this reflects a relaxation of precision weighting of priors that coincides with the increased weight of bottom-up signalling. This allows bottom-up information to have more influence on our conscious experience, and also on the configuration of the hierarchy overall. The REBUS model postulates that the effects of psychedelics on the precision weighting of high-level priors or beliefs may mediate the therapeutic efficacy of these compounds. As the REBUS model states that high-level priors may be sensitised to liberated bottom-up information flow, combining the belief relaxation process with the right intention, care provision and context may help cultivate the revision of such entrenched pathological priors (Carhart-Harris and Friston, 2019). With this in mind, the negative cognitive bias characteristic of severe depression (Beck, 1967; Beck, 1976; Beck and Rush, 1979) is a good example of how over-weighted high-level priors can have devastating consequences. If we go by the REBUS model of psychedelic action, the negative bias underlying depression may be amenable to revision via psychedelic therapy-induced belief relaxation and recalibration processes.

In this chapter, I examine the presence of a negative cognitive bias in patients with treatment-resistant depression (TRD) and the ability of psilocybin therapy to alleviate this. With the REBUS model in mind, the presence of a pessimism bias would indicate excessive precision, weight, or influence of this prior in TRD. Moreover, the alleviation of this negative bias following psilocybin therapy would indicate that the treatment has induced belief relaxation and recalibration processes. As such, it is hypothesised that TRD patients will show a significant pessimism bias that can be effectively treated with psilocybin therapy.

3.2. INTRODUCTION

Major depressive disorder (MDD) affects 350 million people worldwide and is one of the foremost contributors to the overall global burden of disease (Marcus et al., 2012; James et al., 2018). Sadly, much of this burden can be accounted for by treatment-resistant depression (TRD), which afflicts approximately one third of MDD patients (Gaynes, 2009; Greden, 2001). Worse still, even when conventional medications are effective, they often have a delayed onset of action meaning it can take weeks to months before patients experience any clinical benefit (Machado-Vieira et al., 2010) – this can have devastating consequences, especially as MDD accounts for over half of all suicides and is the leading cause of disability in the world (Marcus et al., 2012). As such, elucidating more effective, rapid-acting antidepressant treatments with positive outcomes that sustain long-term is essential.

3.2.1. *Pathophysiology of depression*

Despite great need and substantial effort, no clear or compelling model has been developed to explain the causes and mechanisms of MDD. An extensive body of research has implicated monoamine neurotransmitters and their respective GPCRs in the pathophysiology of MDD, particularly the serotonergic and noradrenergic systems (Delgado, 2000; Hirschfeld, 2000; Kaster et al., 2016; Ferrari and Villa, 2017). Based on this evidence, the monoaminergic hypothesis has primarily directed the development of antidepressant medications to date, with inhibition of serotonin and/or noradrenaline re-uptake as the primary mechanism of action (Lambert and Bourin, 2002). Yet, despite inducing a rapid increase in monoamine levels, clinical response to currently available antidepressants is often delayed and of poor efficacy, with 33% of patients responding similarly to placebos (Brown, 1994; Trivedi and Rush, 1994; Sonawalla and Rosenbaum, 2002; Mann, 2005; Kirsch, 2019). Since the monoamine hypothesis of MDD alone fails to fully explain the mechanisms of the disease or the lack or latency of response to antidepressants, other pathogenic factors must also be considered.

The dysfunction of many neurotransmitter and metabolic systems are involved in the neurobiology of depression. For instance, deficiencies in peptide neurotransmitters and trophic factors as well as hyperactivity in still other neurotransmitter systems involving acetylcholine, corticotropin-releasing factor and substance P have all been implicated in the pathophysiology of MDD (for a review, see Mann et al., 2011). Considerable evidence suggests that genetic predisposition and stress play a key role in the aetiology of MDD and may operate via a number of pathophysiological mechanisms, such as reduced monoaminergic neurotransmission and hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis and inflammatory response system (Duman et al., 2016; Zhang et al., 2013; Hosang et al., 2014; Ota et al., 2014). The hypersecretion of cortisol is an important pathophysiological feature of MDD and is associated with functional and structural abnormalities within cortico-limbic neurocircuits implicated in depression and mood regulation (Liu and Aghajanian, 2008; Magariños and McEwen, 1995). To this end, certain aspects of MDD may result from maladaptive stress-induced neuroplastic

changes, whereby chronically administered antidepressants have been shown to exert some neuroprotective effects (Sheline et al., 2003; Ampuero et al., 2010; Castrén and Hen, 2013).

It is clear from the evidence presented above that MDD has a multifactorial aetiopathogenesis, with biological, psychological and social factors all playing key roles in the development and maintenance of the disorder. As such, medications only addressing certain biological features of MDD (i.e., reduced monoaminergic neurotransmission for example) may not resolve other key underlying factors contributing to the disorder, such as resilience to acute traumatic or chronic stress and hopefulness for the future. To this end, it is imperative that we look beyond the specific biological features of MDD and embrace an all-encompassing approach in the search for more effective and enduring treatments.

3.2.2. Cognitive biases in depression

Psychotherapy and pharmacotherapy are of comparable efficacy in the acute treatment of MDD, albeit with lower rates of attrition for psychological interventions (Cuijpers et al., 2013a). However, evidence suggests that long-term outcomes are significantly better for patients receiving psychotherapy than for those receiving medication (Cuijpers et al., 2013b) and relapse rates are significantly lower (Shea et al., 1992; Kirsch, 2019). This suggests that psychotherapy may be more advantageous relative to medication in the long-term and highlights an important caveat in the ‘medication only’ treatment approach in mental health care.

Cognitive therapy is the most widely studied and practiced psychotherapeutic intervention for MDD (Butler et al., 2006). The cognitive bias model of depression states that patients have an unrealistic negative perspective of themselves and the world more generally (Beck, 1967; Beck, 1976; Beck and Rush, 1979). Somewhat in contrast to this, the depressive realism hypothesis states that depressed patients actually see themselves and the world in a more realistic way than the general population – who, the hypothesis maintains, are somewhat inaccurate and unrealistic in their optimism (Alloy and Abramson, 1979). The cognitive triad—which comprises a negative attitude towards oneself, the environment and the future (Beck, 1967)—partly forms the theory upon which cognitive therapy for MDD is based. A key cognitive feature in MDD is that patients expect the future to be bleak and anticipate unfavourable outcomes when facing life events of unknown emotional impact. Early research has shown that MDD patients have more dysfunctional attitudes, feelings of hopelessness, negative thoughts and pessimism than healthy individuals (Beck et al., 1988; Hill et al., 1989; Hollon et al., 1986; Peterson and Seligman, 1984). More recent findings have since supported the cognitive-bias model of depression over the depressive realism hypothesis, demonstrating, for example, that MDD patients exhibit pessimistic biases when anticipating future life events (Strunk and Adler, 2009; Strunk et al., 2006).

3.2.3. *Psychedelic therapy*

As MDD is associated with excessive pessimism, it is interesting to note that psilocybin has been shown to increase optimism, psychological well-being, trait openness and life satisfaction in an enduring way following just a single dose in healthy populations (Griffiths et al., 2011; MacLean et al., 2011; Griffiths et al., 2008). Increased optimism and well-being have also been found for weeks to months after a controlled administration of LSD to healthy participants (Carhart-Harris et al., 2016b; Liechti, 2017). Functional brain imaging of the psychedelic state has implicated brain regions linked to MDD (Carhart-Harris et al., 2012a), including the subgenual prefrontal cortex (sgPFC) and default mode network (DMN; Hamilton et al., 2015). Moreover, the key receptor associated with the action of psychedelics, the serotonin 2A receptor (5-HT_{2A}R; Glennon et al., 1984), has its highest expression in DMN regions (Beliveau et al., 2017) - and 5-HT_{2A}R binding has been reliably linked with trait pessimism (Bhagwagar et al., 2006; Meyer et al., 2003) and neuroticism (Frokjaer et al., 2008; Frokjaer et al., 2010). It has been hypothesised that deficient signalling at the 5-HT_{2A}R may confer an inflexible and pessimistic mind-set, and that increased 5-HT_{2A}R signalling may serve to 'lubricate the mind', relaxing prior beliefs to aid new learning (Carhart-Harris and Nutt, 2017); see Matias et al. (2017) for more empirical support this.

Recent years have witnessed a resurgence of interest in the ability of classical psychedelic compounds to treat a range of disorders, including MDD (Carhart-Harris and Goodwin, 2017; dos Santos et al., 2016). Randomised controlled trials (RCTs) have demonstrated that psilocybin can rapidly alleviate depression and anxiety in patients with life-threatening cancer, with sustained effects for 6 months (Griffiths et al., 2016; Ross et al., 2016). A recent feasibility study of ours found that psilocybin with psychological support was well tolerated and associated with rapid and marked reductions in depressive symptoms in TRD patients (Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a), thus endorsing its potential as a safe and effective treatment for such clinical populations, albeit with important caveats (Carhart-Harris et al., 2018).

3.3. OBJECTIVES AND HYPOTHESES

The present study sought to investigate the presence of pessimism in TRD patients, in line with the depressive-bias hypothesis, and the ability of psilocybin therapy to alleviate this. It was hypothesised that TRD patients would show a significant pessimism bias that could be effectively treated with psilocybin therapy. Relevant measures of pessimism were given at baseline and one-week post-dosing. To demonstrate robustness to order confounds, TRD patients' data were compared with those from non-treated healthy control subjects matched in terms of age, gender and education tested over an equivalent time period.

3.4. METHODS

3.4.1. *Statement of contributions*

Team contribution: Prof David Nutt and Dr Robin Carhart-Harris conceptualised the study design and coordinated the larger TRD pilot study (of which this study formed part of). Dr Mark Bolstridge was the lead psychiatrist and provided psychological support for the patients alongside other clinically-trained members of our team. Team members recruited the TRD patients, decorated the dosing room, designed the music playlist for the psychedelic sessions, and administered the drug.

Personal contribution: I conducted experiments using a sample of the TRD patients that were recruited for the larger pilot study. To control against test-retest reliability of the outcome measures, Philip Ashton, Jack Stroud and I recruited healthy and non-treated control subjects matched in terms of age, gender and education and tested them over an equivalent time period. I collected, plotted, analysed and interpreted all of the data presented in this chapter.

3.4.2. *Ethical Approvals*

This study received a favourable opinion from NRES London-West London, was sponsored by Imperial College London, and was carried out in accordance with Good Clinical Practice Guidelines. The National Institute for Health Research/Wellcome Trust ICRF provided site-specific approval and the Medicines and Healthcare products Regulatory Agency reviewed and approved this research. All patients provided written informed consent.

3.4.3. *Study design and participants*

The Centre for Psychedelic Research conducted an open-label pilot study with a mixed-model design to assess the safety and feasibility of psilocybin as a treatment for patients with TRD. I conducted experiments using a sample of the TRD patients that were recruited for this larger open-label pilot study, the findings of which are discussed in this chapter. The study participants and research team were not masked to treatment assignment. All TRD patients were administered psilocybin in two dosing sessions: an initial safety dose (10 mg) and a subsequent treatment dose (25 mg) one week later. Although not included in the larger pilot study, I recruited a healthy control group. These control subjects were not administered psilocybin and were primarily included to examine test-retest data on the relevant primary measures that I was interested in.

General practitioners were provided with recruitment information via the North West London Clinical Research Network and were asked to identify potential patients with TRD. TRD patients residing in the

UK were also able to self-refer to the study. In most cases, it was the TRD patients that initiated contact with the research team. After initiating contact, patients were given a study information sheet and were initially screened via a telephone call with the research team's lead psychiatrist to determine eligibility for the study. Each patient's general practitioner or psychiatrist provided written confirmation and documentation of the patient's diagnosis and mental health background.

The inclusion criteria were (i) MDD of a moderate to severe degree (16 on the 21-item Hamilton Depression Rating scale [HAM-D]), and (ii) no improvement despite two adequate courses of antidepressant treatment of different pharmacological classes lasting at least 6 weeks within the current depressive episode. The exclusion criteria were (i) current or previously diagnosed psychotic disorder, (ii) first degree relative with a diagnosed psychotic disorder, (iii) medically significant condition rendering unsuitability for the study, (iv) history of serious suicide attempts (requiring hospitalisation), (v) previous manic episode, (vi) blood or needle phobia; (vii) positive pregnancy test at screening or during the study, and (viii) current drug or alcohol dependence.

Physically and mentally healthy age-matched control subjects were recruited via word of mouth and were subject to the same exclusion criteria outlined above. Non-treated control subjects were included to examine test-retest data on the outcome measure. Informed consent was obtained from all of the study participants.

3.4.4. Drug

Psilocybin was obtained from THC Pharm GmbH (Frankfurt, Germany) and formulated (5 mg psilocybin per size 0 capsule) by Guy's and St Thomas' Hospitals' Pharmacy Manufacturing Unit (London, UK). Home Office approvals for storing and dispensing Schedule One drugs were obtained.

3.4.5. Procedures and dosing sessions

Screening procedures typically lasted 4 hours and consisted of psychiatric and physical health assessments. All patients provided written informed consent. Clinicians carried out a Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) and assessed depression severity using the HAM-D (Hamilton, 1960) and Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979) in patient participants. Physical assessments included an electrocardiogram, routine blood tests, blood pressure and heart rate tests, and a physical examination. Eligible TRD patients were allocated two psychiatrists for support throughout the study.

All patients enrolled in the study attended a pre-dosing preparatory session with the allocated psychiatrists on the next visit after screening. Psychological preparation consisted of discussing the effects of psilocybin and simulating aspects of the dosing session to make patients aware of what to

expect. Patients also shared their personal history and thoughts on the origins of their depression. This session helped to build rapport and trust between the patient and their allocated psychiatrists, thus minimising the risk of adverse reactions to psilocybin (Metzner et al., 1965).

All patients attended two dosing sessions separated by one week at the Imperial Clinical Research Facility (ICRF). A close friend or relative accompanied each patient to and from the ICRF and return transport was arranged before the dosing sessions took place. A urine sample for drugs of abuse (and pregnancy where applicable), breathalyser test for alcohol use and vital signs measurements were collected before each dosing session. Patients were then taken to a decorated dosing room with dim lighting and music where they were invited to lie down and relax. The allocated psychiatrists remained at either side of the bed and adopted a non-directive, supportive approach. Patients were supervised at all times and the psychiatrists checked how they were feeling at approximately one hour intervals and assessed them for discharge approximately 6 hours after drug administration.

3.4.6. Outcome measures

Self-rated depressive symptoms were measured in all study participants using the Beck Depression Inventory (BDI; Beck et al., 1961). Cognitive biases were measured using the Prediction of Future Life Events task (POFLE; Strunk et al., 2006). The POFLE contains 40 different life events (20 desirable events and 20 undesirable events) that patients must predict the probability of occurring within the next 30 days (see Strunk et al. (2006) for the scoring procedures). The POFLE was split into two versions, A and B, each containing 20 items (10 desirable and 10 undesirable). All patients received both versions in balanced order; half received POFLE version A at screening and POFLE version B at the follow-up, and the other half vice versa. Patients were contacted 30 days after completing each version to determine which of the events actually occurred (Figure 3-1). All of the above outcome measures were assessed at screening and then again at the follow-up.

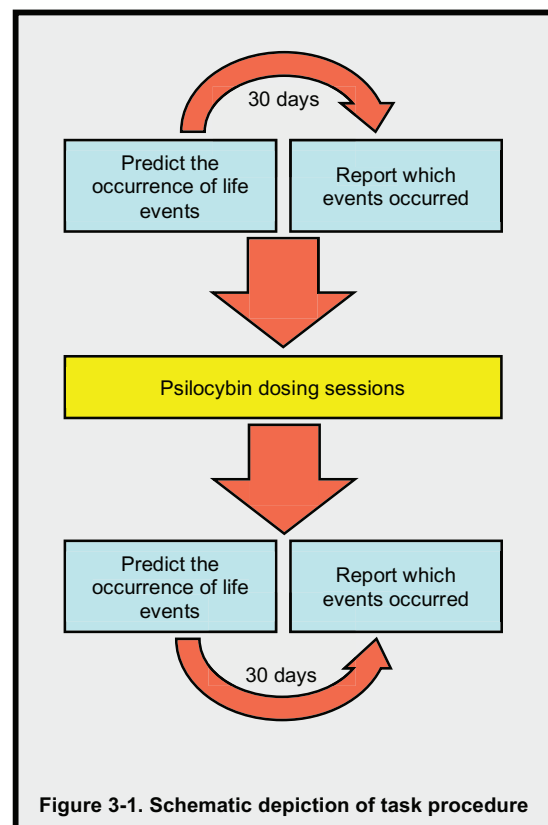


Figure 3-1. Schematic depiction of task procedure

3.4.7. Statistical analysis

All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Within-group comparisons were performed using two-tailed paired t-tests for parametric data and Wilcoxon signed ranks tests for non-parametric data. Between-group comparisons were performed using two-tailed independent t-tests for parametric data and Mann-Whitney U tests for non-parametric data. Correlations were analysed using Pearson's correlation coefficient for parametric data and Spearman's rho for non-parametric data. Results provide 95% CIs around the mean differences. Effect sizes were calculated using the Hedges' *g* formula.

Study power for comparisons on the primary outcome measure (i.e., the POFLE task) was estimated *a priori* using nQuery version 8.6 (Statistical Solutions Ltd., Los Angeles, CA, USA) based on the fixed sample size of *n*=15 per group. Analyses indicated 88% power to detect a difference in means via paired comparisons and 91% power to detect a difference in means between groups with a 5% two-sided significance level. For correlations, power analyses indicated a detectable effect size of 0.56 with a desired power of 80% at the 5% significance level.

3.5. RESULTS

3.5.1. Demographics

Of the total 30 participants who took part in this study, the majority were Caucasian (93.33%) and men (73.33%) with a post-secondary level of education (80%). Of the 15 clinically assessed patients with TRD, the Hamilton Depression Scale scores ranged from 18 to 29 ($M=23.73$, $SD=4.70$) and the Montgomery-Åsberg Depression Rating Scale scores from 24 to 42 ($M=32.33$, $SD=5.14$); thus, all patients recruited were diagnosed with TRD of at least moderate severity, with most meeting criteria for severe depression. Control subjects were not clinically assessed because they were deemed physically and mentally healthy. Self-reported depressive symptoms (BDI) were collected for all study participants. A breakdown of the participant demographic details and depressive scores at baseline can be found in Table 3-1.

Table 3-1. Study participant demographics.

Characteristic	Control subjects (<i>n</i> =15)	TRD patients (<i>n</i> =15)	All participants (<i>n</i> =30)	<i>P</i> value
Gender (% male)	60.0%	73.3%	66.7%	0.439 ^a
Age in years (mean, SEM)	37.6 (3.7)	45.4 (2.9)	41.5 (2.4)	0.109 ^b
Ethnicity (%)				0.309 ^a
White	100.0%	93.3%	96.7%	
Black	0%	6.7%	3.3%	
Education (%)				
Secondary School	6.7%	20.0%	13.3%	0.497 ^a
Undergraduate	53.3%	53.3%	53.3%	
Postgraduate	40.0%	26.7%	33.3%	
Baseline scores (mean, SEM)				
QIDS	3.46 (0.72)	19.13 (0.58)	NA	<0.0001 ^c
BDI	4.07 (1.10)	34.33 (1.92)		<0.0001 ^c

^a Chi-square test

^b *t* test

^c *U* test

3.5.2. Depressive symptoms

At baseline, patients ($M=34.33$, $SD=7.44$) scored significantly higher than controls ($M=3.67$, $SD=3.83$) on the BDI ($U=0.0E0$, $p<0.001$, $g=5.0$). The patients showed a significant decrease in BDI scores one week after the psilocybin sessions ($M=12.13$, $SD=9.80$) compared to baseline ($M=34.33$, $SD=7.44$; $t_{(14)}=7.900$, $p<0.001$; 95% CI [16.17, 28.23], $g=1.9$). There was no significant difference found in the controls' baseline ($M=3.67$, $SD=3.83$) versus follow-up BDI scores ($M=2.73$, $SD=3.41$; $Z=-1.071$, $p=0.284$). These results confirm that depressive symptoms were significantly reduced for the patients one week after psilocybin treatment, but did not change over an equivalent period for the non-treated control subjects.

Despite the significant improvement, patients' post-treatment BDI scores ($M=12.13$, $SD=9.80$) continued to significantly differ from the controls' follow-up values ($M=4.29$, $SD=4.35$; $U=34.50$, $p=0.001$, $g=1.1$). These results suggest that the patients' depressive symptoms were largely reduced but did not quite reach levels comparable with healthy controls one week after psilocybin treatment (Figure 3-2 A).

The patients' depressive symptoms were also measured prior to the administration of psilocybin at the beginning of dosing days 1 and 2. There was a significant reduction in the patients' BDI scores over time ($\chi^2_{(3, N=15)}=32.35$, $p<0.0001$). Post hoc comparisons with Dunn's correction showed no significant difference in baseline ($M=34.33$, $SD=7.44$) versus dosing day 1 ($M=30.53$, $SD=12.36$; $p=0.944$) BDI scores. This confirms that depressive symptoms did not naturally decline from baseline prior to psilocybin treatment. However, BDI scores were significantly reduced on dosing day 2 ($M=21.13$, $SD=12.93$) when compared with baseline ($M=34.33$, $SD=7.44$; $p<0.001$) and dosing day 1 ($M=30.53$, $SD=12.36$; $p=0.033$) scores. This demonstrates a reduction in the patients' depressive symptoms 1 week after receiving the first dose of psilocybin. One-week after both dosing sessions ($M=12.67$, $SD=10.40$), BDI scores were significantly reduced when compared to baseline ($M=34.33$, $SD=7.44$; $p<0.001$), dosing day 1 ($M=30.53$, $SD=12.36$; $p<0.001$) and dosing day 2 ($M=21.13$, $SD=12.93$; $p=0.009$) scores. This confirms that the patients' depressive symptoms were reduced even further following each dose of psilocybin (Figure 3-2 B).

3.5.3. *Predicting future life-events before psilocybin treatment*

Before proceeding to testing the specific hypotheses of this study, some description of the prediction and event occurrence data is warranted. As the POFLE contains both desirable and undesirable life events, it is most informative to consider the data split by the desirability of these life events. When predicting life events at baseline, the patients gave similar probability estimates for desirable ($M=0.29$, $SD=0.15$) and undesirable life events ($M=0.23$, $SD=0.15$, $t_{(14)}=1.037$, $p=0.317$; 95% CI [-0.07, 0.19]). Contrary to their predictions however, patients reported experiencing more desirable ($M=4.60$, $SD=1.76$) than undesirable events ($M=1.40$, $SD=1.35$, $t_{(14)}=5.967$, $p<0.001$; 95% CI [2.05, 4.35], $g=1.5$) within the ensuing 30-day period after their baseline predictions. This demonstrates that the patients predicted a negative future that was not borne out by their experience.

Control subjects gave higher probability estimates for desirable ($M=0.57$, $SD=0.09$) than undesirable life events ($M=0.22$, $SD=0.16$; $t_{(14)}=8.492$, $p<0.001$; 95% CI [0.26, 0.43], $g=2.0$) at baseline and in line with their predictions, reported the occurrence of more desirable ($M=5.60$, $SD=1.45$) than undesirable ($M=2.33$, $SD=1.84$; $t_{(14)}=4.859$, $p<0.001$; 95% CI [1.8, 4.7], $g=1.2$) events within the ensuing 30 days. This suggests that the controls could realistically forecast the occurrence of future life events – most of which were desirable.

Between-group comparisons revealed that patients predicted significantly less desirable events than control subjects at baseline ($M=0.29$, $SD=0.15$ vs $M=0.57$, $SD=0.09$; $t_{(28)}=5.820$, $p<0.001$; 95% CI [0.17, 0.36], $g=2.2$), but there was no significant difference found between patients ($M=0.23$, $SD=0.15$) and controls ($M=0.22$, $SD=0.16$) in their predictions of undesirable events ($U=108.000$, $p=0.852$). Therefore, despite the occurrence of relatively more desirable than undesirable life events occurring within the ensuing 30-day period, the patients (wrongly) expected an equal number of desirable and undesirable life events and predicted significantly less desirable life events than the control subjects. Both results are consistent with an unrealistic pessimism bias in the patients prior to receipt of psilocybin, in which they were especially prone to underestimate the (true) likelihood of desirable future life events.

There were no significant between-groups differences found in the rates at which desirable (patients: $M=4.60$, $SD=1.76$; controls: $M=5.60$, $SD=1.45$; $U=75.000$, $p=0.113$) and undesirable (patients: $M=1.40$, $SD=1.35$ controls: $M=2.33$, $SD=1.84$; $U=78.500$, $p=0.148$) events occurred within the 30-day period from baseline. These findings reflect that both groups were experiencing similar life circumstances, despite patients predicting a more negative reality.

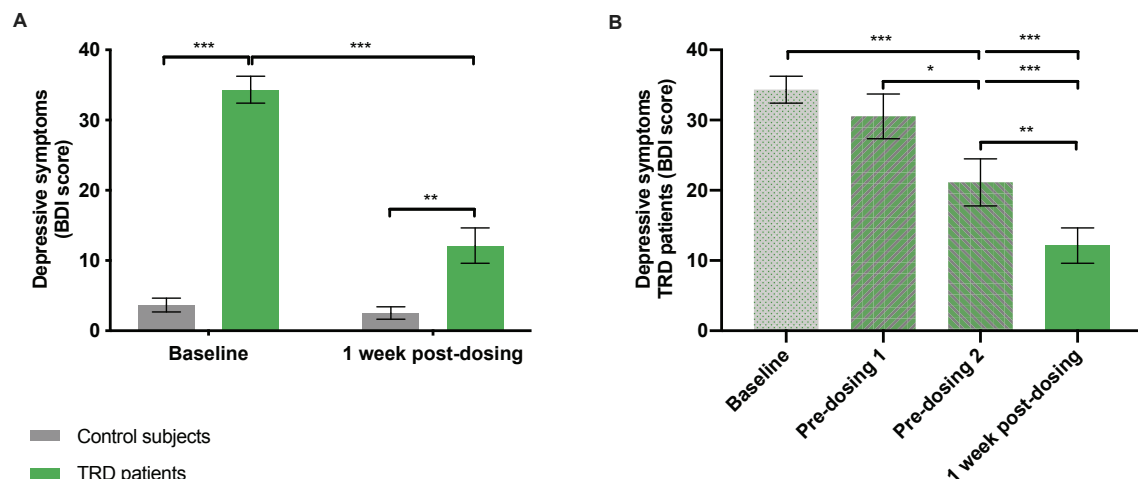


Figure 3-2. Depressive symptoms. (A) Depressive symptoms. Patients had significantly higher BDI scores than controls at baseline ($U=0.0E0$, $p<0.001$; $g=5.0$). The patients' BDI scores were significantly reduced 1 week after psilocybin treatment ($t_{(14)}=7.900$, $p<0.001$; 95% CI [16.17, 28.23], $g=1.9$), but remained significantly greater than the controls ($U=34.50$, $p=0.001$; $g=1.1$). **(B) Depressive symptoms over time in TRD patients.** There was a significant reduction in the patients' BDI scores over time [$\chi^2_{(3, N=15)}=32.35$, $p<0.0001$]. Baseline BDI scores did not significantly differ from scores at the beginning of dosing day 1 ($p=0.944$), confirming that the depressive symptoms had not naturally declined prior to psilocybin treatment. BDI scores were significantly reduced on dosing day 2 when compared with baseline ($p<0.001$) and dosing day 1 ($p=0.033$) scores. One-week after both dosing sessions, BDI scores were significantly reduced when compared to baseline ($p<0.001$), dosing day 1 ($p<0.001$) and dosing day 2 ($p=0.009$) scores. Data expressed as mean $\pm$ SEM ($p<0.05^*$; $p<0.01^{**}$; $p<0.001^{***}$). Note: Time (days) between: (i) baseline and dosing day 1 ($M=35.0$, $SD=23.3$); (ii) dosing day 1 and dosing day 2 ($M=7.9$, $SD=2.6$) (iii) dosing day 2 and one-week post follow-up ($M=7.8$, $SD=2.5$; (iv) baseline and one-week post follow-up ($M=50.8$; $SD=25.3$).

3.5.4. Predicting future life-events after psilocybin-treatment (second 30-day period)

In contrast to their pre-treatment pessimism, post-treatment, patients gave significantly higher probability estimates for desirable ($M=0.44$, $SD=0.20$) than undesirable life events ($M=0.25$, $SD=0.22$, $t_{(14)}=2.322$, $p=0.036$; 95% CI [0.01, 0.35], $g=0.6$). Justifying this difference, the patients also reported a higher percentage of desirable events ($M=3.80$, $SD=2.57$) actually occurring within this (second and post-treatment) 30-day period – relative to undesirable events ($M=1.40$, $SD=1.18$; $t_{(14)}=3.485$, $p=0.004$; 95% CI [0.92, 3.88], $g=1.3$); suggestive of an improved accuracy in predicting future life events (see below for more specific accuracy measures).

As they did at baseline, control subjects gave higher probability estimates for desirable ($M=0.57$, $SD=0.14$) than undesirable ($M=0.20$, $SD=0.12$) life events at the follow-up ($t_{(14)}=8.399$, $p<0.001$; 95% CI [0.28, 0.46], $g=2.0$), and did not differ from baseline in their second predictions of desirable ($t_{(14)}=-0.0001$, $p=1.000$; 95% CI [-0.07, 0.07]) or undesirable events ($t_{(14)}=0.686$, $p=0.504$; 95% CI [-0.06, 0.12]). Consistent with these predictions, control subjects reported the occurrence of more desirable ($M=5.53$, $SD=1.19$) than undesirable ($M=1.80$, $SD=1.78$) events within the ensuing 30 days from follow-up ($t_{(14)}=8.071$, $p<0.001$; 95% CI [2.74, 4.73], $g=2.0$).

Contrary to what was seen at baseline, there were no significant between-groups differences in the predictions of both desirable ($t_{(28)}=1.972$, $p=0.059$; 95% CI [-0.01, 0.25]) and undesirable events ($U=95.000$, $p=0.468$) for the second block of predictions. Further, there were no significant between-groups differences found in the rates at which desirable ($U=70.000$, $p=0.072$) and undesirable ($U=105.000$, $p=0.741$) events actually occurred within the second 30-day period.

In summary, after treatment with psilocybin, consistent with what was actually reported to have happened during the ensuing 30-day period, TRD patients (rightly) expected more desirable than undesirable life events to occur – and thus began to make more accurate predictions of future life events – in line with the behaviour of healthy control subjects.

3.5.5. Accuracy of forecasts

Next, we examined whether there were any changes in the accuracy of participants' forecasts—defined as the magnitude of discrepancy between predictions and actual event occurrence—between baseline and the post-treatment repeat phase. Closely paralleling the above results, patients were significantly more accurate in their predictions post-treatment ($M=0.76$, $SD=0.07$) than at pre-treatment baseline ($M=0.71$, $SD=0.12$; $t_{(14)}=1.857$, $p=0.042$; 95% CI [-0.01, 0.12], $g=0.6$) (Figure 3-3 A).

As predicted, there were no significant changes in accuracy observed in the non-treated healthy control subjects from baseline ($M=0.72$, $SD=0.12$) to the repeat phase ($M=0.74$, $SD=0.09$; $t_{(14)}=0.603$, $p=0.556$; 95% CI [-0.06, 0.09]). There were also no significant between-groups differences found at baseline

($t_{(28)}=-0.196$, $p=0.423$; 95% CI [-0.09, 0.08]) or the follow-up ($t_{(28)}=0.676$, $p=0.252$; 95% CI [-0.04, 0.08]) (Figure 3-3 A).

3.5.6. *Assessing the presence of a pessimism bias in the TRD patients*

Before proceeding to testing specific hypotheses, some description of the computation of bias is warranted. In order to compute cognitive bias, the life events that actually occurred need to be taken into account. Therefore, different base rates for desirable versus undesirable events do not impede the analysis of bias. Bias scores can range from -1 to 1, with a score of 0 indicating that there is neither an optimism nor pessimism bias). The greater the deviation from 0, the greater the pessimism (for negative scores) or optimism (for positive scores) bias.

The mean amount of bias shown by the patients at baseline ($M=-0.10$, $SD=0.12$) was significantly lower than zero; thus confirming a suspected pessimism bias ($t_{(14)}=-3.260$, $p=0.006$; 95% CI [-0.16, -0.03], $g=1.1$). This should be interpreted as consistent with what one what suspect in a study of people with mostly severe depression. Following psilocybin treatment, this pessimism bias was significantly reduced ($M=0.02$, $SD=0.08$; $t_{(14)}=-2.714$, $p=0.017$; 95% CI [-0.21, -0.02], $g=0.7$) to such an extent that the mean score was no longer significantly different from zero ($t_{(14)}=0.768$, $p=0.455$; 95% CI [-0.06, 0.02]) – indicating an effective absence of bias. These results demonstrate that the patients were indeed ‘unjustifiably’ or erroneously pessimistic at baseline and that this cognitive bias was remediated following psilocybin treatment (Figure 3-3 B).

The mean amount of bias for the control subjects was negligible and did not significantly differ from zero at baseline ($M=0.04$, $SD=0.09$, $t_{(14)}=1.823$, $p=0.090$; 95% CI [-0.09, 0.01]) or the repeat phase ($M=0.01$, $SD=0.07$, $t_{(14)}=0.460$, $p=0.653$; 95% CI [-0.05, 0.03]). This demonstrates an effective absence of bias (i.e., no appreciable optimism or pessimism bias) in the control subjects at both baseline and the follow-up (Figure 3-3 B).

Not surprisingly, bias scores were significantly lower (i.e., greater pessimism) for patients ($M=-0.10$, $SD=0.12$) than controls at baseline ($M=0.04$, $SD=0.09$; $t_{(28)}=3.704$, $p<0.001$; 95% CI [0.06, 0.22], $g=1.3$) and it there was no significant difference for the repeat phase ($t_{(28)}=-0.265$, $p=0.793$; 95% CI [-0.07, 0.05]). These results demonstrate that the patients were more pessimistic than the controls at baseline, but this bias was alleviated to control levels following psilocybin treatment (Figure 3-3 B).

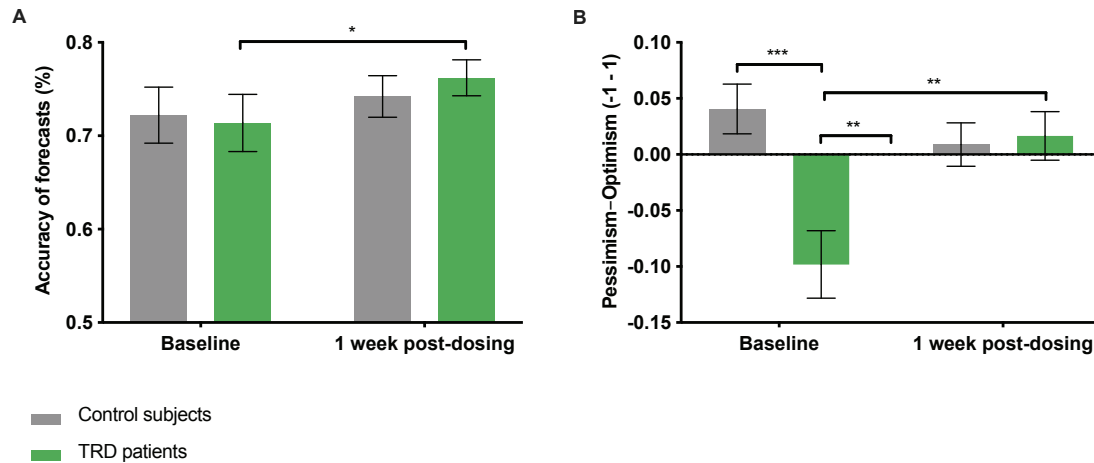


Figure 3-3. Accuracy and biases. (A) Accuracy. Patients were significantly more accurate in their predictions at the follow-up relative to baseline ($t_{(14)}=1.857$, $p=0.042$; 95% CI [-0.01, 0.12], $g=0.6$). There were no significant changes in accuracy for the control subjects ($t_{(14)}=0.603$, $p=0.556$; 95% CI [-0.06, 0.09]). No between-groups differences in accuracy were found at baseline ($t_{(28)}=-0.196$, $p=0.423$) or the follow-up ($t_{(28)}=0.676$, $p=0.252$; 95% CI [-0.04, 0.08]). **(B) Cognitive bias.** The greater the digression from zero on the y-axis, the greater the bias. Patients had a significant pessimism bias at baseline ($t_{(14)}=-3.260$, $p=0.006$; 95% CI [-0.16, -0.03], $g=1.1$). No significant biases were found for the controls ($t_{(14)}=1.823$, $p=0.090$; 95% CI [-0.09, 0.01]). Baseline bias scores were significantly lower (i.e., greater pessimism) for the patients than the controls [$t_{(28)}=3.704$, $p<0.001$; 95% CI [0.06, 0.22], $g=1.3$]. Patients' pessimism bias was significantly reduced ($t_{(14)}=-2.714$, $p=0.017$) and they no longer displayed any biases following psilocybin treatment ($t_{(14)}=0.768$, $p=0.455$; 95% CI [-0.06, 0.02]). No significant biases were found for the controls ($t_{(14)}=0.460$, $p=0.653$; 95% CI [-0.05, 0.03]) and there was no significant between-groups difference ($t_{(28)}=-0.265$, $p=0.793$; 95% CI [-0.07, 0.05]) at the follow-up. Data expressed as mean $\pm$ SEM ($p<0.05^*$; $p<0.01^{**}$; $p<0.001^{***}$). #Only the TRD patients received psilocybin treatment.

3.5.7. Relationship between cognitive biases and depressive symptoms

Next, we investigated any potential relationships between baseline pessimism and depressive symptoms. Before psilocybin, the patients' bias scores were significantly related to their BDI scores ($R_s=-0.55$, $p=0.017$). This finding demonstrates that greater pessimism (i.e., lower bias scores) was associated with more severe depressive symptoms in the patients. No significant relationships were found between BDI and bias scores for the control subjects ($R_s=-0.22$, $p=0.219$) (Figure 3-4 A).

When analysing the magnitude of change in scores between baseline and the follow-up, it was found that the bias ($M=0.11$, $SD=0.16$) and BDI scores ($M=-22.20$, $SD=10.88$) changed significantly more for the patients than the control subjects (bias: $M=-0.03$, $SD=0.73$, $t_{(28)}=-3.167$, $p=0.004$, 95% CI [-0.24, -0.05], $g=1.2$; BDI: $M=-1.13$, $SD=2.99$, $t_{(28)}=7.228$, $p<0.001$, 95% CI [15.09, 27.04], $g=2.6$). The decrease in BDI score was significantly related to the decrease in pessimism for the patients ($R^2=0.57$, $p=0.014$; Figure 3-4 B). There was no significant relationship found between the change in BDI and bias scores ($R_s=-0.007$, $p=0.980$) for the control subjects.

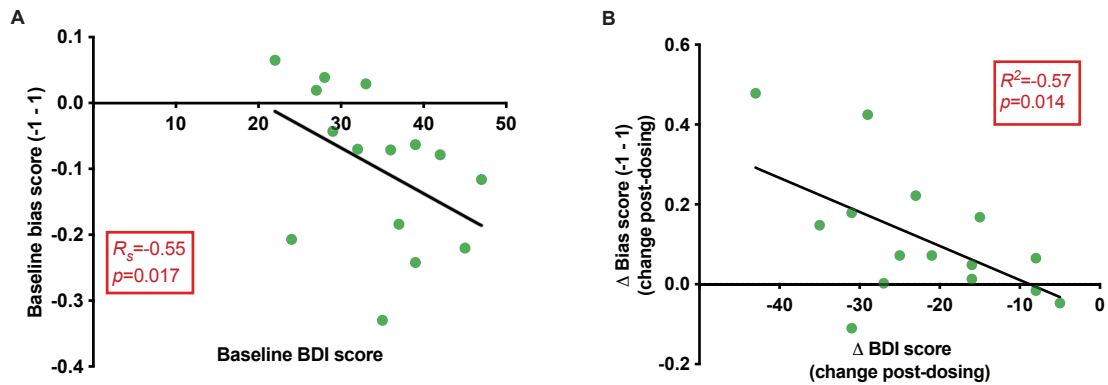


Figure 3-4. Relationship between bias and depression. (A) Relationship between depressive symptoms and bias at baseline. At baseline, the patients' bias scores were moderately and significantly related to their BDI scores ($R_s = -0.55$, $p = 0.017$). No significant relationships were found between BDI and bias scores for the control subjects ($R_s = -0.22$, $p = 0.219$). **(B) Relationship between the change in depressive symptoms and bias.** Bias and BDI scores changed significantly more for the patients than the control subjects' bias ($t_{(28)} = -3.167$, $p = 0.004$; 95% CI [-0.24, -0.05], $g = 1.2$) and BDI scores ($t_{(28)} = 7.228$, $p < 0.001$; 95% CI [15.09, 27.04], $g = 2.6$). The decrease in BDI score was significantly related to the decrease in pessimism for the patients ($R^2 = -0.57$, $p = 0.014$). There was no significant relationship found between the change in BDI and bias scores ($R_s = -0.007$, $p = 0.980$) for the control subjects.

3.6. DISCUSSION

The present study sought to investigate the effects of psilocybin on pessimism bias in patients with TRD. Before treatment with psilocybin, patients were excessively and unrealistically pessimistic when predicting the occurrence of future life events – and this pessimism was significantly correlated with the severity of their depressive symptoms. One week after treatment, patients' pessimism was alleviated and their depressive symptoms greatly improved; moreover, the magnitude of change in both variables was related – such that as their depression improved, so did their ability to accurately forecast their future. No such bias nor change in forecasting was seen in a matched control group assessed over an equivalent time period. Taken together, these findings indicate that the psychologically supportive administration of psilocybin remediates negative cognitive biases characteristic of severe depression – enabling individuals to forecast their futures more accurately, unfettered by unrealistic pessimism.

3.6.1. *Depressive symptoms*

The alleviation of depressive symptoms one week after psilocybin treatment found here is consistent with previous research demonstrating the therapeutic potential of psychedelic compounds (see Carhart-Harris and Goodwin, 2017; Andersen et al., 2020: for reviews). A single dose of the natural psychedelic brew prepared from Amazonian plants, ayahuasca, had rapid and sustained antidepressant effects in patients with recurrent MDD (Osorio Fde et al., 2015). Likewise, a single dose of psilocybin has been shown to induce long-lasting reductions in anxiety and depression as well as increases in quality of life, life meaning, and optimism in patients suffering from depression and anxiety reactive to advanced-stage cancer (Griffiths et al., 2016; Ross et al., 2016; Grob et al., 2011). A recent RCT provides further support for the therapeutic potential of psilocybin, whereby rapid antidepressant effects were sustained for up to four weeks in 71% of MDD patients (Davis et al., 2020). Moreover, enduring improvements in psychological well-being (Griffiths et al., 2008), trait openness and optimism (Carhart-Harris et al., 2016b; MacLean et al., 2011) have also been observed in healthy participants following a single dose of a psychedelic drug.

The pharmacological action of psilocybin might help to explain the reductions in depressive symptoms found here, as well as the positive clinical outcomes found in the studies outlined above⁹. There is a strong interaction between the stress systems and monoamine neurocircuitries involved in mood disorders, including the serotonin system (Duman et al., 2016; Zhang et al., 2013; Hosang et al., 2014; Ota et al., 2014). The 5-HT_{2A}R – the key receptor through which psychedelics elicit their signature effects (Glennon et al., 1984; Nichols, 2017) – is centrally involved in affect regulation and is an important drug target in the treatment of various psychiatric conditions, including MDD (Carhart-Harris et al., 2016a; Carhart-Harris et al., 2017b; Carhart-Harris and Goodwin, 2017; Carhart-Harris and Nutt,

⁹ See Chapter 2: INTRODUCTION, Section 2.2: PHARMACOLOGY OF PSYCHEDELICS.

2017; Ross et al., 2016). It has been proposed that the stress-relieving functions of 5-HT_{1A} receptor signalling combined with 5-HT_{2A}-receptor-mediated flexibility of mind and facilitation of plasticity, under favourable conditions (i.e. context-sensitive), is conducive to positive psychological health (Carhart-Harris and Nutt, 2017). To this end, by enhancing environmental sensitivity and adaptability, 5-HT_{2A}R signalling has been proposed as a crucial mediator of change processes, particularly in conditions of uncertainty or crisis (e.g. Matias et al., 2017). The ability of psychedelics to hijack 5-HT_{2A}R-mediated signalling is in stark contrast to the 5-HT_{1A}R-mediated 'passive coping' mechanisms slowly modulated by the chronic use of conventional antidepressants, and may account for the wealth of evidence demonstrating rapid and enduring post-psychedelic outcomes in this population.

Psychedelics produce profound psychological effects and a principal theory in current psychedelic research is that the quality of the acute experience mediates the long-term beneficial effects of these drugs (Yaden and Griffiths, 2020; Roseman et al., 2017). Consistent with this principle, blockade of the 5-HT_{2A}R not only blocks the subjective effects, but also the mood-improving effects of psychedelics in humans (Preller et al., 2017; Komater et al., 2012). Further supporting evidence stems from a separate study using the same sample of TRD patients reported here, which found that insightfulness during the psilocybin experience predicted subsequent reductions in depressive symptoms up to five week post-dosing (Carhart-Harris et al., 2017a). As such, the key role placed on subjective experience makes psychedelic therapy constitute a very novel treatment paradigm when compared to the conventional pharmacological management of mental health conditions. The potential importance of psychological insight and relationship with neural and psychological outcomes are explored in a later chapter of this thesis (see Chapter 4, STUDY 2).

3.6.2. Cognitive biases and accuracy of predictions

As predicted by the cognitive-bias model of depression (Beck, 1967; Beck, 1976), we found a significant pessimism bias in the TRD patients when predicting the likelihood of future events prior to psilocybin treatment. Consistent with the depressive bias hypothesis—i.e. the theory that as depressive symptoms increase in severity, judgments become more negatively biased (Beck, 1976)—we found greater pessimism among patients with more severe depressive symptoms. This is in line with previous studies demonstrating a relationship between depression and pessimism (Beck et al., 1988; Hill et al., 1989; Hollon et al., 1986; Peterson and Seligman, 1984). It also opens up the possibility of using pessimism in healthy samples as a proxy of depression in clinically depressed samples. The finding here that patients were unrealistically pessimistic about the likelihood of positive life events befalling them prior to psilocybin treatment appeared not to be entirely justified by poor life circumstances, as the patients' predictions (e.g., of the likelihood of desirable life events) underestimated their actual rate of occurrence. Moreover, the rates at which desirable and undesirable events actually occurred did not differ from the control subjects, who lacked such biases. This is consistent with classic depression research demonstrating that depressed patients present with excessively negative perspectives that

are disproportionate to their true life circumstances (Beck, 1967; Strunk and Adler, 2009; Strunk et al., 2006).

One week after psilocybin treatment, the patients' pessimism was alleviated and they no longer showed any cognitive biases. In line with this finding, previous research has demonstrated sustained increases in optimism following psilocybin treatment in patients suffering from depression and anxiety reactive to advanced-stage cancer (Griffiths et al., 2016; Ross et al., 2016; Grob et al., 2011). Long-lasting improvements in optimism have also been observed in healthy participants following a single dose of a psychedelic (Carhart-Harris et al., 2016b; MacLean et al., 2011) and there is also evidence that recreational psychedelic users are more optimistic (or less pessimistic) than non-users (Grob et al., 1996). Psychedelic compounds have previously been shown to increase positive mood (Kraehenmann et al., 2015; Schmid et al., 2015) and often induce lasting changes in attitudes and behaviour associated with a more positive outlook (Griffiths et al., 2008; Griffiths et al., 2006; McGlothlin and Arnold, 1971; Savage, 1962; Studerus et al., 2011). Here, we found that patients predicted significantly more desirable events post-treatment; making more accurate predictions that better reflected their actual life situation. No cognitive biases were found among the control subjects. The lack of cognitive bias in the controls found here is in contrast to previous research suggesting that healthy individuals have a tendency towards an optimism bias (Sharot et al., 2007; Taylor and Brown, 1988; Weinstein, 1989).

Evidence suggests that healthy people are worse at incorporating information about a more negative than expected future, and instead are more likely to update their beliefs in response to better-than-expected information, resulting in an unrealistic optimism (Garrett and Sharot, 2017; Garrett et al., 2014; Izuma and Adolphs, 2011; Korn et al., 2014; Sharot et al., 2011). It has been shown that this unrealistic optimism is maintained due to a selective update failure and the diminished neural coding of undesirable information (Sharot et al., 2011). Dismissing and/or denying undesirable information may serve an adaptive function by enhancing exploratory behaviour and reducing stress and anxiety (Scheier et al., 1989; Taylor et al., 2000; Varki, 2009). This is in line with research showing a relationship between mild depression and accuracy in predictions, with more severe depression being associated with clear pessimism (Strunk et al., 2006). However, an unrealistic optimism has drawbacks as well, e.g. in terms of underestimating risks and so failing to adopt preventative and/or protective measures (Shepperd et al., 2013).

This idea that optimism is conducive to positive mental health has been critiqued recently however, e.g. with arguments made that improved methodologies are required to elucidate such biases and an accurate perception of reality is most important for mental health (Colvin and Block, 1994; Shah et al., 2016) – see also Letheby (2016) in specific relation to the psychedelic experience. Entertaining worst-case scenarios when anticipating future events may help one's ability to cope with negative outcomes, through contingency plans and preparedness. In this sense, there are benefits of having a pessimistic attitude and expecting an undesirable outcome when facing events of unknown emotional impact (Nesse, 2000), such as not engaging in risky behaviour (Gibson and Sanbonmatsu, 2004) and avoiding

disappointment by setting low expectations (Norem and Cantor, 1986; Shepperd and McNulty, 2002). However, an unrealistic pessimism about the future also represents a key cognitive feature in MDD (Lavender and Watkins, 2004; Pyszczynski et al., 1987), as expressed in the cognitive triad (Beck, 1967). It follows that a balanced, reality-focused perspective is optimal for mental health – as unrealistic optimism may encourage negligent and reckless behaviour that could result in catastrophe, and unrealistic pessimism may lead to avoidant behaviour, passivity, low mood, vulnerability to MDD and perhaps related disorders (Hecht, 2013).

The neurobiology of optimism versus pessimism is poorly understood. It is well known however, that serotonergic mechanisms are involved in mood states and cognitive styles (Cowen and Sherwood, 2013; Meneses, 1999; Young and Leyton, 2002); for example, 5-HT_{2A}Rs are upregulated during states of low synaptic 5-HT (Cahir et al., 2007) as well as in unmedicated MDD patients (Bhagwagar et al., 2006) and individuals scoring high on neuroticism (Frokjaer et al., 2008; Frokjaer et al., 2010) and dysfunctional attitudes (Meyer et al., 2003) – an analogue of trait pessimism. Through their serotonergic mechanism of action (i.e. agonism at the 5-HT_{2A}R), it is theorised that psychedelics induce an acute state of heightened entropy that may enable disruption at the level of the system that encodes the precision of priors, beliefs, or assumptions (Carhart-Harris and Friston, 2019). The ability of psychedelics to disrupt reinforced patterns of negative thought and behaviour by disintegrating the patterns of brain activity upon which they rest may account for their positive therapeutic effects (Carhart-Harris et al., 2014; Watts et al., 2017). Psychedelics have been shown to temporarily deconstruct the DMN (Carhart-Harris et al., 2012a; Palhano-Fontes et al., 2015; Speth et al., 2016), a network in the brain associated with ruminative thought (Berman et al., 2011), self-reflection (Johnson et al., 2002) and introspection more generally (Fleming et al., 2010; Qin and Northoff, 2011) – although the DMN appears to recover its integrity as the effects of a psychedelic wear off, with a potential increase in integration post treatment with psilocybin for TRD (Watts et al., 2017). We hope to explore the relationship between DMN properties before, during and after psilocybin in future studies and assess its relationship to optimism/pessimism. A functional imaging paradigm that incorporates the actual forecasting of future life events (prospection) might be interesting to explore in this context.

3.6.3. Methodological strengths

The results reported here substantially extend findings of previous research demonstrating increased optimism following psychedelic drug use (Barbosa et al., 2009; Carhart-Harris et al., 2016b; Griffiths et al., 2008; Griffiths et al., 2011). Previous studies of psychedelics-induced optimism have used subjective measures, whereas the present study utilised a behavioural instrument to more objectively measure optimism/pessimism. By analysing the effects of psilocybin on the prediction of one's personal future, this study addresses an important facet of the cognitive triad, i.e., a negative attitude towards oneself, the environment and the future. To our knowledge, this is the first study to analyse the effects of a psychedelic compound on cognitive biases in patients with TRD and also the first to address psychedelic-induced changes in biases that are not purely subjective and self-referential, but instead

feature a behavioural aspect, requiring the predictions of future life events – the accuracy of which can later be assessed based on what is reported to have actually occurred in the individual's life.

3.6.4. Limitations

It is necessary to address some limitations of this study. It formed part of an open-label clinical trial (Carhart-Harris et al., 2016a) with a small sample size, and although a control group was recruited to examine test re-test reliability on these measures, the controls were healthy subjects not exposed to the same treatment procedures. Since treatment with psilocybin involved more than just drug administration (e.g., psychological support before and after the psilocybin dosing sessions), it is possible that some drug-unrelated factor(s) contributed to the changes in cognitive bias observed here. It should be emphasised however, that such non-drug components are considered an integral part of the therapeutic model and cannot easily be extricated from it without collapsing the model itself (e.g. see Carhart-Harris et al., 2018). It is also unclear whether the reported changes in cognitive bias found here following psilocybin treatment were selective for these outcomes or rather an epiphenomenon of the treatment's core effects on depressive symptoms. It might be that cognitive biases are an inextricable part of depression and so an impact on one of these factors will necessarily affect the other. Additionally, although the use of change scores can be advantageous and were deemed most appropriate for the regression analyses, the role of regression to the mean must also be considered when interpreting the correlations found here (e.g. see Allison, 1990).

3.6.5. Conclusions

In conclusion, this study sought to investigate the effects of psilocybin with psychological support on the prediction of future life events in patients with treatment-resistant depression. To our knowledge, this is the first study using a behavioural measure to objectively address cognitive biases integral to depression and how these change post-treatment with a psychedelic. The present findings suggest that the psilocybin with psychological support treatment model may alleviate pessimism bias in depression, giving patients a clearer, more accurate outlook on their future. Further controlled studies are warranted to better determine the causality, reliability, specificity and durability of these findings.

4. STUDY 2 |

What are the acute and long term effects of psilocybin in healthy participants?

“A little bit of something beats a lot of nothing. Break the largest of difficult tasks into the smallest of steps and it can be done.”

– Dan Millman

4.1. FOREWORD

The findings presented in the previous chapter (STUDY 1) suggest that the psychologically supportive administration of psilocybin remediates negative cognitive biases characteristic of severe depression. The previous chapter therefore provides evidence for a rapid, marked and enduring change in an entrenched pathological belief after a psychedelic experience. If we remind ourselves of REBUS (see Carhart-Harris and Friston, 2019), the model would theorise that the presence of a pessimism bias indicates excessive precision, weight, or influence of this prior in TRD. Moreover, alleviation of this negative bias following psilocybin therapy indicates that the treatment has induced belief relaxation and recalibration processes, enabling belief revision.

If psychedelics facilitate the relaxation of prior assumptions, does this result in heightened openness and receptivity to new perspectives (i.e., the precondition for psychological insight)? The proposition that the relaxation of high-level prior assumptions facilitates psychological insight suggests that insight-related processes operate without conscious awareness; this implies that insight-related processes are more effective following the suspension of executive control. The ability of psychedelics to facilitate psychological insight is a central principle of psychedelic therapy and is largely based on the notion that psychedelic (“mind-manifesting”) drugs permit latent or unconscious aspects of the mind to rise into conscious awareness. Chapter 3 discussed my experiments using a sample of TRD patients recruited for a larger open-label pilot study. Interestingly, the larger pilot study found that insightfulness during the psilocybin experience predicted subsequent long-lasting clinical improvements the same sample of TRD patients (see Carhart-Harris et al., 2017a).

Psychedelics have been shown to acutely increase signal complexity of spontaneous brain activity (Alonso et al., 2015; Lebedev et al., 2016; Scharfner et al., 2017; Viol et al., 2017), which might reflect a major phase of the process through which insight occurs. In this phase, reduced confidence in high-level priors may result in novel perspectives by disinhibiting latent (lower-level) contents of the mind and allowing them to emerge into conscious awareness (Carhart-Harris and Friston, 2019). As such, this may enable the cultivation of changes to the relevant assumptions instantiated by higher levels in the hierarchy (i.e., model refinement). This is in line with a key concept of the REBUS framework, Bayesian Model Reduction (BMR), which is a hypothesised mechanism of model optimisation through which redundant parameters are pruned in order to refine the model (Carhart-Harris and Friston, 2019). In this way, pruning away unnecessary details may allow one to gain an understanding beyond what was immediately apparent (i.e., the bigger picture). To summarise, the REBUS model (Carhart-Harris and Friston, 2019) theorises that increased brain signal complexity with relaxed high-level priors enables the brain to be more amenable to curiosity and BMR processes. This may result in the facilitation of psychological insight and the attainment of novel perspectives, which may ultimately mediate long-term outcomes. In order to test this principle, a structured assessment of psychological insight and how it relates to brain signal complexity and long-term outcomes is required.

In this chapter, I will present my thorough investigation of the acute and enduring effects of psilocybin on the brain and psychology of healthy participants. I include a relatively varied and extensive assessment of psilocybin-induced psychological insight, both in the acute state as well as in the long-term. Moreover, I perform resting-state EEG recordings during the acute drug state to measure psilocybin-induced alterations in brain signal complexity. Psychological outcomes are assessed throughout and potential enduring post-psilocybin changes in the brain are explored.

4.2. INTRODUCTION

Psychedelic (literally translated as “mind-revealing”) drugs possess a unique potential as scientific tools to study the brain and demonstrate great therapeutic potential. One such drug includes psilocybin, a naturally occurring psychedelic derived from the *Psilocybe* genus of mushrooms. A growing body of evidence suggests that as little as one single high dose of psilocybin can have safe and long-lasting positive psychological effects on healthy individuals and patient populations (Carhart-Harris et al., 2016a; Carhart-Harris et al., 2017a; Carhart-Harris and Goodwin, 2017; MacLean et al., 2011; Griffiths et al., 2011). Moreover, psilocybin and related psychedelics can acutely occasion peak experience phenomena that may modulate some of these enduring outcomes (Griffiths et al., 2011; Roseman et al., 2018b; Carhart-Harris et al., 2017a; Haijen et al., 2018; Russ et al., 2019). A model of psychedelic drug action, REBUS, has recently been developed to account for the psychological and neurological effects of psychedelics, both acutely and in the long-term (Carhart-Harris and Friston, 2019). Science is currently gaining an initial understanding of the acute brain action of psilocybin and related psychedelic drugs (Carhart-Harris et al., 2012a; Carhart-Harris et al., 2013; Carhart-Harris et al., 2012b; Carhart-Harris et al., 2016c; Carhart-Harris et al., 2017d; Vollenweider et al., 1997b; Roseman et al., 2016; Lebedev et al., 2016; Lebedev et al., 2015; Muthukumaraswamy et al., 2013; Tagliazucchi et al., 2014; Tagliazucchi et al., 2016), but their potential long-term brain effects are not as well studied nor understood. Accordingly, testing the principles put forward by REBUS and determining the long-term brain effects of psychedelics, as well as the neural correlates of long-term psychological outcomes that they can induce, will help fill this knowledge gap and would hold significant value.

4.2.1. *The essential importance of context*

The REBUS model proposes that psychedelics facilitate the relaxation of prior assumptions such that they become more sensitive to context and amenable to revision (Carhart-Harris and Friston, 2019). This is supported by evidence showing that psychedelics heighten one’s sensitivity to the internal (set) and external (setting) environment (Carhart-Harris et al., 2018). As such, evidence suggests that contextual factors (i.e. set and setting) are crucial determinants of the quality of a psychedelic experience. For instance, trait absorption – defined as how easily and deeply an individual can be immersed in stimuli and/or experiences – has been shown to predict psychedelic-induced challenging experiences (Haijen et al., 2018), visual effects, insightfulness, and mystical-type experiences during the acute drug state (Studerus et al., 2012; Haijen et al., 2018; Russ et al., 2019). Moreover, positive prior expectations have been found to predict more positive experiences under a psychedelic (Metzner et al., 1965), whereas pre-dosing anxiety can reduce the likelihood of having a “peak experience” (Richards et al., 1977) and has been shown to predict negative experiences during a psychedelic session (Metzner et al., 1965). A direct association between state anxiety and therapeutic alliance has been shown previously in non-psychedelic research (Kim et al., 2015; Mundorf et al., 2017). Although not yet demonstrated in psychedelic research, therapeutic alliance is known to be a major predictor of

response in mental healthcare (Kazdin, 2007) and its influence is likely to be greater still in the context of psychedelics. It makes sense that successfully building rapport and trust allows the participant to foster feelings of security, ultimately resulting in a reduction of pre-dosing anxiety. Therefore, it's very likely that the interpersonal environment created by the study guides ("sitters") is a major determinant of one's response to a psychedelic drug (Masters and Houston, 1966; Leary et al., 1964; Johnson et al., 2008) and should be investigated in this context.

4.2.2. Importance of the quality of the acute experience

Through their serotonergic mechanism of action (i.e. agonism at the 5-HT_{2A}R), psychedelics produce profound effects on consciousness and alter perception, cognition and mood in a characteristic way. A principal theory in current psychedelic research is that the nature and quality of the acute psychedelic experience mediates the long-term beneficial effects of these drugs. Indeed, the psychedelic occasioned peak (mystical-type) experience¹⁰ has been likened to a 'quantum change experience' (Griffiths et al., 2018), which describes a deeply profound experience that brings about enduring behavioural, emotional and cognitive changes (Miller, 2004). In naturalistic settings, the magnitude of psychedelic peak experience was predictive of sustained increases in well-being (Russ et al., 2019; Barrett et al., 2015; Haijen et al., 2018). This has been also been demonstrated in patients with TRD, where the occurrence and intensity of oceanic boundlessness was associated with markedly better clinical outcomes five weeks after psilocybin treatment (Roseman et al., 2018b). Moreover, a positive association has been identified between the extent of ego-dissolution experienced and changes in nature-relatedness (Nour et al., 2017). In controlled studies with healthy participants, the positive and enduring effects of psilocybin and LSD on subjective well-being, life satisfaction and shifts in perspective have been attributed to the psychedelic experience for up to 14-months (Griffiths et al., 2008; Griffiths et al., 2011; Schmid and Liechti, 2017; Nicholas et al., 2018). Despite evidence demonstrating long-term psychological effects after a psychedelic experience, potential enduring changes in the brain have not been sufficiently investigated and the purported influence of acute psychedelic experiential components on these potential long-term brain effects has not been studied to date.

4.2.3. Complexity of the psychedelic brain and psychological insight

Entropy is a measure of a system's randomness and is therefore an index of uncertainty. The higher the entropy (uncertainty), the higher the diversity of the signal (Spiegelhalter et al., 2002; Friston, 2010; Crutchfield, 2012). The idea that the human brain acts in an entropic fashion under the influence of psychedelics is a relatively novel theory (Carhart-Harris, 2018; Carhart-Harris et al., 2014; Carhart-Harris and Friston, 2019) that may further our understanding of consciousness itself in addition to

¹⁰ The term "mystical-type experience" is based on Walter Stace's work on mysticism (Stace, 1960), and is synonymous with the term "peak experience" derived from the work of Abraham Maslow (Maslow, 1959, 1964).

psychedelic drug action. The measures listed below are all formally related to entropy, but will be referred to more generally as measures of complexity.

Intriguingly, evidence suggests that brain signal complexity is heightened during the psychedelic state (Alonso et al., 2015; Lebedev et al., 2016; Schartner et al., 2017; Viol et al., 2017). Certain complexity-related measures appear to be particularly informative about the quality of conscious states. For instance, brain signal complexity is reduced in disorders of consciousness, such as the vegetative state (Sitt et al., 2014), as well as when under general anaesthesia (Schartner et al., 2015). In healthy individuals, brain complexity is lowest during deep sleep but at equivalent levels in rapid eye movement (REM) sleep and at wake (Casali et al., 2013). Taken together, this evidence indicates that the complexity (entropy) of brain dynamics is tightly linked to the manifestation of consciousness experience. This suggests that acute psychedelic effects – characterised by entropic brain activity and profound effects on states of consciousness – may reflect a state of enhanced conscious content (Carhart-Harris, 2018; Carhart-Harris et al., 2014) and provides support for a multidimensional conception of conscious states (Bayne and Carter, 2018). Based on their ability to increase brain complexity, psychedelics have been proposed as a potential treatment to increase conscious content in patients with disorders of consciousness (Scott and Carhart-Harris, 2019) – however, this has yet to be tested. As acute changes in consciousness can predict therapeutic outcomes in the long-term after a psychedelic experience (Griffiths et al., 2011; Roseman et al., 2018b; Carhart-Harris et al., 2017a), deepening our understanding of the entropic brain, which may be regarded as a neural proxy for conscious state, may aid with the interpretation of post-psychedelic outcomes.

The REBUS model (Carhart-Harris and Friston, 2019) theorises that an entropic psychedelic brain reduces confidence in high-level priors¹¹. In this way, novel perspectives may be obtained through the disinhibition of latent contents of the mind in lower levels of the hierarchy, thus allowing them to emerge into conscious awareness – this notion represents a key principle of psychedelic therapy, in which psychedelics are literally defined as “mind-manifesting”. To this end, the REBUS model proposes that increased brain signal complexity (entropy) with relaxed high-level priors enables the brain to be more amenable to curiosity and BMR processes, which may reflect a mechanism through which psychological insight occurs (Carhart-Harris and Friston, 2019). Psychological insight may be defined as the procurement of novel perspectives, thoughts or realisations about one’s self, behaviour or assumptions – and is therefore characterised by novelty. Evidence of an association between brain complexity and novel experiences provides support for the REBUS model. For instance, increased complexity has been linked to states of improvisation – a term that suggests a note of uncertainty and diversity – during music playing (Dolan et al., 2018). Relatedly, increased brain complexity in the LSD state was found to predict increases trait openness two weeks later (Lebedev et al., 2016). As openness is an attribute characterised by cognitive exploration and is linked to creativity, aesthetic sensitivity,

¹¹ Hierarchical predictive coding posits that the brain generates mental models that predict upcoming sensory inputs. These predictive models are called “priors” (i.e., “prior beliefs.”) and are layered in a hierarchy. Higher levels send predictions down the hierarchy and lower levels report sensory information upwards. When top-down predictions don’t match the bottom-up sensory input, the model either updates its priors based on the new information or ignores this information and maintains its priors.

unconventionality, imagination and intellectual curiosity (McCrae and Costa, 1997), it may be regarded as a set of traits characterised by an interest in novel experiences. Taken together, it seems plausible to suggest that insight during the psychedelic state might be linked to increased brain signal complexity.

It is well-established that insight can occur acutely in a state-like fashion during a psychedelic experience and recreational users report that gaining greater insight into the human condition is a common intention for engaging in psychedelic use (Passie and Brandt, 2018). Moreover, the facilitation of psychological insight is among the most common benefits of psilocybin use reported by recreational users (Carhart-Harris and Nutt, 2013). This is particularly important in the therapeutic setting given that increased insight is associated with improved life satisfaction and well-being in healthy participants (Harrington and Loffredo, 2010) as well as decreased depression and anxiety in patient populations (Silvia and Phillips, 2011; Grant et al., 2002). In a recent study, 74% of alcoholics rated their psychedelic experience as one of the top 10 most insightful experiences of their lives and attributed their positive clinical outcomes to this insight (Garcia-Romeu et al., 2019). Based on this finding, the authors suggest that psychological insight may represent an important mechanism of behavioural change following a psychedelic experience (Garcia-Romeu et al., 2019). In TRD patients, increases in insightfulness during the acute treatment period predicted decreases in depressive symptoms and positive changes in personality in the long-term (Carhart-Harris et al., 2017a; Erritzoe et al., 2018). Furthermore, significantly increased insightfulness has been observed at 360 minutes post-dose psilocybin (Kometer et al., 2015), but whether this persists is unknown. It is important to note that most studies to date have used a subscale of the 11D-ASC questionnaire to measure insightfulness, which consists of only three statements relating to insight and may be insufficient to measure insight in its entirety. To address this issue, a recent study conducted a more extensive assessment of psychedelic-induced insight, albeit only acutely and not followed up long-term (Carbonaro et al., 2018). In order to test the ideas put forth by the REBUS model, a structured assessment of psychological insight and how it relates to brain signal complexity and long-term outcomes is required, yet has not been studied to date.

4.2.4. Well-being and connectedness

Psychedelics are known to produce a myriad of positive psychological effects in the long-term. For instance, sustained increases in well-being lasting over a year have been observed following a single high-dose psilocybin experience (e.g. Griffiths et al., 2006; Griffiths et al., 2008; Griffiths et al., 2011). While definitions of well-being vary, there is little debate as to whether this psychological construct is a crucial factor related to mental and physical health (Pressman et al., 2013) and the importance of well-being is becoming increasingly recognised. This has led to rapid expansion of the field of positive psychology, with a major shift in focus toward promoting psychological well-being as opposed to only treating mental illness (Slade, 2010). There are many intimately linked factors that influence an individual's well-being and mental health, such as the degree of flourishing, felt presence of meaning in life, psychological flexibility (i.e. reduced experiential avoidance) and connectedness to nature and others. In a recent study by our group, TRD patients were asked to describe their experience of

psilocybin-assisted therapy. The major theme identified through thematic analysis was that of 'connectedness', or the process change of disconnection to connection, which patients believed to be modulating the positive clinical outcomes that they had experienced (Watts et al., 2017). As such, *connectedness* – defined as an empathic and embodied sense of closeness to self, others and world/universe – has been posited as a psychological mechanism by which psilocybin exerts positive psychological effects (Carhart-Harris et al., 2017c).

Social connectedness

The construct 'social connectedness' is used to describe a variety of self-other experiences, which overall reflect an individual's subjective interconnection and closeness with others (Lee and Robbins, 1995). It is well-established that humans have a deep-seated psychological need to be connected to others and possess a sense of 'belongingness'. This sense of belongingness arises from social relationships and is considered to act as a psychological buffer that protects mental health during times of stress (Haslam et al., 2012; Baumeister and Leary, 1995; Praherso et al., 2017). Indeed, empirical evidence supports such theories; self-reports of individuals' perceived social connectedness, as measured by assessing perceived social support, and quality of social relationships, are strongly related to psychological well-being (Yu et al., 2015; Patterson and Veenstra, 2010). These subjective appraisals of individuals' social connectedness are more closely linked with mental health than objective components, such as regularity of social participation, living alone and marital status (Berry and Welsh, 2010; Holt-Lunstad et al., 2010). This highlights the importance of the experiential quality and one's perception of social interactions and relationships in mediating well-being. As such the subjective sense of social connectedness has been conceptualised as "*the 'active ingredient' in social relationships*" (Saeri et al., 2018).

Psychedelics have been associated with a range of acute and enduring prosocial effects and can profoundly alter the way one views others (e.g. Griffiths et al., 2018; Pokorny et al., 2017). Empathy – defined as the ability to feel or understand the emotions of others – has been shown to promote prosocial behaviour (Eisenberg and Miller, 1987) and can be categorised as emotional (sharing the emotion of another individual) or cognitive (understanding the emotion). Emotional empathy, but not cognitive, has been shown to significantly increase during the acute phase of psilocybin effects (Pokorny et al., 2017). Relatedly, LSD has been found to acutely increase feelings of trust, emotional empathy and closeness to others (Schmid et al., 2015; Dolder et al., 2016), with self-reported altruistic social effects lasting up to 12-months after the experience (Schmid and Liechti, 2017). It's possible that acute increases in emotional empathy may endure following a psilocybin experience, but this has not yet been sufficiently investigated.

Compassion is defined as a feeling of concern for others, particularly those who are suffering, with an orientation toward supporting and understanding; on the other hand, self-compassion is extending these feelings to one's self in instances of perceived inadequacy, failure or general suffering. Notably,

elevated self-compassion correlates with lowered depression and anxiety scores (Neff, 2003). Ayahuasca has been demonstrated to promote self-compassion 24-hours after ingestion in an effect known as the 'afterglow', but this is reduced to baseline before two months have elapsed (Sampedro et al., 2017). Unfortunately, no measurements of self-compassion were taken between 24-hours and two months post-dosing, making it difficult to determine the length of time this effect endured. Furthermore, after administration of two high doses of psilocybin (25-40 mg each), self-compassion peaked one week after the second dose and persisted for up to a year (Bogenschutz et al., 2018). Nevertheless, this was only shown in three case studies making it difficult to generalise these results. Moreover, previous studies have focused primarily on self-compassion rather than on compassion as a whole. Taken together, current research suggests that compassion for oneself and possibly others may persist following a psychedelic experience. Despite this, long-term changes in compassion following a single high dose of psilocybin administration have not yet been measured.

Relatedly, gratitude is defined as a feeling of thankfulness or gratefulness and is regarded as an outwardly directed, prosocial behaviour that is important for building relationships (McCullough et al., 2002). An increase in gratitude has been linked to decreases in depressive behaviour as a result of positive emotional reframing (Lambert et al., 2012). Interestingly, increased gratitude has been demonstrated to persist for up to six months in patients given a repeated high dose of psilocybin (Griffiths et al., 2018). Such long persistence may be attributable to multiple dosing regimens, but further research is warranted to observe if a single high dose of psilocybin has similar, long-lasting effects.

In summary, it is clear from clinical research that psilocybin has the potential to induce a variety of prosocial effects – such as increased empathy, compassion, gratitude and social connectedness in general. However, little research has investigated the long-term effects of psilocybin on these measures to date. In order to gain a more comprehensive understanding of the enduring effects of psilocybin, it is important to explore how this drug mediates long-term psychological measures such as these.

Nature connectedness

Nature connectedness is a term used to describe an individual's sense of oneness with the natural world (Mayer and Frantz, 2004). Both trait and state nature connectedness, are intimately linked with enhanced physical health, psychological and social well-being, and positively correlated with mindfulness (Cervinka et al., 2012; Howell et al., 2011; Mayer and Frantz, 2004; Nisbet et al., 2011; Capaldi et al., 2014; Dean et al., 2018). State nature connectedness refers to the extent to which connection to nature is presently felt, which may be mediated by present surroundings. Spending time in nature has been associated with improved general health and enhanced hedonic well-being, including increased life satisfaction, positive affect and lower levels of anxiety (Nisbet and Zelenski, 2011; Nisbet et al., 2011; Diener, 2009; Mayer et al., 2008; Capaldi et al., 2014; White et al., 2019). At the trait level, nature connectedness is defined as the stable sense of oneness and interconnectedness with the natural world (Mayer and Frantz, 2004) and is predictive of environmental concern and pro-

environmental behaviours (Schultz et al., 2004). Higher levels of trait nature connectedness are associated with greater eudaimonic well-being and social functioning (Pritchard et al., 2019). Moreover, there is evidence indicating that the relationship between nature connectedness and psychological well-being is mediated by enhanced meaning in life, which is posited to arise from the sense that one is part of something greater and more enduring than oneself (Howell et al., 2013).

Psychedelic use in naturalistic settings has been associated with increased nature relatedness, concern for the environment and pro-environmental behaviour (Forstmann and Sagioglou, 2017; Nour et al., 2017; Kettner et al., 2019). Additionally, when compared with users of other illicit non-psychedelic drugs, users of psychedelics reported greater concern for the environment and others (Lerner and Lyvers, 2006). Empirically, it has been demonstrated that nature-relatedness increased in TRD patients up to one year after psilocybin-assisted therapy (Lyons and Carhart-Harris, 2018a). While there is evidence suggesting that psychedelics have enduring impacts on nature connectedness, this has not been sufficiently investigated in a controlled setting in healthy participants.

4.2.5. Cognitive flexibility

Cognitive flexibility is defined as the contextually appropriate recruitment of and interaction between executive functioning, attentional mechanisms, object/abstract representations, perception, previous knowledge and conflict monitoring with respect to goal-directed contexts and available resources (Ionescu, 2012). As such, cognitive flexibility encompasses a multidimensional set of cognitive functions, but in short may be regarded as an executive function that includes attentional set-shifting, response inhibition, perseveration and reversal of stimulus-reward associations. The importance of cognitive flexibility for mental health is highlighted by its impairment in a myriad of psychopathological disorders, including eating disorders (Tchanturia et al., 2012), obsessive-compulsive disorder (OCD; Chamberlain et al., 2006), PTSD (Palm and Follette, 2011) and depression (Beck, 1976; Coles and Heimberg, 2002).

It is well-established that serotonin plays a major role in the modulation of cognitive flexibility (see Carhart-Harris and Nutt, 2017: for a review). Notably, agonism at the key site of psychedelic drug action – the 5-HT_{2A}R – is associated with enhanced cognitive flexibility, cortical plasticity and learning (Chen et al., 2003; Harvey, 2003; Romano et al., 2010; King et al., 1974; Boulougouris et al., 2008; Zhang and Stackman, 2015). On the other hand, 5-HT_{2A}R antagonism is associated with impaired cognitive flexibility, learning and working memory (Boulougouris et al., 2008; Clarke et al., 2004; Seymour et al., 2012; Wingen et al., 2007). Intriguingly, a recent optogenetics study found that serotonergic neurons within the dorsal raphe nuclei significantly activate when mice experience a surprising violation of assumptions, irrespective of reward-related implications (Matias et al., 2017). This suggests that serotonergic signalling plays an important role in conditions of uncertainty by enhancing environmental sensitivity and adaptability. 5-HT_{2A}R signalling has been proposed as the key mediator of this effect, particularly in situations of crisis (i.e. promotion of neural plasticity when changes are required; Carhart-

Harris and Nutt, 2017). The claim that 5-HT_{2A}R signalling is a critical mediator of change processes, particularly in uncertain conditions, fits well with the REBUS model of psychedelic drug action (Carhart-Harris and Friston, 2019). According to REBUS, these findings support the notion that the psychedelic brain enters an entropic “hot state” that is more sensitive to context and facilitates synaptic efficacy and plasticity (Ly et al., 2018; Carhart-Harris and Friston, 2019). The model goes further to suggest that, when the acute psychedelic effects begin to subside, the brain returns to its usual regimen of efficient free-energy minimisation and this is reflected by a renewed sense of familiarity and assuredness (Carhart-Harris and Friston, 2019). Importantly however, the brain may not return to how it was before the psychedelic experience. This suggests a potential window of opportunity for psychedelics to induce persistent functional and maybe even anatomical changes in the brain as a result of this period of elevated plasticity (Ly et al., 2018).

5-HT_{2A}Rs are abundantly expressed in cortical high-level association areas, including the highly interconnected Default Mode Network (DMN; Beliveau et al., 2017; Varnas et al., 2004). The DMN acts as a cognitive transit hub and plays a role in functionally high-level behaviour (Finc et al., 2017; Kaplan et al., 2017; Soto et al., 2018; Van Hoeck et al., 2013). Dynamic, adaptive regulation of DMN plays an important role in the facilitation of cognitive processes as well as monitoring the environment for unexpected events (Buckner et al., 2008; Spreng, 2012). Internal or external shifting of context has also been demonstrated to correlate with increased DMN activity within a cognitive set-shift task (Crittenden et al., 2015), indicating a potential facilitative role of the DMN in cognitive flexibility. This is interesting given the evidence showing acute and post-acute modulations of DMN connectivity and dynamics following psychedelic compounds (Carhart-Harris et al., 2012a; Carhart-Harris et al., 2013; Carhart-Harris et al., 2017d). Indeed, a recent study provides tentative evidence that psychedelics acutely enhance a construct approximating cognitive flexibility, namely divergent creative thinking (Kuypers et al., 2016). However, the potential for enduring changes in cognitive flexibility after a psychedelic experience has not been sufficiently investigated to date.

4.2.6. Psychedelic effects on neural networks

Evidence suggests that psychedelics desynchronise and disorganise neuronal activity via 5-HT_{2A}R activation, particularly within high-level brain networks such as the DMN (Carhart-Harris et al., 2016c; Komater et al., 2015; Muthukumaraswamy et al., 2013; Puig et al., 2010; Wood et al., 2012; Schenberg et al., 2015; Riba et al., 2004; Riba et al., 2002; Carhart-Harris et al., 2012a). Additionally, psychedelics facilitate increased coupling between brain regions and networks that are usually functionally segregated (Carhart-Harris et al., 2013; Palhano-Fontes et al., 2015). Increased global integration in cortical association regions (Tagliazucchi et al., 2016) and functional disintegration within high-level networks (Lebedev et al., 2015) have been found to correlate with subjective ratings of ego-dissolution in the acute psychedelic drug state. To this end, a collapse of the usual hierarchy and subsequent decrease in modularity of the brain may represent an important mechanism through which psychedelics induce their subjective effects – this is in line with the ideas put forward by the REBUS model (Carhart-

Harris and Friston, 2019). Recent work suggests that brain changes persist for up to one week following a psychedelic experience. For instance, increased amygdala responsiveness to emotional face stimuli was found one day after psilocybin treatment for depression (Roseman et al., 2018a), yet decreased amygdala responsiveness was found one week post-psilocybin in healthy participants (Barrett et al., 2020). Although we are beginning to understand the acute and post-acute action of psychedelics on the brain, there is much still to learn and many controversies in the results to untangle. Moreover, despite these initial insights, the capacity of psilocybin and related psychedelics to induce persistent brain effects is unknown. Given the period of elevated plasticity (Ly et al., 2018) during the psychedelic “hot state” (Carhart-Harris and Friston, 2019) combined with evidence of positive psychological outcomes in the long-term following a psychedelic experience (see Carhart-Harris and Goodwin, 2017; Elsey, 2017; Carhart-Harris et al., 2017c; Johnson et al., 2019: for reviews), the proposition that psychedelics may induce long-term brain effects is not unlikely. Accordingly, unveiling potential enduring effects of psychedelics on the brain, and neural correlates of the positive psychological changes that they can induce, would significantly contribute to the field.

4.3. OBJECTIVES AND HYPOTHESES

Taken together, it's apparent that psychedelic compounds have the capacity to promote positive changes that sustain long-term, but a growing awareness of other possible phenomenological features and outcomes of the psychedelic experience encourage further research into additional psychological mechanisms at play. For instance, the predictive value of psychedelic peak experiences in promoting positive long-term changes is well-established, but the potential role of psychological insight has yet to be determined. Moreover, science is currently gaining an understanding of the acute and post-acute brain effects of psychedelics, but the long-term brain effects and neurobiological mechanisms underlying long-term psychedelic outcomes are yet to be elucidated. In an attempt to account for the acute and long-term psychological and neurological effects of psychedelics, the REBUS model of psychedelic drug action was recently developed (Carhart-Harris and Friston, 2019). An extensive investigation of a broad repertoire of psilocybin-induced effects on the brain and psychology of healthy participants, both acutely and in the long-term, is required to help fill the knowledge gaps. To this end, testing a key principle of the REBUS model – i.e. a structured assessment of psychological insight and if/how it relates to brain signal complexity and long-term outcomes – and determining the long-term brain effects of psychedelics, as well as the neural correlates of long-term psychological outcomes that they can induce, would hold significant value.

Study objective: To comprehensively investigate the acute and long-term effects of psilocybin on the brain and psychology of healthy participants, with a key focus on the role of psychological insight.

Hypotheses:

- Therapeutic alliance will be negatively associated with pre-dosing anxiety, and both of these contextual factors as well as trait absorption will predict the quality of the psilocybin experience.
- Emotional breakthrough during the acute psilocybin session will positively relate to peak experience phenomena.
- During the acute phase of the psilocybin drug effects, insight will be positively associated with brain signal complexity.
- Psychological insight will continue to be facilitated in the psilocybin integration session and also in the weeks (≤ 4 weeks) following the psilocybin experience.
- The enduring facilitation of psychological insight (and subsequent insight-induced behavioural change) will relate to positive psychological outcomes following the psilocybin experience.
- Cognitive flexibility will increase in the long-term, sustained at four weeks following the psilocybin experience.
- Psilocybin will induce changes in resting-state functional connectivity four weeks post-dosing.
- Psilocybin will alter amygdala responsivity to facial affect stimuli four weeks post-dosing.
- Psilocybin peak experience phenomena will predict long-term neural changes.
- Enduring neural effects will correlate with enduring psychological effects.

4.4. METHODS

4.4.1. *Statement of contributions*

Personal contribution: Dr Robin Carhart-Harris and I conceptualised the study design. I coordinated the study and obtained the Home Office licence, psilocybin, and ethical and site-specific approvals. I recruited all of the participants, designed and decorated the dosing room and provided psychological support for the participants throughout the study. I led all study visits, including screening, dosing, integration and scanning sessions. I collected (including EEG), plotted and interpreted all of the data presented in this chapter. With the exception of the EEG and fMRI data, I analysed all data presented here.

Team contribution: Various team members provided assistance on all study visits; those members that played an essential role include Luigi Espasiano, Victoria Nygart, Manca Peskar, Melissa Shukuroglou and Meg Spriggs. Team psychiatrists, namely David Erritzoe, Roberta Murphy and Jonathan Martell, provided medical cover and support on screening, scanning and dosing days. I supervised MSc Translational Neuroscience students (Gregory Cooper, Pablo Mallaroni, Katie Trinci and Joseph Peill) in their contribution to the data analysis with the help of co-supervisors Christopher Timmermann and Robin Carhart-Harris, as well as all team members, notably Meg Spriggs, Leor Roseman and Fernando Rosas. Gregory Cooper, Pablo Mallaroni, Katie Trinci and Joseph Peill pre-processed the EEG data. Together with Fernando Rosas, Joseph Peill analysed the EEG data. Leor Roseman analysed the fMRI data.

4.4.2. *Ethical approvals*

This study received a favourable opinion from the NRES London-Surrey Research Ethics Committee and was carried out in accordance with Good Clinical Practice Guidelines. The National Institute for Health Research/Wellcome Trust Imperial Clinical Research Facility (ICRF) provided site-specific approvals and a Home Office Licence was obtained for the storage and handling of psilocybin, a Schedule 1 drug. This research was sponsored by Imperial College London. All participants provided written informed consent.

4.4.3. *Drug procurement*

COMPASS Pathways supplied the psilocybin for this study. COMPASS Pathways' stock of psilocybin was synthesised by Onyx Pharmaceuticals UK and encapsulated (size 4 capsules) by Juniper Pharma Services in accordance with Good Manufacturing Practice (GMP) (see appendix: certificate of analysis detailing purity and GMP quality). COMPASS Pathways provided the

psilocybin capsules to Fisher Clinical Services for bottling, labelling and distribution. Following Home Office guidelines for the transport of Scheduled drugs, the psilocybin was transported in a secure temperature-controlled vehicle from Fisher Clinical Services to the Burlington Danes Building of Imperial College London, which holds the required Home Office license. The psilocybin was stored in a robustly steel-sheeted Safety Storage Cabinet within an ID card-controlled and restricted entry only room. Storage conditions were light-protected and temperature-controlled. On dosing days, I dispensed and transported the psilocybin to the dosing room in the presence of a witness. The study psychiatrist subsequently administered the psilocybin capsules to the participants.

4.4.4. Experimental overview and procedures

Study design

This is a one-arm, single-blind, fixed order, within-subjects study investigating the acute and long-term effects of psilocybin on healthy participants. All participants received two oral doses of psilocybin 4-weeks apart. To blind the subjects, participants were informed that they would receive up to 25 mg of psilocybin in each dosing session, however, no further information regarding dosage was provided. In reality and known to the researchers, all participants received 1 mg of psilocybin on the first dosing day and 25 mg of psilocybin on the second dosing day. The 1 mg dose of psilocybin is generally considered to be a subperceptual/subthreshold dose (Madsen et al., 2019). This low (inactive) dose acted as the control in that it would not occasion a full psychedelic experience and would control for participant expectation. The subsequent dose of 25 mg psilocybin is generally considered to be a fully active high dose.

Recruitment and screening

Participants ($N=17$) were recruited via an online advertisement on the Centre for Psychedelic Research website. All participants initiated contact to gain their place on the study. I spoke to potential participants on the telephone to briefly ascertain suitability. After this initial telephone screen, participants were formally screened in the ICRF with the study physician and ICRF nursing staff to determine eligibility for the study. Participants were emailed the participant information sheet outlining the nature, purpose and risks of the study in sufficient time for them to read it prior to the screening visit. At the screening visit, I explained all of the information contained in the sheet and ensured that the information was fully understood. Participants were encouraged to ask questions about the information provided before giving their informed consent.

After providing written informed consent, the participants underwent physical and mental health assessments. Physical assessments included: (i) an alcohol breathalyser test, (ii) a urine screen for drugs of abuse and pregnancy (where applicable), (iii) an electrocardiogram (ECG), (iv) routine

blood tests, and (v) blood pressure, heart rate, height and weight recordings. Mental health assessments included (i) a neurological examination and (ii) a standard psychiatric interview, the Mini-International Neuropsychiatric Interview (MINI) version 5. Participants provided their full medical history and declared previous drug use. Screenings typically lasted 2.5 hours. After the retrieval of screening results, eligible participants were enrolled in the study and invited to return for subsequent visits.

Inclusion criteria

- Physically and mentally healthy
- Males & females
- 18-85 years old
- Good command of the English language
- No prior experience with psychedelic drugs

Exclusion criteria

- Current or previously diagnosed psychiatric disorder
- One or more immediate family members with a current or previously diagnosed psychotic disorder
- Medically significant condition that renders them unsuitable for the study (e.g., diabetes, severe cardiovascular disease, hepatic or renal failure etc.)
- Show MR contraindications (e.g., metal implants, pacemakers, claustrophobia etc.)
- Prior experience with a psychedelic drug
- Blood or needle phobia
- Show a positive pregnancy test at screening or during the study
- Excessive use of alcohol or other drugs (determined by study medic)
- No email access
- Inadequate grasp of English language
- Currently or very recently (within 6 months) involved in other studies
- Use of alcohol within 24 hours or other drugs within 7 days of scans and doses

Study visits and timeline

Following the screening session on visit 1, eligible participants enrolled in the study were required to attend 7 subsequent visits. All participants were instructed to refrain from recreational drug use for the duration of the study and alcohol for at least 24 hours before each study visit (Figure 4-1).

- Visit 1: Screening
- Visit 2: fMRI 1, dosing preparation and computer tasks
- Visit 3: Dosing session 1 (1 mg); including resting-state EEG

- Visit 4: Integration session 1
- Visit 5: fMRI 2, dosing preparation and computer tasks
- Visit 6: Dosing session 2 (25 mg); including resting-state EEG
- Visit 7: Integration session 2
- Visit 8: fMRI 3 and computer tasks

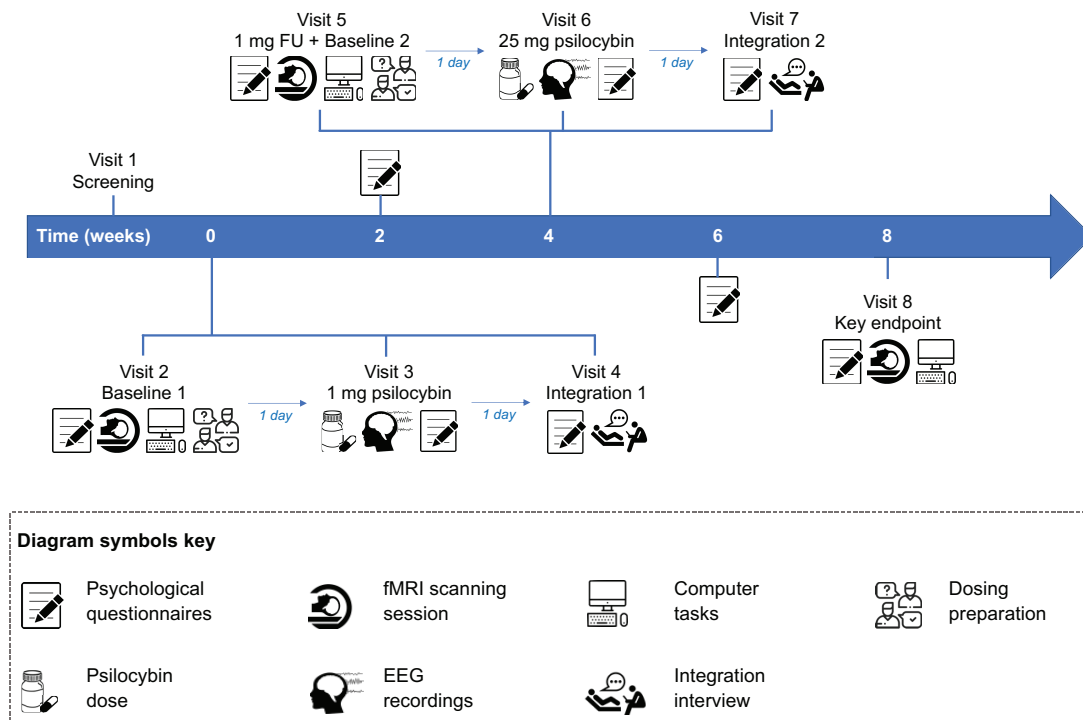


Figure 4-1. INSIGHT study timeline. Schematic diagram illustrating the study timeline in weeks and schedule of study interventions.

4.4.5. fMRI scanning sessions (visits 2, 5 and 8)

Participants attended three separate fMRI scanning sessions at the scanning centre, Invicro, on visits 2, 5 and 8: (i) the first scanning session collected baseline data, (ii) the second scanning session took place four weeks after dosing day 1 and served as the follow-up for 1 mg psilocybin as well as a baseline for dosing day 2, and (iii) the third and final scanning session served as the four week follow-up for 25 mg psilocybin and was the primary study endpoint. For each fMRI visit, participants provided a urine sample for drugs of abuse and pregnancy (where applicable) and were breathalysed on arrival by the Invicro nursing staff. Samples were disposed of immediately following the retrieval of the results. Participants completed an MR safety questionnaire with the Invicro radiographers prior to entering the scanner on the first scanning visit. On subsequent visits, participants were asked to confirm that the information provided in the safety screen remained correct. Each fMRI scanning session lasted 60 minutes and included: (i) BOLD eyes-closed resting-state and (ii) BOLD emotional faces task (3 versions: A, B and C) scans.

Imaging was performed on a 3T Siemens Tim Trio using a 12-channel head coil. Whole-head anatomical images were acquired using the Alzheimer's Disease Neuroimaging Initiative, Grand Opportunity (ADNI-GO; Jack et al., 2008) recommended Magnetisation Prepared Rapid Gradient Echo (MPRAGE) parameters – 1 mm isotropic voxels, TR = 2300 ms, TE = 2.98 ms, 160 sagittal slices, 256 × 256 in-plane FOV, flip angle = 9°, bandwidth = 240 Hz/pixel, parallel imaging (PI) factor = 2, Inversion time = 900 ms.

BOLD fMRI data acquisition

This study used multiband acquisition with two slices acquired simultaneously, also combined with in-plane (slice) acceleration using GRAPPA (i.e. the MB2R2 protocol characterised in (i.e. the MB2R2 protocol characterised in Demetriou et al., 2018). The MB2R2 protocol was based on the multiband EPI WIP v012b provided by the University of Minnesota (Cauley et al., 2014; Setsompop et al., 2012; Xu et al., 2013). T2*-weighted echo-planar images (EPI) were acquired using the MB2R2 protocol with interleaved slice acquisitions for the functional scans using 3 mm isotropic voxels, TR = 1250 ms, TE = 30 ms, 44 axial slices, 192 mm in-plane FOV, flip angle = 80°, bandwidth = 2232 Hz/pixel, GRAPPA acceleration = 2, number of volumes = 384. Both the resting state scan and emotional faces paradigm scan were each 8 minutes in duration.

Resting-state scans were completed with eyes-closed. For the emotional faces paradigm, participants used a mirror mounted on the head-coil to view a screen mounted in the rear of the scanner bore, where visual stimuli were back-projected through a wave-guide in the rear wall of the scanner room. The emotional faces paradigm was a block-design task lasting 8 min. Participants were shown faces with either fearful, happy, or neutral expressions, selected from the Karolinska Directed Emotional Faces set (Goeleven et al., 2008). An equal number of male and female faces were selected for the task. Each face was presented on screen for 3 s, and five faces of the same expression were presented in each 15 s block. Rest blocks (also 15 s) were also included, and there were 8 repetitions of each block type, presented in a pseudo-random sequence (32 blocks in total). Three versions of the task were used and the order of the task versions on each scanning visit was counter-balanced across participants. Participants passively viewed the faces but were instructed to press a single button with their thumb with the presentation of each new face, to confirm that they were paying attention to the stimuli.

BOLD pre-processing

Four different but complementary imaging software packages were used to analyse the fMRI data. Specifically, FMRIB Software Library (FSL; Smith et al., 2004), Analysis of Functional NeuroImages (AFNI; Cox, 1996), Freesurfer (Dale et al., 1999) and Advanced Normalization Tools (ANTS; Avants et al., 2009) were used. The following pre-processing stages were performed: 1) motion correction (3dvolreg, AFNI) by registering each volume to the volume most similar, in the least squares sense, to all others (in-house code); 2) brain extraction (BET, FSL);

3) rigid body registration to anatomical scans (BBR, FSL); 4) non-linear registration to 2 mm MNI brain (Symmetric Normalisation, ANTS); 5) scrubbing (Power et al., 2012) – using a framewise displacement (FD) threshold of 0.90 mm, which is a recommended threshold for task fMRI (Siegel et al., 2014). No significant difference in head movement (mean FD) was found between the scans. Scrubbed volumes were replaced with the mean of the surrounding volumes; 6) spatial smoothing (FWHM) of 6 mm (3dBlurInMask, AFNI); 7) high-pass filter of 0.01Hz; 8) regressing out 6 motion-related nuisance regressors (3 translations, 3 rotations, high-pass filtered with the same 0.01 Hz filter).

Seed-based resting state functional connectivity (rsFC)

Based on prior hypotheses, four seeds were chosen for these analyses: (i) bilateral parahippocampus (PH), (ii) bilateral amygdala, (iii) ventromedial prefrontal cortex (vmPFC), and (iv) subgenual anterior cingulate cortex (sgACC). The PH seed was constructed by combining the anterior and posterior parahippocampal gyrus from the Harvard-Oxford probabilistic atlas, which was then thresholded at 50%. The vmPFC seed was the same as one previously used by our team in analyses of the acute effects of LSD (Carhart-Harris et al., 2016c), psilocybin (Carhart-Harris et al., 2013) and MDMA (Carhart-Harris et al., 2015b). The sgACC seed was a 5 mm sphere centred at MNI coordinates $\pm 2\ 28\ -5$ (Scheidegger et al., 2012). Bilateral amygdala seed was based on Harvard-Oxford probabilistic atlas, threshold at 50%. Mean time-series were derived for these seeds for each resting-state scan. The rsFC analyses were performed using FSL's FEAT for each subject. Pre-whitening (FILM) was applied. A higher level analysis was performed to compare pre-treatment and post-treatment conditions using a mixed-effects GLM (FLAME 1 + 2), cluster corrected ($Z > 2.3$, $p < 0.05$). MRICron was used to display the results.

Resting State Networks (RSN)

RSNs were derived using Independent Component Analysis (ICA) performed on Human Connectome Project data (Van Essen et al., 2013). In summary, 20 independent components (ICs) were derived, of which the same 12 functionally meaningful RSNs were identified, namely: medial visual network (VisM), lateral visual network (VisL), occipital pole network (VisO), auditory network (AUD), sensorimotor network (SMN), default-mode network (DMN), parietal cortex network (PAR), dorsal attention network (DAN), salience network (SAL), posterior opercular network (POP), left frontoparietal network (IFPN) and right frontoparietal network (rFPN).

Network integrity was calculated for each RSN for both pre-treatment and post-treatment. All 20 HCP ICA components were entered into FSL's dual regression analysis (Beckmann et al., 2009). The first step of the dual regression used the components as regressors applied to the 4D BOLD datasets for each subject, resulting in a matrix of time-series for each ICA. The second step involved regressing these time-series into the same 4D scan data to get a subject-specific set of spatial maps (parameter estimate (PE) images). For each subject and for each condition, within

each of the 12 RSNs of interest (threshold = 3), the mean PE across voxels was calculated. This mean PE represents the integrity value. Subsequently, paired t-tests were used to calculate the difference in integrity between conditions for each RSN (Bonferroni corrected for 12 RSNs).

Between-RSN RSFC was calculated in a similar manner to previous analyses involving acute LSD (Carhart-Harris et al., 2016c) and psilocybin (Carhart-Harris et al., 2013). Specifically, a 12×12 matrix was constructed representing rsFC between different RSN pairs. For each subject and for each condition, the time-series for the relevant pair of RSNs, was entered into a GLM, resulting in a PE value representing the strength of functional connectivity between them. GLM was used rather than correlation coefficients because differences between Pearson's correlations could be a result of either signal or noise differences; therefore, it is preferable to perform regression and look for differences on the PE (Friston, 2011). The GLM was estimated twice: 1) each RSN as a dependant variable in one model, and 2) each RSN as an independent variable in the second model. These two PE values were then averaged together, to generate a symmetric 12×12 matrix. Four 12×12 matrices were created as follows: (i) mean PE values for pre-1 mg treatment; (ii) mean PE values for four weeks post-1 mg treatment; (iii) mean PE values for four weeks post-25 mg treatment; and (iv) paired t-tests (two-tailed) to compare the PE values 1 vs 2 and 2 vs 3.

Emotional faces task: ROI analysis

Different approaches were used to investigate changes in amygdala response: (i) calculating mean amygdala signal of left and right amygdala ROIs; and (ii) voxelwise analysis within a bilateral amygdala mask (Harvard-Oxford atlas, probability >50%). For both approaches, a standard GLM was used for the first analysis step, as implemented in the FEAT module in FSL. Regressors derived from the onset times of each stimulus condition were convolved with a Gamma function in order to simulate the Haemodynamic Response Function (HRF). Pre-whitening (FILM) was applied to correct for autocorrelations. Contrasts were defined that isolated activity related to each stimulus condition (fearful, happy, neutral) relative to the baseline, and also compared between two stimulus conditions, as appropriate (fearful > neutral and happy > neutral; five contrasts in total). Two-tailed t-tests were performed to compare before versus four weeks after each dosing day (before any intervention (scan 1), four weeks after 1 mg psilocybin (scan 2) and four weeks after 25 mg psilocybin (scan 3). BOLD responses for the mean ROI analysis and mixed-effects GLM (FLAME-1 2) for the voxelwise analysis. A statistical threshold of $Z > 2.3$, (cluster-corrected for multiple comparisons, $p < 0.050$) was used for all voxelwise analyses. A further voxelwise exploratory analysis was carried out on the whole brain.

4.4.6. Cognitive task

Participants completed a cognitive task on the study laptop (Windows 7 Professional; 64-bit; Intel Core i7-2620M CPU at 2.70 GHz) on visits 2, 5 and 8. The tasks were completed in a quiet room and the participants were instructed to concentrate fully.

Intra-dimensional/extra-dimensional (IDED) task

Cognitive flexibility was measured via the IDED task, which was kindly provided by Adam Hampshire, C3NL, Imperial College London. The format of the task is identical to the *intra-dimensional/extra-dimensional* (IDED) task present in the CANTAB neuropsychological test automated battery (Robbins et al., 1994), with minor aesthetic modifications. The IDED task consists of 9 stages. Two stimuli are presented to the participant at a time. At any given phase, a hidden rule determines the correct response to the trial. Participants are required to determine the rule and chose the correct stimuli in 6 consecutive trials before progressing to the next phase. Participants are not informed when the rule changes, only receiving ticks or crosses as feedback for correct or incorrect responses, respectively. Participants fail the task upon responding incorrectly 30 times within any given phase. Stimuli sets are pseudorandomised (different sets of lines and shapes from a given pool) between timepoints. Errors made at each phase were adjusted to the total number of stimuli presented within each phase, representing a measure of 'phase-accuracy' in order to assess changes in IDED performance across time-points while controlling for individual variability in learning rates.

Stage 1 (simple discrimination [SD]) requires learning of the correct stimulus from a choice of two shapes; at Stage 2 (simple reversal [SR]), the previously irrelevant shape becomes correct. At Stage 3 (compound discrimination [C_D]), a second dimension (line) is introduced alongside the shape dimension with each of the two stimuli containing a shape and line; subjects need to continue selecting the shape dimension. At Stage 4 (compound discrimination [CD]), the lines are superimposed onto shapes in each stimulus, and responding to the previous shape is required. At Stage 5 (compound reversal [CR]), the previously incorrect shape now becomes the correct response. Thus, at stages 1–5, the exemplars are the same, and subjects are required to respond to the same dimension of shape. Stage 6 (intra-dimensional shift [IDS]) tests the veracity of rule learning as new compound line and shape exemplars are introduced, but the same dimension (shape) remains correct. Selection of the previously incorrect shape pattern is required at Stage 7 (intra-dimensional reversal [IDR]). Stage 8 tests attentional set-shifting (extra-dimensional shift [EDS]) as the previously irrelevant dimension (line) now becomes relevant, and one of the line patterns becomes the correct response. At Stage 9 (extra-dimensional reversal [EDR]) the previously incorrect line becomes the required response. The various stages of this task require simple discrimination learning, compound discrimination learning, abstraction, attentional set-shifting, and reversal learning.

4.4.7. Psychedelic dosing preparation (visits 2 and 5)

Participants were carefully prepared for the dosing days on visits 2 and 5 (one day before each dosing session) in accordance with guidelines for the safe management of psychedelic drug sessions (Johnson et al., 2008). In this preparation session, I provided psychoeducation on the subjective effects of psilocybin and introduced the volunteer to the team member assisting me on their dosing days. In addition to providing advice on the effective management of positive and negative emotional responses to the drug, I simulated the dosing procedure and provided guided breathing techniques intended to promote relaxation. Preparation took approximately 2 hours in total.

4.4.8. Dosing sessions (visits 3 and 6)

Procedure

Participants attended two dosing sessions on which they received 1 mg psilocybin on dosing day 1 (visit 3) and 25 mg on dosing day 2 (visit 6). Participants were instructed to refrain from caffeinated products and to consume no more than a very light breakfast on the mornings of dosing. Participants arrived at the ICRF at 9 am on dosing days. For each dosing day visit, participants provided a urine sample for drugs of abuse and pregnancy (where applicable) and were breathalysed on arrival. All samples were disposed of immediately following the retrieval of the results. Whilst waiting on the results, participants were provided with the ICRF menu and asked to choose their meal to be served at the end of the day.

In recognition of the powerful influence of context, classically termed 'set and setting', a strong emphasis was placed on the interpersonal support in preparation for, during and after, the psilocybin-sessions. Before dosing, participants discussed their intentions and expectations with the guides (me and a team member) who would be present with them to provide non-directive support throughout the dosing-sessions. The intention was subsequently written on a white board in the dosing room before dosing.

All participants were dosed at approximately 10:30 am. Dosing days took place in a dimly lit, aesthetically pleasing room within the ICRF facility. A music playlist specifically designed to map onto the psychedelic-experience was created on Spotify and simultaneously played through both headphones (Bose Sound-link II around ear) and speakers (KRK.RP5G3 Rockit Powered Studio). Participants wore an eye mask and were encouraged to lie in supine position on the bed. Participants were asked to verbally report the subjective intensity of the drug effects from 0 (no drug effects) to 10 (most intense drug effects) every hour. When the drug effects subsided and dosing day was complete, the participant was assessed for potential discharge by the study physician. A taxi home was ordered for London-based participants; these participants were

required to phone or send a text message to me when they arrived home safely. For participants traveling from further afield, an overnight stay was arranged onsite at The Cement Danes Housing Association.

Music

Music was played simultaneously through headphones and speakers throughout the psychedelic session, excluding the silent EEG recordings. The playlist contained relaxing music, with a mixture of classical, ambient and world music as well as natural and environmental soundtracks. During the session, users are encouraged to focus their attention 'inward', and it is thought that music may help to facilitate this (Kaelen et al., 2018). It has been demonstrated that music mediates the quality of the acute experience which subsequently mediates therapeutic outcomes with psychedelics (Kaelen et al., 2018). Therefore, music is consistently used in psychedelic studies; playing a central role in the psychedelic experience (Kaelen et al., 2018; Swift et al., 2017; Watts et al., 2017). Although the effects of music on EEG and psychological measures will not be assessed in this study, it is still important to note the central role of music and how it may contribute to the overall outcome of the experiment.

Dosing day questionnaires

Participants completed a variety of questionnaires on dosing days via PsychoPy version 3.2 software on the study computer. Participants completed pre-dosing questionnaires immediately before receiving their psilocybin dose. Post-dosing questionnaires were completed at the end of dosing days immediately before leaving the facility.

Pre-dosing questionnaires

State-Trait Anxiety Inventory (STAI; Spielberger, 1983). The STAI is scored using a 4-point Likert scale and measures two types of anxiety: (i) 20-items measuring state anxiety, or anxiety about an event; and (ii) 20-items measuring trait anxiety, or anxiety level as a personal characteristic. Possible scores on the STAI range from a minimum score of 20 to a maximum score of 80 on both the STAI-Trait and STAI-State subscales. STAI scores are commonly classified as "no or low anxiety" (20-37), "moderate anxiety" (38-44), and "high anxiety" (45-80). As pre-dosing state anxiety was of interest for the purposes of this study, only the state scale of the STAI was used.

Post-dosing questionnaires

Mystical Experience Questionnaire (MEQ; Maclean et al., 2012). The subjective transcendent, spiritual quality of experiences was evaluated using the MEQ, a 30-item measure initially devised for characterising all types of mystical experiences based on Stace's framework on mysticism

(Pahnke and Richards, 1966; Maclean et al., 2012). The MEQ has been verified by factor analysis to retain pertinence to psilocybin-induced spiritual experiences in a laboratory (Maclean et al., 2012) and survey setting (Barrett et al., 2015). The items in the MEQ are categorised into 4 factors associated with different elements of a typical peak experience: (i) *mystical quality* (sacredness, noetic quality and unity), (ii) *transcendence of time/space*, (iii) *positive mood*, and (iv) *ineffability*. Items were scored using a 5-point Likert scale, with answers spanning 0-none (not at all) to 5-extreme (more than ever before in my life).

Altered States of Consciousness Questionnaire (ASC). The ASC was used to measure acute subjective experience phenomena. The ASC can be divided into five (5D-ASC, 94 items; Dittrich, 1998) or eleven dimensions (11D-ASC, 42 items; Studerus et al., 2010). The five dimensions are: (i) *oceanic boundlessness* (OBN), (ii) *dread of ego-dissolution* (DED), (iii) *visionary restructuralisation* (VRS), (iv) *auditory alterations* (AUA), and (v) *vigilance reduction* (VIR). The OBN items (Bodmer et al., 1994; Studerus et al., 2010) were formulated based on six of the nine categories of “mystical experiences” proposed by Stace (1960), in a similar way to the MEQ (Pahnke and Richards, 1966; Maclean et al., 2012). DED is considered to probe negative, aversive experiences in which anxiety is a central aspect. VRS measures altered perception and meaning including visual hallucinations and synesthesia. The eleven sub-dimensions are made only from OBN, DED and VRS – OBN sub-dimensions: *experience of unity*, *spiritual experience*, *blissful state*, *insightfulness*, *disembodiment*; DED sub-dimensions: *impaired control or cognition*, *anxiety*; VRS sub-dimensions: *complex imagery*, *elementary imagery*, *audio/visual synaesthesia*, *changed meaning of percepts*. ASC items were rated using a visual analogue scale (0–100, with incremental units of one) with zero defined as “No, not more than usually”, and 100 defined as “Yes, much more than usually”.

Emotional Breakthrough Inventory (EBI; Roseman et al., 2019). The degree of emotional breakthrough, synonymous with cathartic emotional release, was measured using the EBI. The 8-item EBI is constructed from items pertaining to emotional breakthroughs in previous psychedelic phenomenological literature reports of drug-induced psychocatharses (Watts et al., 2017), such as “*I experienced the resolution of personal conflict/trauma*” or “*I was able to explore challenging emotions and memories*”. Statements were rated by participants through the means of a visual analogue scale composed of 100 units with increments of 1 according to their applicability, with 0 being “not more than usually” and 100 being “Yes entirely, or completely”.

The Ego Dissolution Inventory (EDI; Nour et al., 2016). The EDI is an 8-item scale designed to measure ego-dissolution, a key feature of the psychedelic experience defined as a distorted sense of “self”. Items were rated using a visual analogue scale (0–100, with incremental units of one) with zero defined as “No, not more than usually”, and 100 defined as “Yes, entirely or completely”. A mean score on all 8 items is calculated for a single EDI factor.

Challenging Experience Questionnaire (CEQ; Barrett et al., 2016). The CEQ was used to characterise and quantify adverse psychological reactions during the acute psychedelic experience. In addition to yielding a total score, the CEQ is decomposed of 7 factors which represent different aspects of challenging experience: (i) *grief*, (ii) *fear*, (iii) *death*, (iv) *isolation*, (v) *paranoia*, (vi) *physical distress*, and (vii) *insanity*. Participants are asked to grade how much statements applied to the experience using a 5-point Likert scale, with answers spanning 0-none (not at all) to 5-extreme (more than ever before in my life).

Near-Death Experience (NDE) scale (Greyson, 1983). The NDE scale was used to assess psilocybin-induced near-death type experiences. The NDE scale consists of 16-items, resulting in a total score representing the global intensity of the experience as well as scores for four subscales: (i) *cognitive*, (ii) *affective*, (iii) *transcendental*, and (iv) *paranormal*. The sum of all 16 items gives the total NDE Scale score. A total score greater than or equal to seven is considered the threshold for an NDE. For each of the four subscales, the sum of the corresponding items on the scale yields the total subscale scores. The typology of NDE is then determined by the subscale scores: (i) *Cognitive type*: cognitive subscale score is greater than or equal to five; (ii) *Transcendental type*: cognitive subscale score is less than five and transcendental subscale score is greater than or equal to five; (iii) *Affective type*: cognitive and transcendental subscale scores are each less than five, and affective subscale score is greater than or equal to five; (iv) *Paranormal type*: cognitive, transcendental, and affective subscale scores are each less than five, and paranormal subscale score is greater than or equal to five; and (v) *Unclassifiable*: no subscale score is greater than or equal to five. Items were scored using a 4-point Likert scale, with answers spanning 0 (strongly disagree) to 4 (strongly agree).

Psychotomimetic States Inventory (PSI; Mason et al., 2008). The PSI is a 48-item scale designed to measure drug-induced psychotomimetic symptoms. In the present study, the PSI was used to assess the nature and extent of acute psilocybin-induced psychotomimetic effects. Items are rated on a 4-point scale (from 0 = never to 3 = strongly), with some items reverse scored. As well as yielding a total score, the PSI has six subscales: (i) *delusional thinking*, (ii) *perceptual distortions*, (iii) *cognitive disorganisation*, (iv) *anhedonia*, (v) *mania*, and (vi) *paranoia*.

Resting-state EEG recordings

A 24-channel wireless EEG cap (DSI-24 System, Wearable Sensing) was used to assess the acute brain effects across 21 dry electrodes (0.317 μ V resolution, 300hz sampling rate). Electrodes were positioned according to the 10-20 international format: Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T7/T3, T8/T4, Pz, P3, P4, P7/T5, P8/T6, O1, O2. Electrode Pz served as an online reference, with data re-referenced to electrodes A1 and A2 placed on the earlobes for the subtraction of noise in other channels. All data were recorded using DSI-Streamer-v.1.08.41.

As mentioned previously, participants set an intention for the psilocybin sessions (see procedure section). Participants were instructed to focus on their intention and to stay still with their eyes closed during the resting-state EEG recordings. Four resting-state EEG recordings were completed: (i) baseline, (ii) 1 hr post-dose, (iii) 2 hr post-dose, and (iv) 4.5 hr post dose. Each resting-state EEG recording lasted 11-minutes and included 4 minutes in silence followed immediately by 7 minutes with non-emotional, non-directive music playing (music track: “Sea of Love” by Huerco S).

Following each resting-state EEG recording, participants were given the following questions: Q1A. Please rate the strength of emotion felt during the silent EEG recording; Q1B. Please rate the strength of emotion felt during the music EEG recording; Q2A. Please rate the degree of insight experienced during the silent EEG recording; Q2B. Please rate the degree of insight experienced during the music EEG recording; Q3A. Please rate the richness (vividness and diversity of content) of your eyes-closed visual imagery during the silent EEG recording; and Q3B. Please rate the richness (vividness and diversity of content) of your eyes-closed visual imagery during the music EEG recording. Items were rated using a paper visual analogue scale (VAS) (0–100, with incremental units of one) with zero defined as ‘no more than usual’ and 100 defined as ‘much more than usual’.

EEG pre-processing

Raw EEG data were pre-processed and analysed in MATLAB 2019b (MathWorks, Natick, USA) using the FieldTrip toolbox (Oostenveld et al., 2011). Data were baseline-corrected (demeaned) and notch-filtered to remove line noise. A bandpass filter (1-45 Hz) was applied to account for high and low frequency noise caused by noise disturbances, sweating and skin conductance changes. Artifacts (e.g., eye movement and other muscle activity) were removed via manual visual inspection and subsequently interpolated using neighbouring channels. Independent Component Analysis (ICA) was performed to separate multi-channel EEG data into 20 temporally independent and spatially fixed components; these were then examined based on spatial, temporal and frequency features and components containing noise were manually removed. Only components containing gross and definite artifacts were removed from further analyses, with a maximum of 5 components removed for each recording. The Pz channel served as an online reference and was removed in all measures, leaving a total of 20 channels. Measures were re-referenced to electrodes A1 and A2, which were placed on the earlobes, to subtract noise in other channels. The EEG data was split into 10 second epochs for subsequent analyses. Three of the total 17 participants were unwilling to complete the EEG recordings during dosing day 2. Following visual evaluation, 11 of the 14 participants that completed the EEG recordings generated usable data for both dosing days.

Lempel-Ziv complexity analysis

The Lempel-Ziv Complexity (LZc) algorithm is a universal compression algorithm that can be applied to EEG data to gain an understanding of the diversity and therefore complexity of the signal (Ziv and Lempel, 1978). In previous research, LZc has been shown to be more consistent than other measures of entropy across psychedelic drug states (Schartner et al., 2017). Therefore, LZc was the preferred method of brain complexity analysis for this experiment. Following pre-processing, the LZc between 0 and 1 was computed. The signal was binarised via a Hilbert transform, resulting in a matrix consisting of a row for each channel and column for each observation. The LZ76 compression algorithm was then applied to further divide each binary string for all channels. This resulted in a list of unique, non-overlapping binary substrings. The more complex the initial string of a channel, the more unique substrings were generated by the LZ76 compression, equating to a higher LZc. The total amount of substrings (i.e. the size of the dictionary) was divided by the number of samples over the log of the samples.

4.4.9. Integration sessions (visits 4 and 7)

Integration sessions took place one day after each dosing session. During the integration sessions, participants verbally described their drug experience that took place the previous day. When this verbal account was complete, I asked them questions about their experience in the form of an unstructured interview.

4.4.10. Remote assessments

Data collection

In addition to the pre- and post-dosing questionnaires given onsite at the beginning and end of dosing days, respectively (visits 3 and 6; see dosing sessions section), further assessments were given remotely throughout the study. Psychological questionnaires were remotely completed immediately after visits 2 – 8 as well as two weeks post visits 3 and 6 via the Survey Gizmo platform. The timepoints at which all psychometric measures were administered are outlined in Table 4-1.

The Psychedelic Survey platform (psychedelicsurvey.com) was created by our group to send automatic reminder emails to complete the remote assessments. These emails linked participants to questionnaires contained within the separate Survey Gizmo platform. The Psychedelic Survey platform only had access to volunteer email addresses and phone numbers, no other personal/medical data. This data was considered personal but not sensitive and was therefore in accordance with Schedules I and II of the Data Protection Act. Questionnaire data were stored

only within the Survey Gizmo platform. Volunteer consent was obtained for the use of these platforms and was not compulsory for inclusion in the study.

Psychological measures

Scale to Assess Therapeutic Relationships (STAR; i.e. patient version). The STAR (McGuire-Snieckus et al., 2007) is a 12-item measure that was administered before each dosing day to assess the relationship between participants and study guides. As well as yielding a total score, the STAR contains three subscales: (i) positive collaboration, (ii) positive clinician input, and (iii) non-supportive clinician input (reverse scored). The STAR is rated on a 5-point Likert scale, with answers ranging from 0 to 4. Total subscale scores are obtained by summing scores on the relative subscale items. The total STAR score is obtained by adding the scores for all 12 items (range 0–48), with higher scores suggesting a better therapeutic relationship.

Gains from Psychedelic Integration questionnaire (GPI). I adapted The Gains from Dream Interpretation questionnaire (GDI; Heaton et al., 1998) by replacing the word “dream” with the words “psychedelic experience” in each item to create the GPI (validation publication in preparation). The GPI is a 13-item questionnaire, with items rated using a 9-point scale (from 1 = strongly disagree to 9 = strongly agree). As well as yielding a total score, the GPI has three subscales designed to measure different gains from the integration sessions: (i) *Exploration-Insight gains*, measuring insight as a result of exploring the psychedelic experience, (ii) *Experiential gains*, measuring the participant’s ability to re-experience the psychedelic session and the emotions associated with it, and (iii) *Insight-Action gains*, measuring the participant’s ability to change their cognition and behaviour as a result of the insight.

Mastery-Insight Scale (MIS). The MIS is a 5-item subscale of the Therapeutic Realisations Scale (TRS; Kolden, 1991) that assesses the level of mastery and insight gained from therapy. In the present study, the MIS was used to assess the level of mastery and insight gained from working through the psilocybin session during integration. The five items are rated on a 3-point Likert scale (from 0 = low to 2 = high). A total score is obtained by averaging all five items, with high scores indicating greater mastery and insight achieved during sessions.

Psychological Insight Scale (PIS). The PIS was self-constructed by the Centre for Psychedelic Research Group to measure of how insight affects one’s perspectives and/or behaviours (validation publication in preparation). The PIS is a 7-item questionnaire with two subscales: (i) *insight* subscale to measure psychological insight, and (ii) *insight-behaviour* subscale to measure changes to lifestyle/behaviour as a result of this insight. The PIS is scored using a VAS (0–100, with incremental units of one) with zero defined as ‘no more than usual’ and 100 defined as ‘much more than usual’. Preliminary calculations demonstrate an internal reliability of 0.94 (Cronbach’s alpha) and reliability remains high in our group’s survey studies of over 300 participants (PIS

validation publication in preparation). In the present study, the PIS demonstrates a significant positive correlation (spearman's rank (r_s)= 0.52, $p < 0.05$) with the insightfulness subdimension of the 11D-ASC. Furthermore, the PIS correlates with the SRIS – insightfulness subscale at two weeks post-25 mg psilocybin administration (Pearson's correlation coefficient (r)=0.634).

Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS; Tennant et al., 2007). The WEMWBS is a 14-item questionnaire used to assess psychological well-being. The WEMWBS is designed to assess mental well-being itself and not the determinants of mental well-being – e.g. resilience, problem solving, etc. The WEMWBS includes hedonic (i.e. happiness, life satisfaction) and eudaimonic (i.e. positive relationships, psychological functioning) items which together measure mental well-being. The 14-items are rated on a 5-point Likert scale (from 1 = none of the time to 5 = all of the time) to yield a total summed score, with a minimum possible score of 14 and a maximum score of 70 (population norms: $M=51$, $SD=9$; Morris and Earl, 2017).

Brief Experiential Avoidance Questionnaire (bEAQ; Gamez et al., 2014)). The bEAQ assesses one's tendency to avoid unwanted internal experiences such as negative feelings or cognitions. The bEAQ is a 15-item measure rated on a 6-point Likert scale (from 1 = "strongly disagree" to 6 = "strongly agree"). Scores range from 15 to 90 points (item 6 must be inverted), with higher scores showing greater experiential avoidance.

Life Orientation Test-Revised (LOT-R; Scheier et al., 1994). The LOT-R is a 6-item questionnaire developed to assess individual differences in generalised optimism versus pessimism. The six items are rated on a 5-point Likert scale (from 0 = strongly disagree to 4 = strongly agree). Items 2, 5, 6, and 8 are fillers and are therefore not scored. Responses to "scored" items are coded so that high values imply optimism, with some items being reverse scored.

Meaning in Life Questionnaire (MLQ; Steger et al., 2006). The MLQ assesses meaning in life as defined as an individual's awareness regarding the significance of their existence, and sense made of their life. The MLQ is a 10-item measure rated on a 7-point Likert scale (from 1 = *absolutely untrue* to 7 = *absolutely true*) and is made up of two subscales: (i) *presence of meaning in life* (MLQ-P) and (ii) *search for meaning in life* (MLQ-S).

Connectedness to Nature Scale (CNS; Mayer and Frantz, 2004). The CNS measures an individual's affective and experiential 'oneness' or 'connectedness' with nature. The CNS correlates positively with environmentalism and well-being, and negatively with consumerism. The CNS is a 14-item questionnaire rated on a 7-point Likert scale (from 1 = "strongly disagree" to 7 = "strongly agree").

Social Connectedness Scale (SCS; Lee and Robbins, 1995). The SCS measures an individual's sense of 'belongingness' by assessing subjective interpersonal closeness/connectedness experienced between the self and others. The SCS is an 8-item measure rated on a 6-point Likert scale (from 1 = "agree" to 6 = "disagree").

Santa Clara Brief Compassion Scale (SCBCS; Hwang et al., 2008). The SCBCS is a 5-item measure of compassion – defined as an attitude toward non-intimate other(s), including strangers; containing feelings, cognitions, and behaviors that are focused on concern and an orientation toward supporting and understanding the other(s)" – rated on a 7-point Likert scale (from 1 = "not at all true of me" to 7 = "very true of me").

Gratitude Questionnaire (GQ-6; McCullough et al., 2002). The GQ-6 is a 6-item scale designed to assess individual differences in the proneness to experience gratitude in daily life. The GQ-6 is rated on a 7-point Likert scale (from 1 = "strongly disagree" to 7 = "strongly agree"). Items are summed to yield a total score, with some items reverse scored. Scores range from 6 to 42, with higher scores showing greater gratitude.

Short Suggestibility Scale (SSS; Kotov et al., 2004). The SSS is a 21-item measure of suggestibility. The SSS is rated on a 5-point Likert scale (from 0 = "not at all or very slightly" to 4 = "a lot") and summed to yield a total score. Scores range from 0 to 84, with higher scores representing greater suggestibility.

Short Rosenberg Self-Esteem Scale (RSES-S; Tambs and Røysamb, 2014). The RSES-S is a shortened 4-item version of the 10-item RSES scale developed by (Rosenberg, 1965) to measure self-esteem (defined as one's own feeling of worthiness). Reliability of the 4-item short version is high, correlating at 0.95 with the full scale (Tambs and Røysamb, 2014). The RSES-S is rated on a 4-point Likert scale (from 1 = "strongly disagree" to 4 = "strongly agree"), with two items reversed. Scores are summed to yield a total score, with higher scores indicating greater self-esteem.

Interpersonal Reactivity Index (IRI; Davis, 1983). The IRI is a 28-item measure of empathy rated on a 5-point Likert scale (from 0 = "does not describe me well" to 4 = "describes me very well"), with some items reverse scored. The IRI has four subscales: (i) *Perspective Taking* – the tendency to spontaneously adopt the psychological point of view of others; (ii) *Fantasy* – taps respondents' tendencies to transpose themselves imaginatively into the feelings and actions of fictitious characters in books, movies, and plays; (iii) *Empathic Concern* – assesses "other-oriented" feelings of sympathy and concern for unfortunate others; and (iv) *Personal Distress* – measures "self-oriented" feelings of personal anxiety and unease in tense interpersonal settings. The IRI is particularly useful as it enables distinction between cognitive (*perspective-taking*

subscale) and emotional empathy (*empathic concern* subscale). Items on each subscale are summed to yield total subscale scores ranging from 0 to 28.

Self-Reflection and Insight Scale (SRIS; Grant et al., 2002). The SRIS measures the tendency to reflect on the self and the extent to which individuals have insight into their own behaviour. The SRIS is a 20-item measure rated on a 6-point Likert scale (from 1 = “strongly disagree” to 6 = “strongly agree”). The SRIS has three subscales: (i) *insight* (SRIS-IN) – measures the presence of insight; (ii) *need for self-reflection* (SRIS-NSR) – measures one’s recognition of their need for self-reflection; and (iii) *engagement in self-reflection* (SRIS-ESR) – measures the process of engaging in self-reflection. Scores are summed to yield total subscale scores.

Flourishing Scale (FS; Diener et al., 2010). The FS is a brief 8-item measure of flourishing, defined as self-perceived success in important areas such as relationships, self-esteem, purpose, and optimism. The FS is rated on a 7-point Likert scale (from 1 = “strongly disagree” to 7 = “strongly agree”) and provides a single psychological well-being (flourishing) score. Scores are summed to yield the total ranging from 8 to 56, with higher scores reflecting greater flourishing.

Profile of Mood States (POMS; Grove and Prapavessis, 1992). The 40-item abbreviated version of the POMS was used to measure mood. Items are rated on a 4-point Likert scale (from 0 = “not at all” to 4 = “extremely”), with some items reversed. The POMS has six primary factors: (i) *tension*; (ii) *depression*; (iii) *anger*; (iv) *fatigue*; (v) *confusion*; and (vi) *vigour*. Scores on each factor are summed to yield subscale totals. A Total Mood Disturbance (TMD) score can also be calculated by adding the scores for *tension*, *depression*, *anger*, *fatigue* and *confusion* before subtracting the score for *vigour*.

Various consciousness questions. Six months post-dosing, participants were instructed to reflect on the peak of each dosing session and asked the following three questions: (i) “How profound was the state of consciousness you experienced?”, rated on a 8-point Likert scale (from 1 = “no more profound than routine everyday states of consciousness” to 8 = “the single most profound state of consciousness of my life, that I can recall”); (ii) “How intense was the state of consciousness you experienced?”, rated on a 8-point Likert scale (from 1 = “no more intense than routine everyday states of consciousness” to 8 = “the single most intense state of consciousness of my life, that I can recall”); and (iii) “How unusual was the state of consciousness you experienced?”, rated on a 8-point Likert scale (from 1 = “no more unusual than routine everyday states of consciousness” to 8 = “the single most unusual state of consciousness of my life, that I can recall”).

Table 4-1. Schedule of psychometric assessments.

	PRE-DOSING	ACUTE DOSING	POST-DOSING		
			1 day	2 weeks	4 weeks
STAR	X				
STAI	X				
INTENSITY		X			
PSI		X			
ASC		X			
MEQ		X			
NDE		X			
EDI		X			
EBI		X			
CEQ		X			
GPI			X		
MIS			X		
PIS			X	X	X
MODTAS	X			X	X
WEMWBS	X			X	X
BEAQ	X			X	X
LOT-R	X			X	X
MLQ	X			X	X
CNS	X			X	X
SCS	X			X	X
GQ-6	X			X	X
SSS	X			X	X
IRI	X				X
RSES-S	X			X	X
SRIS	X			X	X
POMS	X			X	X

Abbreviations: STAR, Scale to Assess Therapeutic Relationship; STAI, State-Trait Anxiety Inventory; PSI, Psychotomimetic States Inventory; ASC, Altered State of Consciousness questionnaires; NDE, Near Death Experience; EDI, Ego Dissolution Inventory; MEQ, Mystical Experience Questionnaire; EBI, Emotional Breakthrough Inventory; CEQ, Challenging Experience Questionnaire; MIS, Mastery-Insight Scale; GPI, Gains from Psychedelic Integration; PIS, Psychological Insight Scale; SRIS, Self-Reflection and Insight Scale; WEMWBS, Warwick-Edinburgh Mental Well-being Scale; MLQ, Meaning in Life Questionnaire; FS, Flourishing Scale; CNS, Connectedness to Nature Scale; SCS, Social Connectedness Scale; GQ-6, Gratitude Questionnaire; SCBCS, Santa Clara Brief Compassion Scale; IRI, Interpersonal Reactivity Index; RSES-S, Short Rosenberg Self-Esteem Scale; LOT-R, Revised Life Orientation Test; SSS, Short Suggestibility Scale; POMS, Profile of Mood States; MODTAS, Modified Tellegen Absorption Scale.

4.4.11. Psychometric data analysis

All statistical analyses were performed using either SPSS version 26.0 (IBM Corp., Armonk, NY, USA) or GraphPad Prism version 8.3.1 (GraphPad Software, La Jolla California USA). Simple comparisons were performed using two-tailed paired t-tests for parametric data and Wilcoxon signed ranks tests for non-parametric data. Other data were subjected to one- and two-way repeated measures ANOVAs and followed up on with Bonferroni-corrected *post hoc* multiple comparisons. Where necessary, a linear mixed-effects model (LMM) was used in place of repeated measures ANOVA to account for missing values. Relationships between the measures were analysed using simple linear regressions. Results provide 95% CIs around the mean differences. Effect sizes were calculated using the Hedges' *g* formula. Statistical significance was assumed if the null hypothesis could be rejected at $p \leq 0.050$ probability level.

Study power was estimated *a priori* using nQuery version 8.6 (Statistical Solutions Ltd., Los Angeles, CA, USA) based on the fixed sample size of $N=17$. For the one-way repeated measures ANOVA with a 0.05 significance level on the primary psychological outcome measure (i.e. psychological well-being measured via WEMWBS), analyses indicated 99% power to detect a difference in means across the three primary timepoints – (i) baseline; (ii) four weeks post 1 mg psilocybin; and (iii) four weeks post 25 mg psilocybin – characterised by an effect size of 0.3 (based on a variance of means, $V = \sum(\mu_i - \mu)^2/M$, of 5.556, standard deviation at each level of 8.7, and between level correlation of 0.755). For correlations, power analyses indicated a detectable effect size of 0.59 with a desired power of 80% at the 5% two-sided significance level.

4.5. RESULTS

4.5.1. Demographics

A total of 17 participants completed the study. Participants had an average age of 40 years ($SD=7.76$, age range: 29–59). All participants were psychedelic naïve (100%) and balanced in terms of gender ($\chi^2=0.06$, $p=0.808$), marital status ($\chi^2=3.09$, $p=0.214$), educational attainment ($\chi^2=0.12$, $p=0.943$) and English language proficiency ($\chi^2=3.78$, $p=0.086$). However, participants differed in ethnicity ($\chi^2=8.50$, $p=0.004$), nationality ($\chi^2=18.13$, $p=0.001$) and employment status ($\chi^2=12.80$, $p=0.002$) as the majority were British (76%) Caucasians (88%) in full-time employment (88%). A breakdown of the participant demographic details can be found in Table 4-2.

Table 4-2. Study participant demographics.

Demographic variable	<i>M</i>	<i>SD</i>	Range
Age (in years)	40	7.76	29 – 59
	<i>n</i>	%	<i>p</i>
Gender			
Male	9	53%	0.808
Female	8	47%	
Ethnicity			
White Caucasian	15	88%	0.004**
Undisclosed	2	12%	
Nationality			
British	13	76%	0.001**
South African	1	6%	
Dutch	1	6%	
Hungarian	1	6%	
American	1	6%	
English language proficiency			
Native speaker	13	82%	0.086
Non-native fluent speaker	3	18%	
Education			
Secondary School	6	35%	0.943
Undergraduate	6	35%	
Postgraduate	5	30%	
Employment status			
Full time	14	82%	0.002**
Part time	3	18%	
Unemployed	0	0%	
Marital status			
Married	10	59%	0.214
Never married	5	29%	
Cohabiting with partner	2	12%	
Previous psychedelic experience			
No experience	17	100%	0%
Limited experience (<10 times used)	0	0%	

Age data expressed as mean $\pm$ SD with the age range. The chi-square test was used to determine differences in categorical variables. Data expressed as frequency count (*n*) and percentage (%) of sample ($p<0.01$ **).

4.5.2. Pre-dosing contextual factors

In order to determine predictors of the acute experience, pre-dosing contextual factors were assessed. A paired sample *t*-test showed a trend level difference in the Scale To Assess Therapeutic Relationship (STAR) scores between the 25 mg ($M=39.00$, $SD=5.26$) and 1 mg ($M=35.18$, $SD=5.80$); $t_{(16)}=2.116$, $p=0.051$ ns trend, 95% *CI* [-0.01, 7.66]) psilocybin sessions (Figure 4-2 A). A three (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=7.00$, $p=0.018$) and a main effect of subscale ($F_{(1.50,23.97)}=365.50$, $p<0.0001$), but no interaction between dose and subscale ($F_{(1.32,21.17)}=2.29$, $p=0.140$ ns). Post-hoc comparisons revealed significantly greater *positive clinician input* subscale scores for the 25 mg dose ($M=9.41$, $SD=1.42$) compared with the 1 mg dose of psilocybin ($M=8.06$, $SD=2.05$; $p=0.021$, 95% *CI* [-1.67, 2.02], $g=0.7$). No other subscales differed between the doses of psilocybin (all $p>0.164$ ns). With regards to differences between the subscales, post-hoc multiple comparisons revealed significantly greater *positive collaboration* subscale scores (1 mg: $M=17.47$, $SD=3.43$; 25 mg: $M=19.76$, $SD=3.21$) than *positive clinician input* (1 mg: $M=8.06$, $SD=2.05$; $p<0.0001$, 95% *CI* [-10.98, -7.84], $g=3.17$; 25 mg: $M=9.41$, $SD=1.42$; $p<0.0001$, 95% *CI* [-12.17, -8.54], $g=4.0$) and *non-supportive clinician input* (1 mg: $M=2.35$, $SD=1.90$; $p<0.0001$, 95% *CI* [12.33, 17.91], $g=5.2$; 25 mg: $M=2.18$, $SD=2.90$; $p<0.0001$, 95% *CI* [14.70, 20.48], $g=5.5$) subscale scores, as well as significantly greater *positive clinician input* (1 mg: $M=8.06$, $SD=2.05$; 25 mg: $M=9.41$, $SD=1.42$) than *non-supportive clinician input* (1 mg: $M=2.35$, $SD=1.90$; $p<0.0001$, 95% *CI* [3.81, 7.60], $g=2.7$; 25 mg: $M=2.18$, $SD=2.90$; $p<0.0001$, 95% *CI* [5.04, 9.43], $g=3.0$) subscale scores for both doses of psilocybin.

A Wilcoxon matched pairs test showed no difference in the participants' State-Trait Anxiety Inventory (STAI_state) scores between the 25 mg psilocybin session ($M=27.53$, $SD=8.63$) and the 1 mg psilocybin session ($M=29.12$, $SD=6.99$); $Z=41.0$, $p=0.163$ ns, 95% *CI* [-3.67, 0.49] (Figure 4-2 B). A direct association between state anxiety and therapeutic alliance has been shown previously (Kim et al., 2015; Mundorf et al., 2017), yet it has not been investigated within the context of psychedelics. To address this, a simple linear regression was carried out to determine the relationship between 25 mg psilocybin pre-dosing state anxiety and therapeutic alliance. In line with the hypothesis, the linear regression demonstrated a negative relationship between 25 mg pre-dosing STAI and STAR ($F_{(1, 15)}=8.08$, $R^2=0.383$, $p=0.012$) scores (Figure 4-2 C).

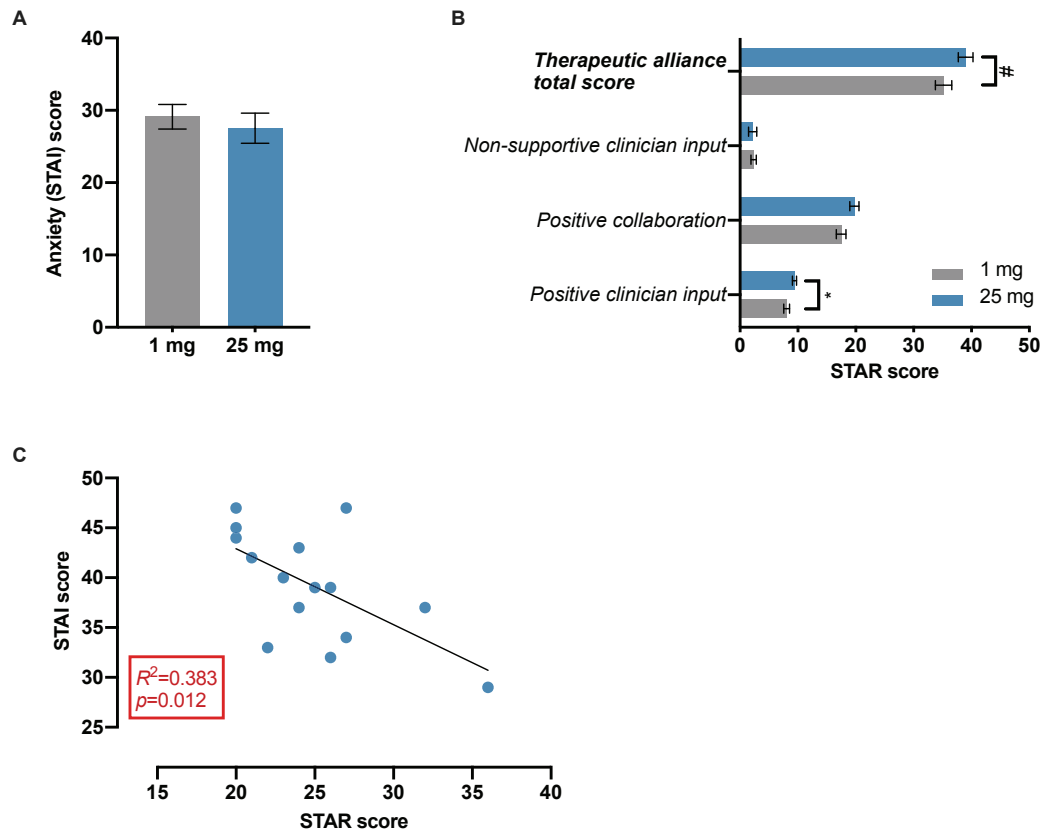


Figure 4-2. Pre-dosing variables | (A) Pre-dosing state anxiety. There was no difference in STAI scores between the 25 mg and 1 mg psilocybin sessions ($Z=41.0$, $p=0.163$ ns, 95% CI [-3.67, 0.49]). **(B) Therapeutic alliance.** A trend level increase was found in the total STAR scores between 25 mg and 1 mg ($t_{(16)}=2.116$, $p=0.051$ ns trend, 95% CI [-0.01, 7.66]) psilocybin sessions. Post-hoc comparisons revealed significantly greater *positive clinician input* subscale scores for the 25 mg dose compared with the 1 mg dose of psilocybin ($p=0.021$, 95% CI [-1.67, 2.02], $g=0.7$). No other subscales differed between the doses of psilocybin. **(C) Relationship between therapeutic alliance and pre-dosing anxiety.** A negative association was found between the 25 mg pre-dosing STAI scores and the STAR ($F_{(1, 15)}=8.08$, $R^2=0.383$, $p=0.012$). | Data are expressed as mean $\pm$ SEM ($p=0.051^{\#}$ $p<0.05^*$). Abbreviations: STAR, Scale to Assess Therapeutic Relationship; STAI, State-Trait Anxiety Inventory.

4.5.3. Acute drug effects

Intensity and time-course of drug effects

Participants were asked to rate the intensity of the drug effects from 0 (no effects) to 10 (most intense) at baseline and every hour post-dosing. The time-course of the participants' self-rated psilocybin intensity scores are illustrated in Figure 4-3 A.

A Wilcoxon matched pairs test showed a significant increase in the participants' overall drug intensity scores for the 25 mg psilocybin session ($M=4.23$, $SD=3.83$) compared with the 1 mg

psilocybin session ($M=0.33$, $SD=0.46$); $Z=36.0$, $p=0.008$, 95% CI [1.26, 6.54], $g=1.4$). An eight (time)×two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=259.70$, $p<0.0001$), a main effect of time ($F_{(2,39)}=70.96$, $p<0.0001$), and an interaction between dose and time ($F_{(2,37)}=54.79$, $p<0.0001$).

Post-hoc multiple comparisons revealed significantly greater intensity following 25 mg versus 1 mg psilocybin at 1 hour (1 mg: $M=1.09$, $SD=1.19$; 25 mg: $M=7.59$, $SD=3.41$; $p<0.0001$, 95% CI [-9.06, -3.94], $g=2.4$); 2 hours (1 mg: $M=1.03$, $SD=1.26$; 25 mg: $M=9.50$, $SD=1.27$; $p<0.0001$, 95% CI [-9.66, -7.28], $g=6.4$); 3 hours (1 mg: $M=0.59$, $SD=0.92$; 25 mg: $M=8.94$, $SD=2.07$; $p<0.0001$, 95% CI [-9.93, -6.78], $g=5.0$); 4 hours (1 mg: $M=0.26$, $SD=0.61$; 25 mg: $M=5.71$, $SD=2.60$; $p<0.0001$, 95% CI [-7.40, -3.48], $g=2.9$); 5 hours (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=3.76$, $SD=2.63$; $p<0.001$, 95% CI [-5.80, -1.73], $g=1.9$); and 6 hours (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=1.79$, $SD=2.25$; $p=0.041$, 95% CI [-3.54, -0.05], $g=1.1$) post-dosing. These results demonstrate that the 25 mg dose of psilocybin produces more intense drug effects than 1 mg psilocybin, as expected (Figure 4-3 A)

Post-hoc multiple comparisons revealed significantly greater intensity following 25 mg compared to baseline (time 0: $M=0.00$, $SD=0.00$) at the following timepoints: 1 hour ($M=7.59$, $SD=3.41$; $p<0.0001$, 95% CI [-10.53, -4.65], $g=3.0$); 2 hours ($M=9.50$, $SD=1.27$; $p<0.0001$, 95% CI [-10.60, -8.40], $g=10.1$); 3 hours ($M=8.94$, $SD=2.08$; $p<0.0001$, 95% CI [-10.73, -7.15], $g=5.8$); 4 hours ($M=5.71$, $SD=2.60$; $p<0.0001$, 95% CI [-7.95, -3.46], $g=2.9$); 5 hours ($M=3.76$, $SD=2.63$; $p<0.001$, 95% CI [-6.03, -1.50], $g=1.9$); and 6 hours ($M=1.79$, $SD=2.25$; $p=0.036$, 95% CI [-3.51, -0.08], $g=1.1$). Significantly greater intensity following 1 mg psilocybin compared to baseline (time 0: $M=0.00$, $SD=0.00$) was only observed at one-hour post-dosing ($M=1.09$, $SD=1.19$; $p=0.033$, 95% CI [-2.11, -0.06], $g=1.2$). These results demonstrate that the 25 mg dose of psilocybin produced significant drug effects up to six hours post-dosing, after which the drug effects subsided to baseline levels. The findings show that the subjective drug effects approached peak intensity at 2 hours post-dosing with 25 mg psilocybin and then gradually declined in the following hours (Figure 4-3 A).

Adverse events

The acute effects of psilocybin were well tolerated by all of the participants and no serious or unexpected adverse events occurred during either dosing session. Mild and transient nausea ($n=3$) and flushing ($n=1$) occurred during the second dosing session (high dose, 25 mg psilocybin), however, these adverse events were expected effects of psilocybin. No participants required tranquilising medications (oral lorazepam) during the dosing sessions and no prolonged psychotic symptoms were observed in any of the participants.

Psychotomimetic symptoms

The Psychotomimetic States Inventory (PSI) was used to assess the nature and extent of psilocybin-induced psychotomimetic effects. As well as yielding a total score, the PSI has subscales of *delusional thinking*, *perceptual distortions*, *cognitive disorganisation*, *anhedonia*, *mania* and *paranoia*.

A Wilcoxon matched pairs test showed a significant increase in the participants' total Psychotomimetic States Inventory (PSI) scores for the 25 mg psilocybin session ($M=0.30$, $SD=0.09$) compared with the 1 mg psilocybin session ($M=0.09$, $SD=0.04$); $Z=153.0$, $p<0.0001$, 95% CI [0.16, 0.24] $g=2.9$). A six (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=106.60$, $p<0.0001$) and a main effect of subscale ($F_{(5,80)}=13.41$, $p<0.0001$), and an interaction between dose and subscale ($F_{(5,80)}=21.76$, $p<0.0001$; Figure 4-3 B).

Post-hoc comparisons revealed that except for *anhedonia* (1 mg: $M=0.22$, $SD=0.06$; 25 mg: $M=0.27$, $SD=0.14$; $p=0.271$ ns, 95% CI [-0.12, -0.02]), all other subscale scores were significantly increased following 25 mg versus 1 mg psilocybin: *perceptual* (1 mg: $M=0.04$, $SD=0.06$; 25 mg: $M=0.34$, $SD=0.14$; $p<0.0001$, 95% CI [-0.37, -0.23], $g=2.6$); *paranoia* (1 mg: $M=0.04$, $SD=0.06$; 25 mg: $M=0.14$, $SD=0.13$; $p=0.001$, 95% CI [-0.17, -0.03], $g=0.9$); *mania* (1 mg: $M=0.15$, $SD=0.06$; 25 mg: $M=0.35$, $SD=0.14$; $p<0.0001$, 95% CI [-0.27, -0.13], $g=1.8$); *delusional* (1 mg: $M=0.07$, $SD=0.06$; 25 mg: $M=0.21$, $SD=0.14$; $p<0.0001$, 95% CI [-0.21, -0.07], $g=1.2$); *cognitive disorganisation* (1 mg: $M=0.08$, $SD=0.07$; 25 mg: $M=0.44$, $SD=0.21$; $p<0.0001$, 95% CI [-0.42, -0.29], $g=2.2$; Figure 4-3 B)

With regards to differences between the PSI subscales, post-hoc multiple comparisons revealed significantly greater *mania* ($M=0.15$, $SD=0.06$) compared with *perceptual* ($M=0.04$, $SD=0.06$; $p<0.001$, 95% CI [-0.19, -0.03], $g=1.7$), *paranoia* ($M=0.04$, $SD=0.06$; $p=0.002$, 95% CI [-0.18, -0.03], $g=1.7$) and *delusional* ($M=0.07$, $SD=0.06$; $p=0.048$, 95% CI [0.0003, 0.15], $g=1.3$) scores for the 1 mg dose of psilocybin. Significantly greater *anhedonia* scores ($M=0.22$, $SD=0.09$) were also observed compared with *perceptual* ($M=0.04$, $SD=0.06$; $p<0.0001$, 95% CI [-0.26, -0.10], $g=2.2$), *delusional* ($M=0.07$, $SD=0.06$; $p<0.0001$, 95% CI [-0.23, -0.07], $g=1.9$) and *cognitive disorganisation* ($M=0.08$, $SD=0.07$; $p<0.0001$, 95% CI [-0.21, -0.06], $g=1.6$) scores for the 1 mg dose of psilocybin. No other differences between the PSI subscales were found for the 1 mg dose of psilocybin (all $p>0.091$ ns).

For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly greater *perceptual* ($M=0.34$, $SD=0.14$) and *cognitive disorganisation* ($M=0.44$, $SD=0.21$) scores compared with *paranoia* ($M=0.14$, $SD=0.13$; *perceptual*: $p<0.0001$, 95% CI [0.12, 0.27], $g=1.4$; *cognitive disorganisation*: $p<0.0001$, 95% CI [-0.37, -0.22], $g=1.6$) and *delusional* ($M=0.21$,

$SD=0.14$; *perceptual*: $p<0.0001$, 95% CI [0.05, 0.21], $g=0.9$; *cognitive disorganisation*: $p<0.0001$, 95% CI [-0.31, -0.15], $g=1.2$) scores. Likewise, significantly greater *cognitive disorganisation* scores ($M=0.44$, $SD=0.21$) compared with *perceptual* ($M=0.34$, $SD=0.14$; $p=0.003$, 95% CI [-0.17, -0.02], $g=0.5$), *mania* ($M=0.35$, $SD=0.14$; $p=0.007$, 95% CI [-0.17, -0.02], $g=0.5$) and *anhedonia* scores ($M=0.27$, $SD=0.14$; $p<0.0001$, 95% CI [0.09, 0.25], $g=0.9$). Significantly greater *mania* scores ($M=0.35$, $SD=0.14$) were also observed compared with *paranoia* ($M=0.14$, $SD=0.13$; $p<0.0001$, 95% CI [-0.28, -0.12], $g=1.5$) and *delusional* ($M=0.21$, $SD=0.14$; $p<0.0001$, 95% CI [0.06, 0.21], $g=0.9$) scores, as well as *anhedonia* ($M=0.27$, $SD=0.14$) compared with *paranoia* ($M=0.14$, $SD=0.13$; $p<0.0001$, 95% CI [-0.20, -0.05], $g=0.9$) scores. No other differences between the subscales were found for the 25 mg dose of psilocybin (all $p>0.051$ ns).

These results show that the 25 mg dose of psilocybin produced significantly more transient psychotomimetic effects than 1 mg psilocybin, with the exception of the phenomenon of anhedonia. Cognitive disorganisation was found to be the most pronounced transient psychotic phenomenon enhanced following 25 mg psilocybin.

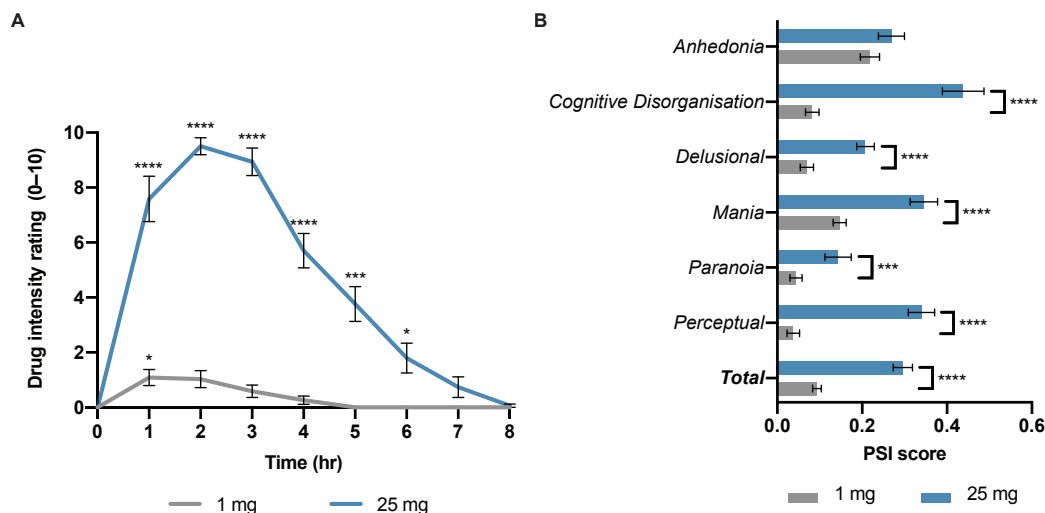


Figure 4-3. Intensity and time-course of the drug effects and psychotomimetic symptoms | (A) Time-course of the drug effects. Overall drug intensity ratings across all timepoints were significantly greater for 25 mg versus 1 mg psilocybin ($Z=36.0$, $p=0.008$, 95% CI [1.26, 6.54], $g=1.4$). Significantly greater intensity was observed following 25 mg versus time 0 (baseline) and 1 mg psilocybin at 1 – 6 hours post-dosing (1 – 4 hours: all $p<0.0001$, $g>2.4$; 5 hours: $p<0.001$, $g=1.9$; 6 hours: $p<0.041$, $g=1.1$). The subjective drug effects approached peak intensity at two hours post-25 mg psilocybin administration and then gradually declined in the following hours. Significantly greater intensity following 1 mg psilocybin compared to time 0 (baseline) was only observed at 1 hour post-dosing ($p=0.033$, 95% CI [-2.11, -0.06], $g=1.2$). **(B) Psychotomimetic symptoms.** Total PSI scores were significantly greater for 25 mg versus 1 mg psilocybin ($Z=153.0$, $p<0.0001$, 95% CI [0.16, 0.24], $g=2.9$). Post-hoc comparisons revealed that all subscale scores were significantly increased except for *anhedonia* ($p=0.271$ ns) following 25 mg versus 1 mg psilocybin (all $p<0.001$; $g>0.9$). | Data are expressed as mean $\pm$ SEM ($p<0.05^*$, $p<0.001^{***}$, $p<0.0001^{****}$). Abbreviations: PSI, Psychotomimetic States Inventory.

Altered states of consciousness

Altered states of consciousness (ASC) represent a marked deviation in the subjective experience or psychological functioning from normal waking consciousness. The ASC questionnaire was used to assess the quality of the acute subjective experience in the psilocybin dosing sessions across five (5D-ASC; Dittrich, 1998) and eleven (11D-ASC; Studerus et al., 2010) dimensions.

5D-ASC: The five dimensions are: *oceanic boundlessness* (OBN), *dread of ego dissolution* (DED), *visionary restructuralisation* (VRS), *auditory alterations* (AUA), and *vigilance reduction* (VIR). The OBN items were formulated based on six of the nine categories of “mystical experiences” proposed by Stace (1960) (Studerus et al., 2010) in a similar way to the MEQ (Maclean et al., 2012). DED is considered to probe negative, aversive experiences in which anxiety is a central aspect. VRS measures altered perception and meaning including visual hallucinations and synaesthesia (Figure 4-4 A).

A five (dimension) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=129.30$, $p<0.0001$), a main effect of dimension ($F_{(4,64)}=19.18$, $p<0.0001$) and a significant interaction between dose and dimension ($F_{(4,64)}=13.95$, $p<0.0001$). Post-hoc multiple comparisons revealed significantly greater alterations in consciousness following 25 mg versus 1 mg psilocybin on all dimensions of the 5D-ASC: OBN (1 mg: $M=0.08$, $SD=0.15$; 25 mg: $M=0.76$, $SD=0.20$; $p<0.0001$, 95% CI [-0.80, -0.56], $g=3.7$); DED (1 mg: $M=0.02$, $SD=0.03$; 25 mg: $M=0.43$, $SD=0.23$; $p<0.0001$, 95% CI [-0.52, -0.28], $g=2.4$); VRS (1 mg: $M=0.11$, $SD=0.12$; 25 mg: $M=0.74$, $SD=0.19$; $p<0.0001$, 95% CI [-0.75, -0.50], $g=3.8$); AUA (1 mg: $M=0.02$, $SD=0.04$; 25 mg: $M=0.38$, $SD=0.26$; $p<0.0001$, 95% CI [-0.47, -0.23], $g=1.8$); VIR (1 mg: $M=0.16$, $SD=0.15$; 25 mg: $M=0.45$, $SD=0.23$; $p<0.0001$, 95% CI [-0.41, -0.17], $g=1.4$).

With regards to differences between the dimensions, post-hoc multiple comparisons revealed significantly more VIR ($M=0.16$, $SD=0.15$) than DED ($M=0.02$, $SD=0.03$; $p=0.040$, 95% CI [-0.27, -0.003], $g=1.2$) and AUA ($M=0.02$, $SD=0.04$; $p=0.039$, 95% CI [-0.27, -0.004], $g=1.2$) for the 1 mg dose of psilocybin. There were no other significant differences between the dimensions for 1 mg dose of psilocybin (all $p>0.427$ ns). For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly greater OBN ($M=0.76$, $SD=0.20$) and VRS ($M=0.74$, $SD=0.19$) scores compared with all other dimensions: DED ($M=0.43$, $SD=0.23$; OBN: $p<0.0001$, 95% CI [0.20, 0.47], $g=1.5$; VRS: $p<0.0001$, 95% CI [0.18, 0.44], $g=1.4$); AUA ($M=0.38$, $SD=0.26$; OBN: $p<0.0001$, 95% CI [0.25, 0.52], $g=1.7$; VRS: $p<0.0001$, 95% CI [0.23, 0.49], $g=1.5$); and VIR ($M=0.45$, $SD=0.23$; OBN: $p<0.0001$, 95% CI [0.18, 0.44], $g=1.4$; VRS: $p<0.0001$, 95% CI [0.15, 0.42], $g=1.3$). There were no other significant differences between the dimensions for 25 mg dose of psilocybin (all $p>0.680$ ns).

These results show that the 25 mg dose of psilocybin produced significant alterations in consciousness on all five ASC dimensions, especially the OBN and VRS dimensions, compared with the 1 mg dose.

11D-ASC: The eleven sub-dimensions are made only from OBN, DED and VRS. The OBN sub-dimensions are *experience of unity*, *spiritual experience*, *blissful state*, *insightfulness*, and *disembodiment*. The DED sub-dimensions are *impaired control or cognition*, and *anxiety*. The VRS sub-dimensions are *complex imagery*, *elementary imagery*, *audio/visual synaesthesia*, and *changed meaning of percepts* (Figure 4-4 B).

An eleven (dimension) × two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=202.30$, $p<0.0001$), a main effect of dimension ($F_{(10,160)}=12.69$, $p<0.0001$), and an interaction between dose and dimension ($F_{(10,160)}=4.49$, $p<0.0001$). Post-hoc multiple comparisons revealed significantly greater alterations in consciousness following 25 mg versus 1 mg psilocybin on all dimensions of the 11D-ASC: *changed meaning of percepts* (1 mg: $M=0.04$, $SD=0.08$; 25 mg: $M=0.66$, $SD=0.28$; $p<0.0001$, 95% CI [-0.77, -0.47], $g=2.9$); *audio-visual synaesthesia* (1 mg: $M=0.19$, $SD=0.25$; 25 mg: $M=0.91$, $SD=0.14$; $p<0.0001$, 95% CI [-0.87, -0.57], $g=3.4$); *elementary imagery* (1 mg: $M=0.20$, $SD=0.22$; 25 mg: $M=0.85$, $SD=0.26$; $p<0.0001$, 95% CI [-0.80, -0.50], $g=2.6$); *complex imagery* (1 mg: $M=0.11$, $SD=0.14$; 25 mg: $M=0.80$, $SD=0.26$; $p<0.0001$, 95% CI [-0.84, -0.54], $g=3.1$); *anxiety* (1 mg: $M<0.01$, $SD<0.01$; 25 mg: $M=0.37$, $SD=0.29$; $p<0.0001$, 95% CI [-0.52, -0.21], $g=1.7$); *impaired control and cognition* (1 mg: $M=0.04$, $SD=0.06$; 25 mg: $M=0.48$, $SD=0.25$; $p<0.0001$, 95% CI [-0.60, -0.30], $g=2.3$); *disembodiment* (1 mg: $M=0.09$, $SD=0.22$; 25 mg: $M=0.81$, $SD=0.23$; $p<0.0001$, 95% CI [-0.86, -0.56], $g=3.0$); *insightfulness* (1 mg: $M=0.07$, $SD=0.19$; 25 mg: $M=0.70$, $SD=0.22$; $p<0.0001$, 95% CI [-0.77, -0.47], $g=2.9$); *blissful state* (1 mg: $M=0.09$, $SD=0.14$; 25 mg: $M=0.78$, $SD=0.28$; $p<0.0001$, 95% CI [-0.85, -0.54], $g=3.0$); *spiritual experience* (1 mg: $M=0.06$, $SD=0.11$; 25 mg: $M=0.68$, $SD=0.26$; $p<0.0001$, 95% CI [-0.77, -0.46], $g=3.0$); *experience of unity* (1 mg: $M=0.06$, $SD=0.19$; 25 mg: $M=0.69$, $SD=0.26$; $p<0.0001$, 95% CI [-0.78, -0.48], $g=2.6$). Compared with normal waking consciousness (drug-free), 25 mg psilocybin induced pronounced alterations with significant increases in all dimensions of the 11D-ASC ($p<0.0001$), whereas 1 mg psilocybin only induced a significant increase in *Elementary Imagery* ($p=0.020$).

With regards to differences between the dimensions, post-hoc multiple comparisons revealed significantly more *audio-visual synaesthesia* ($M=0.19$, $SD=0.25$; $p=0.037$, 95% CI [0.005, 0.36], $g=1.0$) and *elementary imagery* ($M=0.20$, $SD=0.22$; $p=0.021$, 95% CI [0.01, 0.37], $g=1.2$) than *anxiety* ($M=0.01$, $SD=0.01$) for the 1 mg dose of psilocybin. There were no other significant differences between the dimensions for 1 mg psilocybin (all $p>0.152$ ns). For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly less *anxiety* ($M=0.37$, $SD=0.29$) and *impaired control and cognition* ($M=0.48$, $SD=0.25$) compared with the following dimensions: *changed meaning of percepts* ($M=0.66$, $SD=0.28$; *anxiety*: $p<0.0001$, 95% CI [0.11, 0.47], $g=0.9$;

impaired control and cognition: $p=0.049$, 95% CI [0.0002, 0.36], $g=0.6$); *audio-visual synaesthesia* ($M=0.91$, $SD=0.14$; *anxiety*: $p<0.0001$, 95% CI [0.36, 0.71], $g=2.3$; *impaired cognition and control*: $p<0.0001$, 95% CI [0.24, 0.60], $g=2.0$); *complex imagery* ($M=0.80$, $SD=0.26$; *anxiety*: $p<0.0001$, 95% CI [0.25, 0.60], $g=1.5$; *impaired cognition and control*: $p<0.0001$, 95% CI [0.14, 0.49], $g=1.2$); *elementary imagery* ($M=0.85$, $SD=0.26$; *anxiety*: $p<0.0001$, 95% CI [0.30, 0.66], $g=1.7$; *impaired cognition and control*: $p<0.0001$, 95% CI [0.19, 0.54], $g=1.4$); *disembodiment* ($M=0.81$, $SD=0.23$; *anxiety*: $p<0.0001$, 95% CI [0.61, 0.26], $g=1.6$; *impaired cognition and control*: $p<0.0001$, 95% CI [0.50, 0.14], $g=1.3$); *insightfulness* ($M=0.70$, $SD=0.22$; *anxiety*: $p<0.0001$, 95% CI [0.50, 0.15], $g=1.2$; *impaired cognition and control*: $p=0.004$, 95% CI [0.39, 0.04], $g=0.9$); *blissful state* ($M=0.78$, $SD=0.28$; *anxiety*: $p<0.0001$, 95% CI [0.59, 0.23], $g=1.4$; *impaired cognition and control*: $p<0.0001$, 95% CI [0.47, 0.12], $g=1.1$); *spiritual experience* ($M=0.68$, $SD=0.26$; *anxiety*: $p<0.0001$, 95% CI [0.48, 0.13], $g=1.1$; *impaired cognition and control*: $p=0.018$, 95% CI [0.37, 0.01], $g=0.7$); and *experience of unity* ($M=0.69$, $SD=0.26$; *anxiety*: $p<0.0001$, 95% CI [0.50, 0.15], $g=1.1$; *impaired cognition and control*: $p=0.006$, 95% CI [0.39, 0.03], $g=0.8$). There was no significant difference between *anxiety* ($M=0.37$, $SD=0.29$) and *impaired control and cognition* ($M=0.48$, $SD=0.25$; $p=0.839$ ns) for the 25 mg dose of psilocybin.

These results show that the 25 mg dose of psilocybin produced significant alterations in consciousness on all eleven ASC dimensions compared with the 1 mg dose, as expected. Alterations in *anxiety* and *impaired control and cognition* dimensions were less pronounced compared with the other dimensions following 25 mg psilocybin.

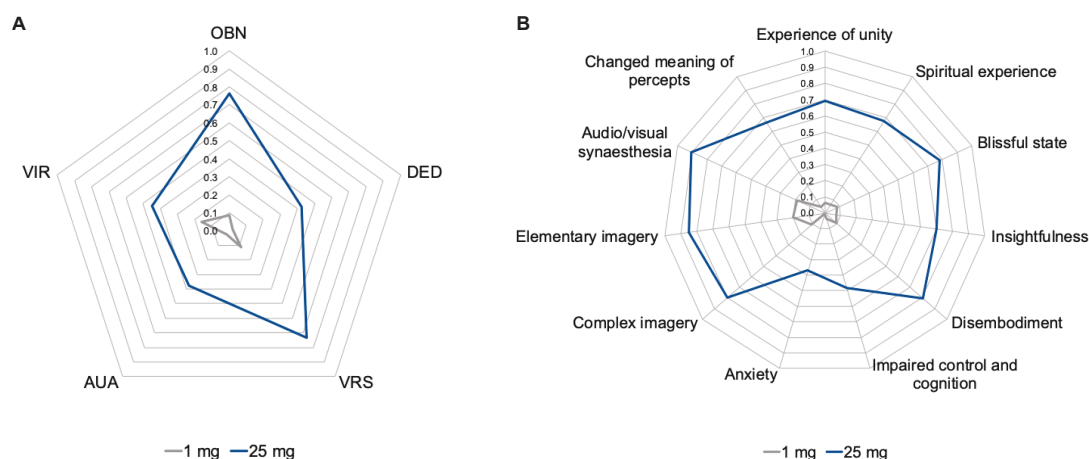


Figure 4-4. Altered states of consciousness | (A) 5D-ASC. The five dimensions are: *oceanic boundlessness* (OBN), *dread of ego dissolution* (DED), *visionary restructuralisation* (VRS), *auditory alterations* (AUA), and *vigilance reduction* (VIR). There were significantly greater alterations in consciousness following 25 mg versus 1 mg psilocybin on all dimensions of the 5D-ASC (all $p<0.0001$, $g>1.4$). Post-hoc multiple comparisons revealed significantly more VIR than DED ($p=0.040$, 95% CI [-0.27, -0.003], $g=1.2$) and AUA ($p=0.039$, 95% CI [-0.27, -0.004], $g=1.2$) for the 1 mg dose of psilocybin. For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly greater OBN and VRS scores

compared with all other dimensions (all $p < 0.0001$, $g > 1.3$). **(B) 11D-ASC.** The eleven sub-dimensions are made only from OBN, DED and VRS. The OBN sub-dimensions are *experience of unity*, *spiritual experience*, *blissful state*, *insightfulness*, and *disembodiment*. The DED sub-dimensions are *impaired control or cognition*, and *anxiety*. The VRS sub-dimensions are *complex imagery*, *elementary imagery*, *audio/visual synaesthesia*, and *changed meaning of percepts*. Compared with normal waking consciousness (drug-free), 25 mg psilocybin induced pronounced alterations with significant increases in all dimensions of the 11D-ASC (all $p < 0.0001$), whereas 1 mg psilocybin only induced a significant increase in *Elementary Imagery* ($p = 0.020$). Abbreviations: 5D-ASC, 5-Dimensional Altered State of Consciousness; OBN, oceanic boundlessness; DED, dread of ego dissolution; VRS, visionary restructuralisation; AUA, auditory alterations; VIR, vigilance reduction; 11D-ASC, 11-Dimensional Altered State of Consciousness.

Mystical-type experiences

The subjective transcendent, spiritual quality of experiences was evaluated using the 30-item Mystical Experience Questionnaire (MEQ). The MEQ contains yields a total score consisting of the average of all items as well as four subscales associated with different elements of a typical peak experience: (i) mystical (sacredness, noetic quality and unity), (ii) transcendence of time/space, (iii) positive mood, and (iv) ineffability.

A Wilcoxon matched pairs test showed a significant increase in the participants' total Mystical Experience Questionnaire (MEQ) scores for the 25 mg psilocybin session ($M = 85.12$, $SD = 17.15$) compared with the 1 mg psilocybin session ($M = 13.16$, $SD = 16.11$); $Z = 153.0$, $p < 0.0001$, 95% CI [61.96, 81.95], $g = 4.1$). A four (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)} = 232.80$, $p < 0.0001$), a main effect of subscale ($F_{(3,48)} = 4.54$, $p = 0.007$), and an interaction between dose and subscale ($F_{(3,48)} = 4.43$, $p = 0.008$). Post-hoc multiple comparisons revealed significantly more mystical experiences following 25 mg versus 1 mg psilocybin on all subscales of the MEQ: mystical quality (1 mg: $M = 8.94$, $SD = 16.06$; 25 mg: $M = 73.88$, $SD = 25.07$; $p < 0.0001$, 95% CI [-74.94, -54.95], $g = 2.9$); positive mood (1 mg: $M = 19.53$, $SD = 22.65$; 25 mg: $M = 84.76$, $SD = 23.10$; $p < 0.0001$, 95% CI [-75.23, -55.24], $g = 2.7$); transcendence of space and time (1 mg: $M = 13.12$, $SD = 16.43$; 25 mg: $M = 89.76$, $SD = 18.90$; $p < 0.0001$, 95% CI [-86.64, -66.65], $g = 4.1$); ineffability (1 mg: $M = 11.06$, $SD = 16.54$; 25 mg: $M = 92.06$, $SD = 18.99$; $p < 0.0001$, 95% CI [-91.00, -71.00], $g = 4.3$; Figure 4-5 C).

With regards to differences between the subscales, post-hoc multiple comparisons revealed significantly more positive mood ($M = 19.53$, $SD = 22.65$) than mystical quality ($M = 8.94$, $SD = 16.06$) for the 1 mg dose of psilocybin ($p = 0.024$, 95% CI [0.89, 20.29], $g = 0.5$). There were no other significant differences between the subscales for the 1 mg dose of psilocybin (all $p > 0.130$ ns). For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly more ineffability ($M = 92.06$, $SD = 18.99$; $p < 0.0001$, 95% CI [8.48, 27.88], $g = 0.8$), positive mood ($M = 84.76$, $SD = 23.10$; $p = 0.018$, 95% CI [1.18, 20.58], $g = 0.4$) and transcendence of space and time ($M = 89.76$, $SD = 18.90$; $p < 0.001$, 95% CI [6.18, 25.58], $g = 0.7$) than mystical quality ($M = 73.88$, $SD = 25.07$).

These results demonstrate that the 25 mg dose of psilocybin produced a greater mystical-type (peak) experience compared with the 1 mg dose.

Ego dissolution

The Ego Dissolution Inventory (EDI) was used to assess the experience of a compromised sense of self occasioned by psychedelic drugs. A Wilcoxon matched pairs test showed a significant increase in the participants' total EDI scores for the 25 mg psilocybin session ($M=80.18$, $SD=23.43$) compared with the 1 mg psilocybin session ($M=8.53$, $SD=18.33$); $Z=153.0$, $p<0.0001$, 95% CI [57.43, 85.88], $g=3.2$; Figure 4-5 B).

Next, I explored the eight items comprising the EDI in order to assess specific phenomenological features. An eight (items) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=114.00$, $p<0.0001$) and a main effect of the items ($F_{(7,112)}=2.86$, $p=0.009$), but no interaction ($F_{(7,112)}=1.76$, $p=0.102$ ns). Post-hoc multiple comparisons revealed significantly more ego dissolution following 25 mg versus 1 mg psilocybin on all items of the EDI: “All notion of self and identity dissolved away” (1 mg: $M=7.24$, $SD=20.38$; 25 mg: $M=78.00$, $SD=31.67$; $p<0.0001$, 95% CI [-82.45, -59.08], $g=2.5$); “I lost all sense of ego” (1 mg: $M=7.77$, $SD=19.69$; 25 mg: $M=73.18$, $SD=33.45$; $p<0.0001$, 95% CI [-77.10, -53.73], $g=2.3$); “I felt far less absorbed by my own issues and concerns” (1 mg: $M=12.71$, $SD=21.36$; 25 mg: $M=88.71$, $SD=19.02$; $p<0.0001$, 95% CI [-87.68, -64.32], $g=3.6$); “I experienced a disintegration of my “self” or “ego”” (1 mg: $M=8.18$, $SD=19.87$; 25 mg: $M=82.94$, $SD=25.21$; $p<0.0001$, 95% CI [-86.45, -63.08], $g=3.1$); “I experienced a decrease in my sense of self-importance” (1 mg: $M=8.24$, $SD=18.37$; 25 mg: $M=86.94$, $SD=20.40$; $p<0.0001$, 95% CI [-90.39, -67.02], $g=3.9$); “I felt a sense of union with others” (1 mg: $M=9.18$, $SD=18.73$; 25 mg: $M=74.88$, $SD=31.18$; $p<0.0001$, 95% CI [-77.39, -54.02], $g=2.4$); “I felt at one with the universe” (1 mg: $M=8.00$, $SD=17.75$; 25 mg: $M=73.29$, $SD=31.80$; $p<0.0001$, 95% CI [-76.98, -53.61], $g=2.4$); “I experienced a dissolution of my “self” or “ego”” (1 mg: $M=6.94$, $SD=16.11$; 25 mg: $M=83.53$, $SD=27.65$; $p<0.0001$, 95% CI [-88.27, -64.90], $g=3.2$).

For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly greater scores on the item “I felt far less absorbed by my own issues and concerns” ($M=88.71$, $SD=19.02$) compared with the items “I lost all sense of ego” ($M=73.18$, $SD=33.45$; $p=0.010$, 95% CI [-28.95, -2.11], $g=0.5$), “I felt a sense of union with others” ($M=74.88$, $SD=31.18$; $p=0.037$, 95% CI [0.40, 27.25], $g=0.5$) and “I felt at one with the universe” ($M=73.29$, $SD=31.80$; $p=0.010$, 95% CI [1.99, 28.83], $g=0.5$). Significantly greater scores were also observed on the item “I experienced a decrease in my sense of self-importance” ($M=86.94$, $SD=20.40$) compared with the items “I lost all sense of ego” ($M=73.18$, $SD=33.45$; $p=0.039$, 95% CI [-27.19, -0.34], $g=0.5$) and “I felt at one

with the universe" ($M=73.29$, $SD=31.80$; $p=0.042$, 95% CI [0.22, 27.07], $g=0.5$). No other differences between the items were found for the 25 mg dose of psilocybin (all $p>0.129$ ns).

Near death (type) experiences

All participants scored above the conventional threshold (≥ 7) for a (psilocybin-induced) near-death (type) experience (Greyson, 1983) following 25 mg psilocybin. The total NDE score was significantly greater following 25 mg ($M=16.41$, $SD=7.41$) versus 1 mg ($M=2.82$, $SD=3.23$) psilocybin; $t_{(16)}=8.696$, $p<0.0001$, $g=2.3$. Therefore, the mean score following 25 mg psilocybin exceeds the cut-off point and may be regarded as inducing a near-death (type) experience. The mean score following 1 mg psilocybin does not reach the cut-off point and may not be regarded as inducing a near-death (type) experience (Figure 4-5 A).

A four (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=75.62$, $p<0.0001$) and a main effect of subscale ($F_{(3,48)}=9.63$, $p<0.0001$), with no interaction between dose and subscale ($F_{(3,48)}=0.22$, $p=0.884$ ns). Post-hoc comparisons revealed that all subscales were significantly increased following 25 mg versus 1 mg psilocybin: cognitive (1 mg: $M=0.71$, $SD=0.92$; 25 mg: $M=4.06$, $SD=2.25$; $p<0.0001$, 95% CI [-4.53, -2.18], $g=1.9$); affective (1 mg: $M=1.41$, $SD=1.42$; 25 mg: $M=5.12$, $SD=2.55$; $p<0.0001$, 95% CI [-4.88, -2.53], $g=1.7$); paranormal (1 mg: $M=0.59$, $SD=1.06$; 25 mg: $M=3.89$, $SD=2.12$; $p<0.0001$, 95% CI [-4.47, -2.12], $g=1.9$); transcendental (1 mg: $M=0.12$, $SD=0.33$; 25 mg: $M=3.35$, $SD=2.40$; $p<0.0001$, 95% CI [-4.41, -2.06], $g=1.8$). These results show that near-death experience phenomena were significantly enhanced following 25 mg psilocybin administration. As the cognitive and transcendental component scores are each less than five and the affective component score exceeds five, the 25 mg psilocybin near-death (type) experience may be classified as affective-type.

Next, I analysed the 16 items comprising the NDE scale in order to assess the specific phenomenological features of the psilocybin experience. A 16 (items) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=75.62$, $p<0.0001$) and item ($F_{(15,240)}=11.28$, $p<0.0001$), as well as a significant interaction between dose and item ($F_{(15,240)}=3.46$, $p<0.0001$). Post-hoc comparisons revealed that 12 of the 16 items were scored significantly higher under 25 mg than 1 mg: altered time perception (1 mg: $M=0.29$, $SD=0.27$; 25 mg: $M=1.53$, $SD=0.62$; $p<0.0001$, 95% CI [-1.77, -0.71], $g=2.5$); speeded thoughts (1 mg: $M=0.06$, $SD=0.24$; 25 mg: $M=0.88$, $SD=0.93$; $p<0.0001$, 95% CI [-1.35, -0.29], $g=1.1$); understanding everything (1 mg: $M=0.06$, $SD=0.24$; 25 mg: $M=1.06$, $SD=0.75$; $p<0.0001$, 95% CI [-1.53, -0.47], $g=1.7$); peace/pleasantness (1 mg: $M=0.59$, $SD=0.71$; 25 mg: $M=1.59$, $SD=0.62$; $p<0.0001$, 95% CI [-1.53, -0.47], $g=1.4$); feelings of joy (1 mg: $M=0.53$, $SD=0.51$; 25 mg: $M=1.47$, $SD=0.62$; $p<0.0001$, 95% CI [-1.47, -0.41], $g=1.6$); harmony/unity (1 mg: $M=0.24$, $SD=0.56$; 25 mg: $M=1.24$, $SD=0.83$; $p<0.0001$, 95% CI [-1.53, -0.47], $g=1.3$); bright light (1 mg: $M=0.06$, $SD=0.24$; 25 mg:

$M=0.82$, $SD=0.95$; $p<0.001$, 95% CI [-1.29, -0.23], $g=1.0$); heightened senses (1 mg: $M=0.29$, $SD=0.47$; 25 mg: $M=1.71$, $SD=0.47$; $p<0.0001$, 95% CI [-1.94, -0.88], $g=2.9$); separation from the body (1 mg: $M=0.18$, $SD=0.39$; 25 mg: $M=1.18$, $SD=0.64$; $p<0.0001$, 95% CI [-1.53, -0.47], $g=1.8$); unearthly environment (1 mg: $M=0.18$, $SD=0.39$; 25 mg: $M=1.47$, $SD=0.62$; $p<0.0001$, 95% CI [-1.88, -0.82], $g=2.4$); encountering mystical being/presence (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=0.76$, $SD=0.90$; $p<0.001$, 95% CI [-1.29, -0.23], $g=1.1$); border/point of no return (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=0.59$, $SD=0.87$; $p=0.017$, 95% CI [-1.12, -0.06], $g=0.9$). There was no significant difference between the following items: encountering deceased/religious spirits (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=0.53$, $SD=0.87$; $p=0.051$ ns trend, 95% CI [-1.06, 0.00]); precognitive visions (1 mg: $M=0.06$, $SD=0.24$; 25 mg: $M=0.53$, $SD=0.87$; $p=0.131$ ns, 95% CI [-1.00, 0.06]); extrasensory perception (1 mg: $M=0.06$, $SD=0.24$; 25 mg: $M=0.47$, $SD=0.87$; $p=0.295$ ns, 95% CI [-0.94, 0.12]); and life review (1 mg: $M=0.29$, $SD=0.47$; 25 mg: $M=0.59$, $SD=0.71$; $p=0.814$ ns, 95% CI [-0.82, 0.24]).

These results show that altered time perception, speeded thoughts, understanding everything, peace/pleasantness, feelings of joy, harmony/unity, bright light, heightened senses, separation from the body, unearthly environment, encountering mystical being/presence and border/point of no return are the specific NDE phenomena enhanced following 25 mg psilocybin administration. Life review, extrasensory perception, precognitive visions and encountering deceased/religious spirits are NDE phenomena that are not enhanced following 25 mg psilocybin administration. This demonstrates that there are specific phenomenological features of the affective-type NDE occasioned by 25 mg psilocybin.

Emotional breakthrough

A Wilcoxon matched pairs test showed a significant increase in the participants' Emotional Breakthrough Inventory (EBI) scores for the 25 mg psilocybin session ($M=0.75$, $SD=0.27$) compared with the 1 mg psilocybin session ($M=0.05$, $SD=0.10$); $Z=153.0$, $p<0.0001$, 95% CI [0.56, 0.84], $g=3.3$; Figure 4-6 A).

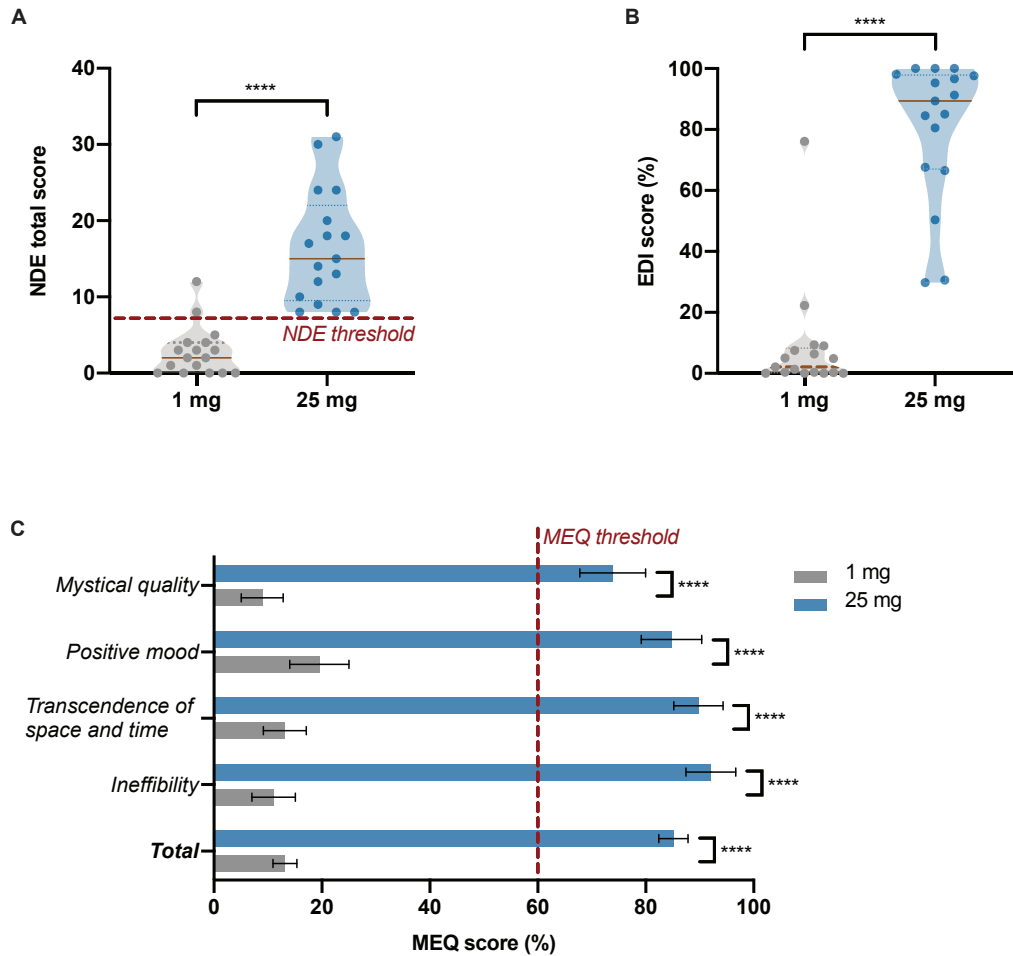


Figure 4-5. Peak experience phenomena | (A) Near-death experience. All participants scored above the conventional threshold (≥ 7) for a (psilocybin-induced) near-death (type) experience following 25 mg psilocybin. The NDE score was significantly greater following 25 mg versus 1 mg psilocybin ($t_{(16)}=8.696$, $p<0.0001$, $g=2.3$). The mean score following 25 mg psilocybin exceeds the cut-off point and may be regarded as inducing a near-death (type) experience. **(B) Ego-dissolution.** The EDI score was significantly greater following 25 mg versus 1 mg psilocybin ($Z=153.0$, $p<0.0001$, 95% CI [57.43, 85.88], $g=3.2$). **(C) Mystical experience.** The majority of participants scored above the threshold ($n=14$; $\geq 60\%$) for a psilocybin-induced mystical-type experience following 25 mg psilocybin. MEQ scores were significantly greater following 25 mg versus 1 mg psilocybin ($Z=153.0$, $p<0.0001$, 95% CI [61.96, 81.95], $g=4.1$). Post-hoc multiple comparisons revealed significantly greater scores following 25 mg versus 1 mg psilocybin on all subscales of the MEQ (all $p<0.0001$, $g>2.7$). | Data are expressed as mean $\pm$ SEM ($p<0.0001$ ****). Abbreviations: NDE, Near Death Experience; EDI, Ego Dissolution Inventory; MEQ, Mystical Experience Questionnaire.

Challenging experiences

The Challenging Experience Questionnaire (CEQ) (Barrett et al., 2016) was used to assess unpleasant reactions to psychedelic drugs, or “challenging” experiences. The CEQ yields a total score as well as seven subscales scores to assess specific phenomenological features of the

challenging experience (i.e., fear, grief, physical distress, insanity, isolation, death, and paranoia), covering acute affective, cognitive, and somatic symptoms.

A Wilcoxon matched pairs test showed a significant increase in the participants' overall Challenging Experience Questionnaire (CEQ) scores for the 25 mg psilocybin session ($M=0.35$, $SD=0.21$) compared with the 1 mg psilocybin session ($M=0.05$, $SD=0.05$); $Z=151.0$, $p<0.0001$, 95% CI [0.56, 0.84], $g=1.9$). A seven (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=31.75$, $p<0.0001$), a main effect of subscale ($F_{(6,96)}=5.67$, $p<0.0001$), and an interaction between dose and subscale ($F_{(6,96)}=3.33$, $p=0.005$; Figure 4-6 B).

Post-hoc comparisons revealed that the *paranoia* subscale scores did not change (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=0.06$, $SD=0.13$; $p=0.906$ ns, 95% CI [-0.23, 0.10]), but all other subscale scores were significantly increased following 25 mg versus 1 mg psilocybin: *death* (1 mg: $M=0.00$, $SD=0.00$; 25 mg: $M=0.32$, $SD=0.36$; $p<0.0001$, 95% CI [-0.49, -0.16], $g=1.2$); *isolation* (1 mg: $M=0.06$, $SD=0.14$; 25 mg: $M=0.46$, $SD=0.42$; $p<0.0001$, 95% CI [-0.57, -0.23], $g=1.2$); *grief* (1 mg: $M=0.10$, $SD=0.14$; 25 mg: $M=0.48$, $SD=0.30$; $p<0.0001$, 95% CI [-0.55, -0.22], $g=1.5$); *insanity* (1 mg: $M=0.02$, $SD=0.08$; 25 mg: $M=0.30$, $SD=0.33$; $p<0.0001$, 95% CI [-0.44, -0.11], $g=1.1$); *fear* (1 mg: $M=0.03$, $SD=0.04$; 25 mg: $M=0.31$, $SD=0.32$; $p<0.0001$, 95% CI [-0.45, -0.11], $g=1.2$); *physical distress* (1 mg: $M=0.06$, $SD=0.08$; 25 mg: $M=0.33$, $SD=0.25$; $p<0.0001$, 95% CI [-0.44, -0.11], $g=1.4$; Figure 4-6 C).

With regards to differences between the subscales, post-hoc multiple comparisons revealed that there was no significant difference between the CEQ subscales for the 1 mg dose of psilocybin (all $p>0.923$ ns). For the 25 mg dose of psilocybin, post-hoc multiple comparisons revealed significantly less *paranoia* ($M=0.06$, $SD=0.13$) than all other subscales: *death* ($M=0.32$, $SD=0.36$; $p<0.001$, 95% CI [-0.45, -0.07], $g=0.9$); *isolation* ($M=0.46$, $SD=0.42$; $p<0.0001$, 95% CI [-0.59, -0.21], $g=1.2$); *grief* ($M=0.48$, $SD=0.30$; $p<0.0001$, 95% CI [-0.60, -0.23], $g=1.7$); *insanity* ($M=0.30$, $SD=0.33$; $p=0.003$, 95% CI [-0.42, -0.05], $g=0.9$); *fear* ($M=0.31$, $SD=0.32$; $p=0.002$, 95% CI [-0.43, -0.06], $g=1.0$); *physical distress* ($M=0.33$, $SD=0.25$; $p<0.001$, 95% CI [-0.46, -0.08], $g=1.3$). No other differences between the subscales were found for the 25 mg dose of psilocybin (all $p>0.087$ ns).

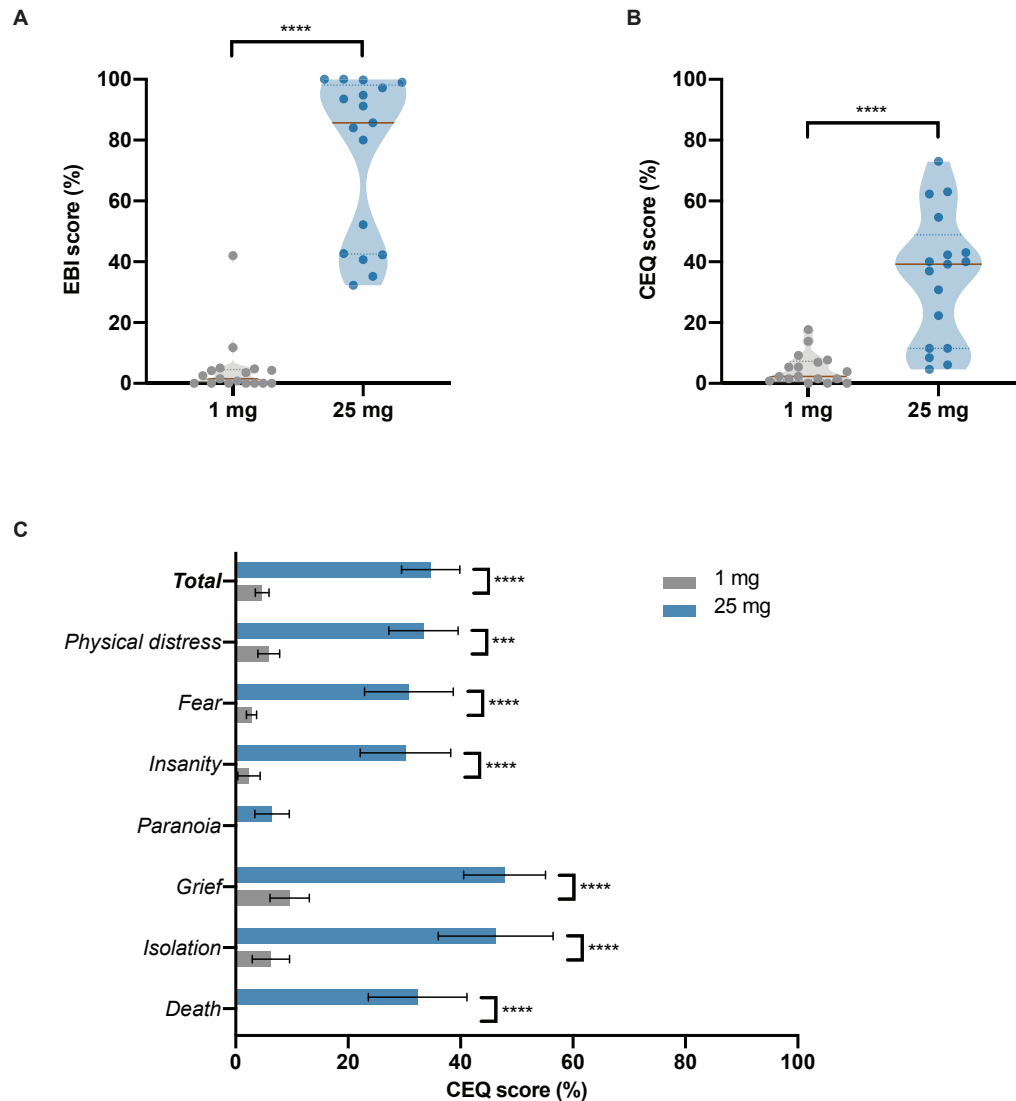


Figure 4-6. Emotional breakthrough and challenging experiences | (A) Emotional breakthrough. There was a significant increase in EBI scores for 25 mg versus 1 mg psilocybin ($Z=153.0$, $p<0.0001$, 95% CI [0.56, 0.84], $g=3.3$). **(B) Challenging experience.** There was a significant increase in total CEQ scores for the 25 mg versus 1 mg psilocybin ($Z=151.0$, $p<0.0001$, 95% CI [0.56, 0.84], $g=1.9$). **(C) CEQ subscales.** Post-hoc comparisons revealed that all subscale scores (except for *paranoia*; $p=0.906$ ns) were significantly increased following 25 mg versus 1 mg psilocybin (all $p<0.001$, $g>1.1$). | Data are expressed as mean $\pm$ SEM ($p<0.001$ ***; $p<0.0001$ ****). Abbreviations: EBI, Emotional Breakthrough Inventory; CEQ, Challenging Experience Questionnaire.

4.5.4. Acute brain effects

After each EEG recording, participants rated the degree to which they experienced (i) insight, (ii) emotional and (iii) visual imagery during the EEG using visual analogue scales (VAS). There were significantly higher VAS-insight, VAS-emotion and VAS-visual imagery scores for 25 mg versus

1 mg psilocybin at 1-hour ($p<0.001$) and 2-hours ($p<0.0001$,) after administration of psilocybin. Scores returned to baseline levels at 4.5 hours post-dosing (see Figure 4-7 A-C).

A two (dose; 1 mg vs 25 mg)×twelve ($3 \times$ VAS at EEG recording timepoints; time 0, 1, 2, and 4.5 hrs post-dosing) LMM revealed a significant main effect of dose ($F_{(1,16)}=110.00$, $p<0.0001$), a main effect of timepoint ($F_{(11,176)}=22.76$, $p<0.0001$) and a significant interaction between dose and timepoint ($F_{(11,163)}=28.87$, $p<0.0001$). Post-hoc comparisons revealed significantly greater VAS-insight at one hour (1 mg: $M=17.68$, $SD=17.19$; 25 mg: $M=59.41$, $SD=34.76$; $p<0.0001$, 95% CI [-60.81, -20.43]), two hours (1 mg: $M=20.61$, $SD=20.53$; 25 mg: $M=88.18$, $SD=12.81$; $p<0.0001$, 95% CI [-87.67, -47.92]) and four hours 30 mins (1 mg: $M=16.93$, $SD=23.32$; 25 mg: $M=41.57$, $SD=32.01$; $p=0.002$, 95% CI [-45.62, -6.58]) after the administration of 25 mg compared with 1 mg psilocybin (Figure 4-7 A). Similar findings were obtained for VAS-emotion, whereby significantly greater scores were found at one hour (1 mg: $M=31.96$, $SD=20.37$; 25 mg: $M=69.80$, $SD=29.48$; $p<0.0001$, 95% CI [-56.47, -19.22]), two hours (1 mg: $M=19.38$, $SD=15.94$; 25 mg: $M=89.70$, $SD=12.26$; $p<0.0001$, 95% CI [-88.94, -51.69]) and four hours 30 mins (1 mg: $M=17.26$, $SD=18.57$; 25 mg: $M=44.68$, $SD=36.63$; $p<0.001$, 95% CI [-46.04, -8.79]) after the administration of 25 mg compared with 1 mg psilocybin (Figure 4-7 B). Likewise, significantly greater VAS-visual imagery was found at one hour (1 mg: $M=28.00$, $SD=21.12$; 25 mg: $M=81.59$, $SD=26.47$; $p<0.0001$, 95% CI [-56.47, -19.22]), two hours (1 mg: $M=16.17$, $SD=19.48$; 25 mg: $M=92.33$, $SD=13.10$; $p<0.0001$, 95% CI [-88.94, -51.69]) and four hours 30 mins (1 mg: $M=13.53$, $SD=19.91$; 25 mg: $M=44.10$, $SD=40.31$; $p<0.001$, 95% CI [-46.04, -8.79]) after the administration of 25 mg compared with 1 mg psilocybin (Figure 4-7 C). No difference between the doses was found at time 0 for any VAS scale (all $p>0.146$ ns).

A two (dose; 1 mg vs 25 mg)×four (EEG recording timepoint; time 0, 1, 2, and 4.5 hrs post-dosing) LMM revealed a significant main effect of dose ($F_{(1,11)}=10.22$, $p=0.008$), a main effect of EEG recording timepoint ($F_{(1.58,17.41)}=6.40$, $p=0.012$) and a significant interaction between dose and EEG recording timepoint ($F_{(2.12,14.15)}=5.52$, $p=0.016$). Post-hoc comparisons revealed significantly greater LZc values two hours post dosing with 25 mg ($M=0.47$, $SD=0.03$) relative to 1 mg psilocybin ($M=0.42$, $SD=0.03$; $p=0.024$, 95% CI [-0.08, -0.01], $g=1.6$). No other significant differences in LZc values were found between the doses of psilocybin at any other EEG recording timepoints (all $p>0.102$ ns). With regards to differences between the EEG recording timepoints, post-hoc multiple comparisons revealed significantly greater LZc values at one hour ($M=0.46$, $SD=0.04$; $p=0.010$, 95% CI [-0.08, -0.01], $g=1.1$) and two hours ($M=0.47$, $SD=0.03$; $p=0.009$, 95% CI [-0.08, -0.01], $g=1.6$) post-dosing with 25 mg psilocybin relative to baseline ($M=0.42$, $SD=0.02$; Figure 4-7 D-E). No other significant differences were found between the EEG recording timepoints for the 25 mg dose of psilocybin. With regards to the 1 mg dose of psilocybin, no differences were observed between any EEG recording timepoints (all $p>0.455$ ns).

The REBUS model (Carhart-Harris and Friston, 2019) theorises that increased brain signal complexity with relaxed high-level priors enables the psychedelic brain to be more amenable to

curiosity and BMR processes, which may result in the facilitation of psychological insight and the attainment of novel perspectives. Therefore, a key question of this research was: is insightfulness related to brain signal complexity during the acute 25 mg psilocybin peak experience? To address this question, a simple linear regression was carried out between the two hours post-dosing LZc and VAS-insight scores. In line with the hypothesis, greater insight experienced during the recording positively correlated with increased LZc ($F_{(1,9)}=6.12$, $R^2=0.405$, $p=0.035$) two hours after the administration of 25 mg psilocybin (Figure 4-7 F). No other associations were found between the VAS ratings and LZc.

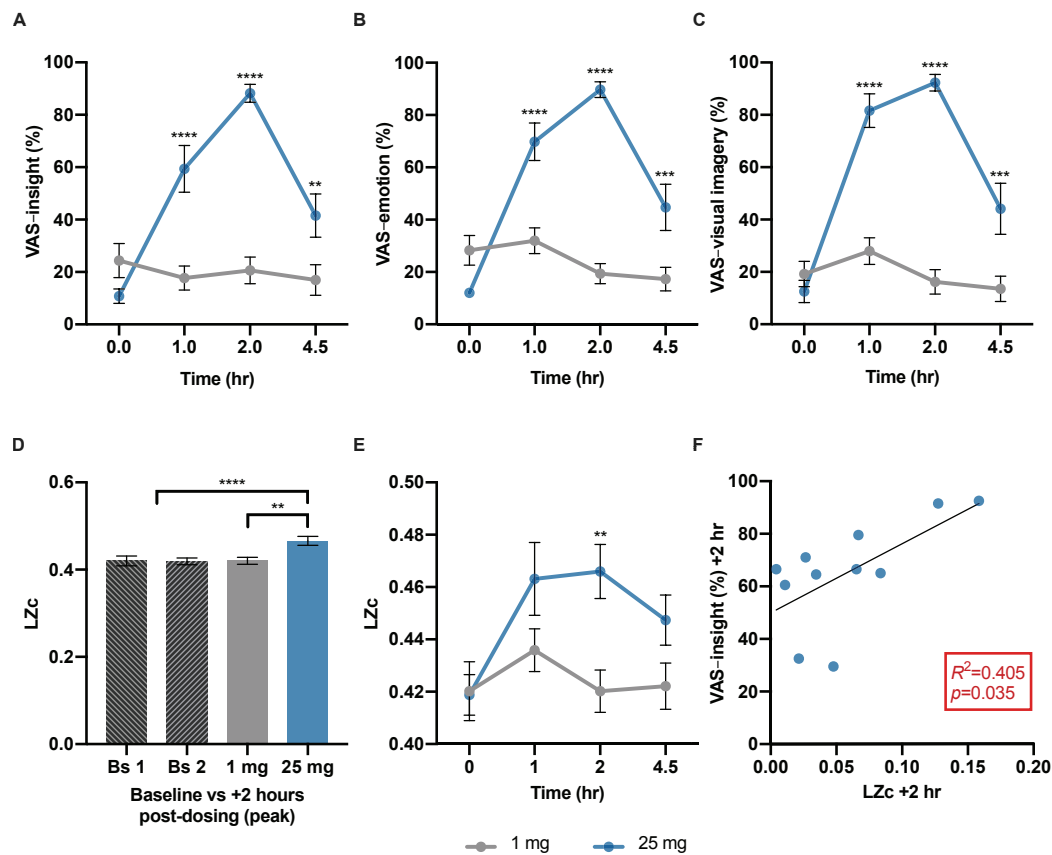


Figure 4-7. Acute brain effects | (A) VAS-insight. Significantly greater VAS-insight was observed at one hour ($p<0.0001$, 95% CI [-60.81, -20.43]), two hours ($p<0.0001$, 95% CI [-87.67, -47.92]) and four hours 30 mins ($p=0.002$, 95% CI [-45.62, -6.58]) after the administration of 25 mg compared with 1 mg psilocybin. **(B) VAS-emotion.** Significantly greater VAS-emotion was observed at one hour ($p<0.0001$, 95% CI [-56.47, -19.22]), two hours ($p<0.0001$, 95% CI [-88.94, -51.69]) and four hours 30 mins ($p<0.001$, 95% CI [-46.04, -8.79]) after the administration of 25 mg compared with 1 mg psilocybin. **(C) VAS-visual imagery.** Significantly greater VAS-visual imagery was observed at one hour ($p<0.0001$, 95% CI [-56.47, -19.22]), two hours ($p<0.0001$, 95% CI [-88.94, -51.69]) and four hours 30 mins ($p<0.001$, 95% CI [-46.04, -8.79]) after the administration of 25 mg compared with 1 mg psilocybin. **(D) Lempel-Ziv complexity at two hours post-dosing versus baseline.** Significantly greater LZc scores were observed two hours post-dosing with 25 mg relative to two hours post-dosing with 1 mg psilocybin ($p=0.024$, 95% CI [-0.08, -0.01], $g=1.6$) and baseline ($p=0.009$, 95% CI [-0.08, -0.01], $g=1.6$). **(E) Time-course of Lempel-Ziv complexity.** Significantly greater

LZc values were found two hours post-dosing with 25 mg relative to 1 mg psilocybin ($p=0.024$, 95% *CI* [-0.08, -0.01], $g=1.6$). No other significant differences in LZc values were found between the doses of psilocybin at any other EEG recording timepoints. With regards to differences between the EEG recording timepoints, post-hoc multiple comparisons revealed significantly greater LZc values at one hour ($p=0.010$, 95% *CI* [-0.08, -0.01], $g=1.1$) and two hours ($p=0.009$, 95% *CI* [-0.08, -0.01], $g=1.6$) post-dosing with 25 mg psilocybin relative to baseline. No other significant differences were found between the EEG recording timepoints for the 25 mg dose of psilocybin. No differences were observed between any EEG recording timepoints for the 1 mg dose of psilocybin. **(F) Relationship between complexity and insight.** A positive association was found between LZc and VAS-insight scores ($F_{(1,9)}=6.12$, $R^2=0.405$, $p=0.035$) two hours after the administration of 25 mg psilocybin. | Data are expressed as mean $\pm$ SEM ($p<0.01^{**}$; $p<0.001^{***}$; $p<0.0001^{****}$). Abbreviations: VAS, visual analogue scale; LZc, Lempel-Ziv complexity; Bs 1, baseline 1 - pre-dosing 1 mg); Bs 2, baseline 2 - pre-dosing 25 mg).

4.5.5. *Psilocybin integration sessions*

A Wilcoxon matched pairs test showed a significant increase in the participants' Mastery-Insight Scale (MIS) scores one day after the 25 mg psilocybin session ($M=1.48$, $SD=0.37$) compared with one day after the 1 mg psilocybin session ($M=0.33$, $SD=0.50$; $Z=152.0$, $p<0.0001$, 95% *CI* [0.89, 1.41], $g=2.5$; Figure 4-8 A). Self-ratings of gains from participation in the integration sessions were also assessed using the Gains from Psychedelic Integration (GPI) questionnaire. A Wilcoxon matched pairs test showed a significant increase in the participants' overall GPI scores one day after the 25 mg psilocybin session ($M=6.38$, $SD=1.18$) compared with one day after the 1 mg psilocybin session ($M=3.25$, $SD=1.99$; $Z=150.0$, $p<0.0001$, 95% *CI* [1.94, 4.33], $g=1.8$) Figure 4-8 B). Next, analyses were completed for the three separate subscales contained in the GPI. A three (subscale) $\times$ two (dose) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,16)}=30.93$, $p<0.0001$), no effect of subscale ($F_{(1.07,17.17)}=2.73$, $p=0.115$ ns) and an interaction between dose and subscale ($F_{(1.24,19.90)}=5.81$, $p=0.020$). Post-hoc comparisons revealed significantly greater experiential gains (1 mg: $M=3.18$, $SD=2.10$; 25 mg: $M=5.71$, $SD=1.80$; $p=0.003$, 95% *CI* [-4.23, -0.83], $g=1.2$), exploration-insight gains (1 mg: $M=3.34$, $SD=2.05$; 25 mg: $M=6.75$, $SD=1.34$; $p<0.0001$, 95% *CI* [-4.93, -1.90], $g=1.9$) and insight-action gains (1 mg: $M=3.24$, $SD=2.06$; 25 mg: $M=6.69$, $SD=1.29$; $p<0.0001$, 95% *CI* [-4.96, -1.96], $g=1.9$) one day after the 25 mg dose compared with one day after the 1 mg dose of psilocybin Figure 4-8 C).

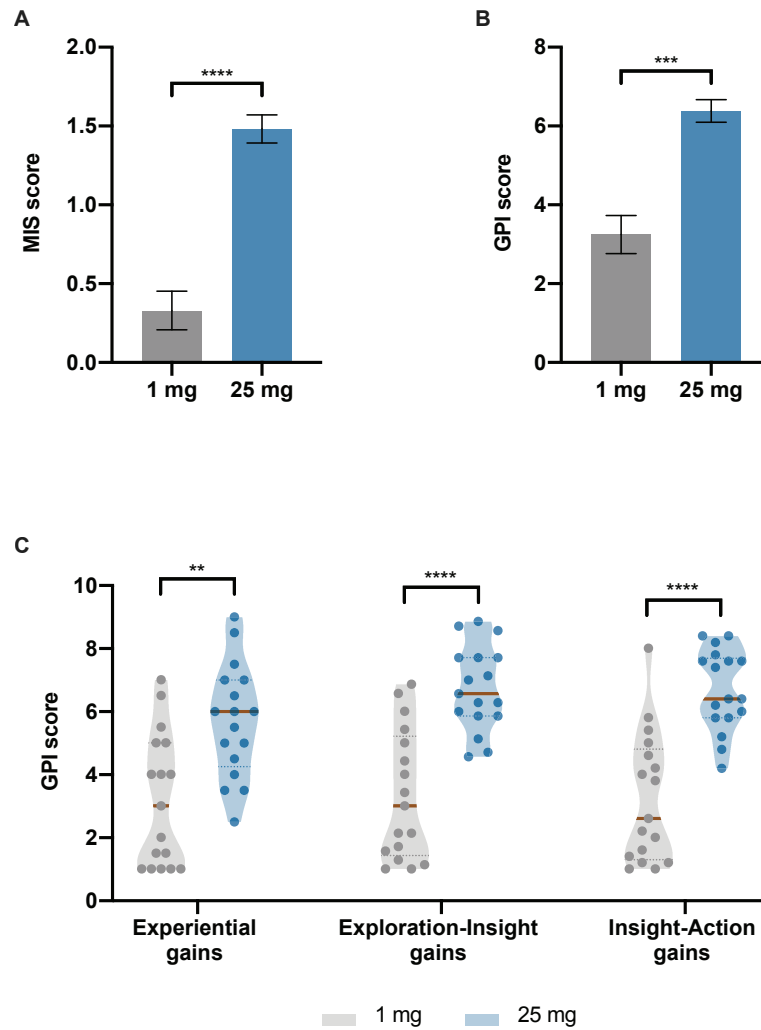


Figure 4-8. Integration sessions (+1 day post-dosing) | (A) Mastery-Insight. MIS scores were significantly increased following the 25 mg psilocybin integration compared with the 1 mg psilocybin integration ($Z=152.0$, $p<0.0001$, 95% CI [0.89, 1.41], $g=2.5$). **(B) Total gains from psychedelic integration.** A significant increase in overall GPI scores was found following the 25 mg psilocybin integration compared with the 1 mg psilocybin integration ($Z=150.0$, $p<0.0001$, 95% CI [1.94, 4.33], $g=1.8$). **(C) Experiential, exploration-insight and insight-action gains from psychedelic integration.** Post-hoc comparisons of the GPI revealed significantly greater *experiential gains* ($p=0.003$, 95% CI [-4.23, -0.83], $g=1.2$), *exploration-insight gains* ($p<0.0001$, 95% CI [-4.93, -1.90], $g=1.9$) and *insight-action gains* ($p<0.0001$, 95% CI [-4.96, -1.96], $g=1.9$) following the 25 mg psilocybin integration compared with the 1 mg psilocybin integration. | Data are expressed as mean $\pm$ SEM ($p<0.01^{**}$, $p<0.001^{***}$, $p<0.0001^{****}$). Abbreviations: MIS, Mastery-Insight Scale; GPI, Gains from Psychedelic Integration.

4.5.6. Long-term outcomes

Psychological insight

The Psychological Insight Scale (PIS) was used to assess the extent of psychological insight and subsequent behavioural changes facilitated by psilocybin. The PIS has two subscales: the *psychological insight (PI)* subscale to assess the insight and the *behavioural change (BC)* subscale to assess insight-induced changes in behaviour.

A two (subscale)×six (timepoint) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1.98,31.64)}=42.35$, $p<0.0001$), no effect of subscale ($F_{(1,16)}=0.06$, $p=0.817$ ns), and a significant interaction between dose and subscale ($F_{(2.73,43.75)}=4.71$, $p=0.008$). Post-hoc comparisons revealed that both subscales were significantly increased one day (PIS-PI, 1 mg: $M=9.70$, $SD=19.56$; 25 mg: $M=66.87$, $SD=24.05$, $p<0.0001$, 95% CI [-75.55, -38.80], $g=2.5$; PIS-BC, 1 mg: $M=9.65$, $SD=23.32$; 25 mg: $M=53.41$, $SD=28.43$; $p<0.0001$, 95% CI [-65.42, -22.11], $g=1.6$), 14 days (PIS-PI, 1 mg: $M=9.98$, $SD=23.44$; 25 mg: PIS $M=45.91$, $SD=25.89$, $p<0.001$, 95% CI [-54.61, -17.26], $g=1.4$; PIS-BC, 1 mg: $M=11.06$, $SD=23.05$; 25 mg: $M=56.35$, $SD=29.83$, $p<0.0001$, 95% CI [-67.02, -23.57], $g=1.6$) and four weeks (PIS-PI, 1 mg: $M=13.14$, $SD=19.27$; 25 mg: $M=48.54$, $SD=22.78$, $p<0.0001$, 95% CI [-52.41, -18.40], $g=1.6$; PIS-BC, 1 mg: $M=13.71$, $SD=24.79$; 25 mg: $M=53.06$, $SD=29.44$, $p=0.002$, 95% CI [-65.98, -12.72], $g=1.4$) following the 25 mg psilocybin session versus the 1 mg psilocybin session (Figure 4-9 A-B).

Self-reflection and insight

A five (timepoint)×three (subscale) repeated-measures ANOVA revealed a significant main effect of timepoint ($F_{(4,64)}=9.66$, $p<0.0001$) and a main effect of subscale ($F_{(2,32)}=19.28$, $p<0.0001$), but no interaction between timepoint and subscale ($F_{(8,128)}=0.35$, $p=0.943$ ns; Figure 4-9 C-E).

Post-hoc comparisons revealed that there was no significant difference in the Engagement in Self-Reflection subscale scores at 14 days ($M=4.90$, $SD=0.82$; $p=0.998$ ns, 95% CI [-0.38, 0.31]) or four weeks ($M=4.92$, $SD=0.77$; $p=0.989$ ns, 95% CI [-0.40, 0.29]) after the 1 mg psilocybin session compared to baseline ($M=4.86$, $SD=0.76$). However, significantly greater Engagement in Self-Reflection subscale scores were observed at 14 days ($M=5.40$, $SD=0.48$; $p=0.015$, 95% CI [-0.74, -0.05], $g=0.7$) and four weeks ($M=5.49$, $SD=0.56$; $p=0.018$, 95% CI [-0.39, -0.05], $g=0.8$) after the 25 mg psilocybin session compared to the second baseline ($M=4.92$, $SD=0.77$; Figure 4-9 E). Relatedly, no significant difference was found in the Need for Self-Reflection subscale scores at 14 days ($M=5.02$, $SD=0.72$; $p=0.999$ ns, 95% CI [-0.32, 0.37]) or four weeks ($M=5.08$, $SD=0.65$; $p=0.999$ ns, 95% CI [-0.37, 0.32]) after the 1 mg psilocybin session compared to baseline ($M=5.05$, $SD=0.75$). However, significantly greater Need for Self-Reflection subscale scores were observed at four weeks ($M=5.31$, $SD=0.56$; $p=0.011$, 95% CI [-0.76, -0.07], $g=0.4$), but only at

trend level at 14 days ($M=5.32$, $SD=0.57$; $p=0.078$ (ns trend), 95% CI [-0.67, 0.02], $g=0.4$), after the 25 mg psilocybin session compared to the second baseline ($M=5.08$, $SD=0.65$; (Figure 4-9 D). No significant difference in the Insight subscale scores was found at 14 days ($M=4.20$, $SD=0.77$; $p=0.995$ ns, 95% CI [-0.39, 0.29]) or four weeks ($M=4.17$, $SD=0.90$; $p>0.999$ ns, 95% CI [-0.36, 0.33] after the 1 mg psilocybin session compared to baseline ($M=4.15$, $SD=0.80$). However, significantly greater Insight subscale scores were observed at 14 days ($M=4.73$, $SD=0.74$; $p\leq 0.0001$, 95% CI [-0.91, -0.22], $g=0.6$) and four weeks ($M=4.58$, $SD=0.80$; $p=0.012$, 95% CI [-0.75, -0.06], $g=0.5$) after the 25 mg psilocybin session compared to the second baseline ($M=4.17$, $SD=0.90$; Figure 4-9 C).

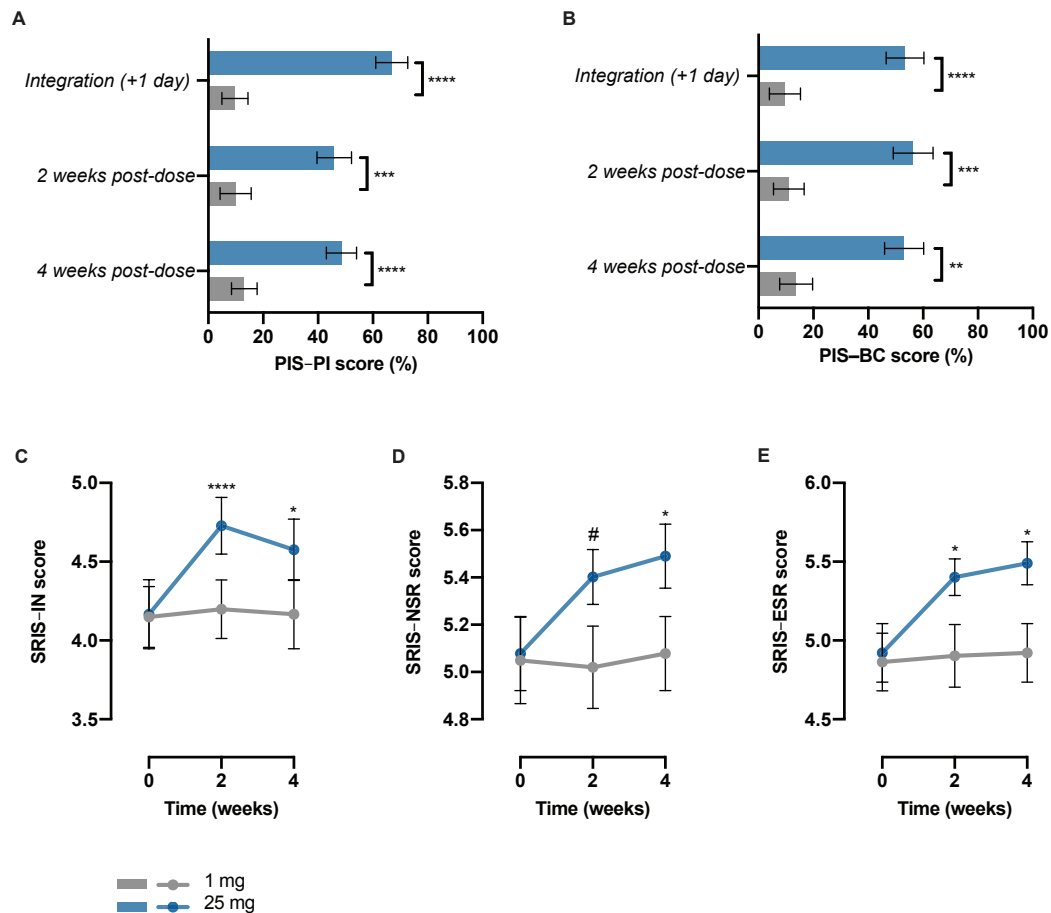


Figure 4-9. Insight | (A) Psychological insight. PIS-PI scores were significantly increased one day ($Z=152.0$, $p<0.0001$, 95% CI [0.89, 1.41], $g=2.5$), 14 days ($p<0.001$, 95% CI [-54.61, -17.26], $g=1.4$) and four weeks ($p<0.0001$, 95% CI [-52.41, -18.40], $g=1.6$) after 25 mg versus 1 mg psilocybin. **(B) Insight-induced behavioural change.** Scores on the PIS-BC subscale were significantly increased one day ($p<0.0001$, 95% CI [-65.42, -22.11], $g=1.6$), 14 days ($p<0.0001$, 95% CI [-67.02, -23.57], $g=1.6$) and four weeks ($p=0.002$, 95% CI [-65.98, -12.72], $g=1.4$) after 25 mg versus 1 mg psilocybin. **(C) SRIS – Insight.** Significantly greater SRIS-IN scores were observed at 14 days ($p\leq 0.0001$, 95% CI [-0.91, -0.22], $g=0.6$) and four weeks ($p=0.012$, 95% CI [-0.75, -0.06], $g=0.5$) after 25 mg psilocybin. No differences were found following 1 mg psilocybin (all $p>0.994$ ns). **(D) SRIS – Need for self-reflection.** Significantly greater SRIS-NSR scores were observed

at four weeks ($p=0.011$, 95% CI [-0.76, -0.07], $g=0.4$), but only at trend level at 14 days ($p=0.078$ (ns trend), 95% CI [-0.67, 0.02], $g=0.4$), after the 25 mg psilocybin session. No differences were found following 1 mg psilocybin (all $p>0.988$ ns). **(E) SRIS – engagement in self-reflection.** Significantly greater SRIS-ESR scores were observed at 14 days ($p=0.015$, 95% CI [-0.74, -0.05], $g=0.7$) and four weeks ($p=0.018$, 95% CI [-0.39, -0.05], $g=0.8$) after 25 mg psilocybin. No differences were found following 1 mg psilocybin (all $p>0.998$ ns). | Data are expressed as mean $\pm$ SEM ($p=0.078^{\#}$ (ns trend), $p<0.05^*$, $p<0.01^{**}$, $p<0.001^{***}$, $p\leq 0.0001^{****}$). Abbreviations: PIS, Psychological Insight Scale; PIS-PI, Psychological Insight subscale; PIS-BC, Behavioural Change subscale; SRIS, Self-Reflection and Insight Scale; IN, insight; SRIS-NSR, SRIS need for self-reflection subscale; SRIS-ESR, SRIS engagement in self-reflection subscale.

Psychological well-being

A one-way repeated-measures ANOVA revealed a significant difference in the Warwick-Edinburgh Mental Well-being Scale (WEMWBS) scores between the timepoints ($F_{(2.73,42.05)}=7.59$, $p<0.001$). Post-hoc multiple comparisons revealed that there was no significant difference in WEMWBS scores at 14 days ($M=47.82$, $SD=6.71$; $p=0.424$ ns, 95% CI [-1.67, 6.49]) or four weeks ($M=47.88$, $SD=6.93$; $p=0.339$ ns, 95% CI [-1.31, 6.01]) after the 1 mg psilocybin session compared with baseline ($M=50.24$, $SD=6.84$). However, significantly greater WEMWBS scores were observed at 14 days ($M=54.94$, $SD=5.73$; $p=0.007$, 95% CI [-12.39, -1.73], $g=1.1$) and four weeks ($M=53.53$, $SD=6.49$; $p=0.012$, 95% CI [-10.23, -1.06], $g=0.8$) after the 25 mg psilocybin session compared with the second baseline ($M=47.88$, $SD=6.93$; Figure 4-10 A).

Meaning in life

A five (timepoint) $\times$ two (subscale) repeated-measures ANOVA revealed a significant main effect of timepoint ($F_{(3.05,48.76)}=3.29$, $p=0.027$) and a main effect of subscale ($F_{(1,16)}=30.48$, $p<0.0001$), and an interaction between timepoint and subscale ($F_{(2.76,44.11)}=7.34$, $p<0.001$).

Post-hoc comparisons revealed that there was no significant difference in the Presence of Meaning subscale scores at 14 days ($M=17.47$, $SD=5.42$; $p>0.999$ ns, 95% CI [-2.72, 2.48]) or four weeks ($M=16.59$, $SD=5.26$; $p=0.857$ ns, 95% CI [-1.61, 3.14]) after the 1 mg psilocybin session compared to baseline ($M=17.35$, $SD=5.33$). However, significantly greater Presence of Meaning subscale scores were observed at 14 days ($M=21.76$, $SD=4.84$; $p=0.015$, 95% CI [-9.56, -0.79], $g=1.0$) and four weeks ($M=21.17$, $SD=5.79$; ($p=0.032$, 95% CI [-8.85, 0.29], $g=0.8$) after the 25 mg psilocybin session compared to the second baseline ($M=16.59$, $SD=5.26$).

There was no significant difference in the Search for Meaning subscale scores at 14 days (1 mg: $M=27.12$, $SD=3.79$, $p>0.999$ ns, 95% CI [-3.04, 3.63]; 25 mg: $M=25.82$, $SD=5.49$, $p=0.986$ ns, 95% CI [-1.81, 3.34]) or four weeks (1 mg: $M=26.82$, $SD=5.00$, $p=0.998$ ns, 95% CI [-2.84, 4.49]; 25 mg: $M=26.65$, $SD=3.90$, $p>0.999$ ns, 95% CI [-3.77, 3.65]) after the 1 mg or 25 mg psilocybin sessions compared to their relative baseline scores (1 mg: $M=27.41$, $SD=4.93$; 25 mg $M=26.82$, $SD=5.00$; Figure 4-10 B-C).

Flourishing

A one-way repeated-measures ANOVA revealed a significant difference in The Flourishing Scale (FS) scores between the timepoints ($F_{(4,64)}=6.14$, $p<0.001$). Post-hoc multiple comparisons revealed that there was no significant difference in FS scores at 14 days ($M=41.88$, $SD=5.59$; $p>0.999$ ns, 95% CI [-3.55, 3.66]) or four weeks ($M=40.76$, $SD=7.45$; $p=0.876$ ns, 95% CI [-2.43, 4.78]) after the 1 mg psilocybin session compared with baseline ($M=41.94$, $SD=6.55$). However, significantly greater FS scores were observed at 14 days ($M=46.35$, $SD=6.41$; $p<0.001$, 95% CI [-9.19, -1.98], $g=0.8$) and four weeks ($M=45.47$, $SD=6.74$; $p=0.005$, 95% CI [-8.31, -1.10], $g=0.6$) after the 25 mg psilocybin session compared with the second baseline ($M=40.76$, $SD=7.45$; Figure 4-10 D).

Connectedness to nature

A one-way repeated-measures ANOVA revealed a significant difference in the Connectedness to Nature Scale (CNS) scores between the timepoints ($F_{(2.07,31.95)}=11.60$, $p\leq0.0001$). Post-hoc multiple comparisons revealed that there was no significant difference in CNS scores at 14 days ($M=4.58$, $SD=1.11$; $p>0.999$ ns, 95% CI [-0.36, 0.39]) or four weeks ($M=4.51$, $SD=0.96$; $p=0.988$ ns, 95% CI [-0.41, 0.56]) after the 1 mg psilocybin session compared with baseline ($M=4.59$, $SD=1.09$). However, significantly greater CNS scores were observed at 14 days ($M=5.26$, $SD=0.94$; $p<0.001$, 95% CI [-1.14, -0.34], $g=0.7$) and four weeks ($M=5.20$, $SD=0.86$; $p<0.0001$, 95% CI [-1.00, -0.37], $g=0.7$) after the 25 mg psilocybin session compared with the second baseline ($M=4.51$, $SD=0.96$; Figure 4-10 E).

Social Connectedness

A one-way repeated-measures ANOVA revealed a significant difference in the Social Connectedness Scale (SCS) scores between the timepoints ($F_{(2.73,43.75)}=8.79$, $p<0.001$). Post-hoc multiple comparisons revealed that there was no significant difference in SCS scores at 14 days ($M=32.71$, $SD=11.66$; $p>0.999$ ns, 95% CI [-2.45, 4.92]) or four weeks ($M=33.71$, $SD=10.09$; $p>0.999$ ns, 95% CI [-4.36, 4.83]) after the 1 mg psilocybin session compared with baseline ($M=33.94$, $SD=10.23$). However, significantly increased SCS scores were observed at 14 days ($M=39.65$, $SD=8.79$; $p=0.006$, 95% CI [-10.31, -1.58], $g=0.6$), and increased at trend level at four weeks ($M=37.88$, $SD=8.96$; $p=0.066$ (ns trend level), 95% CI [-8.56, 0.21], $g=0.4$), after the 25 mg psilocybin session compared with the second baseline ($M=33.71$, $SD=10.09$; Figure 4-10 F).

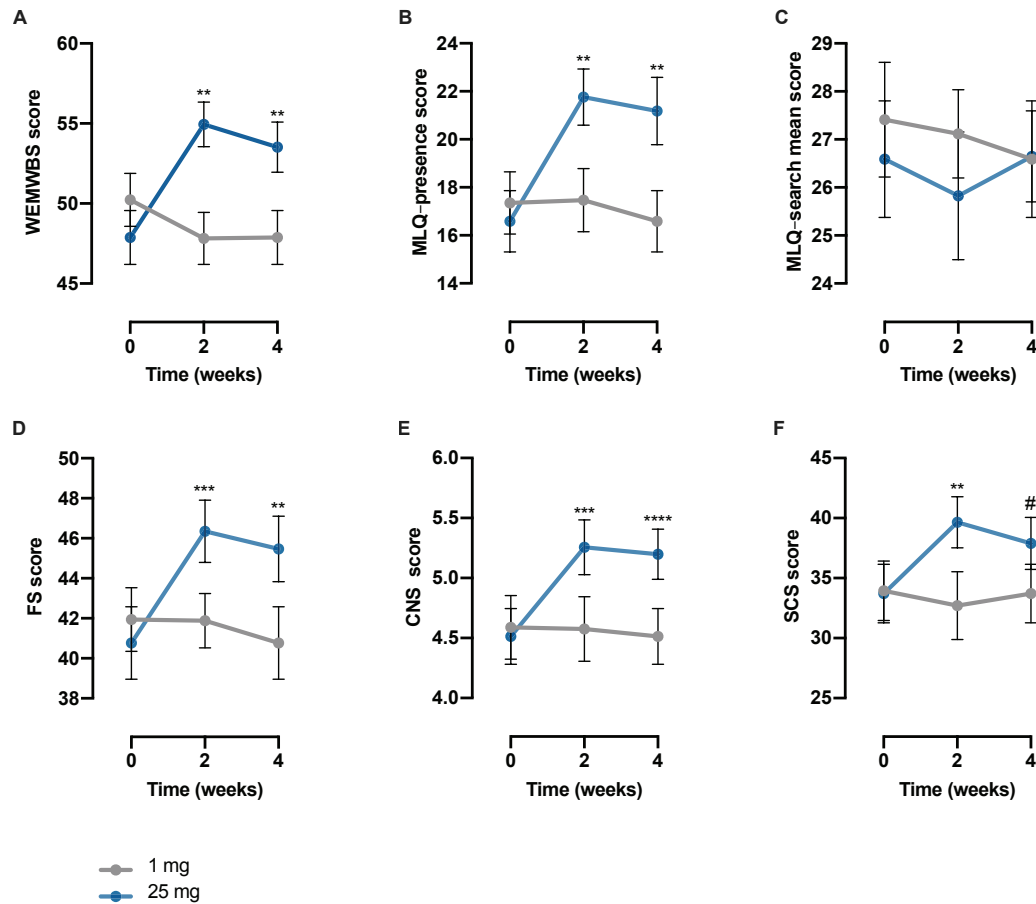


Figure 4-10. Well-being and connectedness | (A) Psychological well-being. WEMWBS scores were significantly increased 14 days ($p < 0.001$, 95% CI [-54.61, -17.26], $g = 1.4$) and four weeks ($p < 0.0001$, 95% CI [-52.41, -18.40], $g = 1.6$) after 25 mg psilocybin. No differences were found in WEMWBS scores after 1 mg psilocybin. **(B–C) Meaning in life.** MLQ-presence scores were significantly increased 14 days ($p = 0.015$, 95% CI [-9.56, -0.79], $g = 1.0$) and four weeks ($p = 0.032$, 95% CI [-8.85, 0.29], $g = 0.8$) after 25 mg psilocybin. No differences were found in MLQ-presence scores after 1 mg psilocybin. There were no significant differences in MLQ-search scores. **(D) Flourishing.** Significantly greater FS scores were observed 14 days ($p < 0.001$, 95% CI [-9.19, -1.98], $g = 0.8$) and four weeks ($p = 0.005$, 95% CI [-8.31, -1.10], $g = 0.6$) after 25 mg psilocybin. There was no significant difference in FS scores after 1 mg psilocybin. **(E) Connectedness to nature.** Significantly greater CNS scores were observed 14 days ($p < 0.001$, 95% CI [-1.14, -0.34], $g = 0.7$) and four weeks ($p < 0.0001$, 95% CI [-1.00, -0.37], $g = 0.7$) after 25 mg psilocybin. No significant differences in CNS scores were found after 1 mg psilocybin. **(F) Social connectedness.** Significantly increased SCS scores were observed at 14 days ($p = 0.006$, 95% CI [-10.31, -1.58], $g = 0.6$), and increased at trend level at four weeks ($M = 37.88$, $SD = 8.96$; $p = 0.066$ ns trend, 95% CI [-8.56, 0.21], $g = 0.4$), after 25 mg psilocybin. There was no significant difference in SCS scores after 1 mg psilocybin. | Data are expressed as mean $\pm$ SEM ($p = 0.066^{\#}$ (ns trend), $p < 0.01^{**}$, $p < 0.001^{***}$, $p < 0.0001^{****}$). Abbreviations: WEMWBS, Warwick-Edinburgh Mental Well-being Scale; MLQ, Meaning in Life Questionnaire; FS, Flourishing Scale; CNS, Connectedness to Nature Scale; SCS, Social Connectedness Scale.

Gratitude

A non-parametric Friedman one-way repeated-measures ANOVA test revealed a significant difference in the Gratitude Questionnaire (GQ-6) scores between the timepoints ($\chi^2(4)=19.38$, $p<0.001$). Post-hoc multiple comparisons revealed that there was no significant difference in GQ-6 scores at 14 days ($M=5.92$, $SD=0.93$; $p>0.999$ ns) or four weeks ($M=5.92$, $SD=0.95$; $p>0.999$ ns) after the 1 mg psilocybin session compared with baseline ($M=5.88$, $SD=1.03$). However, significantly greater GQ-6 scores were observed at 14 days ($M=6.47$, $SD=0.62$; $p=0.003$, $g=0.6$) and at trend level at four weeks ($M=6.28$, $SD=0.80$; $p=0.079$ ns (trend level), $g=0.4$) after the 25 mg psilocybin session compared with the second baseline ($M=5.92$, $SD=0.95$; Figure 4-11 A).

Compassion

A one-way repeated-measures ANOVA revealed a significant difference in The Santa Clara Brief Compassion Scale (SCBCS) scores between the timepoints ($F_{(3.14,48.28)}=6.47$, $p<0.0001$). Post-hoc multiple comparisons revealed that there was no significant difference in SCBCS scores at 14 days ($M=4.71$, $SD=1.03$; $p=0.616$ ns, 95% CI [-0.20, 0.56]) or four weeks ($M=4.54$, $SD=1.29$; $p=0.350$ ns, 95% CI [-0.21, 0.89]) after the 1 mg psilocybin session compared with baseline ($M=4.88$, $SD=0.90$). However, significantly greater SCBCS scores were observed at 14 days ($M=5.40$, $SD=0.87$; $p=0.006$, 95% CI [-1.49, -0.22], $g=0.7$) and four weeks ($M=5.25$, $SD=0.93$; $p=0.009$, 95% CI [-1.25, -0.16], $g=0.6$) after the 25 mg psilocybin session compared with the second baseline ($M=4.54$, $SD=1.29$; Figure 4-11 B).

Interpersonal reactivity

A one-way repeated-measures ANOVA revealed no significant difference in the IRI total scores four weeks after the 1 mg ($M=65.94$, $SD=12.82$; $p=0.413$ ns, 95% CI [-2.13, 6.48]) or 25 mg ($M=68.06$, $SD=12.00$; $p=0.585$ ns, 95% CI [-7.56, 3.32]) psilocybin sessions compared to their relative baselines (1 mg: $M=68.12$, $SD=13.14$; 25 mg: $M=65.94$, $SD=12.82$). A four (subscale)×three (timepoint) repeated-measures ANOVA revealed a significant main effect of subscale ($F_{(2.28,36.54)}=275.30$, $p<0.0001$), but no main effect of timepoint ($F_{(1.80,28.88)}=0.92$, $p=0.401$ ns). A significant interaction between timepoint and subscale was also found ($F_{(3.70,59.15)}=2.98$, $p=0.029$).

Post-hoc comparisons revealed that there was no significant difference in the Fantasy subscale scores four weeks ($M=16.65$, $SD=7.62$; $p=0.818$ ns, 95% CI [-1.72, 2.78]) after the 1 mg psilocybin session compared to baseline ($M=17.18$, $SD=6.94$). Likewise, there was no significant difference in the Fantasy subscale scores four weeks ($M=17.00$, $SD=6.96$; $p=0.923$ ns, 95% CI [-2.73, 2.03]) after the 25 mg psilocybin session compared to the second baseline ($M=16.65$, $SD=7.62$). Similarly, there was no significant difference in the Perspective Taking subscale scores four

weeks after the 1 mg ($M=19.94$, $SD=4.62$; $p=0.689$ ns, 95% CI [-1.11, 2.17]) or 25 mg ($M=21.29$, $SD=4.93$; $p=0.104$ ns, 95% CI [-2.95, 0.24]) psilocybin sessions compared to their relative baseline scores (1 mg: $M=20.47$, $SD=4.82$; 25 mg: $M=19.94$, $SD=4.62$). There was no significant difference in the Empathic Concern subscale scores four weeks after the 1 mg psilocybin session ($M=19.24$, $SD=4.74$; $p=0.840$ ns, 95% CI [-1.47, 2.29]) compared to baseline ($M=19.65$, $SD=4.87$). However, a trend level increase in the Empathic Concern subscale scores were observed four weeks after the 25 mg psilocybin session ($M=21.65$, $SD=4.21$; $p=0.065$ (ns trend level), 95% CI [-4.96, 0.14], $g=0.5$) compared to the second baseline ($M=19.24$, $SD=4.74$). With regards to the Personal Distress subscale, there was no significant difference in four weeks after the 1 mg psilocybin session ($M=10.12$, $SD=4.59$; $p=0.468$ ns, 95% CI [-0.81, 2.22]) compared to baseline ($M=10.82$, $SD=4.77$). However, a significant decrease in the Personal Distress subscale scores were observed four weeks after the 25 mg psilocybin session ($M=8.12$, $SD=3.99$; $p=0.013$, 95% CI [0.40, 3.60], $g=0.4$) compared to the second baseline ($M=10.12$, $SD=4.59$; Figure 4-11 C).

Self-esteem

A one-way repeated-measures ANOVA revealed a significant difference in the shortened Rosenberg Self-Esteem Scale (RSES-S) scores between the timepoints ($F_{(4,64)}=4.17$, $p=0.005$). Post-hoc multiple comparisons revealed that there was no significant difference in RSES-S scores at 14 days ($M=7.35$, $SD=1.83$; $p=0.784$ ns, 95% CI [-0.64, 1.46]) or four weeks ($M=7.59$, $SD=1.84$; $p=0.988$ ns, 95% CI [-0.87, 1.22]) after the 1 mg psilocybin session compared with baseline ($M=7.76$, $SD=1.95$). Significantly greater RSES-S scores were observed at 14 days ($M=8.77$, $SD=2.19$; $p=0.022$, 95% CI [-2.23, -0.13], $g=0.6$), but not at four weeks ($M=8.41$, $SD=2.15$; $p=0.180$, 95% CI [-1.87, 0.22]), after the 25 mg psilocybin session compared with the second baseline ($M=7.59$, $SD=1.84$; Figure 4-12 A).

Optimism and suggestibility

A non-parametric Friedman one-way repeated-measures ANOVA test revealed no significant differences in the Revised Life Orientation Test (LOT-R) scores between the timepoints ($\chi^2(4)=2.91$, $p=0.574$ ns; Figure 4-12 B). Likewise, a non-parametric Friedman one-way repeated-measures ANOVA test revealed no significant differences in the Short Suggestibility Scale (SSS) scores between the timepoints ($\chi^2(4)=2.44$, $p=0.655$ ns; Figure 4-12 C).

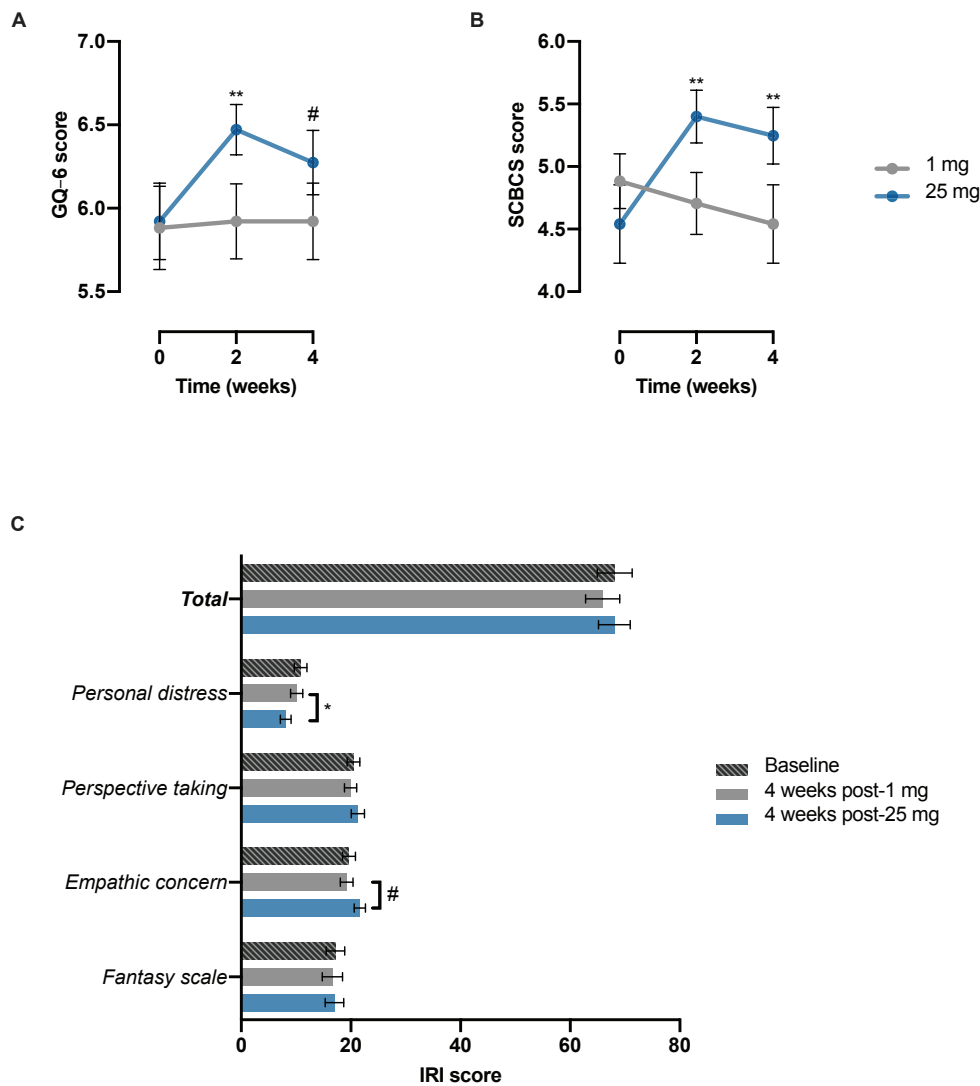


Figure 4-11. Prosociality and personal distress | (A) Gratitude. GQ-6 scores were significantly increased 14 days ($p=0.003$, $g=0.6$) and increased at trend level four weeks ($p=0.079$ ns (trend level), $g=0.4$) after 25 mg psilocybin. No differences were found in GQ-6 scores after 1 mg psilocybin. **(B) Compassion.** SCBCS scores were significantly increased 14 days ($p=0.006$, 95% CI [-1.49, -0.22], $g=0.7$) and four weeks ($p=0.009$, 95% CI [-1.25, -0.16], $g=0.6$) after 25 mg psilocybin. No differences were found in SCBCS scores after 1 mg psilocybin. **(C) Interpersonal reactivity.** A significant decrease in personal distress ($p=0.013$, 95% CI [0.40, 3.60], $g=0.4$) and a trend level increase in empathic concern ($p=0.065$ (ns trend), 95% CI [-4.96, 0.14], $g=0.5$) was found four weeks after 25 mg psilocybin. No differences in personal distress or empathic concern were found after 1 mg psilocybin. No differences in fantasy or perspective-taking were found at any timepoint. | Data are expressed as mean $\pm$ SEM ($p=0.065$ # (ns trend), $p<0.05$ *, $p<0.01$ **). Abbreviations: GQ-6, Gratitude Questionnaire; SCBCS, Santa Clara Brief Compassion Scale; IRI, Interpersonal Reactivity Index.

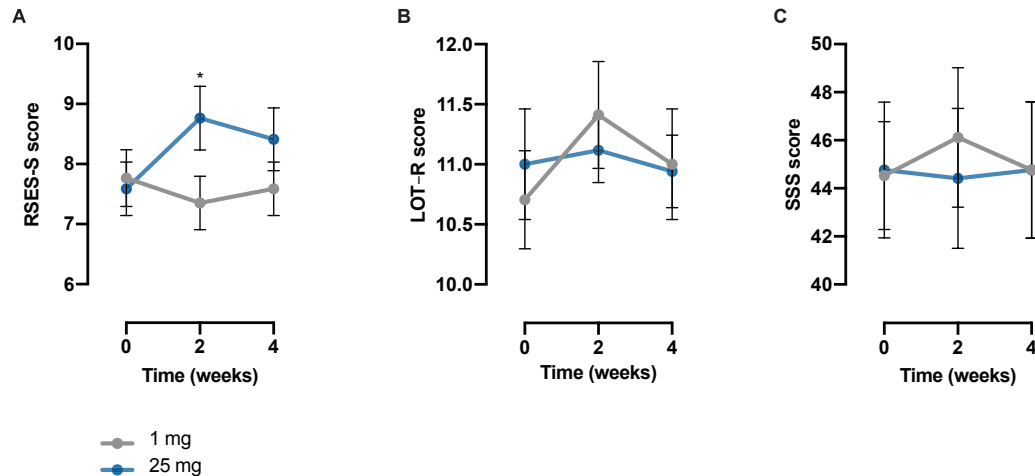


Figure 4-12. Self-esteem, optimism and suggestibility | (A) Self-esteem. RSES-S scores were significantly increased at 14 days ($p=0.022$, 95% CI [-2.23, -0.13], $g=0.6$) but not at four weeks ($p=0.180$ ns) after 25 mg psilocybin. No differences were found in RSES-S scores after 1 mg psilocybin. **(B) Optimism.** No differences were found in LOT-R scores after 1 mg or 25 mg psilocybin. **(C) Suggestibility.** No differences were found in SSS scores after 1 mg or 25 mg psilocybin. | Data are expressed as mean $\pm$ SEM ($p<0.05^*$). Abbreviations: RSES-S, Short Rosenberg Self-Esteem Scale; LOT-R, Revised Life Orientation Test; SSS, Short Suggestibility Scale.

Mood states

A five (timepoint) $\times$ seven (mood states) repeated-measures ANOVA revealed a significant main effect of timepoint ($F_{(2,64,42,30)}=3.62$, $p=0.025$) and a main effect of mood state ($F_{(1,81,29,05)}=39.60$, $p<0.0001$), and an interaction between timepoint and mood state ($F_{(24,384)}=17.03$, $p<0.0001$; Figure 4-13).

Tension: Post-hoc comparisons revealed that there was no significant difference in tension scores at 14 days ($M=9.12$, $SD=3.76$; $p=0.167$ ns, 95% CI [-3.62, 0.68]) or four weeks ($M=5.47$, $SD=4.72$; $p=0.062$ ns, 95% CI [-0.12, 4.48]) after the 1 mg psilocybin session compared to baseline ($M=7.65$, $SD=5.12$). There was a significant decrease in tension scores at 14 days ($M=4.00$, $SD=3.77$; $p=0.037$, 95% CI [0.10, 2.84], $g=0.3$), but not four weeks ($M=4.12$, $SD=3.87$; $p=0.058$ ns trend, 95% CI [-0.11, 0.88], $g=0.3$), after the 25 mg psilocybin session compared to the second baseline ($M=5.47$, $SD=4.72$).

Anger: Post-hoc comparisons revealed that there was a significant increase in anger scores at 14 days ($M=8.53$, $SD=3.39$; $p<0.001$, 95% CI [-5.47, -1.71], $g=1.0$), but not at four weeks ($M=4.00$, $SD=2.42$; $p=0.310$ ns, 95% CI [-0.96, 2.84]), after the 1 mg psilocybin session compared to baseline ($M=4.94$, $SD=3.32$). There was a significant decrease in anger scores at 14 days ($M=1.94$, $SD=1.78$; $p<0.001$, 95% CI [0.99, 3.13], $g=0.9$), but not four weeks ($M=2.59$, $SD=3.52$; $p=0.134$ ns, 95% CI [-0.49, 3.31]), after the 25 mg psilocybin session compared to the second baseline ($M=4.00$, $SD=2.42$).

Fatigue: Post-hoc comparisons revealed that there was no significant difference in fatigue scores at 14 days ($M=5.71$, $SD=4.36$; $p=0.774$ ns, 95% CI [-1.47, 1.95]) or four weeks ($M=5.06$, $SD=3.19$; $p=0.323$ ns, 95% CI [-0.95, 2.72], after the 1 mg psilocybin session compared to baseline ($M=4.94$, $SD=3.32$). There was a significant decrease in fatigue scores at 14 days ($M=3.47$, $SD=2.81$; $p=0.006$, 95% CI [0.51, 2.66], $g=0.2$), but not four weeks ($M=4.18$, $SD=2.94$; $p=0.096$ ns, 95% CI [-0.18, 1.94]), after the 25 mg psilocybin session compared to the second baseline ($M=4.00$, $SD=2.42$).

Depression: Post-hoc comparisons revealed that there was a significant increase in depression scores at 14 days ($M=9.23$, $SD=4.34$; $p=0.003$, 95% CI [-6.07, -1.46], $g=0.8$), but not at four weeks ($M=4.23$, $SD=4.56$; $p=0.240$ ns, 95% CI [-0.91, 3.38], after the 1 mg psilocybin session compared to baseline ($M=5.47$, $SD=5.36$). There was no significant difference in depression scores at 14 days ($M=2.71$, $SD=4.15$; $p=0.053$ ns trend, 95% CI [-0.02, 3.08], $g=0.4$) or four weeks ($M=4.12$, $SD=5.02$; $p=0.892$ ns, 95% CI [-1.69, 1.92]) after the 25 mg psilocybin session compared to the second baseline ($M=4.00$, $SD=2.42$).

Esteem-related affect: Post-hoc comparisons revealed that there was a significant decrease in esteem-related affect scores at 14 days ($M=6.47$, $SD=5.21$; $p<0.0001$, 95% CI [5.39, 12.02], $g=2.0$), but not at four weeks ($M=14.18$, $SD=3.83$; $p=0.158$ ns, 95% CI [-0.43, 2.43], after the 1 mg psilocybin session compared to baseline ($M=15.18$, $SD=2.77$). There was a significant increase in esteem-related affect scores at 14 days ($M=17.41$, $SD=3.76$; $p=0.001$, 95% CI [-5.01, -1.46], $g=0.8$) and four weeks ($M=16.41$, $SD=3.89$; $p=0.011$, 95% CI [-3.90, -0.57], $g=0.6$) after the 25 mg psilocybin session compared to the second baseline ($M=14.18$, $SD=3.83$).

Vigour: Post-hoc comparisons revealed that there was a significant decrease in vigour scores at 14 days ($M=7.23$, $SD=1.56$; $p=0.008$, 95% CI [0.84, 4.81], $g=1.0$), but not at four weeks ($M=9.29$, $SD=4.50$; $p=0.486$ ns, 95% CI [-1.51, 3.04], after the 1 mg psilocybin session compared to baseline ($M=10.06$, $SD=3.66$). There was no significant difference in vigour scores at 14 days ($M=10.94$, $SD=3.80$; $p=0.123$ ns, 95% CI [-3.79, 0.49]) or four weeks ($M=10.35$, $SD=4.36$; $p=0.287$ ns, 95% CI [-3.09, 0.98]) after the 25 mg psilocybin session compared to the second baseline ($M=9.29$, $SD=4.50$).

Confusion: Post-hoc comparisons revealed that there was no significant difference in confusion scores at 14 days ($M=7.12$, $SD=3.06$; $p=0.056$ ns trend, 95% CI [-4.06, 0.06], $g=0.6$) or four weeks ($M=4.88$, $SD=2.67$; $p=0.796$ ns, 95% CI [-1.66, 2.13], after the 1 mg psilocybin session compared to baseline ($M=5.12$, $SD=2.85$). There was a significant decrease in confusion scores at 14 days ($M=3.29$, $SD=2.78$; $p=0.006$, 95% CI [0.51, 2.66], $g=0.6$), but not four weeks ($M=4.41$, $SD=2.89$; $p=0.577$ ns, 95% CI [-1.28, 2.22]), after the 25 mg psilocybin session compared to the second baseline ($M=4.88$, $SD=2.67$).

Next, I analysed differences between mood states at each timepoint. At baseline, post-hoc multiple comparisons revealed significantly greater esteem-related affect compared with all other mood states: tension ($p=0.007$, 95% CI [-13.28, -1.78], $g=1.7$); anger ($p<0.0001$, 95% CI [-14.22, -6.25], $g=3.2$); fatigue ($p<0.0001$, 95% CI [-12.94, -5.53], $g=2.8$); depression ($p<0.001$, 95% CI [0.51, 2.66], $g=2.2$); vigour ($p<0.0001$, 95% CI [2.97, 7.27], $g=1.5$), and; confusion ($p<0.0001$, 95% CI [6.84, 13.28], $g=3.4$). Significantly more vigour than anger ($p=0.034$, 95% CI [-9.94, -0.29], $g=1.4$) was also observed. Two weeks after the 1 mg psilocybin session, participants showed significantly greater tension than fatigue ($p<0.001$, 95% CI [1.59, 5.23], $g=0.8$) and esteem-related affect ($p=0.008$, 95% CI [0.59, 4.70], $g=0.6$). Significantly less fatigue than depression ($p=0.002$, 95% CI [-5.92, -1.14], $g=0.8$) and anger ($p=0.001$, 95% CI [1.05, 4.60], $g=0.7$), as well as significantly less esteem-related affect than depression ($p=0.033$, 95% CI [0.17, 5.36], $g=0.5$), was also observed. Four weeks after the 1 mg psilocybin session, post-hoc multiple comparisons revealed significantly greater esteem-related affect compared with all other mood states: tension ($p=0.002$, 95% CI [-14.57, -2.84], $g=1.9$); anger ($p<0.0001$, 95% CI [-14.76, -5.59], $g=3.0$); fatigue ($p<0.001$, 95% CI [-13.92, -4.32], $g=2.5$); depression ($p=0.001$, 95% CI [-16.27, -3.61], $g=2.2$); vigour ($p=0.002$, 95% CI [1.64, 8.12], $g=1.1$), and; confusion ($p<0.0001$, 95% CI [5.06, 13.53], $g=2.7$). Significantly more vigour than anger ($p=0.013$, 95% CI [-9.70, -0.89], $g=1.4$) and confusion ($p=0.012$, 95% CI [0.80, 8.03], $g=1.1$) was also observed.

Two weeks after the 25 mg psilocybin session, post-hoc multiple comparisons revealed significantly greater esteem-related affect [tension ($p<0.0001$, 95% CI [-18.79, -8.03], $g=3.3$); anger ($p<0.0001$, 95% CI [-19.58, -11.36], $g=5.0$); fatigue ($p<0.0001$, 95% CI [-18.11, -9.77], $g=4.0$); depression ($p<0.0001$, 95% CI [-20.82, -8.59], $g=3.5$); vigour ($p<0.0001$, 95% CI [4.32, 8.62], $g=1.6$), and; confusion ($p<0.0001$, 95% CI [9.21, 19.02], $g=4.1$)] and vigour [tension ($p=0.003$, 95% CI [-11.78, -2.10], $g=1.7$); anger ($p<0.0001$, 95% CI [-13.10, -4.90], $g=2.9$); fatigue ($p<0.0001$, 95% CI [-11.22, -3.72], $g=2.1$); depression ($p=0.003$, 95% CI [-14.08, -2.39], $g=2.0$), and; confusion ($p<0.001$, 95% CI [3.39, 11.90], $g=2.2$)] compared with all other mood states. Four weeks after the 25 mg psilocybin session, there was significantly greater esteem-related affect compared with all other mood states: tension ($p<0.0001$, 95% CI [-17.64, -6.95], $g=3.0$); anger ($p<0.0001$, 95% CI [-18.85, -8.79], $g=3.5$); fatigue ($p<0.0001$, 95% CI [-16.53, -7.94], $g=3.4$); depression ($p<0.001$, 95% CI [-18.86, -5.73], $g=2.6$); vigour ($p<0.0001$, 95% CI [3.63, 8.49], $g=1.4$), and; confusion ($p<0.0001$, 95% CI [7.49, 16.51], $g=3.3$). Significantly more vigour than all other mood states except for depression and esteem-related affect was also observed: tension ($p=0.011$, 95% CI [-11.31, -1.16], $g=1.4$); anger ($p=0.001$, 95% CI [-12.71, -2.82], $g=1.9$); fatigue ($p<0.001$, 95% CI [-10.40, -1.95], $g=1.6$); and; confusion ($p=0.012$, 95% CI [1.09, 10.79], $g=1.5$).

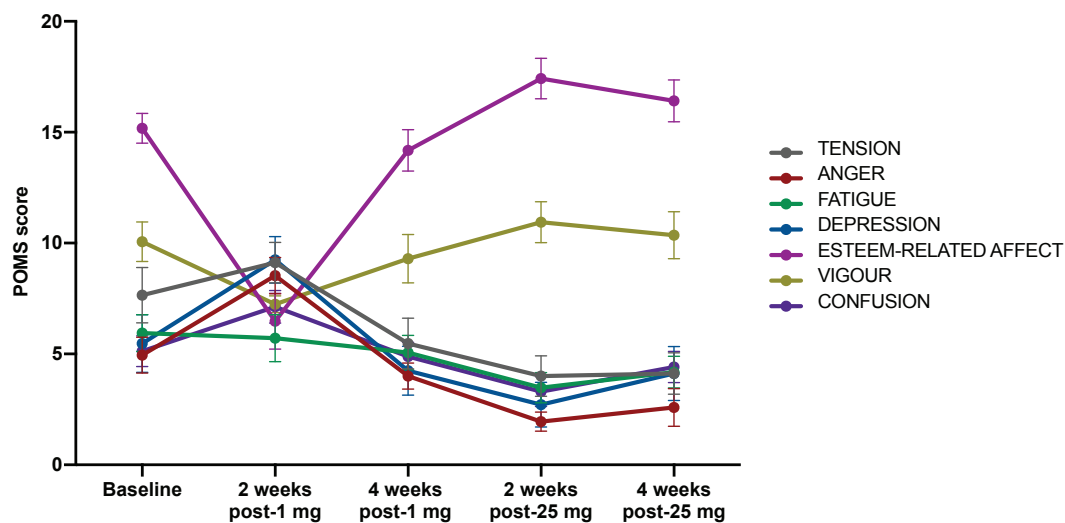


Figure 4-13. Profile of mood states over time. There was an increase in anger ($p<0.001$, 95% CI [-5.47, -1.71], $g=1.0$) and depression ($p=0.003$, 95% CI [-6.07, -1.46], $g=0.8$) as well as a decrease in esteem-related affect ($p<0.0001$, 95% CI [5.39, 12.02], $g=2.0$) and vigour ($p=0.008$, 95% CI [0.84, 4.81], $g=1.0$) 14 days after 1 mg psilocybin compared to baseline. Relative to baseline, no other significant differences were found after 14 days. There were no significant differences found in any of the mood states 4 weeks after 1 mg psilocybin, demonstrating a return to baseline for the previously altered mood states. There was a decrease in tension ($p=0.037$, 95% CI [0.10, 2.84], $g=0.3$), anger ($p<0.001$, 95% CI [0.99, 3.13], $g=0.9$), fatigue ($p=0.006$, 95% CI [0.51, 2.66], $g=0.2$) and confusion ($p=0.006$, 95% CI [0.51, 2.66], $g=0.6$) 14 days after 25 mg psilocybin compared to baseline; these mood states all returned to baseline levels four weeks after 25 mg psilocybin. Relative to baseline, esteem-related affect was increased after 14 days ($p=0.001$, 95% CI [-5.01, -1.46], $g=0.8$) and remained increased four weeks ($p=0.011$, 95% CI [-3.90, -0.57], $g=0.6$) after 25 mg psilocybin. There was no difference in depression or vigour 14 days or four weeks after 25 mg psilocybin compared to baseline. Abbreviations: POMS, Profile of Mood States.

Absorption

A one-way repeated-measures ANOVA revealed a significant difference in the overall Modified Tellegen Absorption Scale (MODTAS) scores between the timepoints ($F_{(2.47, 39.48)}=4.94$, $p=0.008$). Post-hoc multiple comparisons revealed that there was no significant difference in MODTAS scores at 14 days ($M=32.29$, $SD=17.14$, $p=0.591$ ns, 95% CI [-3.23, 9.11]) or four weeks ($M=31.88$, $SD=19.49$; $p=0.702$ ns, 95% CI [-4.71, 11.42]) after the 1 mg psilocybin session compared with baseline ($M=35.24$, $SD=17.63$). However, significantly greater MODTAS scores were observed at 14 days ($M=39.00$, $SD=19.36$; $p=0.010$, 95% CI [-12.71, -1.52], $g=0.4$) and four weeks ($M=40.94$, $SD=19.59$; $p<0.001$, 95% CI [-14.35, -3.77], $g=0.4$) after the 25 mg psilocybin session compared with the second baseline ($M=31.88$, $SD=19.49$; Figure 4-14).

Next, separate analyses were completed for the five subscales contained in the MODTAS. A five (timepoint)×five (subscale) repeated-measures ANOVA revealed a significant main effect of

timepoint ($F_{(2.47,39.48)}=4.93$, $p=0.008$) and a main effect of subscale ($F_{(1.58,25.23)}=44.22$, $p<0.0001$), and an interaction between timepoint and subscale ($F_{(5.20,83.17)}=2.85$, $p=0.018$).

Post-hoc comparisons revealed that there was no significant difference in the Aesthetic Involvement in Nature (AIN) subscale scores at 14 days ($M=9.47$, $SD=4.43$; $p=0.998$ ns, 95% CI [-1.76, 2.11]) or four weeks ($M=9.12$, $SD=5.82$; $p=0.889$ ns, 95% CI [-1.25, 2.31]) after the 1 mg psilocybin session compared to baseline ($M=9.65$, $SD=5.23$). However, significantly greater Aesthetic Involvement in Nature subscale scores were observed at 14 days ($M=10.59$, $SD=5.73$; $p=0.019$, 95% CI [-2.73, -0.21], $g=0.2$) and four weeks ($M=11.18$, $SD=5.91$; $p=0.015$, 95% CI [-3.77, -0.35], $g=0.3$) after the 25 mg psilocybin session compared to the second baseline ($M=9.12$, $SD=5.82$). Similarly, there was no significant difference in the Altered States of Consciousness (ASC) subscale scores at 14 days ($M=3.29$, $SD=3.26$; $p=0.986$ ns, 95% CI [-1.22, 1.69]) or four weeks ($M=3.53$, $SD=3.50$; $p>0.999$ ns, 95% CI [-2.12, 2.12]) after the 1 mg psilocybin session compared to baseline ($M=3.53$, $SD=3.45$). However, significantly greater ASC subscale scores were observed at 14 days ($M=5.88$, $SD=3.53$; $p=0.008$, 95% CI [-4.15, -0.55], $g=0.6$) and four weeks ($M=6.18$, $SD=3.43$; $p=0.002$, 95% CI [-4.41, -0.89], $g=0.7$) after the 25 mg psilocybin session compared to the second baseline ($M=3.53$, $SD=3.50$). Likewise, there was no significant difference in the Imaginative Involvement (II) subscale scores at 14 days ($M=14.47$, $SD=8.23$; $p=0.979$ ns, 95% CI [-2.90, 4.19]) or four weeks ($M=14.35$, $SD=8.23$; $p=0.986$ ns, 95% CI [-3.86, 5.39]) after the 1 mg psilocybin sessions compared to baseline ($M=15.12$, $SD=8.09$). There was also no significant difference in the Imaginative Involvement subscale scores at 14 days ($M=16.41$, $SD=8.32$; $p=0.243$ ns, 95% CI [-4.98, 0.86]) after the 25 mg psilocybin session compared to the second baseline ($M=14.35$, $SD=8.23$). However, the Imaginative Involvement subscale scores were significantly greater four weeks after the 25 mg psilocybin session ($M=17.59$, $SD=9.05$; $p=0.025$, 95% CI [-6.13, -0.34], $g=0.4$).

There was no significant difference in the Extrasensory Perception Experiences (ESP) subscale scores at 14 days (1 mg: $M=3.00$, $SD=1.94$, $p=0.104$ ns, 95% CI [-0.13, 1.90]; 25 mg: $M=3.23$, $SD=1.95$, $p=0.875$ ns, 95% CI [-1.50, 0.79]) or four weeks (1 mg: $M=2.88$, $SD=2.03$; $p=0.090$ ns, 95% CI [-1.61, 3.14]; 25 mg: $M=2.94$, $SD=2.38$, $p=0.999$ ns, 95% CI [-1.61, 3.14]) after the 1 mg or 25 mg psilocybin sessions compared to their relative baseline scores (1mg: $M=3.88$, $SD=1.90$; 25 mg: $M=2.88$, $SD=2.03$). Similar results were found for the Synaesthesia subscale, as there was also no significant difference in the scores at 14 days (1 mg: $M=2.06$, $SD=2.36$; $p=0.517$ ns, 95% CI [-0.91, 2.91]; 25 mg: $M=2.88$, $SD=2.18$; $p=0.365$ ns, 95% CI [-2.32, 0.55]) or four weeks (1 mg: $M=2.00$, $SD=2.06$; $p=0.314$ ns, 95% CI [-0.57, 2.69]; 25 mg: $M=3.06$, $SD=2.16$; $p=0.105$ ns, 95% CI [-2.28, 0.16]) after the 1 mg or 25 mg psilocybin sessions compared to their relative baseline scores (1mg: $M=3.06$, $SD=2.70$; 25 mg: $M=2.00$, $SD=2.06$).

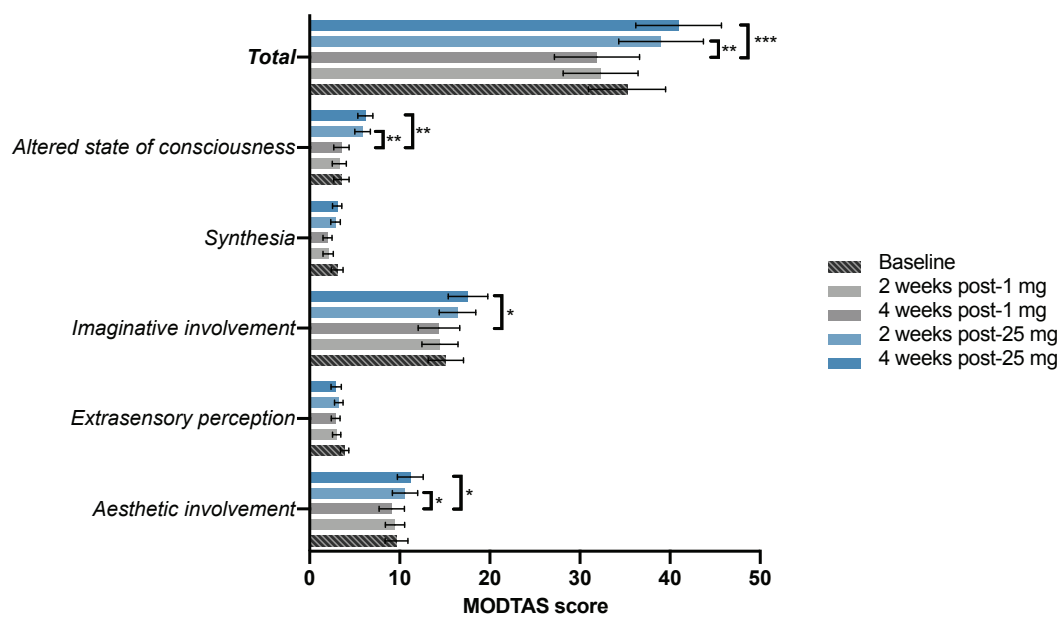


Figure 4-14. Absorption. Total MODTAS scores were significantly increased 14 days ($p=0.010$, 95% CI [-12.71, -1.52], $g=0.4$) and four weeks ($p<0.001$, 95% CI [-14.35, -3.77], $g=0.4$) after 25 mg psilocybin. Absorption in altered states of consciousness was significantly increased 14 days ($p=0.008$, 95% CI [-4.15, -0.55], $g=0.6$) and four weeks ($p=0.002$, 95% CI [-4.41, -0.89], $g=0.7$) after 25 mg psilocybin. Similarly, aesthetic involvement in nature was significantly increased 14 days ($p=0.019$, 95% CI [-2.73, -0.21], $g=0.2$) and four weeks ($p=0.015$, 95% CI [-3.77, -0.35], $g=0.3$) after 25 mg psilocybin. Imaginative involvement was significantly increased four weeks after 25 mg psilocybin ($p=0.025$, 95% CI [-6.13, -0.34], $g=0.4$), but not after 14 days. No differences were found in synaesthesia or extrasensory perception experiences after 25 mg psilocybin. No differences were found in MODTAS total or any subscale scores after 1 mg psilocybin. | Data are expressed as mean $\pm$ SEM ($p<0.05^*$, $p\leq 0.01^{**}$; $p<0.001^{***}$). Abbreviations: MODTAS, Modified Tellegen Absorption Scale.

Experiential avoidance

A one-way repeated-measures ANOVA revealed a significant difference in the Brief Experiential Avoidance Questionnaire (BEAQ) scores between the timepoints ($F_{(2.64, 42.19)}=5.49$, $p=0.004$). Post-hoc multiple comparisons revealed that there was no significant difference in BEAQ scores at 14 days ($M=37.24$, $SD=8.87$; $p=0.602$ ns, 95% CI [-6.99, 2.52]) or four weeks ($M=37.18$, $SD=10.48$; $p=0.714$ ns, 95% CI [-7.50, 3.15]) after the 1 mg psilocybin session compared with baseline ($M=35.00$, $SD=11.54$). However, significantly decreased BEAQ scores were observed at 14 days ($M=30.88$, $SD=9.49$; $p=0.006$, 95% CI [1.67, 10.92], $g=0.6$) and four weeks ($M=31.24$, $SD=10.92$; $p=0.016$, 95% CI [0.98, 10.91], $g=0.5$) after the 25 mg psilocybin session compared with the second baseline ($M=37.18$, $SD=10.48$; A).

Cognitive flexibility

Errors made at each phase were adjusted to the total number of stimuli presented within each stage, representing a measure of ‘phase-accuracy’ in order to assess changes in IDED performance across time-points while controlling for individual variability in learning rates. Performance on the EDS subtask is considered to be the most robust marker of cognitive flexibility, as it demands attentional set-shifting and reversal learning simultaneously. The EDS was therefore the only task stage of interest for the purposes of this study. Accordingly, only adjusted error from the EDS stage of the task was analysed further (see Table 4-3 for results of all stages). In line with previous studies (Vatansever et al., 2015), average adjusted EDS error was higher than errors made at other stages at each time point, suggesting that the variability in total adjusted error rate can mostly be accounted for by this subtask.

A linear mixed-effects model (LMM) was used in place of a one-way repeated-measures ANOVA to account for missing values at baseline ($N=15$). The LMM revealed a significant difference in EDS errors between the timepoints ($F_{(1.75,26.22)}=4.83$, $p=0.019$). Post-hoc comparisons revealed no significant difference in EDS errors four weeks after 1 mg ($M=0.32$, $SD=0.12$) relative to baseline ($M=0.35$, $SD=0.13$; $p=0.664$ ns, 95% CI [-0.07, 0.15]). However, a significant decrease in EDS errors was found four weeks after the 25 mg ($M=0.23$, $SD=0.12$) relative to the second baseline ($M=0.32$, $SD=0.12$; $p=0.031$, 95% CI [0.01, 0.16], $g=0.7$; B).

Table 4-3. IDED task stages and EDS errors.

IDED task stages		Baseline $N=15^{\dagger}$	1 mg + 4 weeks $N=17$	25 mg + 4 weeks $N=17$
		Adjusted Errors (μ)*		
		$\mu \pm SEM$		
Discrimination	SD	0.069 $\pm$ 0.027	0.138 $\pm$ 0.027	0.054 $\pm$ 0.017
	C_D	0.112 $\pm$ 0.028	0.114 $\pm$ 0.032	0.072 $\pm$ 0.023
	CD	0.037 $\pm$ 0.016	0.055 $\pm$ 0.019	0.065 $\pm$ 0.026
	IDS	0.076 $\pm$ 0.019	0.063 $\pm$ 0.020	0.068 $\pm$ 0.026
	EDS	0.354 $\pm$ 0.033	0.317 $\pm$ 0.026	0.232 $\pm$ 0.030
Reversal	SR	0.187 $\pm$ 0.022	0.156 $\pm$ 0.009	0.143 $\pm$ 0.000
	CR	0.155 $\pm$ 0.019	0.169 $\pm$ 0.012	0.193 $\pm$ 0.019
	IDR	0.156 $\pm$ 0.009	0.178 $\pm$ 0.023	0.165 $\pm$ 0.013
	EDR	0.354 $\pm$ 0.033	0.317 $\pm$ 0.026	0.141 $\pm$ 0.034

Abbreviations: IDED, Intra-dimensional/extra-dimensional task; SD, simple discrimination; C_D, compound discrimination with second dimension; CD, compound discrimination; IDS, intra-dimensional shift; EDS, extra-dimensional shift; SR, simple reversal; CR, compound reversal; IDR, intra-dimensional reversal; EDR, extra-dimensional reversal.

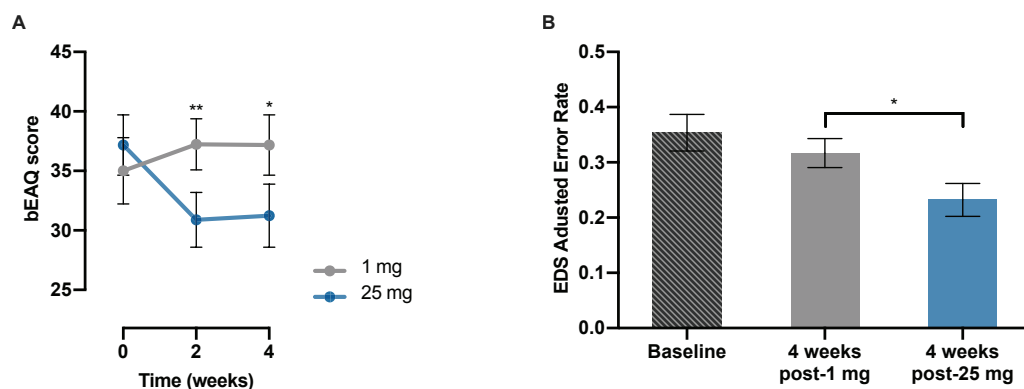


Figure 4-15. Experiential avoidance and cognitive flexibility | (A) Experiential avoidance. bEAQ scores were significantly decreased 14 days ($p=0.006$, 95% CI [1.67, 10.92], $g=0.6$) and four weeks ($p=0.016$, 95% CI [0.98, 10.91], $g=0.5$) after 25 mg psilocybin. No differences in bEAQ scores were found after 1 mg psilocybin. **(B) Cognitive flexibility.** A significant decrease in EDS errors (i.e. increase in cognitive flexibility) was found four weeks after 25 mg psilocybin ($p=0.031$, 95% CI [0.01, 0.16], $g=0.7$). No differences in EDS errors were found after 1 mg psilocybin. | Data are expressed as mean $\pm$ SEM ($p<0.05^*$, $p<0.01^{**}$).

Abbreviations: bEAQ, Brief Experiential Avoidance Questionnaire; EDS, extra-dimensional shift.

Profound, intense and unusual state of consciousness

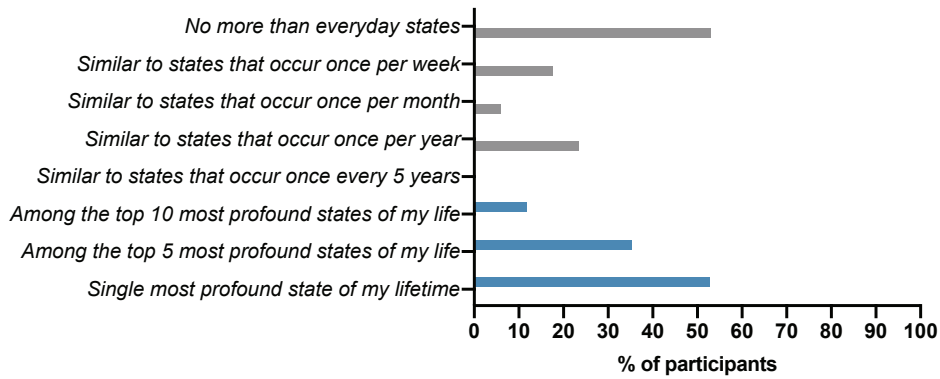
Six months post-dosing, participants were instructed to reflect on the peak of each dosing session and asked the following three questions: (i) “How profound was the state of consciousness you experienced?” (Figure 4-16 A); (ii) “How intense was the state of consciousness you experienced?” (Figure 4-16 B); and (iii) “How unusual was the state of consciousness you experienced?” (Figure 4-16 C). A two (dose; 1 mg vs 25 mg) $\times$ three (questions) repeated-measures ANOVA revealed a significant main effect of dose ($F_{(1,14)}=270.90$, $p<0.0001$) and a significant interaction between dose and question ($F_{(1.99,27.85)}=6.74$, $p=0.004$), but no main effect of question ($F_{(1.46,20.49)}=1.18$, $p=0.312$ ns).

Post-hoc comparisons revealed significantly greater scores on question 1 “how profound was the state of consciousness you experienced?” ($M=7.53$, $SD=0.71$; $p<0.0001$, 95% CI [-6.38, -4.42], $g=5.1$), question 2 “how intense was the state of consciousness you experienced?” ($M=7.59$, $SD=0.62$; $p<0.0001$, 95% CI [-6.70, -4.76], $g=5.3$) and question 3 “how unusual was the state of consciousness you experienced?” ($M=7.93$, $SD=0.24$; $p<0.0001$, 95% CI [-6.78, -4.96], $g=6.1$) six months post dosing with 25 mg relative to 1 mg psilocybin (Q1: $M=2.00$, $SD=1.27$; Q2: $M=1.88$, $SD=1.32$; Q3: $M=1.94$, $SD=1.30$). With regards to question 1, 53% of participants rated the 1 mg psilocybin experience as “no more profound than routine everyday states of consciousness” and the 25 mg psilocybin experience as “the single most profound state of consciousness in their lifetime”. For question 2, 59% of participants rated the 1 mg psilocybin experience as “no more intense than routine everyday states of consciousness” and 69% rated the 25 mg psilocybin experience as “the single most intense state of consciousness in their lifetime”. For question 3,

59% of participants rated the 1 mg psilocybin experience as *“no more unusual than routine everyday states of consciousness”* and 94% rated the 25 mg psilocybin experience as *“the single most unusual state of consciousness in their lifetime”*. With regards to differences between the questions, post-hoc multiple comparisons revealed no differences in scores between any questions six months after the 1 mg psilocybin session (all $p > 0.705$ ns). However, significantly greater scores were observed on question 3 *“how unusual was the state of consciousness you experienced?”* ($M=7.93$; $SD=0.24$) relative to question 1 *“how profound was the state of consciousness you experienced?”* ($M=7.53$, $SD=0.71$; $p=0.025$, 95% CI [-0.75, -0.04], $g=0.6$) six months after the 25 mg psilocybin session. No other significant differences between the questions were observed six months after the 25 mg psilocybin session (all $p > 0.227$ ns).

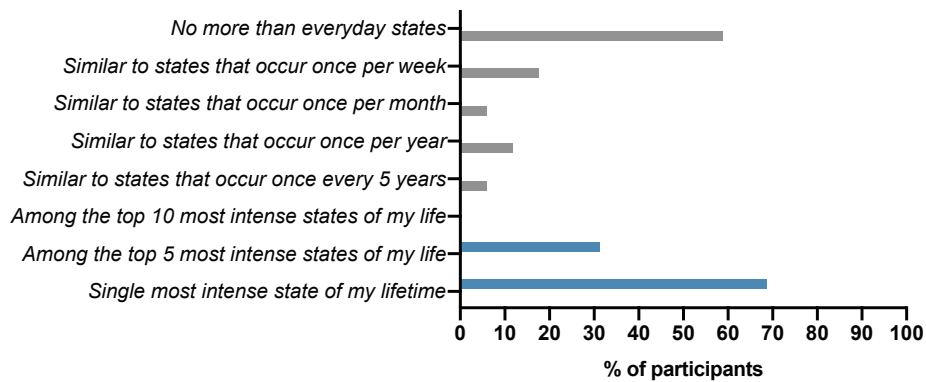
A

Q1: "How profound was your state of consciousness?"



B

Q2: "How intense was your state of consciousness?"



C

Q3: "How unusual was your state of consciousness?"

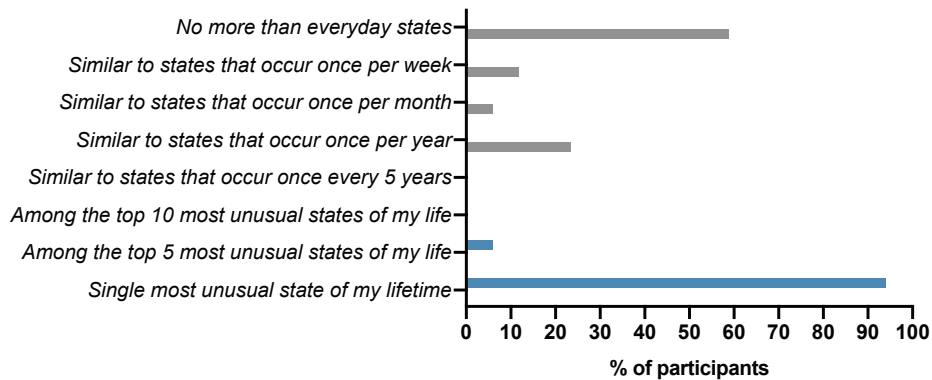


Figure 4-16. State of consciousness (rated 6 months post-dosing) | (A) 53% of participants rated the 1 mg psilocybin experience as "no more profound than routine everyday states of consciousness" and the 25 mg psilocybin experience as "the single most profound state of consciousness in their lifetime". **(B)** 59% of participants rated the 1 mg psilocybin experience as "no more intense than routine everyday states of consciousness" and 69% rated the 25 mg psilocybin experience as "the single most intense state of consciousness in their lifetime". **(C)** 59% of participants rated the 1 mg psilocybin experience as "no more unusual than routine everyday states of consciousness" and 94% rated the 25 mg psilocybin experience as "the single most unusual state of consciousness in their lifetime".

4.5.7. Long-term brain effects

Emotional faces task

A five (stimulus condition)×three (timepoint) repeated-measures ANOVA revealed a significant main effect of stimulus condition ($F_{(1.72,27.59)}=9.01$, $p=0.002$) and a main effect of timepoint ($F_{(1.94,30.99)}=4.07$, $p=0.028$), but no significant interaction between timepoint and stimulus condition ($F_{(3.04,48.57)}=0.88$, $p=0.457$ ns; Figure 4-17 A-B).

Post-hoc multiple comparisons revealed no significant differences in BOLD response across any of the timepoints in left or right amygdala for the following stimulus conditions: (i) neutral (all $p>0.361$ ns); (ii) fear>neutral (all $p>0.186$ ns); and (iii) happy>neutral (all $p>0.116$ ns). A significant increase in BOLD response was found in left and right amygdala for happy faces four weeks after 1 mg psilocybin (Right amygdala: $M=1.30$, $SD=0.69$; Left amygdala: $M=1.05$, $SD=0.55$) relative to baseline (Right amygdala: $M=0.42$, $SD=0.95$; $p=0.001$, 95% CI [-1.04, 0.37], $g=1.0$); Left amygdala: $M=0.32$, $SD=0.90$; $p=0.019$, 95% CI [-1.33, -0.12], $g=0.9$). A trend level increase in left amygdala for happy faces was also observed four weeks after 25 mg psilocybin ($M=1.06$, $SD=1.15$) relative to baseline ($M=0.32$, $SD=0.90$; $p=0.058$ ns trend, 95% CI [-1.49, 0.02], $g=0.7$). No other significant differences in BOLD response for happy faces were found (all $p>0.080$). No difference in BOLD response was found in the left or right amygdala for fearful faces four weeks after 1 mg psilocybin relative to baseline (all $p>0.459$). However, a significant decrease in BOLD response was observed in the left amygdala for fearful faces four weeks after 25 mg psilocybin ($M=0.51$, $SD=0.89$) compared to the relative baseline ($M=1.24$, $SD=0.81$; $p=0.013$, 95% CI [-0.15, 1.30], $g=0.9$).

Resting-state functional connectivity

Next, seed-based RSFC analyses were performed using the BOLD data on four regions of interest (ROIs): (i) the subgenual anterior cingulate cortex (sgACC), (ii) the ventromedial prefrontal cortex (vmPFC), (iii) the bilateral amygdala, and (iv) the bilateral parahippocampus.

Increased sgACC rsFC was observed with regions largely corresponding to the left frontoparietal network (IFPN) four weeks after the 25 mg psilocybin session (Table 4-4; Figure 4-18). Likewise, increased vmPFC rsFC was observed with regions largely corresponding to the FPN four weeks after the 25 mg psilocybin session (Table 4-4; Figure 4-18). No other differences in rsFC were observed after the 25 mg psilocybin session. No differences in rsFC were observed for any ROIs four weeks after the 1 mg psilocybin session. No within-RSN (integrity) or between-RSN (segregation) rsFC changes were observed at any timepoint.

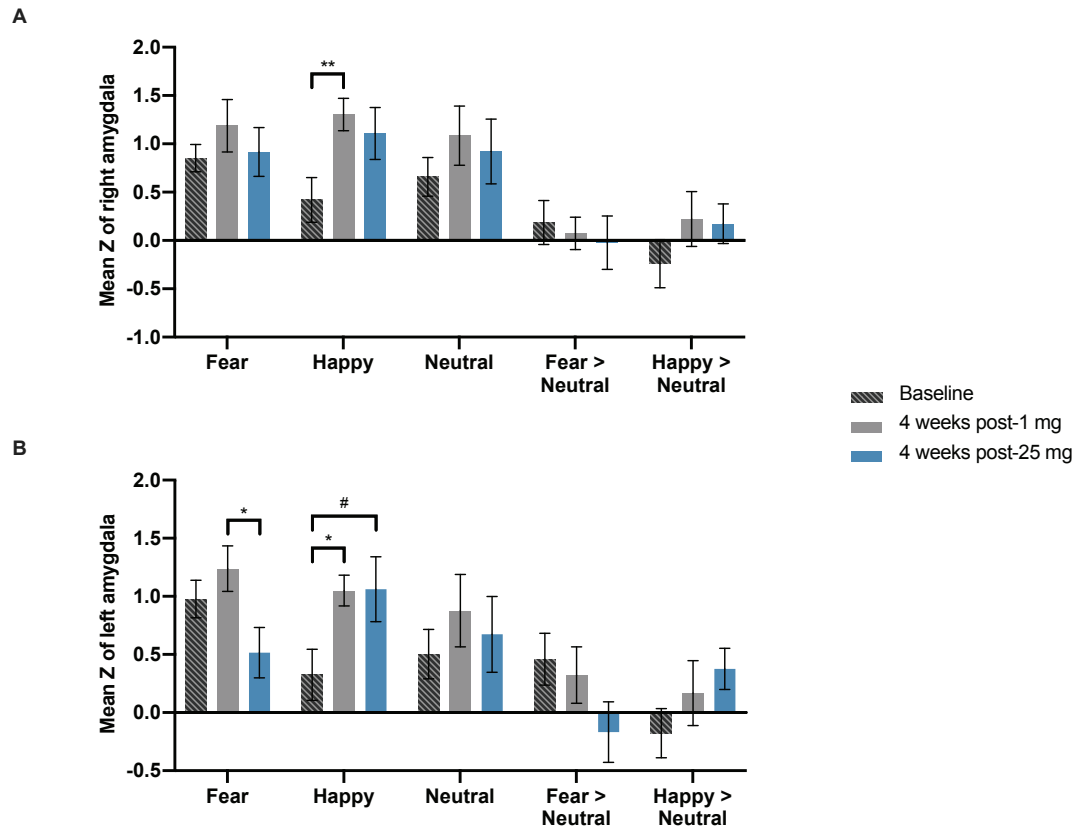


Table 4-4. Whole-brain seed-based rsFC changes four weeks after 25 mg psilocybin.

Seed	Region	Hemisphere	Network	Cluster size	Peak Z	Peak MNI (mm)		
						X	Y	Z
vmPFC	Inferior frontal gyrus	Left	IFPN	679	3.61	-50	16	18
	Frontal pole	Right	rFPN	389	4.61	6	58	36
sgACC	Angular gyrus	Left	IFPN	801	3.80	-46	-60	34
	Frontal pole	Left	IFPN	578	3.87	-14	54	32
	Postcentral gyrus	Left	SMN	436	3.55	-40	-22	42

Abbreviations: MNI, the Montreal Neurological Institute and Hospital coordinate system; vmPFC, ventromedial prefrontal cortex; sgACC, subgenual anterior cingulate cortex; IFPN, left frontoparietal network; rFPN, right frontoparietal network; SMN, somatosensory network.

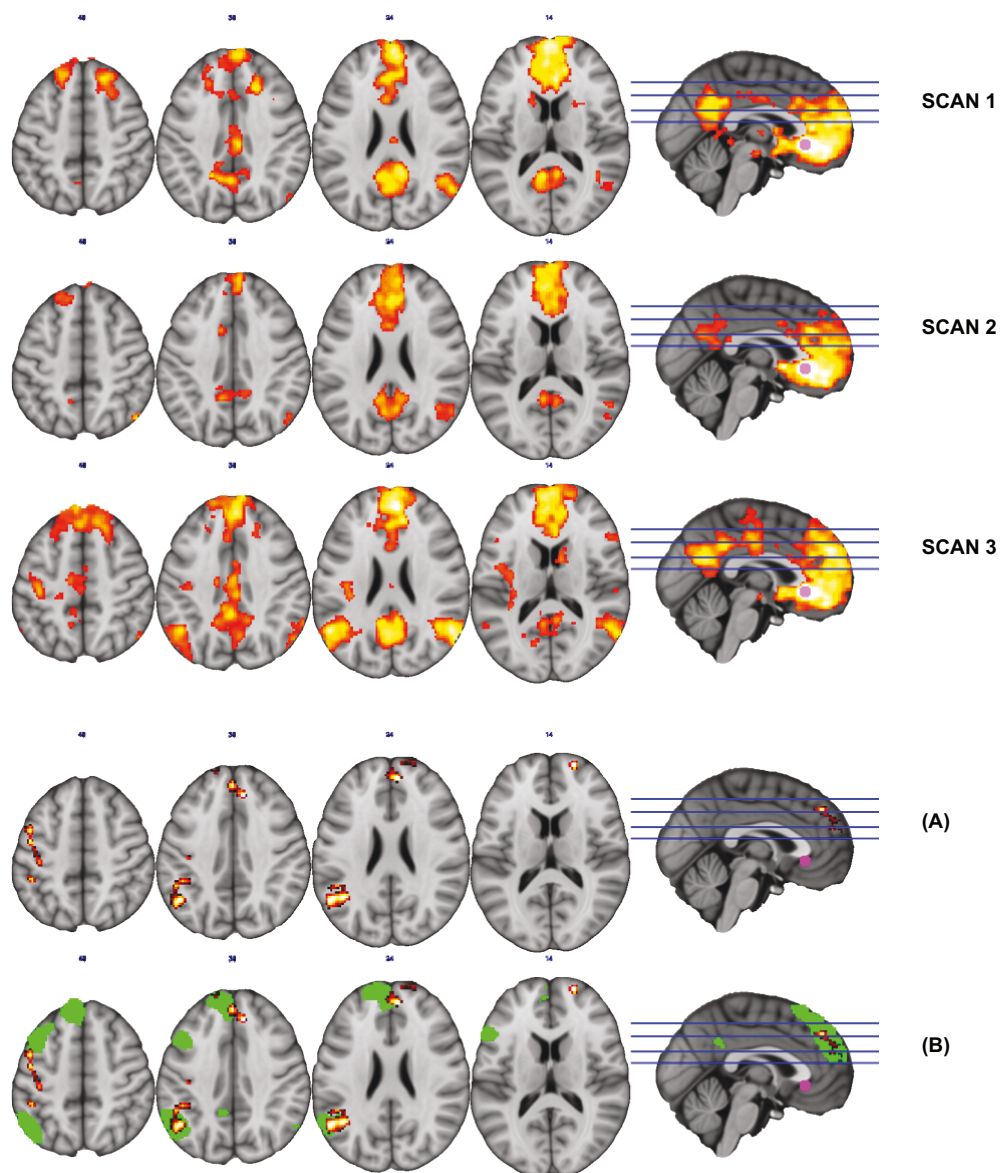


Figure 4-18. Seed-based resting-state functional connectivity analysis of the sgACC. Brain regions showing increased coupling with the sgACC (pink seed) are displayed in yellow/orange (Cluster-corrected; $Z > 2.3$, $p < 0.050$). Scan 1: baseline, scan 2: four weeks after 1 mg psilocybin, scan 3: four weeks after 25 mg psilocybin. No differences were found four weeks after 1 mg psilocybin. **(A)** The contrast: scan 3 (four weeks after 25 mg psilocybin) > scan 2 (four weeks after 1 mg psilocybin) with **(B)** IFPN overlay. Increased sgACC rsFC to regions largely corresponding to the IFPN (see Table 4-4) observed four weeks after the 25 mg psilocybin session. Abbreviations: sgACC, subgenual anterior cingulate cortex; IFPN, left frontoparietal network.

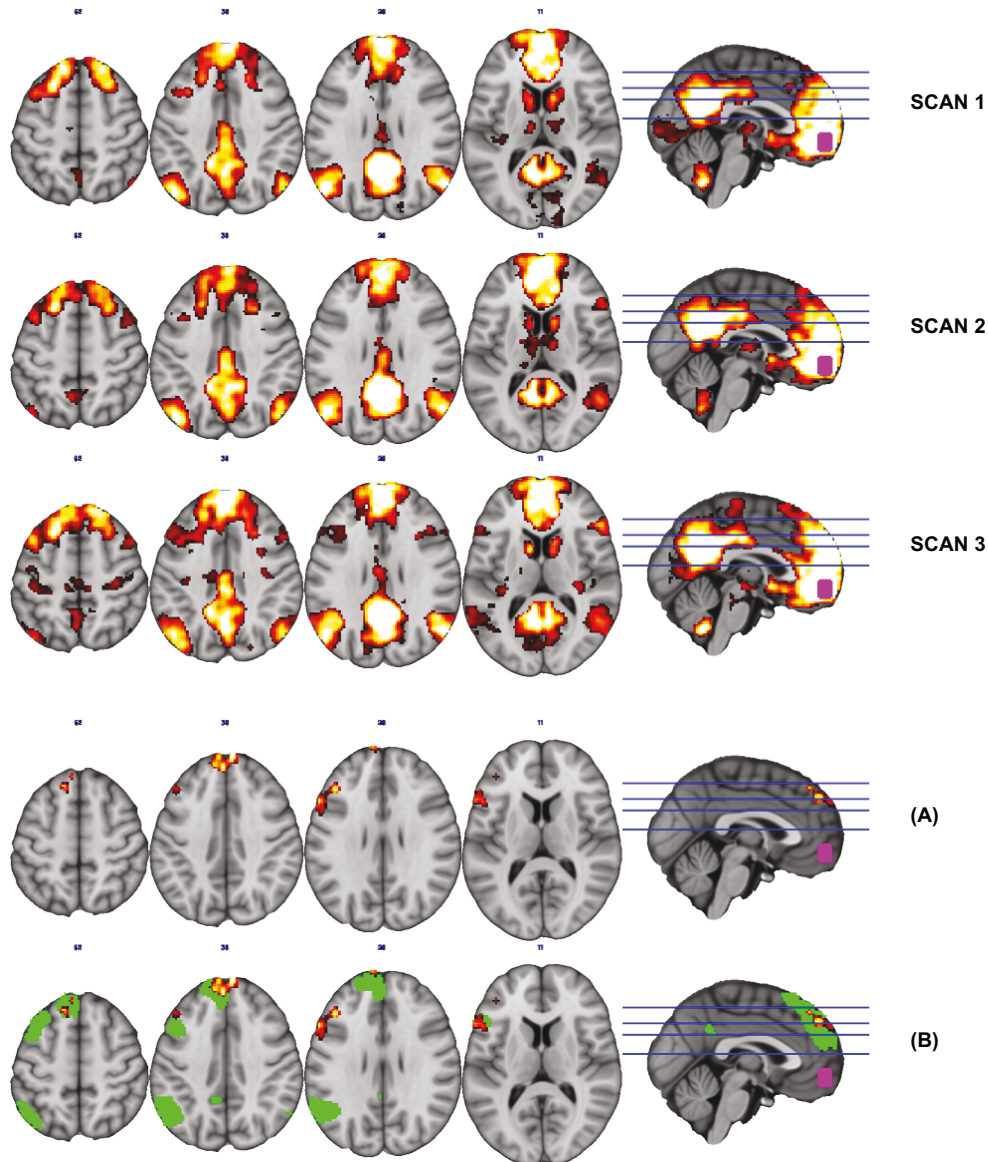


Figure 4-19. Seed-based resting-state functional connectivity analysis of the vmPFC. Brain regions showing increased coupling with the vmPFC (pink seed) are displayed in yellow/orange (Cluster-corrected; $Z > 2.3$, $p < 0.050$). Scan 1: baseline, scan 2: four weeks after 1 mg psilocybin, scan 3: four weeks after 25 mg psilocybin. No differences were found four weeks after 1 mg psilocybin. **(A)** The contrast: scan 3 (four weeks after 25 mg psilocybin) > scan 2 (four weeks after 1 mg psilocybin) with **(B)** IFPN overlay. Increased vmPFC rsFC to regions largely corresponding to the FPN (see Table 4-4) observed four weeks after the 25 mg psilocybin session. Abbreviations: vmPFC, ventromedial prefrontal cortex; FPN, frontoparietal network.

4.5.8. Predictive factors

Contextual factors predict the quality of acute experience

The importance of contextual factors in determining the quality of an acute psychedelic experience is well-established, yet the influence of some factors remain to be investigated in a controlled setting. In a recent qualitative study conducted by our group, patients with treatment-resistant depression reported their felt importance of the relationship with the dosing “guides” or “sitters” in post-psychedelic treatment interviews. However, the importance of the therapeutic alliance in predicting the acute psychedelic experience has not been systematically investigated. Therefore, a key question of this research was: is therapeutic alliance positively related to acute 25 mg psilocybin peak experience phenomena (i.e. OBN dimension of the 5D-ASC; MEQ; EDI)?

Pre-dosing anxiety has been shown to significantly predict anxious or other negative experiences during a psychedelic session (Metzner et al., 1965) and trait absorption has been shown to predict psychedelic-induced visual effects, insightfulness and peak experience phenomena (Studerus et al., 2012; Haijen et al., 2018; Russ et al., 2019). The experience of insightfulness was a key focus of this research. Therefore, key questions of this research were: (i) is pre-dosing anxiety negatively related to insightfulness and positively related to a negative component (i.e. DED dimension of 5D-ASC) of the acute 25 mg psilocybin experience? (ii) is trait absorption positively related to insightfulness during the acute 25 mg psilocybin experience?

Simple linear regressions were carried out to address the questions outlined above. In line with the hypotheses, a positive association was found between 25 mg pre-dosing MODTAS total score of absorption and the *insightfulness* ($F_{(1,15)}=5.98$, $R^2=0.285$, $p=0.027$) dimension of the 11D-ASC (Figure 4-20 F). With regards to therapeutic alliance, the 25 mg pre-dosing STAR score was positively associated with scores on the MEQ ($F_{(1,15)}=10.74$, $R^2=0.417$, $p=0.005$), EDI ($F_{(1,15)}=7.56$, $R^2=0.335$, $p=0.015$) and OBN subscale of the 5D-ASC ($F_{(1,15)}=7.52$, $R^2=0.334$, $p=0.015$) (Figure 4-20 A-C). With regards to pre-dosing anxiety, the 25 mg pre-dosing STAI score was positively associated with the score on the DED dimension of the 5D-ASC ($F_{(1,15)}=6.49$, $R^2=0.302$, $p=0.022$) and negatively associated with the *insightfulness* dimension score of the 11D-ASC ($F_{(1,15)}=7.11$, $R^2=0.322$, $p=0.018$; Figure 4-20 D-E).

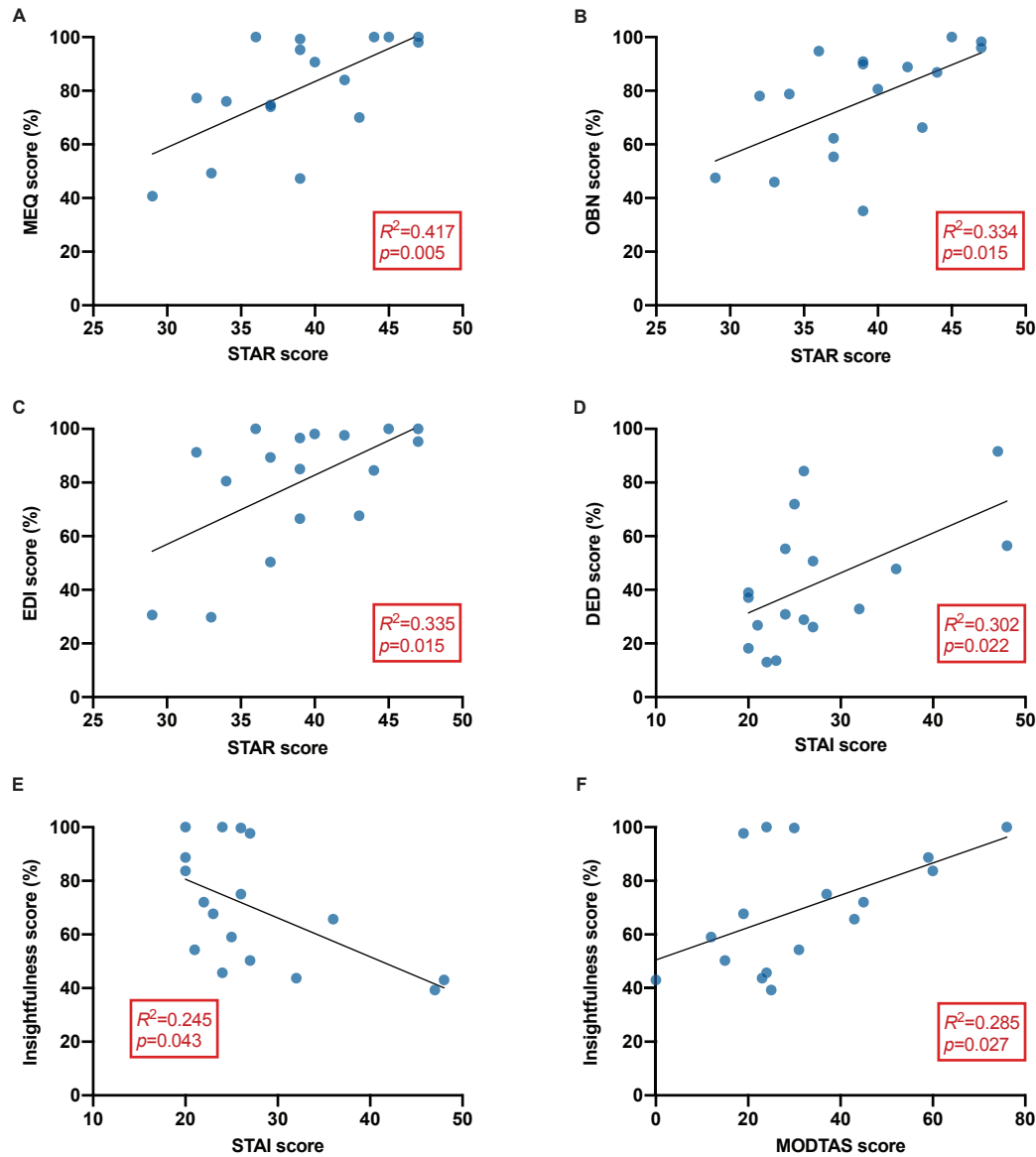


Figure 4-20. Pre-dosing predictors of the acute experience | (A–C) Therapeutic alliance. Positive associations were found between the 25 mg pre-dosing STAR and the MEQ ($F_{(1,15)}=10.74$, $R^2=0.417$, $p=0.005$), EDI ($F_{(1,15)}=7.56$, $R^2=0.335$, $p=0.015$) and OBN subscale of the 5D-ASC ($F_{(1,15)}=7.52$, $R^2=0.334$, $p=0.015$). **(D–E) Pre-dosing anxiety.** A positive association was found between the 25 mg pre-dosing STAI and the DED dimension of the 5D-ASC ($F_{(1,15)}=6.49$, $R^2=0.302$, $p=0.022$). A negative association was found between 25 mg pre-dosing STAI and the *insightfulness* dimension of the 11D-ASC ($F_{(1,15)}=7.11$, $R^2=0.322$, $p=0.018$). **(F)** A positive association was found between 25 mg pre-dosing MODTAS and the *insightfulness* ($F_{(1,15)}=5.98$, $R^2=0.285$, $p=0.027$) dimension of the 11D-ASC. Abbreviations: MODTAS, Modified Tellegen Absorption Scale; STAI, State-Trait Anxiety Inventory; STAR, Scale to Assess Therapeutic Relationship; 5D-ASC, 5-Dimensional Altered State of Consciousness; 11D-ASC, 11-Dimensional Altered State of Consciousness; OBN, oceanic boundlessness; DED, dread of ego dissolution; EDI, Ego Dissolution Inventory; MEQ, Mystical Experience Questionnaire.

Emotional breakthrough correlates with peak experience phenomena

Overcoming psychological resistance and achieving emotional breakthrough has been argued to be a requirement for the occurrence of peak experience phenomena (Jung, 2014). However, determining the importance of emotional breakthrough as a mediator of psychedelic peak experience phenomena has been precluded by a lack of sufficiently sensitive tools to measure its occurrence. With the recent development of the Emotional Breakthrough Inventory (EBI; Roseman et al., 2019), which is a new scale specifically designed to measure emotional breakthrough following the resolution of psychological struggle, determining its significance as a predictor of peak experience phenomena is now possible. As such, a key question of this research was: is emotional breakthrough positively related to acute 25 mg psilocybin peak experience phenomena (i.e. OBN dimension of the 5D-ASC and MEQ)? Simple linear regressions were carried out to address this question. In line with the hypothesis, positive associations were found between 25 mg psilocybin EBI score and scores on the OBN ($F_{(1,15)}=10.55$, $R^2=0.413$, $p=0.005$; Figure 4-21 A) and MEQ ($F_{(1,15)}=9.34$, $R^2=0.384$, $p=0.008$; Figure 4-21 B).

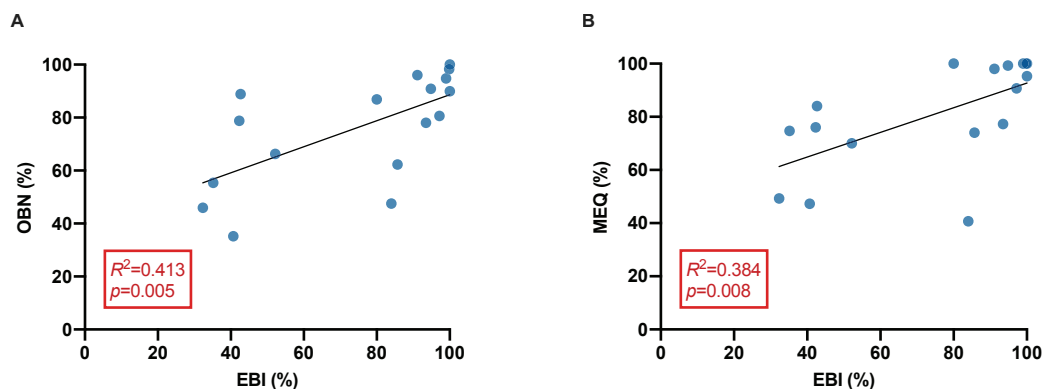


Figure 4-21. Emotional breakthrough relates to peak experience phenomena | (A) Oceanic boundlessness. A positive association was found between the 25 mg psilocybin EBI and OBN ($F_{(1,15)}=10.55$, $R^2=0.413$, $p=0.005$). **(B) Mystical-type experience.** A positive association was found between the 25 mg psilocybin EBI and MEQ ($F_{(1,15)}=9.34$, $R^2=0.384$, $p=0.008$). Abbreviations: OBN, Oceanic Boundlessness; MEQ, Mystical Experience Questionnaire; EBI, Emotional Breakthrough Inventory.

Quality of acute experience predicts enduring change in resting-state functional connectivity

The importance of psychedelic peak experience phenomena in determining post-psychedelic long-term psychological outcomes is well-established. Yet, the importance of peak experience phenomena on post-psychedelic long-term brain outcomes has never been investigated. Therefore, a key question of this research was: can acute 25 mg psilocybin peak experience phenomena predict subsequent long-term changes in resting-state functional connectivity? To address this question, simple linear regressions were carried out between the 25 mg psilocybin

5D-ASC OBN subdimensions of the 11D-ASC (i.e. (i) *experience of unity*, (ii) *spiritual experience*, (iii) *blissful state*, (iv) *insightfulness*, and (v) *disembodiment*) and the changes in sgACC and vmPFC rsFC four weeks later. Positive associations were found between the *experience of unity* dimension and the change in sgACC ($F_{(1,15)}=7.40$, $R^2=0.330$, $p=0.016$) and vmPFC ($F_{(1,15)}=23.29$, $R^2=0.608$, $p<0.001$) rsFC four weeks later (Figure 4-22 C-D). With regards to the *spiritual experience* dimension, a positive association was found with the change in vmPFC rsFC ($F_{(1,15)}=10.92$, $R^2=0.421$, $p=0.005$) four weeks later (Figure 4-22 B). With regards to the *disembodiment* dimension, a positive association was also found with the change in vmPFC rsFC ($F_{(1,15)}=14.91$, $R^2=0.498$, $p=0.001$) four weeks later (Figure 4-22 A). A positive association was found between the *insightfulness* dimension scores and the change in sgACC rsFC four weeks later ($F_{(1,15)}=6.07$, $R^2=0.288$, $p=0.026$; Figure 4-22 E). The association between brain signal complexity during the peak of the 25 mg psilocybin experience (i.e. LZc two hours post dosing) and changes in resting-state functional connectivity four weeks later was also assessed; however, no significant associations were found (all $p>0.659$ ns).

Quality of the integration session predicts long-term outcomes

Early psychedelic therapists suggested that it's essential to properly integrate psychological material that comes up during a psychedelic session (McCabe, 1977; Grof, 1980). Based on case studies, reports and the views of prominent psychedelic therapists, it is hypothesised that the quality of the post-25 mg psilocybin integration session may predict long-term psychological outcomes. To investigate this, simple linear regressions were carried out between the 25 mg psilocybin integration measures (i.e. MIS, GPI and PIS) and the change in experiential avoidance (bEAQ) and well-being (WEMWBS) four weeks later. The change in bEAQ score was negatively associated with the MIS ($F_{(1,15)}=6.23$, $R^2=0.294$, $p=0.025$; Figure 4-23 A) and GPI Insight-Action ($F_{(1,15)}=5.23$, $R^2=0.259$, $p=0.037$; Figure 4-23 B) scores, whereas PIS-PI ($F_{(1,15)}=8.00$, $R^2=0.381$, $p=0.014$; Figure 4-23 D) and PIS-BC ($F_{(1,15)}=6.19$, $R^2=0.323$, $p=0.027$; Figure 4-23 C) scores were positively associated with the change in WEMWBS score four weeks later.

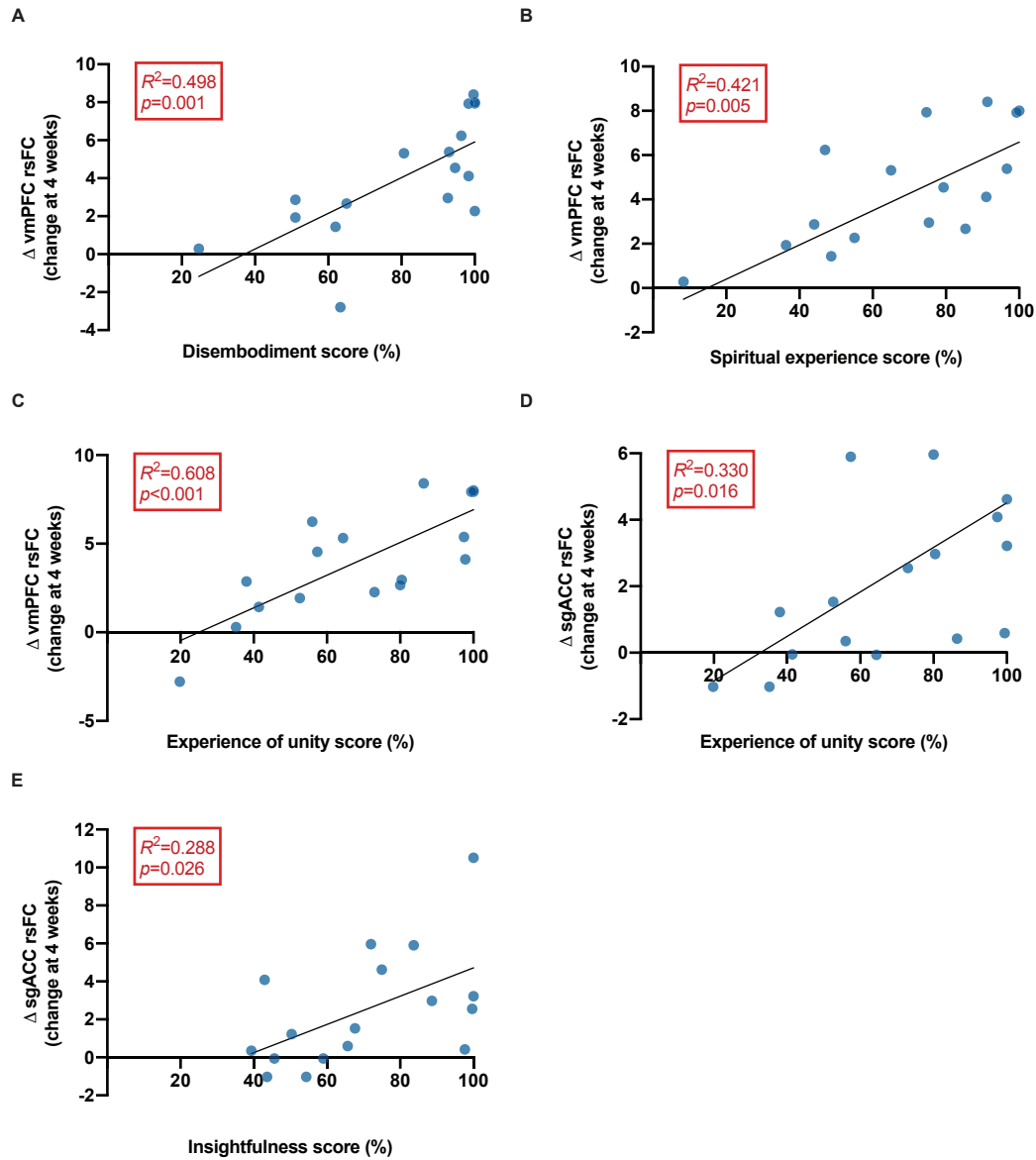


Figure 4-22. Peak experience predictors of change in resting-state functional connectivity | (A) Disembodiment. The 25 mg *disembodiment* score was positively associated with the change in vmPFC rsFC ($F_{(1,15)}=14.91$, $R^2=0.498$, $p=0.001$) four weeks later. **(B) Spiritual experience.** The 25 mg *spiritual experience* score was positively associated with the change in vmPFC rsFC ($F_{(1,15)}=10.92$, $R^2=0.421$, $p=0.005$) four weeks later. **(C–D) Experience of unity.** The 25 mg *experience of unity* score was positively associated with the change in sgACC ($F_{(1,15)}=7.40$, $R^2=0.330$, $p=0.016$) and vmPFC ($F_{(1,15)}=23.29$, $R^2=0.608$, $p<0.001$) rsFC four weeks later. **(E) Insightfulness.** The 25 mg *insightfulness* score was positively associated with the change in sgACC rsFC four weeks later ($F_{(1,15)}=6.07$, $R^2=0.288$, $p=0.026$). Abbreviations: sgACC, subgenual anterior cingulate cortex; vmPFC, ventromedial prefrontal cortex; rsFC, resting-state functional connectivity.

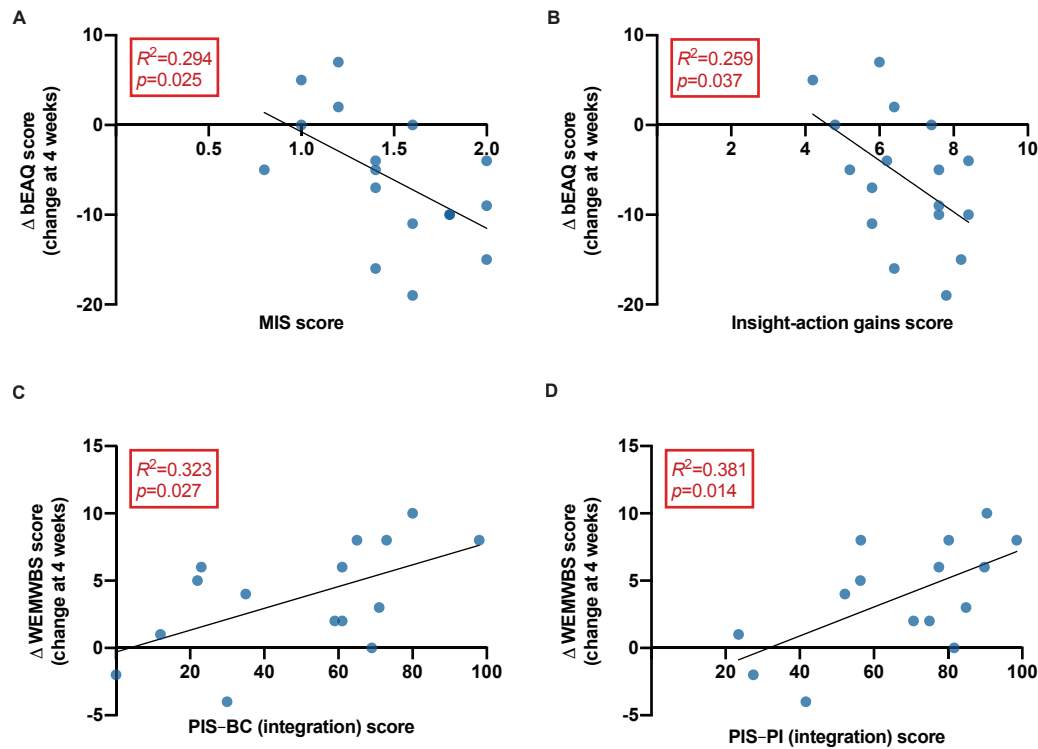


Figure 4-23. Integration outcome predictors of long-term outcomes | (A–B) Experiential avoidance. The 25 mg integration session MIS ($F_{(1,15)}=6.23$, $R^2=0.294$, $p=0.025$) and GPI Insight-Action scores ($F_{(1,15)}=5.23$, $R^2=0.259$, $p=0.037$) were negatively associated with the change in bEAQ scores **(C–D) Psychological well-being.** The PIS-PI ($F_{(1,15)}=8.00$, $R^2=0.381$, $p=0.014$) and PIS-BC ($F_{(1,15)}=6.19$, $R^2=0.323$, $p=0.027$) scores after the 25 mg integration session were positively associated with the change in WEMWBS scores four weeks later. Abbreviations: MIS, Mastery-Insight Scale; GPI, Gains from Psychedelic Integration; PIS, Psychological Insight Scale; PIS-PI, Psychological Insight subscale; PIS-BC, Behavioural Change subscale; bEAQ, Brief Experiential Avoidance Questionnaire; WEMWBS, Warwick-Edinburgh Mental Well-being Scale.

4.5.9. Relationships between long-term outcomes

Experiential avoidance and well-being

A wealth of evidence suggests that experiential avoidance¹² represents one of the most prominent transdiagnostic psychological processes with a known role in a wide variety of psychological disorders. Based on this, a key question of this research was: is experiential avoidance a psychological mechanism underlying positive psychological outcomes following a psychedelic experience? To address this question, a simple linear regression was carried out between the four weeks post-25 mg psilocybin change in bEAQ and WEMWBS scores. To delve in further, the change in bEAQ score was also examined in relation to various facets of well-being, i.e. the

¹² Experiential avoidance is defined as the tendency to rigidly escape or avoid private psychological experiences (thoughts, emotions, sensations, memories, urges), even when doing so is futile or interferes with valued actions (Hayes et al., 1996).

four weeks post-25 mg psilocybin change in MLQ-presence, FS and SCS scores. In line with the hypothesis, the change in bEAQ score was negatively associated with the change in WEMWBS ($F_{(1,15)}=5.27$, $R^2=0.260$, $p=0.036$), SCS ($F_{(1,15)}=8.26$, $R^2=0.355$, $p=0.012$), MLQ-presence ($F_{(1,15)}=5.54$, $R^2=0.2696$, $p=0.033$) and FS ($F_{(1,15)}=12.32$, $R^2=0.468$, $p=0.003$) scores four weeks post-25 mg psilocybin (Figure 4-24).

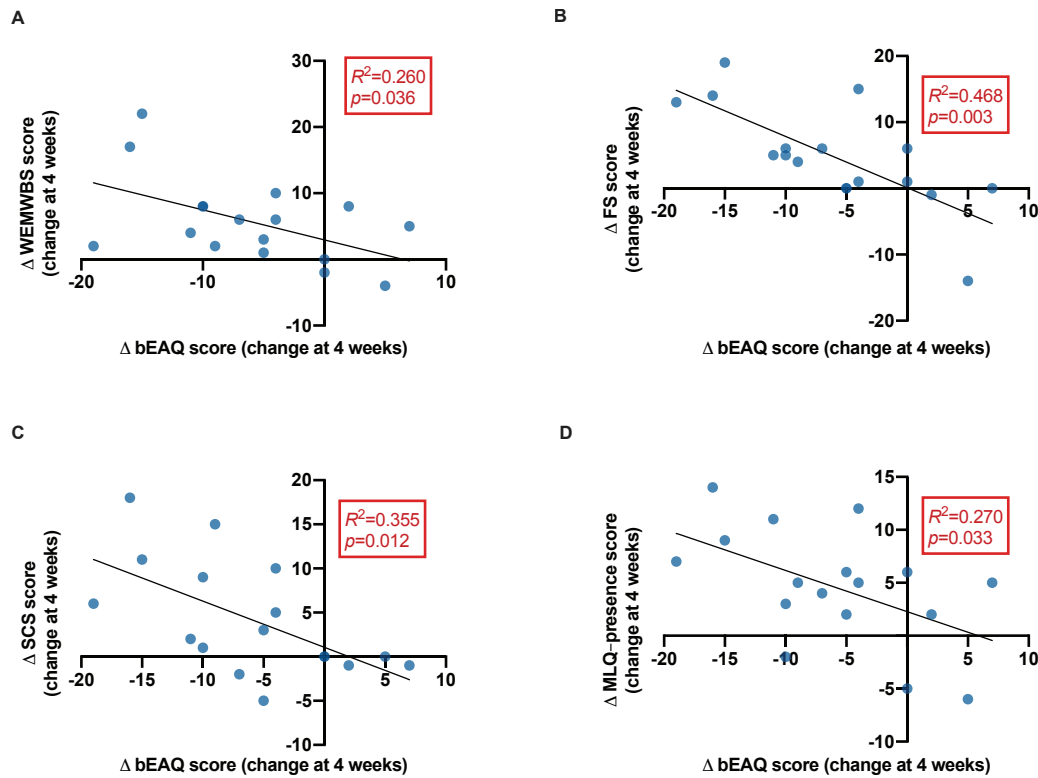


Figure 4-24. Experiential avoidance is related to well-being. | (A) Psychological well-being. The change in bEAQ score was negatively associated with the change in WEMWBS score ($F_{(1,15)}=5.27$, $R^2=0.260$, $p=0.036$) four weeks post-25 mg psilocybin. **(B) Flourishing.** The change in bEAQ score was negatively associated with the change in FS score ($F_{(1,15)}=12.32$, $R^2=0.468$, $p=0.003$) four weeks post-25 mg psilocybin. **(C) Social connectedness.** The change in bEAQ score was negatively associated with the change in SCS score ($F_{(1,15)}=8.26$, $R^2=0.355$, $p=0.012$) four weeks post-25 mg psilocybin. **(D) Presence of meaning in life.** The change in bEAQ score was negatively associated with the change in MLQ-presence score ($F_{(1,15)}=5.54$, $R^2=0.2696$, $p=0.033$) four weeks post-25 mg psilocybin. Abbreviations: WEMWBS, Warwick-Edinburgh Mental Well-being Scale; FS, Flourishing Scale; bEAQ, Brief Experiential Avoidance Questionnaire; SCS, Social Connectedness Scale; MLQ, Meaning in Life Questionnaire.

Psychological insight and insight-induced behavioural change mediate positive outcomes

Psychological insight was a key outcome of this study and was hypothesised to play an important influential role in long-term changes following 25 mg psilocybin. It is well-established that insight can occur acutely in a state-like fashion during a psychedelic experience. Yet, this study has

shown that insight can also be more protracted, crystallising in the integration and also over the course of a few weeks following a psychedelic experience (see Figure 4-9). The influence of psychological insight gained in the integration session on long-term outcomes has already been outlined in a preceding section (see Figure 4-23). As such, a further key question of this study was: is the enduring facilitation of psychological insight (and subsequent insight-induced behavioural change) a mechanism underlying positive psychological outcomes following a psychedelic experience? To address this question, simple linear regressions were carried out between the four weeks post-25 mg psilocybin change in: (i) PIS-PI score and the change in WEMWBS and bEAQ scores; (ii) PIS-BC score and the change in WEMWBS and bEAQ scores; and (iii) SRIS-IN score and the change in WEMWBS and bEAQ scores.

Four weeks post-25 mg psilocybin, the change in bEAQ score was negatively associated with the change in PIS-PI ($F_{(1,15)}=5.23$, $R^2=0.258$, $p=0.037$), PIS-BC ($F_{(1,15)}=11.00$, $R^2=0.423$, $p=0.005$) and SRIS-IN scores ($F_{(1,15)}=4.79$, $R^2=0.242$, $p=0.045$; Figure 4-25 D-F). The change in WEMWBS score four weeks post-25 mg psilocybin was positively associated with the change in PIS-BC (trend level association; $F_{(1,15)}=4.44$, $R^2=0.228$, $p=0.052$ ns trend; Figure 4-25 A). No associations were found with the change in PIS-PI or SRIS-IN scores (all $p>0.309$ ns). As the relationship between the change in WEMWBS and PIS-BC was at trend level and did not reach full significance, simple linear regressions were carried out between the change in FS (a facet of well-being) and both PIS subscales. The change in FS score was positively associated with the change in PIS-PI ($F_{(1,15)}=8.22$, $R^2=0.370$, $p=0.012$) score four weeks after 25 mg psilocybin (Figure 4-25 C). This was also the case for insight-induced behaviour change, as the change in PIS-BC score was positively associated with the change in FS ($F_{(1,15)}=7.01$, $R^2=0.318$, $p=0.018$) score four weeks after 25 mg psilocybin (Figure 4-25 B).

Cognitive flexibility

Enhancements in constructs approximating cognitive flexibility, such as divergent creative thinking (Kuypers et al., 2016), have been demonstrated following the administration of psychedelics. However, enduring post-psychedelic enhancement of cognitive flexibility has not previously been demonstrated. This study found a reduction in EDS errors (i.e. an increase in cognitive flexibility) four weeks after the 25 mg psilocybin experience. It has been postulated that impairments in cognitive flexibility may contribute to psychopathological behaviour. For instance, reduced cognitive flexibility has been associated with the development and perseverance of depression (Beck, 1976; Coles and Heimberg, 2002). As such, a key question of this study was: is cognitive flexibility a mechanism underlying positive psychological outcomes following a psychedelic experience? To address this question, simple linear regressions were carried out between the four weeks post-25 mg psilocybin change in EDS errors and the change in WEMWBS, bEAQ and PIS scores. Contrary to hypotheses, the change in EDS errors was not associated with the change in any of these measures (all $p>0.153$ ns).

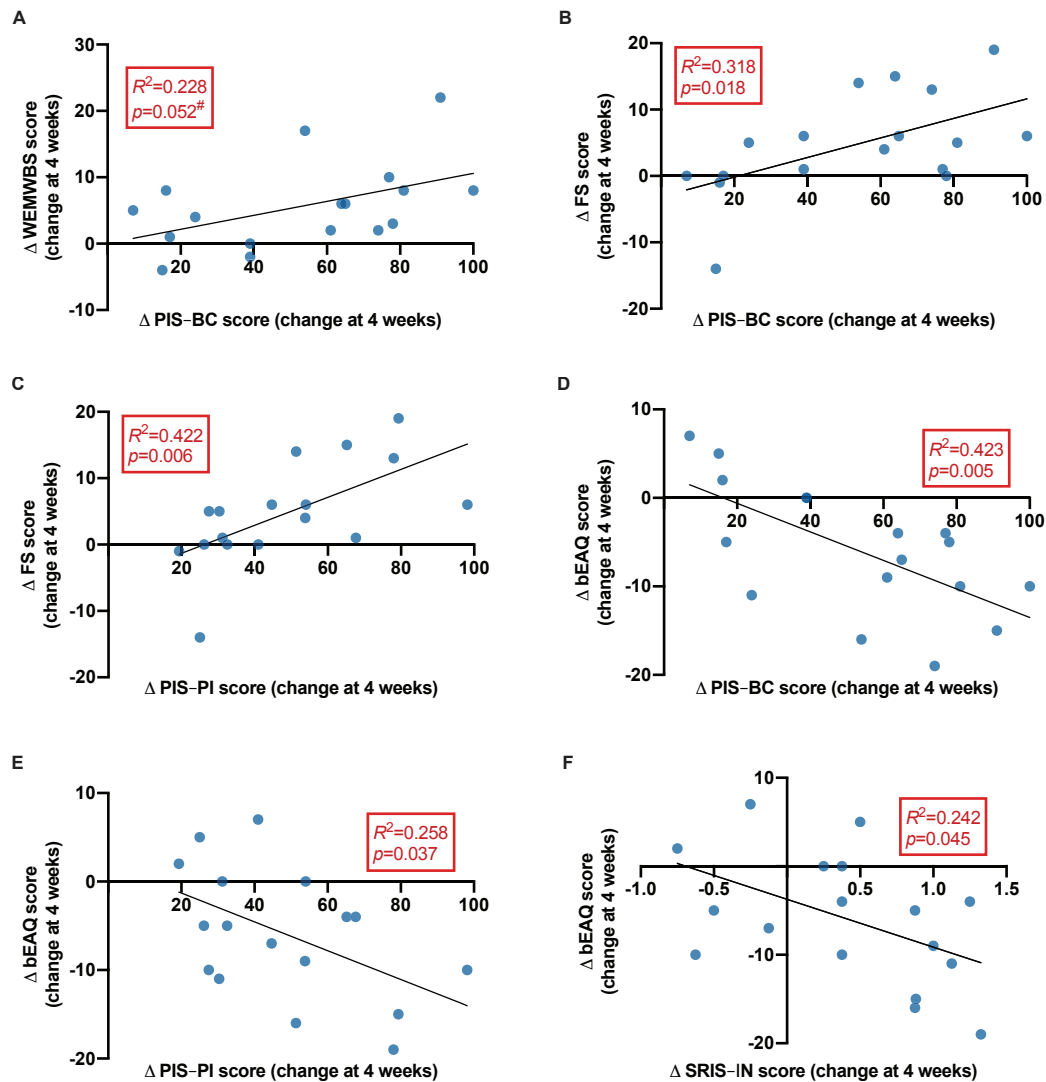


Figure 4-25. Insight mediates change in well-being and experiential avoidance. | **(A) Psychological well-being.** The four weeks post-25 mg psilocybin change in WEMWBS score was positively associated with the change in PIS-BC (trend level association; $F_{(1,15)}=4.44$, $R^2=0.228$, $p=0.052$ ns trend). **(B–C) Flourishing.** The change in FS score was positively associated with the change in PIS-PI ($F_{(1,15)}=8.22$, $R^2=0.370$, $p=0.012$) and PIS-BC ($F_{(1,15)}=7.01$, $R^2=0.318$, $p=0.018$) scores four weeks after 25 mg psilocybin. **(D–F) Experiential avoidance.** Four weeks post-25 mg psilocybin, the change in bEAQ score was negatively associated with the change in PIS-PI ($F_{(1,15)}=5.23$, $R^2=0.258$, $p=0.037$), PIS-BC ($F_{(1,15)}=11.00$, $R^2=0.423$, $p=0.005$) and SRIS-IN ($F_{(1,15)}=4.79$, $R^2=0.242$, $p=0.045$) scores. Abbreviations: PIS, Psychological Insight Scale; PIS-PI, Psychological insight subscale of the PIS; PIS-BC, Behavioural change subscale of the PIS; SRIS-IN, Insight subscale of the Self-Reflection and Insight Scale; WEMWBS, Warwick-Edinburgh Mental Well-being Scale; FS, Flourishing Scale; bEAQ, Brief Experiential Avoidance Questionnaire.

Neural correlates of long-term outcomes

The findings of this study support the well-established view that psychedelic experiences have the potential to promote enduring psychological outcomes. In addition to broadening the scope of post-psychedelic outcomes under investigation, this study is the first to also investigate long-term brain changes following a psychedelic experience. To this end, the present study is well placed to investigate the neural correlates of the long-term psychological outcomes induced by psychedelics. As such, a key question of this study was: are the enduring changes in resting-state functional connectivity and left amygdala responsivity to fearful faces related to the four weeks post-25 mg psilocybin change in well-being and experiential avoidance? As the amygdala and vmPFC are known to be critically involved in social cognition, their association with the change in social connectedness (a facet of well-being) was also assessed. To address this question, simple linear regressions were carried out between the four weeks post-25 mg psilocybin change in (i) sgACC rsFC and the change in WEMWBS and bEAQ scores; (ii) vmPFC rsFC and the change in WEMWBS, SCS and bEAQ scores; (iii) left amygdala responsivity to fearful faces and the change in WEMWBS, SCS and bEAQ scores. The association between cognitive flexibility (i.e. EDS errors) and changes in resting-state functional connectivity and left amygdala responsivity to fearful faces four weeks after the 25 mg psilocybin experience was also assessed; however, no significant associations were found (all $p > 0.120$ ns).

Four weeks after 25 mg psilocybin, the change in sgACC rsFC was positively associated with the change in WEMWBS scores ($F_{(1,15)}=6.62$, $R^2=0.321$, $p=0.022$; Figure 4-26 C). Moreover, the change in vmPFC rsFC was positively associated with the change in SCS ($F_{(1,15)}=5.98$, $R^2=0.285$, $p=0.027$) score four weeks after the 25 mg psilocybin session (Figure 4-26 D). As for the emotional faces paradigm, the change in left amygdala response to fearful faces was negatively associated with the change in WEMWBS ($F_{(1,15)}=5.82$, $R^2=0.294$, $p=0.030$) and SCS ($F_{(1,15)}=6.66$, $R^2=0.308$, $p=0.021$) scores four weeks after the 25 mg psilocybin session (Figure 4-26 A-B). No associations were found between the brain measures and the four weeks post-25 mg psilocybin change in bEAQ score (all $p > 0.080$ ns).

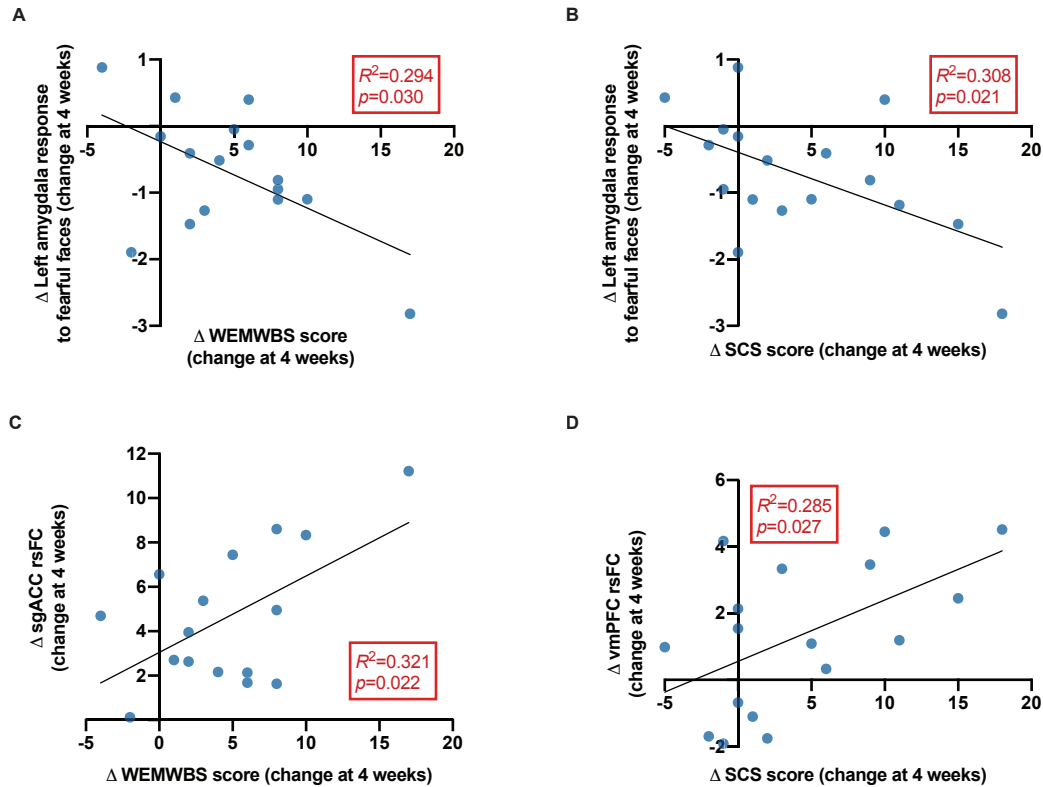


Figure 4-26. Neural correlates of enduring post-psychedelic psychological outcomes. | (A–B) Amygdala response to emotional faces. The change in left amygdala response to fearful faces was negatively associated with the change in WEMWBS ($F_{(1,15)}=5.82$, $R^2=0.294$, $p=0.030$) and SCS ($F_{(1,15)}=6.66$, $R^2=0.308$, $p=0.021$) scores four weeks after the 25 mg psilocybin session. **(C) sgACC resting-state functional connectivity.** Four weeks after 25 mg psilocybin, the change in sgACC rsFC was positively associated with the change in WEMWBS ($F_{(1,15)}=6.62$, $R^2=0.321$, $p=0.022$) score. **(D) vmPFC resting-state functional connectivity.** The change in vmPFC rsFC was positively associated with the change in SCS ($F_{(1,15)}=5.98$, $R^2=0.285$, $p=0.027$) score four weeks after the 25 mg psilocybin session. Abbreviations: WEMWBS, Warwick-Edinburgh Mental Well-being Scale; SCS, Social Connectedness Scale; sgACC, subgenual anterior cingulate cortex; vmPFC, ventromedial prefrontal cortex.

4.6. DISCUSSION

The present study combined multimodal neuroimaging with extensive measurement of a broad repertoire of psychological outcomes. To this end, the novel results considerably broaden the field's knowledge of the acute and long-term psychological and neurological effects of psilocybin and provide inspiration for future work. As the findings of this study are considerable owing to the wide range of assessments utilised, the key findings are summarised in bullet-point below for the sake of clarity and simplicity. Further details pertaining to each bullet point are provided in the corresponding sections of this discussion.

4.6.1. *Summary of the key findings*

- The time-course and intensity levels of acute psilocybin effects were in line with expectations and consistent with previous studies that have administered similar doses of psilocybin.
- Therapeutic alliance and pre-dosing anxiety are interrelated contextual factors that predict the quality of the acute 25 mg psilocybin experience
- 25 mg psilocybin induced peak experience phenomena
- 25 mg psilocybin increased brain signal complexity during the peak intensity of the experience (i.e. two hours post-dosing), and this was related to the degree of insightfulness experienced during the same EEG recording.
- Trait absorption predicted insightful experiences during the 25 mg psilocybin experience
- The 25 mg psilocybin experience induced an afterglow period (<2 weeks post-dosing) characterised by elevated positive mood states and self-esteem. On the other hand, 1 mg psilocybin induced somewhat of an “after-dim” period (<2 weeks post-dosing) characterised by increased negative mood states.
- 25 mg psilocybin enhanced trait absorption and a variety of prosocial effects (e.g. emotional empathy, compassion and gratitude) sustained long-term.
- Experiential avoidance and psychological well-being are interrelated and are improved four weeks after 25 mg psilocybin.
- The 25 mg psilocybin dosing and integration sessions enhanced the facilitation of acute, post-acute and enduring psychological insight, which mediated positive long-term changes in experiential avoidance and well-being.
- 25 mg psilocybin enhanced cognitive flexibility in the long-term.
- Psilocybin did not alter optimism or suggestibility.
- 25 mg psilocybin induced long-term changes in resting-state functional connectivity and left amygdala responsivity to fearfully-expressive facial stimuli, and these brain effects were related to long-term changes in social connectedness and overall well-being.
- 25 mg psilocybin peak experience phenomena predicted changes in resting-state functional connectivity four weeks later.

4.6.2. *Acute subjective effects of psilocybin*

Time-course and intensity of the drug effects

The results of this study show that 25 mg psilocybin produced significant drug effects up to six hours post-dosing. The subjective drug effects approached peak intensity at two hours post-25 mg psilocybin administration and then gradually declined in the following hours. On the other hand, 1 mg psilocybin had negligible drug effects only observed at one hour post-dosing compared to baseline. This is in line with expectations, as 1 mg psilocybin is generally considered to be a subperceptual /subthreshold dose (Madsen et al., 2019) and was used in place of a 'placebo' – i.e. would not occasion a full psychedelic experience and would act as a control for participant expectation. The time-course of acute 25 mg psilocybin effects were consistent with previous studies that have administered similar doses of psilocybin to healthy participants under comparable conditions (Griffiths et al., 2006; Griffiths et al., 2011; Griffiths et al., 2018; Barrett et al., 2018; Madsen et al., 2019; Hasler et al., 2004). These results are in line with expectations as the intake of psilocybin leads to dose-related 5-HT_{2A}R occupancy in the human brain, and both psilocin plasma levels and 5-HT_{2A}R occupancy correlate (non-linear) with subjective intensity ratings (Madsen et al., 2019).

Psilocybin alters consciousness and induces peak experience phenomena

The findings presented here show that 25 mg psilocybin induced ego dissolution and near-death and mystical-type experiences among the majority of participants, whereas 1 mg did not. Moreover, 25 mg psilocybin altered all dimensions on the 5D- and 11D-ASC. In particular, greater scores were found on the OBN and VRS dimensions of the 5D-ASC and reduced scores on the anxiety and impaired cognition and control dimensions of the 11D-ASC relative to the other dimensions on both ASC scales. Relatedly, >90% participants rated the 25 mg psilocybin experience as the single most intense, profound and unusual state of consciousness of their lifetime six months post-dosing, whereas the majority rated the 1 mg psilocybin experience as no more intense, profound or unusual than everyday states. The 25 mg dose of psilocybin also produced significantly more transient psychotomimetic effects than 1 mg psilocybin, with the exception of the phenomenon of anhedonia. In particular, cognitive disorganisation was found to be the most pronounced transient psychotomimetic state phenomenon enhanced following 25 mg psilocybin. Significantly more challenging experiences were reported following the 25 mg dose. That being said, it should be noted that the CEQ overall mean and subscale means were all less than 40% of the maximum score, suggesting that challenging experiences were relatively mild-to-moderate and well-managed. Relatedly, significantly more emotional breakthroughs were reported following 25 mg psilocybin, indicating that psychological struggles were worked through and resolved.

The acute subjective effects presented here are consistent with previous research and are thus in line with expectations. The MEQ, CEQ, ASC and EDI are often used to assess the acute subjective effects of psychedelics (Dittrich, 1998; Studerus et al., 2010; Nour et al., 2016; Barrett et al., 2015; Maclean et

al., 2012; Barrett et al., 2016). Psychedelics are known to alter the perception of time (Wittmann et al., 2007; Wackermann et al., 2008) as well as perception across different sensory modalities, especially at high doses (Studerus et al., 2011; Schmid et al., 2015; Carhart-Harris et al., 2016b). A highly vivid imagination and eyes-closed elementary (i.e. colours and patterns) and complex (i.e. concrete objects, landscapes or entities) imagery are among the most commonly reported acute subjective effects (Carhart-Harris et al., 2011; Studerus et al., 2011; Schmid et al., 2015). Ego-dissolution, which refers to a temporary dissolution of the boundaries between the self (i.e. the “me”) and other (i.e. the “not me”), is a commonly reported effect elicited by psychedelic compounds (Nour et al., 2016) and consistent with the present findings. The loss of the sense of self is an important feature of peak experiences occasioned by psychedelics. Psychedelic peak experiences are characterised by feelings of unity with all that exists. This unitive experience is therefore explicitly related to disturbed ego-boundaries between the self (i.e. the “me”) and the other/object (i.e. the “not me”). In addition to high scores on the EDI (Carhart-Harris et al., 2016b; Lebedev et al., 2015; Nour et al., 2017; Nour et al., 2016; Tagliazucchi et al., 2016), previous studies have also indicated prominent effects of psychedelics on ego-functioning via high ratings on the OBN dimension of the 5D-ASC, defined as “positively experienced derealisation and depersonalisation” (Dittrich, 1998; Studerus et al., 2010). The 25 mg psilocybin-induced psychotomimetic symptoms presented here are in line with a previous LSD study. Carhart-Harris and colleagues (2016) also found that all factors of the PSI, except for ‘anhedonia’, were significantly increased under LSD, with the most marked effect on ‘cognitive disorganisation’ (Carhart-Harris et al., 2016b).

This study found significantly more challenging experiences following 25 mg versus 1 mg psilocybin. That being said, the CEQ overall mean and subscale means were all less than 40% of the maximum score, suggesting that challenging experiences were relatively mild-to-moderate and well-managed. This is in line with the literature, as challenging psychedelic experiences may be minimised and successfully managed in research contexts that recognise the importance of set and setting¹³ (Metzner et al., 1965; Studerus et al., 2012; Johnson et al., 2008), yet can still occur in controlled and supportive conditions (Studerus et al., 2011). Evidence suggests a possible profile of psychedelic challenging experiences that includes the following categories of experience¹⁴: (i) fear or panic; (ii) paranoia; (iii) sadness or depressed mood; (iv) anger; (v) cognitive effects, such as confusion, distressing loss of ego, loss of sanity, delusions, dissociation and depersonalisation; (vi) perceptual effects, such as illusions; and (vii) physiological symptoms, such as increased heart rate, nausea and/or emesis and sympathetic system response (Frecka and Luna, 2006; Johnson et al., 2008; McCabe, 1977).

It has been suggested that the intensity of challenging psychedelic experiences may predict enduring positive outcomes, whereas the duration of struggle, on the other hand, is predictive of negative outcomes (Carbonaro et al., 2016). Relatedly, proponents of psychoanalysis argue that one must

¹³ *Set and setting* is a term used to refer to various internal and external contextual factors that shape one’s response to a psychedelic drug. The ‘set’ typically refers to the participant’s mindset, mood, traits and expectations. The ‘setting’ typically refers to external environmental factors, such as interpersonal relationships with others present and comfortable surroundings.

¹⁴ Important note: Although these challenging experience categories appear in aggregate in the literature, they may not all appear in any single instance of a psychedelic challenging experience.

overcome psychological resistance in order to achieve emotional breakthrough and resolve psychological struggle (Grof, 1980; Fischer, 2015; Eisner, 1958; Crocket et al., 1963). The high levels of emotional breakthrough found here following the 25 mg dose indicate that psychological struggles were indeed worked through and resolved. A recent study demonstrated that emotional breakthroughs – which are closely related to, but qualitatively different from, peak and challenging experience phenomena – represent an essential component of the psychedelic experience (Roseman et al., 2019). Overcoming psychological resistance and achieving emotional breakthrough has been argued to be a requirement for the occurrence of peak experience phenomena (Jung, 2014). Consistent with this notion, the present study found that the degree of emotional breakthrough is related to the occurrence of peak experience phenomena, as measured via the MEQ and the OBN dimension of the 5D-ASC. It should be noted that the specific assessment of emotional breakthrough following the resolution of psychological struggle was precluded until the very recent development of the EBI (Roseman et al., 2019). Accordingly, substantial research efforts are required to better understand the importance and implications of psychedelic-induced emotional breakthrough.

All participants exceeded the threshold for a near-death (type) experience following 25 mg psilocybin, yet it should be noted that two participants also surprisingly exceeded this threshold following 1 mg psilocybin. The 25 mg psilocybin finding is consistent with previous reports of high-dose psychedelic-induced near-death (type) experiences (Martial et al., 2019). Interestingly, the NDE occasioned here by 25 mg psilocybin was of the affective-type with only specific NDE phenomena enhanced. This is consistent with a recent DMT study, which also found DMT-induced NDEs were of the affective-type (Timmermann et al., 2018). Importantly, the overall mean NDE score following 1 mg psilocybin did not reach the cut-off point; thus, 1 mg psilocybin was not regarded as inducing a near-death (type) experience. As for the two participants that exceeded the NDE threshold following the 1 mg dose, the reason for this is unclear but may indicate the following possibilities: (i) the NDE is prone to false positives, thus thwarting our confidence in all findings obtained using this measure; or (ii) the context of the dosing session facilitated the induction of a near-death (type) experience. If we consider the second possibility, participants were instructed to treat each dosing day equally as a kind of “music meditation” to reduce further variability beyond the drug dosage given between the dosing sessions (i.e. varying levels of talking, engaging in other activities, interactions with the “sitters” etc.). This welcomed the participants to engage with and immerse themselves in the music, thereby encouraging them to remain in the bed wearing the eyeshades and headphones regardless of the dose given. With this in mind, experienced Buddhist meditators have been shown to induce near-death (type) experiences during meditation practices (Van Gordon et al., 2018), which may help to explain the findings here following 1 mg psilocybin. Be that as it may, meditation-induced near-death experiences have only been reported in highly advanced practitioners and none of this study’s participants would fall into this category. As such, the findings presented here invite further research in order to disentangle the relative contribution of drug effects and contextual factors in producing psychedelic NDEs.

4.6.3. *Acute brain effects of psilocybin*

Psilocybin increases brain signal complexity

It has been suggested that the increase in brain signal complexity (entropy) under psychedelics reflects an increase in ‘richness’ of experience, in which conscious content becomes more diverse (Carhart-Harris, 2018). The present study found an increase in the complexity (LZc) of spontaneous brain activity two hours after the administration of 25 mg psilocybin – this corresponds to the “peak” of the experience, when subjective ratings of drug intensity were at their highest. The increase in signal complexity found here is consistent with a vast body of literature demonstrating heightened brain signal complexity under psychedelics (e.g. Alonso et al., 2015; Lebedev et al., 2016; Schartner et al., 2017; Viol et al., 2017). Notably, however, here we show a positive association between increased brain complexity and the degree of insightfulness experienced during the same recording, potentially revealing neural basis for this experience – this is the first study to explore and find this relationship.

The correlation between insightfulness and brain complexity (entropy) found here provides substantial support for a key principle of the REBUS model: namely, that increased brain signal complexity with relaxed high-level priors enables the brain to be more amenable to curiosity and BMR processes (Carhart-Harris and Friston, 2019). By inducing a “hot state” in the brain characterised by heightened entropy (and thus uncertainty), 25 mg psilocybin may have facilitated insightfulness by disinhibiting lower-level (latent) priors and enabling them to emerge into conscious awareness. Within the REBUS framework, BMR is a hypothesised mechanism of model optimisation through which redundant parameters are pruned in order to refine the model (Carhart-Harris and Friston, 2019). As such, with high-level priors relaxed, these “insights” may then encourage the cultivation of changes to the relevant assumptions instantiated by higher levels in the hierarchy. In this way, pruning away unnecessary details may allow one to gain an understanding beyond what was immediately apparent (i.e., insight into “the bigger picture”).

The proposition that the relaxation of high-level prior assumptions facilitates psychological insight suggests that insight-related processes are more effective following the suspension of executive control. Relatedly, the emergence of insight on cessation of ego-centred consciousness is a key component of Buddhist philosophy and practice. Consistent with the present findings, a recent study found increased brain entropy in experienced practitioners of Vipassanā – a form of meditation that is sometimes translated as “insight” or more accurately “special-seeing” (Kakumanu et al., 2018). As increased brain entropy in the psychedelic state has been found to correlate with subjective ratings of ego-dissolution (Lebedev et al., 2016), this finding is particularly interesting – this is because Vipassanā meditation encourages insightfulness through a “no self” state, and the Kakumanu et al. (2018) study therefore helps to interpret and integrate this study’s results with the existing literature. Taken together, these findings provide support for heightened brain complexity as a potential neural phase through which insight might occur.

4.6.4. *Predicting the quality of the acute experience*

Response to a psychedelic is highly related to, but not limited to, dosage of the drug (e.g., Studerus et al. (2012). Particular personality traits (e.g. Studerus et al., 2012), mental state at the time of dosing (e.g. Hartogsohn, 2016; Leary et al., 1963; Phelps, 2017; Studerus et al., 2012) and characteristics of the setting in which the experience takes place (e.g. Leary et al., 1963; Hartogsohn, 2016) all influence the response to a psychedelic. Thus, responses to psychedelics strongly vary between and within subjects, even when the drug dose is kept constant (Studerus et al., 2011; Nichols, 2004). It has therefore been postulated that a large proportion of inter- and intraindividual differences in reactions to psychedelics is determined by contextual factors, often referred to as the ‘set’ and ‘setting’ (Carhart-Harris et al., 2018). A limited number of studies have attempted to assess factors that may be predictive of acute or longer-term responses to psychedelics (Russ et al., 2019; Studerus et al., 2012; Haijen et al., 2018). Factors related to traits and mental state at time of dosing are less clear, and those associated with adverse experiences are generally linked to greater pre-dosing anxiety and apprehension as well as a lack of trust in the other people present (e.g. Barrett and Griffiths, 2017; Leary et al., 1963; Studerus et al., 2012; Phelps, 2017).

Therapeutic alliance and pre-dosing anxiety are interrelated and can predict the acute subjective effects of psilocybin.

The importance of contextual factors in determining the quality of an acute psychedelic experience is well-established (see Carhart-Harris et al., 2018), yet the influence of some factors have not previously been investigated in a controlled setting. Therapeutic alliance is known to be a major predictor of response in mental healthcare (Kazdin, 2007), and its influence is likely to be greater still in the context of psychedelics given the influence of set and setting on their effects. Yet, the importance of therapeutic alliance in predicting the acute psychedelic experience has not previously been systematically investigated. To this end, the present study hypothesised that the quality of the therapeutic relationship between the participant and study guides (“sitters”)¹⁶ would be predictive of peak experience phenomena. Moreover, based on the direct association between state anxiety and therapeutic alliance shown previously in non-psychedelic research (Kim et al., 2015; Mundorf et al., 2017), this study hypothesised that 25 mg psilocybin pre-dosing anxiety and therapeutic alliance would be negatively correlated.

Consistent with hypotheses, the present study demonstrated that therapeutic alliance predicted pre-dosing state anxiety. In particular, 25 mg psilocybin pre-dosing state anxiety negatively correlated with therapeutic alliance, demonstrating that a better therapeutic relationship is related to reduced participant anxiety levels prior to the psychedelic session. This finding most likely reflects how “safe” or “comfortable” the participant felt in the care of the sitters. It is likely that the dosing preparation sessions

¹⁶ The terms “sitters” and “guides” are synonymous. In this thesis, “sitters” or “guides” refer to the pair of researchers that care for the participant before, during and after the psilocybin sessions. The role of a sitter is to provide non-directive psychological support as required. As such, the role is not overly active (i.e., as in a therapist) or inactive (i.e., as in passively observing).

aided these feelings of security, as a thorough discussion on the study procedures, risks, drug effects, potential subjective experiences and techniques for navigating such experiences may reduce fear or uncertainty. Further, reviewing personal history and feelings during the preparation is not only important for the development of a trusting relationship, but also because related personal material may “emerge” under the effects of the drug. Prior knowledge of the participant’s circumstances enables the sitters to better understand the participant’s experience and thus provide appropriate interpersonal support should strong emotions arise – thus, it is imperative that additional attention is paid to participant rapport, beyond customary levels in research, in the context of psychedelic studies.

Therapeutic alliance is a major predictor of response in mental healthcare (Kazdin, 2007) and a number of studies have linked it to a significant portion of variance in psychotherapeutic treatment outcomes (Horvath and Symonds, 1991; Horvath et al., 2011; Horvath and Bedi, 2002; Martin et al., 2000). Evidence suggests that the experiential component of psychedelics necessitates and facilitates the development of a strong therapeutic bond between the participant and their sitters (Johnson et al., 2008; Richards, 2016; Carhart-Harris et al., 2018). Therefore, it’s likely that the interpersonal environment created by the sitters is of great importance in influencing a participant’s response to a psychedelic drug (Masters and Houston, 1966; Leary et al., 1964; Johnson et al., 2008). In line with this notion, the present study demonstrated that a better therapeutic alliance reduces pre-dosing anxiety and facilitates acute ego- and boundary-dissolution as well as mystical-type experiences. It makes sense that supportive interpersonal interactions with the sitters enable the participant to trust that their needs are met and that they will be cared for during their experience. Successfully building rapport and trust allows the participant to foster feelings of security, ultimately resulting in a reduction of pre-dosing anxiety and influencing the quality of the psychedelic experience.

Pre-dosing anxiety has been shown to significantly predict anxious or other negative experiences during a psychedelic session (Metzner et al., 1965). In line with this evidence, the present study found a positive association between pre-dosing anxiety and scores on the DED dimension of the 5D-ASC. It is worth noting that the 5D-ASC DED dimension measures an unpleasant and anxious state characterised by cognitive impairment, loss of self-control, feelings of separation from oneself and the world, and anxiety or panic (Dittrich, 1998; Dittrich et al., 2010; Studerus et al., 2010). Relatedly, previous research suggests that positive prior expectations predict more positive experiences under a psychedelic (Metzner et al., 1965) and greater anxiety can reduce the likelihood of having a peak experience (Richards et al., 1977). As the experience of insight was a key focus of this research, this study hypothesised that 25 mg psilocybin pre-dosing anxiety would be negatively associated with scores on the *insightfulness* dimension of the 11D-ASC. In line with the hypothesis and prior literature, this study showed greater 25 mg psilocybin pre-dosing anxiety predicted reduced insightfulness.

Trait absorption predicts the acute subjective effects of psilocybin.

The importance of trait absorption in determining the quality of an acute psychedelic experience is well-established (Studerus et al., 2012; Haijen et al., 2018; Russ et al., 2019). Based on this evidence base and conforming to this study's focus on insight, it was hypothesised that pre-dosing trait absorption would predict scores on the *insightfulness* dimension of the 11D-ASC. Consistent with this, the present study found pre-dosing trait absorption predictive of 25 mg psilocybin-induced ratings on the insightfulness dimension of the 11D-ASC, such that greater trait absorption was associated with greater insightfulness.

The personality trait absorption is related to how easily and deeply an individual can be immersed in stimuli and/or experiences. The present finding that pre-dosing absorption predicted acute 25 mg psilocybin-induced insightfulness is consistent with previous research, as trait absorption has previously been shown to predict psychedelic-induced visual effects, insightfulness and mystical-type experiences (Studerus et al., 2012; Haijen et al., 2018; Russ et al., 2019). Moreover, a large number of studies have shown that absorption is associated with differential responsivity to various other non-psychedelic ASC induction procedures, including hypnosis, meditation, marijuana intoxication, and electromyograph biofeedback (Vaitl et al., 2005; Pekala et al., 1985).

Absorption has been related to genetic variants in the 5-HT_{2A}R, with a specific variant relating to greater 5-HT_{2A}R signalling being associated with higher absorption scores (Ott et al., 2005). Here, the finding that absorption predicted insightfulness during the acute psychedelic experience can therefore be interpreted psychologically or neurobiologically, i.e. in terms of greater immersion in the psychedelic experience or in terms of potential differences in 5-HT_{2A}R functioning facilitating both absorption and psychedelia, respectively. These interpretations are of course not mutually exclusive. The relevance of personality traits in shaping psychedelic responses has been noted previously (Savage et al., 1966; Studerus et al., 2012) and it is intriguing to think these may have a serotonergic underpinning (Frokjaer et al., 2008).

4.6.5. The Psilocybin Afterglow

Psilocybin alters mood states

This study demonstrated an increase in anger, depression and confusion coinciding a decrease in esteem-related affect and vigour two weeks after the 1 mg psilocybin session; these mood states returned to baseline four weeks later. The increase in anger, depression and confusion coinciding a decrease in esteem-related affect and vigour observed two weeks after the 1 mg psilocybin session cannot be explained by the literature. The reason for this increase in negative and decrease in positive mood states is unclear and cannot be explained by the literature, but it is possible that this finding reflects the participants' "disappointment" after receiving a sub-perceptual dose and therefore not

experiencing subjective drug effects. The return of these mood states to baseline levels four weeks later suggests that participants were either no longer disappointed or perhaps anticipated receiving a larger dose on the second dosing session the following day. It should be noted that these suggested possibilities are highly speculative, and the present results highlight the need for further research to examine the potential mood effects of subthreshold doses of psychedelic compounds.

On the other hand, a trend level decrease in depression and a significant decrease in tension, anger, fatigue and confusion together with an increase in esteem-related affect was observed two weeks after the 25 mg psilocybin session. Tension, anger, fatigue and confusion all returned to baseline at four weeks post 25 mg, but the increased esteem-related affect remained four weeks post-dosing. It has been argued that after-effects subsequent to a psychedelic experience constitute a distinct phase, referred to as the 'afterglow' (Albaugh and Anderson, 1974; Krupitsky and Grinenko, 1997; Pahnke, 1969; Sherwood et al., 1962; Pahnke et al., 1970; Metzner, 1998). The psychedelic afterglow refers to positive physical and mental effects that persist after the acute psychedelic effects have subsided, or after the peak experience has subsided (see Majic et al., 2015: for a review). The afterglow phenomenon typically lasts approximately two weeks and is characterised by elevated mood, reduced stress, increased willingness to enter into close interpersonal relationships and enhanced efficacy of psychotherapeutic interventions (Grob et al., 1996; Albaugh and Anderson, 1974; Pahnke, 1969; Winkelman, 2014). This state is often characterised by feelings of psychological clarity and contrasts with the phenomenon of hangovers following the use of various other substances, such as alcohol (Pahnke, 1969; Pahnke et al., 1970; Winkelman, 2014; Majic et al., 2015). In line with the present results showing elevated mood states following the 25 mg psilocybin session, Pahnke described the psychedelic afterglow as an "elevated and energetic mood with a relative freedom from concerns of the past and from guilt and anxiety" (Pahnke, 1969). The decrease in confusion and trend level decrease in depression observed here two weeks after 25 mg psilocybin is also consistent with research showing a reduction of these two mood states in regular ritualistic ayahuasca users (Barbosa et al., 2016).

Psilocybin improves self-esteem

In line with the concept of the 'psychedelic afterglow', the present study found increased self-esteem up to two weeks after the 25 mg psilocybin session, with a subsequent return to baseline levels at four weeks post. Among recreational psychedelic drug users, commonly reported benefits of the psychedelic afterglow include increased confidence (self-esteem), a state of inner peace and feeling "cleansed" (see psychedelic user reports; e.g. erowid.org and shroomery.org). As the psychedelic afterglow typically lasts approximately two weeks (Grob et al., 1996), it's not surprising that the increase in self-esteem found here did not persist beyond the two-week follow-up. Consistent with the present study, accounts of ayahuasca-induced improvements in self-esteem have been reported in patients with addiction (Mabit, 2007; Loizaga-Velder and Pazzi, 2013; Cruz and Nappo, 2018) and eating (Lafrance et al., 2017) disorders. Moreover, an early study found that 71% of participants experienced sustained improvements in self-esteem following LSD therapy (Savage et al., 1964).

4.6.6. *Enduring subjective effects*

Psilocybin does not alter suggestibility or optimism.

Previous research has shown increased suggestibility under psychedelics (Carhart-Harris et al., 2015a), yet the potential long-term enhancement of suggestibility has not been sufficiently investigated. As evidence suggests that psychedelic drug use can lead to long-term increases in openness to experience (MacLean et al., 2011), then it's possible that this is a consequence of greater willingness to entertain unusual ideas. If so, might this represent a manifestation of a long-term increase in suggestibility? To address this question, the present study investigated the potential for long-term enhancements in suggestibility following 25 mg psilocybin. Contrary to hypotheses, the present study did not find a change in suggestibility at any timepoint post-psilocybin treatment.

Suggestibility has been associated with enhanced treatment outcomes for a number of conditions, including pain (Patterson and Jensen, 2003) and depression (Kirsch and Low, 2013). It has therefore been proposed that the psychedelic-induced increase in suggestibility may have important implications for their therapeutic use. The potential of LSD to enhance suggestibility was first noted by clinicians working with the drug in the 1950s (Goolker, 1961; Weitzenhoffer and Hilgard, 1962; Sjöberg and Hollister, 1965). Early studies showed that suggestibility was significantly enhanced by hypnosis, LSD, mescaline and three drugs (LSD, mescaline and psilocybin) in combination, but not by psilocybin alone (Sjöberg and Hollister, 1965). This early study provides support for the findings shown here as it suggests that psilocybin alone is insufficient to alter suggestibility, even in the acute drug state. To this end, it's possible that other psychedelic drugs not investigated here, such as LSD, have the potential to induce long-term increases in suggestibility – this should be explored further in future research. Nevertheless, given the ability of psilocybin to induce enduring positive effects on the psychology of both healthy and patient populations (Andersen et al., 2020; Carhart-Harris and Goodwin, 2017), it's unlikely that a psychedelic-induced increase in suggestibility represents a key mediator of therapeutic outcomes.

The present study did not find changes in optimism following psilocybin. This is in contrast to previous research showing enduring post-psychedelic increases in optimism in healthy participants (Smigielski et al., 2019; Carhart-Harris et al., 2016b). Recreational and ritualistic psychedelic drug users have also been shown to exhibit greater optimism (or reduced pessimism) than non-users (Grob et al., 1996). In patient populations, a single dose of psilocybin has been shown to induce sustained reductions in anxiety and depression as well as increases in optimism in patients suffering from depression and anxiety reactive to advanced-stage cancer (Griffiths et al., 2016; Ross et al., 2016). Psilocybin has also been shown to induce long-lasting reductions in depressive symptoms and pessimism in patients suffering from treatment-resistant depression (Lyons and Carhart-Harris, 2018b), and PET imaging studies have provided support for a positive association between 5-HT_{2A}R signalling and pessimism (Bhagwagar et al., 2006; Meyer et al., 2003). It has been postulated that increased 5-HT_{2A}R binding and associated pessimistic thinking (Bhagwagar et al., 2006; Meyer et al., 2003) may be a corollary of

deficient 5-HT_{2A}R signalling, and that enduring post-psychedelic increases in optimism (or reduced pessimism) may be viewed as evidence of extreme 5-HT_{2A}R signalling having a lasting impact on positive thinking (Carhart-Harris et al., 2016b).

Based on the large body of evidence demonstrating increased optimism (or reduced pessimism) in a variety of samples, it is surprising that the present study did not replicate this well-established finding. One possible explanation for the present study's results may include high scores for optimism at baseline. This effect, also known as the ceiling effect, renders little chance for a significant increase after the intervention. Overall, further research examining the long-term changes in optimism, with particular efforts to avoid ceiling effects, is warranted.

Psilocybin enhances absorption

Absorption has been described as a type of focused attention characterised by deep involvement with particular aspects of subjective experience (Dal Cin et al., 2017). For instance, high trait absorption is related to intense engagement with nature and aesthetic involvement with art (Wild et al., 1995). The present study found a long-term increase in trait absorption up to four weeks after the 25 mg psilocybin session. Consistent with this, a very recent study also found increased absorption four weeks after a high-dose psilocybin experience (Barrett et al., 2020). Furthermore, increased absorption has also been observed following a psychedelic microdosing regime, indicating that participants became more involved in imaginative experiences after microdosing (Polito and Stevenson, 2019).

As mentioned in previous sections, studies indicate that individuals with higher pre-existing levels of trait absorption are more likely to experience stronger effects from psychedelic substances. Absorption is closely linked to one's capacity to experience altered states of consciousness and has been identified as the most important trait predictor of acute boundary dissolution and visual alterations following administration of high doses of psilocybin (Studerus et al., 2012). In line with these findings, the present study also found that baseline trait absorption was predictive of 25 mg psilocybin-induced insightfulness. The implication from the finding presented here – in line with a very recent study (Barrett et al., 2020) – is that the 25 mg psilocybin session also increased one's capacity for absorption in the long-term.

The idea of systematically increasing absorption is not new. Religious rituals and practices are designed to enhance trait levels of absorption in order to increase the capacity to have more intense religious experiences (Luhmann et al., 2010). Whereas microdosing may have a comparable effect, whereby regularly inducing subtle alterations in consciousness may slowly increase sensitivity and responsiveness to future consciousness alterations (Polito and Stevenson, 2019), the present results suggest that a single high dose of psilocybin may be sufficient to bring about this effect. Further research is required to replicate and confirm this finding.

Psilocybin increases emotional empathy, compassion and gratitude

The present study demonstrated that 25 mg psilocybin significantly increased emotional, but not cognitive, empathy for up to four weeks post-dosing in healthy participants. Relatedly, previous research has also shown that psilocybin increases emotional, but not cognitive, empathy in the acute drug state (Pokorny et al., 2017). Taken together, this implies that increased emotional empathy may persist beyond the acute phase of psilocybin effects. Furthermore, the present findings indicate that psilocybin may be useful for the treatment of mental illnesses that phenotypically display impaired empathy, such as autism, bipolar disorder, MDD and psychopathy – this could be explored in future work, which might hypothesise enduring improvements in empathy correlating with positive clinical outcomes in these populations after psilocybin therapy.

In addition to feelings of sympathy (i.e. concern for another), the experience of empathy can sometimes result in personal distress – defined as an aversive, self-focused emotional reaction to the apprehension or comprehension of another's emotional state or condition – when there is confusion between self and other. It is argued that personal distress reduces the likelihood of prosocial behaviour and is therefore detrimental to empathic concern (Decety and Lamm, 2009). Consistent with this, the present study also demonstrated significantly decreased personal distress four weeks post-25 mg psilocybin. This is not surprising given that previous studies have shown significantly decreased psychological distress and suicidal thinking after psilocybin administration (Hendricks et al., 2015). Taken together, the findings suggest that psilocybin may be useful in healthy participants as well as patient populations that are suffering with increased psychological distress. Further investigations are required to replicate and confirm these findings.

This study is the first to show significantly elevated compassion up to four weeks following 25 mg psilocybin, suggesting that positive changes in compassion sustained long-term may be attributable to the psilocybin experience. In line with this finding, previous accounts have demonstrated elevated self-compassion after the psychedelic experience (Bogenschutz et al., 2018; Sampedro et al., 2017). Increased self-compassion appears to be an important contributor to relief from depression and anxiety-related symptoms (Neff, 2003), and is now encouraged as an avenue in psychotherapy (Hofmann et al., 2011). Similarly, increased compassion may positively contribute to depression and anxiety-related symptoms and would be worth exploring in the context of psychedelics and psychotherapy.

The present results demonstrate that increased gratitude persisted up to four weeks post-25 mg psilocybin. In line with this finding, a previous study found increased gratitude six months after the administration of two high doses of psilocybin (Griffiths et al., 2018). The enduring increase in gratitude observed here is a promising result as increased gratitude has been linked to reductions in depressive behaviour via positive emotional reframing (Lambert et al., 2012). Furthermore, gratitude uniquely contributes to increased well-being and life-satisfaction (Wood et al., 2009; Emmons and McCullough, 2003). Taken together, these findings suggest that psilocybin-induced increases in gratitude may

contribute to a long-term decrease in depressive behaviour. Future work may hypothesise that after a psilocybin experience, gratitude would persist in the long-term and negatively correlate with depression scores in this population.

Psilocybin enhances psychological well-being

The present study found increased psychological well-being up to four weeks after the 25 mg psilocybin session. Moreover, the mean post-25 mg psilocybin well-being scores found here were greater than that of the UK average (Morris and Earl, 2017). In addition to psychological well-being in general, this study also observed increases in specific facets of well-being, having meaning in life, flourishing and social and nature connectedness four weeks after 25 mg psilocybin. No changes were observed following the 1 mg psilocybin experience.

In line with the present findings, previous research has demonstrated increases in well-being sustained for more than one year following a single high dose of psilocybin (Griffiths et al., 2008; MacLean et al., 2011). Meaning in life and flourishing are key facets of psychological well-being. The present study found that flourishing and the presence of meaning in life significantly increased up to four weeks post-dosing with 25 mg psilocybin. These results build on previous studies in which the psychedelic experience is rated among the most meaningful and spiritually significant experiences in people's lives (Griffiths et al., 2018; Griffiths et al., 2011; Griffiths et al., 2008). Those with higher meaning in life have been shown to possess greater self-esteem and experience less anxiety and depression (Harlow et al., 1986; Steger et al., 2006). Notably, life meaning has been implicated as a mediating factor between spirituality and well-being (Ivtzan et al., 2013; Fry, 2000). Relatedly, a previous study using psilocybin in combination with spiritual practices identified a synergistic effect of the two in enhancing purpose in life (Griffiths et al., 2018).

The present study demonstrated that 25 mg psilocybin significantly increased social and nature connectedness up to four weeks post-dosing in healthy participants. The CNS used here, although designed to assess emotional and experiential connection to nature (Mayer and Frantz, 2004), was later found to measure cognitive beliefs rather than an emotional connection (Perrin and Benassi, 2009). Taking this into account, the current study demonstrates that psilocybin sessions altered participants beliefs about their connection to nature, beliefs which have been found to correlate with environmental concern and pro-environmental behaviours (Hoot and Friedman, 2011). In line with this finding, naturalistic use of psychedelics has been associated with beliefs of oneness with the universe and greater concern for others compared with non-drug users and users of other illicit substances (Lerner and Lyvers, 2006). Psilocybin has also been found to increase nature-relatedness in the long-term in patients with treatment-resistant depression (Lyons and Carhart-Harris, 2018a). Post-psychedelic increases in nature relatedness have been found to positively correlate with concomitant increases in psychological well-being (Kettner et al., 2019). Moreover, increases in nature relatedness were found to depend on the extent of ego-dissolution and perceived influence of natural surroundings

during the acute psychedelic state (Kettner et al., 2019). Further work is required to examine how serotonergic psychedelics impact emotional response, and emotional connectedness to nature.

This study also found enhanced social connectedness up to four weeks after the 25 mg psilocybin session. Previous studies have highlighted a variety of acute and enduring prosocial effects of psychedelics (Griffiths et al., 2008; Griffiths et al., 2018; Griffiths et al., 2011), however social connectedness has not been explicitly measured as an outcome after psychedelics. Although the increase in social connectedness found here was not significant beyond two weeks, ratings remained at trend level at four weeks. Social connectedness and social functioning is impaired in a variety of mood and anxiety-related disorders, impairments which have historically been regarded as an outcome of mental illnesses (Saeri et al., 2018). However, there is a growing evidence base supporting a bi-directional relationship between social connectedness and mental health which indicate that poor social connectedness is a risk factor for many disorders (Saeri et al., 2018; Yu et al., 2015; De Silva et al., 2005).

Psilocybin reduces experiential avoidance

The present study demonstrated that 25 mg psilocybin significantly decreased experiential avoidance up to four weeks post-dosing in healthy participants. This is in line with a recent theory based on findings obtained from qualitative interviews following psilocybin-assisted psychotherapy in depressed patients (Watts et al., 2017). More specifically, this qualitative evidence suggests that psilocybin-assisted psychotherapy may be promoting sustainable transitions from experiential avoidance to acceptance (Watts et al., 2017).

Experiential avoidance has been defined as an unwillingness to engage with distressing emotions, thoughts, memories, and physical sensations, despite this avoidance creating more harm in the long-term (Hayes et al., 2012). The paradoxical result is that the more unpleasant events are avoided, the more they tend to occur, resulting in increased psychological suffering (Hayes, 2002). A wealth of evidence suggests that experiential avoidance represents one of the most prominent transdiagnostic psychological processes with a known role in a wide variety of psychological disorders. Experiential avoidance is therefore a functional process that has been linked to a wide range of psychopathology, including depressive, anxiety, and externalising disorders (Hayes et al., 1996; Chawla and Ostafin, 2007). Essentially, the concept of experiential avoidance has marked a conceptual shift toward the behavioural responses to distress (avoidance) instead of the content of the distress itself when characterising psychopathologies.

Psychedelics can open people up to both positive and negative feelings that may have been long suppressed. A high-dose psychedelic experience can be challenging, intensely emotional, shake one's worldview, or bring up painful experiences from the past (Barrett et al., 2016). Upon reflection on the present study findings, I theorise that the 25 mg psilocybin experience may have reduced experiential

avoidance via participant exposure to typically avoided stimuli, such as distressing memories, fears and/or traumatic experiences. This possibility is in line with the rationale of exposure-based treatments for various disorders, including PTSD, panic disorder and phobias (Kline et al., 2018; Barlow et al., 2000; Norton and Price, 2007; Ost, 1989).

Psilocybin facilitates psychological insight

Among experienced recreational psychedelic drug users, the facilitation of psychological insight and improved well-being are the most commonly reported benefits of psilocybin use (Carhart-Harris and Nutt, 2013). Extensively investigating the ability of psilocybin to facilitate psychological insight was a key aim of this research. To this end, the present study brought together various different assessments to grasp a measurement of insight at particular timepoints throughout the investigation. Relative to the 1 mg psilocybin integration session, the present study found that the integration session one day after the 25 mg psilocybin experience facilitated increased experiential, personal insight, and insight-induced action gains as measured by the GPI. This study also found that the 25 mg psilocybin integration session led to increased scores on the MIS. Moreover, the present findings highlight the potential for psilocybin to induce a more long-term facilitation of insight. For instance, results obtained using the PIS demonstrated increased psychological insight and subsequent insight-induced behavioural changes up to four weeks post-25 mg versus 1 mg psilocybin. Moreover, SRIS findings demonstrated an increased need for, and engagement in, self-reflection as well as increased insight up to four weeks post-25 mg versus 1 mg psilocybin.

It is well-established that insight can occur acutely in a state-like fashion during a psychedelic experience. Yet, this study has shown that insight can also be more protracted, crystallising in the integration and also over the course of a few weeks following a psychedelic experience. The present results are the first to demonstrate that 25 mg psilocybin significantly increases long-term insight up to four weeks post-dosing in healthy participants. Previous research indicates that increased insight contributes to improved well-being and overall life satisfaction (Harrington and Loffredo, 2010). Additionally, increased insight has been linked to decreases in depression and anxiety in patient populations (Silvia and Phillips, 2011; Grant et al., 2002). In line with this, our group recently found that insightfulness experienced during a psilocybin experience predicted long-lasting clinical improvements in patients with treatment-resistant depression (Carhart-Harris et al., 2017a). This indicates that insight, as a key component of psychedelic therapy, may contribute to long-term improvements in psychopathological disorders. As the present results demonstrated psilocybin-induced improvements in insight that were sustained long-term, future work may look to investigate to what extent insight (sustained after the psilocybin experience) contributes to improvements in patient populations.

Insight has been proposed as an important contributor to behavioural changes after a psychedelic experience (Garcia-Romeu et al., 2019). Consistent with this, the present study found a significant increase in self-reported positive behavioural changes immediately following and up to four weeks post-

25 mg versus 1 mg psilocybin. This suggests a rapid and enduring effect of insight on positive behavioural change. This promising result requires further investigation, such as a deeper assessment of different aspects of post-psychedelic behavioural change and its relation to insightful experiences. For example, psilocybin has been shown to decrease addictive behaviour in tobacco smokers (Johnson et al., 2014). Therefore, future work should investigate the extent of which psychedelic-induced insight contributes to behavioural changes observed in patient populations, such as a decrease in addictive behaviour.

The evaluation of insight within this study is relatively varied and extensive. Many previous psychedelic studies have reported on insight using the ASC-insightfulness sub-scale (Kometer et al., 2015; Carhart-Harris et al., 2017a; Erritzoe et al., 2018). This scale consists of three statements evaluating insight e.g. “I felt particularly profound”, “I had particularly inventive ideas” and “I had insights into the ways the world works that were mysterious to me before”. Taken together, these statements do not appear to be particularly specific to the construct of insight as it is ordinarily understood. Insight, more generally, is the procurement of a novel understanding or perspective about one’s self, behaviour or assumptions. Therefore, it is arguable that statements such as those in the ASC are not adequate to fully evaluate the phenomenon of psychological insight. It is important, therefore, to be cautious when evaluating the outcome of such studies. This is an encouragement for the need of more extensive and accurate insight scales. Hence, multiple scales were used in this study to evaluate the psychedelic-induced facilitation of insight more thoroughly. Future studies investigating insight may look to adopt this approach to gain a more extensive evaluation of this phenomenon. In line with this, one group recently employed a more extensive approach by exploring memories, beliefs and emotions related to psychological insight during the acute psychedelic state, albeit without measuring enduring effects (Carbonaro et al., 2018).

Insight predicts long-term change in well-being and experiential avoidance

A wealth of evidence suggests that experiential avoidance – defined as the tendency to rigidly escape or avoid private psychological experiences even when doing so is futile or interferes with valued actions – represents one of the most prominent transdiagnostic psychological processes with a known role in a wide variety of psychological disorders. Based on this, a key question of this research was: is experiential avoidance a psychological mechanism underlying positive psychological outcomes following a psychedelic experience? To address this question, the relationship between experiential avoidance and various well-being measures was assessed. This study showed that the change in experiential avoidance was related to the change in social connectedness, flourishing, sensed presence of meaning in life and psychological well-being overall four weeks after 25 mg psilocybin. As a key focus of this research was on the ability of facilitated psychological insight to mediate long-term positive change, these findings led to a further question: is insight a psychological mechanism underlying post-25 mg psilocybin positive change in both well-being and experiential avoidance? Furthermore, this study hypothesised that providing a supported integration session for participants to consolidate and reflect on their experience may facilitate new insights into the experience’s personal significance and

meaning. In line with the hypotheses, this study showed that the degree of psychological insight and insight-induced behavioural change, gained at the one-day post-25 mg psilocybin integration (and sustained change four weeks later), predicted the long-term change in well-being and experiential avoidance.

It is well-established that insight can occur acutely during a psychedelic experience, yet an extensive investigation of psychedelic-induced insight (i.e. acutely, post-acutely and in the long-term) has not yet been reported. This study has shown that insight may crystallise in the integration session and also over the course of a few weeks following a 25 mg psilocybin experience, and that this is associated with long-term improvements in well-being and experiential avoidance. This is in line with previous mental health research suggesting that insight contributes to improved well-being and overall life satisfaction in healthy participants (Harrington and Loffredo, 2010) and is linked to decreases in depression and anxiety in patient populations (Silvia and Phillips, 2011; Grant et al., 2002). The present findings also support and extend the notion that insight, as a key component of psychedelic therapy (and now extended to include the post-psychedelic crystallisation of insight and subsequent insight-induced behavioural change), may contribute to long-term improvements in psychopathological disorders (Carhart-Harris et al., 2017a; Noorani et al., 2018; Watts et al., 2017).

Although the occurrence of peak experience phenomena may mediate long-term outcomes observed following a psychedelic experience, the present findings highlight the importance of insight and successful psychedelic integration. Participants attended an integration session the day after each dosing session in the present study. Psychedelic integration sessions are essential to ensure psychological stability, provide an opportunity for the participant to discuss their thoughts and provide a degree of “closure” given the profundity of experiences occasioned by psilocybin and related psychedelic compounds. As with any intense or profound experience, participants often feel the need for, and benefit from, one or more sessions for discussing and consolidating novel thoughts and feelings that may have emerged under the acute drug effects. It is possible that psychedelic integration facilitates engagement with a broader repertoire of perspectives that enables the consolidation of insight, for instance into personal thought and/or behavioural patterns, thus encouraging the implementation of subsequent behavioural changes. This may contribute to the sustained improvements in experiential avoidance and well-being. Notably, it is likely that participants would be relatively more comfortable discussing their experiences with the study guides (sitters) due to their psychedelic knowledge and presence during dosing. To this end, the provision of one or more supportive integration sessions and an investigation of their subsequent outcomes is imperative for future psychedelic studies.

4.6.7. *Enduring behavioural effects*

Psilocybin enhances cognitive flexibility

Significant decreases in adjusted EDS errors (i.e. increased cognitive flexibility) were observed four weeks post-25 mg, but not 1 mg, psilocybin. Importantly however, practice effects cannot be ruled out due to the fixed order design of this study. Lowe & Rabbit (1998) reported an acceptable test-retest correlation ($R=0.7$) for the EDS phase of the task measured in four week intervals, yet test-retest reliability was only determined across two timepoints (Lowe and Rabbitt, 1998). As such, the absence of evidence of learning effects between the first and second IDED does not preclude the absence of learning effects between the second and third, independent of drug effects. Furthermore, cognitive flexibility encompasses a multidimensional set of cognitive functions (Ionescu, 2012), of which abstracted set-shifting and reversal learning may not be fully representative. With this in mind, further research incorporating additional measures of cognitive flexibility with efforts to avoid learning effects is required to interpret the reliability of the present results.

Above limitations considered, the present finding of increased cognitive flexibility four weeks after 25 mg psilocybin is consistent with the literature. Agonism at the 5-HT_{2A}R – the key site of action for psychedelic compounds – is associated with synaptic plasticity (Chen et al., 2003), enhanced associative learning (Harvey, 2003; Romano et al., 2010) and cognitive flexibility in animals (King et al., 1974). Specifically, reversal (Boulougouris et al., 2008) and extinction learning appear to be mediated by 5-HT_{2A}R activation (Boulougouris et al., 2008; Zhang and Stackman, 2015). On the other hand, 5-HT_{2A}R antagonism impairs cognitive flexibility, associative learning and working memory in animals (Boulougouris et al., 2008; Clarke et al., 2004) and in humans (Seymour et al., 2012; Wingen et al., 2007). Enhancements in constructs approximating cognitive flexibility, such as divergent creative thinking, have previously been shown following the administration of psychedelics (Kuypers et al., 2016). However, enduring post-psychedelic enhancement of cognitive flexibility has not previously been demonstrated and should be investigated further. The present study failed to find an association between the change in cognitive flexibility and long-term outcomes, suggesting a possible psilocybin-induced enhancement of a broader construct of psychobehavioural flexibility that mediates the observed psychological and neural changes and IDED performance.

If future studies can replicate and extend these results, the enduring increase in cognitive flexibility observed here is a promising finding. Impaired cognitive flexibility is associated with myriad of psychopathological disorders, including eating disorders (Tchanturia et al., 2012), obsessive-compulsive disorder (OCD; Chamberlain et al., 2006) and PTSD (Palm and Follette, 2011). Moreover, it has been postulated that impairments in cognitive flexibility may contribute to affective inflexibilities and biases associated with the development and perseverance of depression (Beck, 1976; Coles and Heimberg, 2002). To this end, it would be interesting to explore the potential mediating role of cognitive flexibility in promoting post-psychedelic clinical outcomes in patient populations.

4.6.8. Enduring brain effects

Psilocybin alters resting-state functional connectivity

Unveiling the role of interactions among distributed neuronal populations and brain areas in enabling flexible cognitive operations and complex behaviour is a central goal of neuroscience (McIntosh, 1999; Bressler and Menon, 2010). Technological advancements in neuroimaging techniques have resulted in comprehensive network maps of the anatomical connections among neural elements (Sporns et al., 2005), as well as the simultaneous recording of patterned neural activity. Representing and quantifying the structural and functional connectivity of the brain allows us make inferences about its organisational properties (Sporns, 2014). Functional connectivity – defined as statistical dependence between the time series of measured neurophysiological signals – refers to the basic concept that two locations can be said to be functionally connected if they have coherent or synchronised dynamics. Functional connectivity can be examined during the performance of tasks as well as during resting state. At rest, the brain intrinsically originates spontaneous activity that is not attributable to specific inputs or to the generation of specific output. Correlated activity at rest therefore is likely to reflect a combination of conscious activity and internal neural dynamics.

A number of brain networks have been established to have specific cognitive implications that can be replicated across independent samples (Power et al., 2011). For instance, the DMN is associated with self-reflection and mind-wandering thought (Buckner, Andrews-Hanna, & Schacter, 2008; Raichle et al., 2001) and the frontoparietal network (FPN) is associated with executive function (Power et al., 2011). To investigate possible long-term changes in resting-state functional connectivity (rsFC) induced by a psilocybin experience, this study placed seeds in the vmPFC and sgACC of the DMN (see Table 4-4). The results of the present study show increased vmPFC rsFC with regions of the FPN, as well as a relationship between this increased vmPFC-FPN coupling and the increase in social connectedness found four weeks after the 25 mg psilocybin experience. The findings of this study also show increased sgACC rsFC with the left postcentral gyrus (IPoCG) and regions of the IFPN as well as a relationship between this increased sgACC-IFPN/IPoCG coupling and the increase in well-being found four weeks after the 25 mg psilocybin experience. No rsFC changes were observed four weeks after 1 mg psilocybin.

Increased sgACC-IFPN/IPoCG rsFC correlates with increased psychological well-being four weeks after 25 mg psilocybin.

Psilocybin has been shown to effectively treat MDD (Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a) as well as induce long-term increases in psychological well-being in healthy participants (Griffiths et al., 2008, 2011; Garcia-Romeu et al., 2014). Although the present study recruited a healthy sample, the measure of well-being represents a useful proxy that may be generalisable to findings observed in patient populations. With this in mind, the relationship between the increase in sgACC-

IFPN/IPoCG coupling and well-being found here four weeks after 25 mg psilocybin is consistent with previous MDD research.

In line with the current findings, evidence suggests an important link between sgACC-IPoCG rsFC and the severity of depressive symptoms, whereby reduced coupling was associated with worse outcomes (Hirshfeld-Becker et al., 2019; McIntosh et al., 2018; Lee et al., 2018). In a recent longitudinal study, researchers investigated whether rsFC could predict the onset of MDD in children with familial risk for developing the disorder in adolescence (Hirshfeld-Becker et al., 2019). The study found weaker sgACC rsFC with the IPoCG among the children that later developed MDD compared with the children that didn't (Hirshfeld-Becker et al., 2019). Interestingly, the children that didn't develop MDD exhibited greater sgACC-IPoCG connectivity than both the MDD sufferers and the control groups – this suggests that increased sgACC-IPoCG rsFC may represent a possible resilience factor to the disorder (Hirshfeld-Becker et al., 2019). Moreover, they found high predictive accuracy in the classification between MDD-developers and non-developers using a prediction model based on baseline rsFC. In this model, sgACC-IPoCG rsFC was among the features carrying the most predictive power in the classification between MDD-developers and non-developers, potentially providing a useful neuromarker for early identification and treatment (Hirshfeld-Becker et al., 2019). Relatedly, HIV patients have been shown to present with depressive symptoms associated with reduced sgACC-IPoCG rsFC compared to control subjects (McIntosh et al., 2018) and young males suffering from internet gaming disorder (IGD) with comorbid depression also display reduced sgACC rsFC with the IPoCG than IGD subjects without comorbid depression (Lee et al., 2018).

Evidence of decreased DMN-FPN rsFC has been reported in MDD (Kaiser et al., 2015; Mulders et al., 2015) and is also in line with the present findings. For instance, reduced sgACC rsFC with the frontal pole—a key structure involved in cognitive control in affective contexts (Dichter et al., 2010)—has been associated with increased depressive symptoms (van Duinkerken et al., 2016). In a study examining risk and resilience in maltreated children, greater Orthodenticle homeobox 2 (*OTX2*) methylation and lower depression scores were both associated with increased rsFC vmPFC connectivity with the frontal pole (Kaufman et al., 2018). This is interesting given that the transient downregulation of the *OTX2* gene was recently associated with the development of depressive-like behaviours in a mouse model of early life stress (Pena et al., 2017). Reduced sgACC rsFC with the angular gyrus has also been associated with increased depressive symptoms (Strikwerda-Brown et al., 2015). The left angular gyrus is a brain hub that processes information by connecting distinct, functional specialised regions, including the amygdala, vmPFC, sgACC and other areas related to emotion regulation (Achard et al., 2006). In addition to its role in many cognitive tasks (Cabeza et al., 2008; Reineberg et al., 2015; Staresina and Davachi, 2006), many studies have shown that abnormalities of the left angular gyrus are associated with depression. For example, left angular gyrus volume has been associated with suicide attempts in MDD (Lee et al., 2016) and functional connectivity strength of left angular gyrus is lower in MDD patients relative to healthy controls (Lai et al., 2017).

Taken together, as the increase in well-being found here in healthy participants may be regarded as synonymous with (or a proxy of) a decrease in depressive-like symptoms in patients, the above MDD research lends some support to the present results. To this end, a long-term functional imaging follow-up in a depressed population following psilocybin treatment would be interesting to explore in future research. Additionally, the explorative nature of this study warrants further research to replicate these results in healthy participants.

Increased vmPFC-FPN rsFC correlates with increased social connectedness four weeks after 25 mg psilocybin.

The present study observed that the increase in vmPFC rsFC with FPN regions (IFG and frontal pole) was associated with the increase in social connectedness four weeks after 25 mg psilocybin. This is consistent with what we know about these brain regions with regards to social cognition. The DMN has been found to be involved in various domains of social processing, including the vmPFC which has been shown to play a key role in the social understanding of others (Li et al., 2014). Moreover, connectivity between the vmPFC and various emotion regions are mainly associated with emotion engagement during social interactions (Li et al., 2014).

A growing body of evidence indicates that both the IFG and the vmPFC are involved in emotional and cognitive empathy, respectively (Shamay-Tsoory et al., 2009). Although the IFG has been clearly related to social cognition, it is not a region which is typically associated with the emotional aspects. Importantly, the IFG is key part of the human ‘mirror neuron system’ (Kilner et al., 2009). The mirror neuron system is involved in detecting the intentions of actors while watching their actions and is thus a building block for empathy (Decety and Jackson, 2004; Preston and de Waal, 2002; Gallese and Goldman, 1998). The IFG has been implicated in emotion recognition tasks, such as identification of emotional intonation (Wildgruber et al., 2005) and judgement of facial expressions (Kesler-West et al., 2001). Furthermore, IFG damage has been shown to result in deficits in emotion recognition from faces and from prosody (Shamay-Tsoory et al., 2009). This makes sense when we consider that experiencing emotions may encourage and motivate imitation, and therefore depends on an intact mirror neuron system. In summary, there is strong support for the IFG and vmPFC representing two behavioural systems for understanding others—i.e. an early social-emotional matching/mirroring system involving the IFG and a more advanced system for cognitive understanding of mental states, involving the vmPFC.

Emotion-regulation disorders, such as social-anxiety disorder and psychopathy, often result in social isolation and diminished occupational opportunities for those suffering from them. It is clear that the regulation of emotional behaviour is essential for human social interactions and successful participation in society (Hare, 2017). Yet, regulating social-emotional actions is cognitively complex as it requires the effectiveness of ongoing behaviour to be successfully assessed and compared with alternative action strategies. The frontal pole has been associated with many cognitive functions, such as relational

reasoning (Hartogsveld et al., 2018; Vendetti and Bunge, 2014), counterfactual choice (Mansouri et al., 2017; Boorman et al., 2009) and exploration behaviour (Zajkowski et al., 2017; Daw et al., 2006). The frontal pole is involved in the most complex goal-oriented events, including long-term goals and integration of multistage event complexes, which is likely why it is so heavily involved in complex moral judgments (Berthoz et al., 2002; Moll and de Oliveira-Souza, 2007; Moll et al., 2002; Moll et al., 2005).

It is well established that serotonin plays an important role in regulating social and emotional processes (Canli and Lesch, 2007). Research has shown that the risk of developing social psychopathologies is increased in short-allele carriers of the serotonin transporter gene (Caspi et al., 2010). Intriguingly, effective connectivity studies have shown that this increased vulnerability to social disorders in short-allele carriers is due to decreased top-down control from the frontal pole over emotional processing (Volman et al., 2013). It is now widely accepted that the frontal pole coordinates cognitive and affective information in order to initiate cognitive control over social-emotional behaviour (Bramson et al., 2018; Tyborowska et al., 2016; Volman et al., 2011; Volman et al., 2013). However, a recent study extended this knowledge by showing that frontal pole contribution to social-emotional control arises specifically from the lateral frontal pole, which is able to influence other brain structures via its connections to both medial and lateral circuits with parietal, cingulate, and temporal regions (Bramson et al., 2019).

A recent study using Human Connectome Project data from a large dataset of healthy participants found that greater self-control was associated with stronger vmPFC-frontal pole rsFC (Cona et al., 2019). Stronger self-control was found to be associated with positive and desirable qualities, such as increased intelligence, agreeableness and openness as well as decreased antisocial behaviours. On the other hand, weaker self-control was associated with undesirable qualities, such as increased aggression, antisocial behaviour, social withdrawal and anxiety, as well as reported feelings of social rejection. As such, they found that increased vmPFC-frontal pole rsFC was associated with greater self-control, and greater self-control was associated with desirable qualities related to sociability (Cona et al., 2019). Although Cona et al (2019) didn't attempt to find associations between variables beyond self-control and vmPFC-frontal pole rsFC, their findings are interesting considering the association between vmPFC-frontal pole rsFC and social connectedness found here in the present study.

Consistent with the present findings, previous studies have highlighted a variety of acute and enduring prosocial effects of psychedelics (Griffiths et al., 2008; Griffiths et al., 2018; Griffiths et al., 2011). For instance, psilocybin has been shown to alter social rejection processing by reducing feelings of social exclusion, whereby the neural response to social exclusion correlated with psilocybin-induced alterations in self-processing (Preller et al., 2016). This is in line with a study showing that psychedelic-induced alterations in self-processing and social cognition are intertwined (Preller et al., 2018). Moreover, LSD has been shown to increase adaptation to opinions expressed by a norm group (Preller et al., 2019b). Interestingly, Preller et al (2019) showed that increases in BOLD signal in the mPFC were associated with altered social feedback processing (Preller et al., 2019a). In a recent study by our group, TRD patients reported that they experienced a distressing sense of disconnection from others

prior to psilocybin-assisted treatment (Watts et al., 2017). After psilocybin treatment, many patients reported to be able to “re-connect” with family members, friends, strangers, and even people who had wronged them (Watts et al., 2017). Interestingly, TRD patients reported that this increase in social connectedness was a major factor contributing to their treatment response (Watts et al., 2017); this supports the idea that the influence of psychedelics on social cognition and behaviour may be an important mechanism underlying their clinical efficacy. Taken together, the above evidence suggests an important link between vmPFC-FPN rsFC and social-emotional processes and is consistent with the relationship between the increase in social connectedness and vmPFC-FPN rsFC found here four weeks after 25 mg psilocybin.

Psilocybin alters amygdala responsivity to the presentation of emotionally-expressive faces

This study found a decrease in left amygdala response to fearful faces four weeks after 25 mg psilocybin. Interestingly, the decrease in left amygdala response to fearful faces was associated with the change in well-being and social connectedness four weeks after 25 mg psilocybin. On the other hand, this study unexpectedly found an increase in bilateral amygdala response to happy faces four weeks after the 1 mg psilocybin session. As 1 mg psilocybin failed to induce significant acute or long-term effects in all other measures of this study, the validity of this finding is questionable and requires further investigation.

The amygdala is a complex subcortical structure located in the medial portion of the temporal lobe and is involved in multiple tasks, including the generation of emotional behaviour, formation of emotional memories related to fear and anxiety and modulation of the consolidation of explicit memories for emotionally arousing events (Aggleton, 2000; Whalen and Phelps, 2009). Notably, 5-HT_{2A}Rs are densely expressed in the amygdala (Pralong et al., 2002) and play a crucial role in regulating the activity of amygdalar microcircuits and projections (Pralong et al., 2002; Bombardi, 2014). 5-HT₂Rs in the amygdala play an important neuromodulatory role in fear and stress responses (see Bombardi, 2014: for a review), suggesting dysregulation of amygdalar 5-HT₂R signalling may result in psychopathology. Previous research demonstrates the involvement of the amygdala in psychedelic drug action. For instance, psychedelics have previously been shown to modulate the excitability of amygdalar pyramidal cells and GABAergic interneurons through their 5-HT_{2A}R-mediated mechanism of action (Jiang et al., 2009; Rainnie, 1999; Sokal et al., 2005; Stein et al., 2000). Moreover, a previous study found decreased right amygdala responses to negative emotional stimuli during the acute psilocybin experience in healthy participants, which was found to be associated with psilocybin-induced increases in positive mood state (Kraehenmann et al., 2015). Nonetheless, it is important to consider that observations of decreased amygdala response during the acute phase of the psilocybin drug effects (Kraehenmann et al., 2015) might be better explained by participants’ potential lack of engagement with the task and its stimuli. Taken together, it is clear that psychedelics may have the capacity to alter amygdala functioning which might be relevant to the treatment of disorders characterised by dysfunction in this brain structure.

Decreased left amygdala response correlates with increased well-being and social connectedness four weeks after 25 mg psilocybin.

In line with the present findings, a recent study found decreased negative affect and amygdala responsiveness to emotional faces one week after a psilocybin (25 mg/70 kg) experience in healthy participants (Barrett et al., 2020). However, in contrast to the findings presented here, Barrett et al (2020) found a return to baseline levels in amygdala response four weeks post-psilocybin. As such, the present study may be regarded as the first to demonstrate long-term¹⁷ changes in amygdala responsivity to facial affect stimuli and associated psychological outcomes (i.e. four weeks post-dosing).

In line with the association between decreased left amygdala response to fearful faces and increased social connectedness found here, the amygdala and several of its strongly connected targets, particularly the vmPFC, are consistently implicated in social cognition (Frith, 2007). As such, the amygdala is considered to be a hub of the “social brain” based on the distributed topography of its anatomical connections to regions involved in social processing (Freese and Amaral, 2009). Patients with amygdalar damage tend to make less eye-contact (Spezio et al., 2007), are insensitive to personal boundaries (Kennedy et al., 2009), have difficulty spontaneously orienting to (Adolphs et al., 2005) and interpreting signals from the eye region of others’ faces (Kennedy and Adolphs, 2010) and do not exhibit the normal fMRI signal response in the fusiform gyrus and superior temporal sulcus to facial expressions – whereby the magnitude of reduced fMRI signal in these regions compared to controls correlates with the degree of amygdalar damage (Vuilleumier et al., 2004). Moreover, amygdala-damaged patients also exhibit markedly diminished social apprehension, which is a deficit in motivated interpersonal behaviour (Adolphs et al., 1995; Bechara et al., 1999).

The amygdala is also known to be sensitive to emotional salience, regardless of the emotional valence of stimuli (Adolphs, 2010; Santos et al., 2011). As well as being involved in psychedelic drug action (e.g. Bernasconi et al., 2014; Mertens et al., 2020; Kraehenmann et al., 2015; Spain et al., 2015), the amygdala has previously been implicated in the pathophysiology of depression (Drevets et al., 1992) and plays a role in the action of certain antidepressant medications (Ma, 2015). In particular, decreased amygdala responsivity has been observed after effective treatment with conventional antidepressants, namely selective serotonin reuptake inhibitors (SSRIs; Ma, 2015). As such, decreased amygdala response to negative emotional stimuli under SSRIs has been suggested to be a key component of their therapeutic action (Harmer et al., 2017). This is in line with the present study’s findings of an association between decreased left amygdala responsiveness to fearful faces and increased well-being and social connectedness four weeks after 25 mg psilocybin. It is important to highlight a significant discrepancy between the present study’s findings and previous results from our group, which observed increased right amygdala responsiveness to fearful faces post-actively in TRD patients (Roseman et

¹⁷ Barrett et al (2020) found sustained (i.e., one week) decreases in amygdala responsiveness to emotional faces after a psilocybin (25 mg/70 kg) experience, yet this effect returned to baseline levels four weeks post-dosing. One week post-dosing may not be regarded as sufficiently long enough to constitute interpreting these findings as long-term changes. Hence, the present study might be regarded as the first to demonstrate a psilocybin-induced change in amygdala responsivity to facial affect stimuli that is truly sustained in the long-term.

al., 2018a). The reasons for this discrepancy are unclear, yet consistent with the ability of LSD to induce various opposing acute and longer-term effects (Carhart-Harris et al., 2016b). It's possible that this discrepancy is merely a function of the different populations studied (i.e. depressed patients versus healthy volunteers). Alternatively, the increase in amygdala responsiveness found post-acutely (Roseman et al., 2018a) might reflect a transient alteration in serotonergic signalling due to 5-HT_{2A}R receptor internalisation – to this end, if long-term scanning was completed in the depression study, amygdala responsiveness may have also subsequently decreased one week post-dosing in the depressed patients, as in the healthy volunteers study by Barrett et al. (2020). It should also be noted that the increased amygdala reactivity toward fearful faces observed by Roseman et al (2018) was measured prior to any psychological integration work, thus supporting the need for future studies to determine long-term amygdala effects of psilocybin and clinical relevance. Future work should aim to address these discrepancies. To this end, an investigation of the long-term effects of psilocybin on brain function in different populations – with a particular focus on scanning at different post-treatment phases – would be interesting to explore in this context.

4.6.9. *Predicting long-term outcomes*

Quality of the psilocybin experience predicts long-term change in resting-state functional connectivity

The importance of acute psychedelic experiential components in determining long-term psychological outcomes is well-established, yet their ability to predict long-term brain effects has not been studied to date. Accordingly, this study aimed to address this knowledge gap by assessing the predictive potential of peak experience phenomena in determining enduring changes in resting-state functional connectivity. This study found that the occurrence and magnitude of 25 mg psilocybin-induced peak experience phenomena, measured via four OBN sub-dimensions (i.e. *insightfulness*, *experience of unity*, *spiritual experience*, and *disembodiment*) predicted changes in resting-state functional connectivity four weeks later. The present findings are consistent with a basic principle of the psychedelic treatment model, which posits that the quality of the acute experience mediates long-term outcomes.

Psychedelic peak experiences have been reported as among the most meaningful events experienced in one's life (Griffiths et al., 2018). A wealth of evidence suggests that psychedelic peak experiences may mediate some of the enduring positive psychological effects observed following psychedelic use in both healthy and patient populations (see Barrett and Griffiths, 2017: for a review). As such, psychedelic peak experiences have been likened to “*quantum change experiences*” (Griffiths et al., 2018), which are described as sudden and often profoundly meaningful experiences that are said to result in dramatic and enduring behavioural, emotional and cognitive changes (Miller, 2004). In line with this concept, recent research by our group found that greater psilocybin-induced OBN predicted positive long-term clinical outcomes in a sample of TRD patients (Roseman et al., 2018b). Likewise, previous studies have shown that psychedelic-induced peak experiences measured via the MEQ are predictive

of positive long-term outcomes, including increased well-being (Griffiths et al., 2016; MacLean et al., 2011; Ross et al., 2016; Bogenschutz et al., 2015; Haijen et al., 2018).

Given the ability of psychedelic peak experience phenomena to predict subsequent long-term psychological changes, the proposition that psychedelic peak experience phenomena may predict long-term brain changes is not unlikely. Notably, this study found that the quality of the acute 25 mg psilocybin experience predicted neural changes four weeks later. This is the first study to demonstrate the importance of peak experience phenomena in determining long-term neural outcomes after a psychedelic experience, and substantially extends previous research demonstrating the ability of psychedelic peak experience phenomena to predict subsequent long-term psychological changes, including increased well-being. Relatedly, this study found that the change in sgACC rsFC was positively associated with the change in well-being four weeks after the 25 mg psilocybin experience. As psychedelic peak experience phenomena have been linked with sustained changes in well-being, the finding here that *insightfulness* and *experience of unity* scores predicted the change in sgACC rsFC is notable. A basic principle of the psychedelic treatment model is that the treatment's efficacy critically depends on the occurrence of a profound psychological experience. In line with this principle, the present findings suggest that, in addition to the psychological effects, the enduring brain effects of psilocybin may be *experience dependent* and not a simple product of isolated pharmacological action. As such, the importance of promoting a greater quality psychedelic experience deserves appropriate attention in future investigations, as well as the predictive potential of such experiential components in determining long-term brain effects of psychedelics.

4.6.10. Methodological strengths and limitations

This study had many key advantages, including multimodal neuroimaging and the extensive measurement of a broad repertoire of psychological outcomes not previously investigated in psychedelic research, both acutely and in the long term. To highlight the multifaceted approach taken by this study, the various complimentary techniques included the following: (i) an extensive battery of questionnaires provided a subjective assessment of the acute and long-term effects of psilocybin on human cognition, emotions and behaviour; (ii) computer-based tasks (i.e. IDED) provided an objective assessment of enduring post-psilocybin effects on cognitive flexibility; (iii) EEG was used to directly measure neural activity during the acute drug state and enabled an objective view of the psychedelic brain; (iv) fMRI was used to measure enduring neural effects one month post-dosing and enabled an objective view of sustained brain effects (i.e. resting-state functional connectivity and amygdala responsivity to facial affect stimuli) after a psychedelic experience. To this end, the analytical techniques employed in this study have helped to improve understanding of a wide range of acute and long-term psychological and neurological effects of psilocybin in healthy participants. A further advantage concerns the within-subjects design of this study, which minimises random noise due to individual variability and compensates for the reduced sample size due to increased statistical power compared with between-subjects designs.

Study design considerations

A key limitation of the present study was the sample size, which was influenced by various factors, such as: (i) recruitment, (ii) assessment compliance, (iii) availability of psilocybin, and (iv) costs and budgeting. Sample size was reduced further in the context of the acute EEG recordings; this was due to a few participants not complying with the recordings during the high dose (25 mg psilocybin) session as well as EEG artefacts in the data obtained. In specific cases, post-EEG recording VAS ratings were not completed by all of the participants. For instance, some participants were unable to complete the VAS due to the profound effects of psilocybin during the peak intensity of the experience, lowering the number of results. To compensate for this, other approaches could include 'experience sampling' – a technique that cues the user to talk about their experience at distinct time intervals (Csikszentmihalyi and Larson, 1987). This could be a useful way to assess acute insightful experiences as it would enable distinct timings of the 'insightful' event to be correlated with EEG measures, such as brain complexity. With regards to data obtained, three participants had to be excluded from analysis due to excessive artefacts. Reasons for this and possibilities for how to overcome this problem will be discussed later in this section. Finally, three participants withdrew for reasons unrelated to the study. In future, the use of reserve participants could be implemented up until the first dose of psilocybin to compensate for this.

The present study had a single-blind, fixed order, within-subjects study design. All participants received two doses of psilocybin four weeks apart. To blind the subjects, participants were informed that they would receive up to 25 mg of psilocybin in each dosing session, however no further information regarding dosage was provided. In reality and known to the researchers, all participants received 1 mg psilocybin – considered to be a subperceptual/subthreshold dose (Madsen et al., 2019) – on the first dosing day and 25 mg psilocybin – considered to be a fully active high dose – on the second dosing day. As such, the subthreshold dose of 1 mg psilocybin acted in place of a 'placebo' in that it would not occasion a full psychedelic experience yet would control for participant expectation.

Although this design has the advantage of controlling for participant expectation, there are limitations to consider. This study chose 1 mg psilocybin as a control because it is suggested to be pharmacologically 'inert', thus producing effects via expectancy alone similar to a non-active placebo. Positive expectation is the major psychological factor underlying the 'placebo' effect, so the merit of using 1 mg psilocybin as a control is that participants can be briefed to expect psilocybin in both dosing sessions. Participants were encouraged to expect and prepare for up to 25 mg psilocybin, thus standardising preparation and expectations prior to each dosing session. Psilocybin is expected to induce subjectively detectable sympathomimetic effects between approximately 3-5 mg, depending on the individual (Hasler et al., 1997). As such, it was hypothesised that 1 mg psilocybin would not induce psychological or brain effects, either acutely or in the long-term. Although the present results are consistent with this, the effects of 1 mg psilocybin have not been studied to date and it is possible that this dose is sufficient to produce effects in measures unaccounted for in this study. Future research may wish to use an active placebo, such as methylphenidate; however, it should be noted that the

blinding of participants is particularly difficult in psychedelic research due to the characteristic and profound effects of these drugs on perception and cognition. With regards to the fixed-order design of this study, balancing the order of 25 mg and 1 mg psilocybin sessions would be one method to control for potential order effects. However, this was not possible in the current design as 25 mg psilocybin was hypothesised to induce long-term neural and psychological outcomes. As such, post-25 mg psilocybin long-term outcomes would confound post-1 mg psilocybin data if 25 mg was administered first, thus contradicting the methodological reasons for employing a fixed-order design.

Psilocybin and related psychedelic compounds produce a wide range of responses to identical milligram per kilogram (mg/kg) dosing. This is the case between individuals, but also within individuals across various psychedelic experiences. As such, fixed dosing regimens are the norm in the context of psychedelic drug research. This is supported by a recent report of psilocybin subjective effects and clearance, whereby a fixed dose of 25 mg psilocybin was found to approximate circulating levels of psilocin following a weight-adjusted dose of 0.3 mg/kg (Brown et al., 2017). As the present study used a fixed dose of 25 mg psilocybin, this eliminated the need for participant-specific compounding which may be viewed as a further methodological advantage. Nonetheless, despite support for fixed dosing designs, future research may wish to consider weight-based dosing. However, future weight-based studies should consider that this dosing regimen may be unnecessary (e.g. Brown et al., 2017) and dramatically complicates standardisation and validation of the compound and its distribution, particularly for a scheduled drug such as psilocybin.

Participants were instructed to treat each dosing day equally as a kind of “music meditation”, which has the advantage of reducing further variability beyond drug dosage between the dosing sessions (i.e. varying levels of talking, engaging in other activities, interactions with the “sitters” etc.). Further, as outlined in the guidelines for safety (Johnson et al., 2008), it is imperative that study guides (sitters) provide psychological support if required in the context of psychedelic research. Notably, high doses typically occasion more intense, profound and potentially challenging experiences, and therefore a greater need for psychological support provision, than low doses. With this in mind, another limitation of this study concerns its lack of ability to differentiate between the psilocybin drug effects and contextual factors (i.e. the effects of psychological support and music and/or music meditation. Nevertheless, reducing participant comfort and neglecting their needs for support may be deemed unethical and may carry further limitations that outweigh the benefits. To this end, great consideration was given to the potential merits and flaws of each decision made in each step of this study’s design process, with considerable efforts to determine reasonable trade-offs in the approach.

Psychometric considerations

A key advantage of this study includes the extensive assessment of a broad repertoire of psychological outcomes, most of which were measured using validated scales. Moreover, self-constructed measures were developed to compensate for the few instances where validated measures were unavailable or

deemed insufficient. Yet, although self-constructed measures used in this study are currently undergoing validation processes, a further limitation arises from the fact that they remain unvalidated at the time of writing this thesis. Moreover, the majority of psychological outcomes were based on subjective questionnaires. This poses another limitation, as self-reported questionnaires are problematic due to the influence of responder bias. To this end, further work is required to objectively measure these constructs and their associated behavioural changes. This study also used only one objective measure of cognitive flexibility, the IDEED task, which specifically measures abstracted set-shifting and reversal learning. Cognitive flexibility encompasses a multidimensional set of cognitive functions (Ionescu, 2012), of which abstracted set-shifting and reversal learning may not be fully representative. With this in mind, future studies should consider incorporating additional measures of cognitive flexibility. Interpretation of the strength of correlations found here should consider the *a priori* power calculation, which indicated a detectable effect size of 0.56 with a desired power of 80% at the 5% significance level. Further, although the use of change scores can be advantageous and were deemed most appropriate for the regression analyses, the role of regression to the mean must also be considered when interpreting the correlations found here (e.g. see Allison, 1990).

Neurometric and imaging considerations

The present study utilised a wireless EEG cap with 21 electrodes. This limited the volume of available data compared to high density EEG cap alternatives. During analysis, EEG channel removal, ICA and artefact removal decreased this volume further. In some cases, over five ICAs were removed due to excessive artefacts. Furthermore, up to two channels were removed as excessive artefacts rendered them unusable. Increased artefacts during the psilocybin experience were most likely due to increased perspiration and jaw clenching, a problem that has been noted in previous psychedelic studies (e.g. Muthukumaraswamy et al., 2013). To this end, the high dose (25 mg psilocybin) was considerably more affected than the low dose (1 mg psilocybin) recordings. This may be unavoidable in some cases, but steps can be taken prior to EEG recording to ensure cleaner EEG data. For example, high density EEG electrode analysis could be performed, but in the case of this study, it was more desirable to use the wireless EEG cap and increase participant comfort.

There are some important factors to consider when measuring LZc. LZc increases with age in healthy participants (Fernandez et al., 2011). Furthermore, clinical populations demonstrate differences in LZc trends with age. For example, schizophrenic patients demonstrate an opposing trend to the healthy population (Fernandez et al., 2011). This shows that caution must be taken when comparing LZc measurements between different ages and clinical populations. It should be noted, however, that all participants were healthy and the age range was relatively narrow in this study. Nonetheless, future research should look to account for age by introducing age as a covariate during analysis – due to a small number of participants, this analytical technique was not viable in the present study. The LZc algorithm takes into account binary sequences during a given timeframe. If there was little diversity in binary code during the start of the EEG recording but large diversity at the end, this would not be evident

using LZc. Although unlikely, this is one limitation of LZc. To avoid this, LZc could be calculated for multiple, smaller timeframes revealing how complexity changes over time. It is possible to calculate LZc using a length of binary code of 1000 or greater (Amigo et al., 2004), something that is achievable with 4 seconds of EEG data in this case. Future work may look to determine changes in LZc over time during the psychedelic experience in a greater powered study. Furthermore, this may aid in pinpointing psychological correlates to particular timepoints. Finally, LZc has previously been attributed to the level of conscious experience (Casali et al., 2013; Schartner et al., 2015), but is it possible to categorise human consciousness into one number? Given the broad repertoire of experiences in the psychedelic state, the idea of a number to represent conscious experience seems basic. The ability of psychedelics to differentially affect various aspects of consciousness does not support a unidimensional conception. Rather, the effects of psychedelics on consciousness strongly support a multidimensional conception of conscious states instead (see Bayne and Carter, 2018). Accordingly, others have categorised LZc as a reflection of the ‘richness’ of conscious experience (Carhart-Harris, 2018). Taking a multivariate analysis approach of EEG (e.g. LZc and spectral analysis) may also help to extend the findings present here. Consistently, psilocybin induces widespread reduction in alpha oscillatory power (Thatcher et al., 1971; Komater et al., 2015), with patterns different to that of LZc. Moreover, local changes in delta oscillations have been linked to increases in insightfulness during the psychedelic experience (Komater et al., 2015). Combining different analytical methods, such as LZc and spectral analysis, could give a more extensive evaluation of the neurobiological effects during the psilocybin experience.

Additional limitations arise from the use of BOLD-based fMRI measures, which indirectly measure brain activity. More specifically, the dynamics of the BOLD response are dependent on changes in cerebral blood flow, cerebral blood volume and the cerebral metabolic rate of oxygen consumption (Ogawa et al., 1990). There are several limitations of using fMRI to study changes in brain function, including poor temporal resolution. Changes in blood flow show a slow peak after approximately five seconds from the initial increase (referred to as the haemodynamic response function; HRF), thereby significantly limiting the temporal resolution of the BOLD signal. BOLD-signal changes are also non-absolute and susceptible to a significant amount of noise, such as motion artefacts for example. Further, although fMRI measures have relatively better spatial resolution than other neuroimaging modalities, BOLD-signal measured per 3 mm voxel still represents the mixed activity of approximately one million neurons (Arthurs and Boniface, 2002; Logothetis, 2008; Logothetis and Wandell, 2004). Other limitations of fMRI include potential confounders of the data, such as the participants experiencing MRI-induced anxiety (e.g. claustrophobia in the scanner), other mental states (e.g. excitement, frustration, boredom) and falling asleep (especially during resting-state scans). Further limitations arise when investigating network connectivity due to variability in the literature regarding brain networks definitions and the regions within them. It is also important to consider the validity of the fMRI results found here, as fMRI analyses can result in considerably high rates of false positives (Eklund et al., 2016) and commonly used task-fMRI measures (including the emotional faces paradigm used here) lack sufficient test-retest reliability (Elliott et al., 2020). As such, these considerations further highlight the importance of

replicating the current findings in future work as well as the need for more reliable and validated statistical methods in neuroimaging research.

Although fMRI was not performed acutely in this study, it is important to highlight an additional limitation that concerns BOLD-based neuroimaging studies of the acute psychedelic state. As mentioned in the previous section, neuroimaging measures such as BOLD-based fMRI indirectly measure brain activity via blood flow parameters and might therefore partially be confounded by local and/or cardiovascular bottom-up effects (e.g., van't Ent et al., 2014; de Munck et al., 2008). Although any transient changes in receptor dynamics or other residual pharmacological effects related to psilocybin administration would be resolved before the one month post-dosing scans performed here, 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action deserves particular experimental scrutiny, especially in the context of acute and post-acute neuroimaging studies. To enable correct interpretation of neuronal activity by vascular signals, future research needs to deepen our understanding of the basic mechanisms of neurovascular coupling and the specific characteristics of disturbed neurovascular coupling in the psychedelic state. To this end, the next chapter attempts to address this knowledge gap by investigating the 5-HT_{2A}-mediated regulation of systemic circulation using the most 5-HT_{2A}-selective psychedelic yet discovered, 25CN-NBOH.

4.6.11. Conclusions

This study used a multifaceted approach to comprehensively study the psychological and neurological effects of psilocybin in healthy participants. Notably, the results provide evidence for persistent brain effects – which are predicted by psychedelic peak experience phenomena – that correlate with positive long-term changes in prosociality and overall well-being after a high-dose psilocybin experience. The results identified psychological insight – both acutely and as it crystallises in the days to weeks following a high-dose psilocybin experience – as a potential mediator of mental health outcomes based on its ability to promote positive long-term changes in well-being and experiential avoidance. Critically, this study also provides evidence for a direct association between a specific neuronal mechanism (i.e. signal complexity of spontaneous brain activity) and insightfulness experienced during the peak intensity of a high-dose psilocybin experience. Taken together, the results of this study provide support for the recently developed REBUS model of psychedelic drug action. The REBUS model posits that an entropic brain with relaxed high-level priors may facilitate curiosity and BMR processes that encourage the attainment of psychological insight and novel perspectives, which may ultimately mediate long-term outcomes.

In addition to the core conclusions presented above, a few crucial points require consideration. This study highlights the importance of developing a strong therapeutic alliance in psychedelic research and suggests that, in addition to the therapeutic effects, enduring brain effects of psilocybin may be experience-dependent. This emphasises the importance of contextual and experiential components of psychedelic administration and highlights the need for future studies to take this on board. The results

also stress the importance of providing a supported integration session in future psychedelic studies, which facilitates further insight and positive changes in the long-term.

To conclude, this study offers a comprehensive account on the acute and long-term effects of psilocybin on the brain and psychology of healthy participants. The results support and extend the notion that insight, as a key component of psychedelic therapy, may contribute to long-term improvements in psychopathological disorders and mental health in general. This study also demonstrates a neural mechanism underlying the acute experience of insight during a high-dose psilocybin experience, as well as persistent neural changes that correlate with well-being outcomes long after that experience. To this end, these novel results considerably broaden the field's knowledge of the acute and long-term psychological and neurological effects of psilocybin and provide inspiration for future work.

5. STUDY 3 |

Can we untangle neuro- and haemodynamic mechanisms in the psychedelic brain state?

“Follow your heart... but take your brain with you!”

– Alfred Adler

5.1. FOREWORD

The previous chapter presented acute and long-term effects of psilocybin on the brain and psychology of healthy human participants. The findings highlight the importance of contextual factors (i.e., set and setting) in determining the response to a psychedelic compound. The quality of the acute psilocybin experience and the integration of that experience influenced post-psilocybin long-term outcomes. With regards to the brain measures, 25 mg psilocybin induced increased brain signal complexity during the “peak” of the acute drug effects and this was found to correlate with the degree of insightfulness experienced during the recording. Enduring changes in amygdala responsivity to emotional faces expressing fear and resting-state functional connectivity were also observed, and these were found to correlate with positive long-term changes in psychological well-being and social-connectedness.

The evidence presented in this thesis thus far suggests that psychedelics have therapeutic potential, yet very little is known about how they work in the brain mechanistically to give rise to their profound effects. It should be noted that our understanding of the general neurophysiology of (cortical) 5-HT_{2A}Rs – the key site of action for psychedelic compounds – is limited by the lack of highly selective 5-HT_{2A}R agonists. Moreover, our knowledge of the neurophysiological correlates of the human psychedelic brain state largely stems from BOLD-fMRI based research, which indirectly measures neuronal activity by extraction of blood-flow related parameters. BOLD-fMRI based brain functioning studies, thought to mainly reflect neuronal activity, might therefore partially be confounded by local and/or cardiovascular bottom-up effects (e.g., van’t Ent et al., 2014; de Munck et al., 2008). Although there is general effort to control for these complex interactions (Müller et al., 2018; Carhart-Harris et al., 2012a) it appears essential to learn how 5-HT_{2A}R activation influences the brain’s supply of blood when disentangling neuro- and haemodynamic mechanisms involved in the psychedelic brain state. Cardiovascular 5-HT_{2(A)} receptors have been subject to extensive research (see Kermorgant et al., 2018; McCall and Clement, 1994; Nagatomo et al., 2004; Ramage, 2001), yet understanding of how they regulate systemic circulation has largely been occluded by the unavailability of selective agonists. As such, 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action deserves particular experimental scrutiny considering the use of BOLD-fMRI in human studies. Furthermore, unveiling the effects of psychedelics with cell-type and circuit specificity might provide insight into the mechanisms underlying the therapeutic effects of psychedelics as well as many psychiatric disorders. A cell-type specific and circuit-centric investigation of the effects of psychedelics on brain activity holds exciting opportunities and has never been performed.

In this chapter, I present an examination of the suitability of 25CN-NBOH – a novel psychedelic that is the most selective agonist ligand for the 5-HT_{2A}R yet discovered – in mice for prospective *in vivo* optical imaging studies. Using the head twitch response (HTR) as a behavioural readout of central 5-HT_{2A} activity, the following factors are determined: (i) the dose range of 25CN-NBOH required for parenteral administration to induce central 5-HT_{2A} activation, (ii) the time-course of the central action, (iii) sensitivity to 5-HT_{2A} antagonism and experimental familiarity, (iv) a possible involvement of 5-HT_{2C} receptors in

25CN-NBOH induced HTRs, and (v) potential development of tachyphylaxis and tolerance. To address the question of how psychedelic-5-HT_{2A} interactions translate into the brain (haemo-)dynamics characteristic for psychedelia, I examine the effects of 25CN-NBOH on heart rate, blood flow, blood oxygenation, and respiratory rate via non-invasive pulse oximetry drawn from the animals' brain-imminent neck arteries. Since 5-HT_{2A}-related vasoconstriction is thought to be a main effector site of serotonergic thermoregulation (e.g. Ootsuka et al., 2004), I investigate the cardiovascular effects of 25CN-NBOH by varying the temperature of a heat-pad.

5.2. INTRODUCTION

Psychedelics have a history of use in psychotherapy and research shows that psychedelics can have long-lasting benefits on the psychology of healthy individuals as well as patient populations (see Carhart-Harris and Goodwin, 2017; dos Santos et al., 2016; Rucker et al., 2018). Although these compounds have great therapeutic potential, very little is known about how they work in the brain mechanistically to give rise to their intriguing effects. To this end, elucidating the acute and potentially enduring effects of psychedelics on specific cell-types and neural circuitry in rodents may help to explain the effects seen on the psychology and brain activity of human participants. Moreover, understanding the neural mechanisms underlying psychedelic drug action and any possible enduring effects that these drugs have may provide insight into the mechanisms underlying many psychiatric disorders, thus helping to elucidate novel targets for therapeutic intervention.

5.2.1. *Psychedelic-induced head-twitch behaviour*

Modelling psychedelic-induced neuropsychological effects in rodents remains controversial due to the subjective effects produced by these drugs, which often seem uniquely human. However, although one may question the validity of animal models of psychedelic drug action, evidence suggests that behavioural abnormalities induced by psychedelics in rodents may predict such effects in humans (see (see Hanks and González-Maeso, 2013; Canal and Morgan, 2012)). Moreover, since human studies are limited in their ability to probe neurochemical mechanisms, the use of animal models may enable a better understanding of the molecular basis through which psychedelics produce their effects.

Determining which rodent behaviours predict specific types of neuropsychological effects in humans holds significant value. Ideally, suitable rodent models that are analogous to specific behavioural characteristics induced by psychedelic drugs in humans may be used as tools to investigate the anatomy and molecular mechanisms of action underlying such behavioural outcomes. The head-twitch response (HTR) is widely used as a mouse behavioural proxy of human psychedelic action, mostly due to its predictive validity (Canal and Morgan, 2012; Halberstadt et al., 2020). It is important to note that head-twitch behaviour is occasionally observed at baseline, albeit at a much lower frequency than that induced by psychedelic drugs. The HTR is induced in mice by all psychedelic drugs yet studied, and is not induced by non-psychedelic drugs such as lisuride and ergotamine (Gonzalez-Maeso et al., 2007). The HTR is also distinct from other drug-induced behavioural responses in rodents, such as dissociative-induced head-weaving and opiate withdrawal-induced wet-dog shakes (Gonzalez-Maeso and Sealfon, 2009; Martin et al., 1963). In summary, head-twitch behaviour in mice can be used with a high degree of reliability to predict psychedelic potential in humans.

5.2.2. Pharmacology of psychedelics

There are two major classes of psychedelic drugs: (i) the indoleamines, such as LSD, psilocybin and DMT, and (ii) the phenethylamines, such as mescaline and the 2C (2,5-dimethoxy, 4-substituted phenethylamines) series (Nichols, 2004). In contrast with the high selectivity of phenethylamines for the 5-HT₂ family of receptors, indoleamines are relatively non-selective at 5-HT sites (see Table 2-1; Pierce and Peroutka, 1989; Titeler et al., 1988; McKenna et al., 1990). Despite differences in their chemical structure (see

Figure 2-2), the indoleamines and phenethylamines induce cross-tolerance and produce remarkably similar experiences in humans (Abramson and Rolo, 1965; Abramson et al., 1958; Balestrieri and Fontanari, 1959; Hollister et al., 1969; Isbell et al., 1961; Wolbach et al., 1962b).

The serotonin 2A receptor (5-HT_{2A}R) is a member of the 5-HT₂ seven-transmembrane receptor family and is expressed in various cells and tissues across the mammalian body, with highest expression levels in the brain (see GPCR database; Regard et al., 2008). 5-HT_{2A}Rs are specifically enriched in the cerebral cortex, particularly on the apical dendrites of pyramidal cells in layer 5 (Weber and Andrade, 2010). By enhancing cortical glutamate release, 5-HT_{2A}Rs may modulate cognitive processes, such as working memory and attention (Williams et al., 2002; Mirjana et al., 2004). Based on a large body of evidence, it is widely accepted that the unitary mechanism responsible for the effects of psychedelics is activation of the 5-HT_{2A}R. Indoleamines and phenethylamines both act as agonists at this common site (i.e. the 5-HT_{2A}R) of action (Nichols, 2004). Moreover, the strong positive correlation between the affinity of these drugs for the 5-HT_{2A}R and psychedelic potency (Glennon et al., 1984) suggests that the stimulation of this receptor subtype is important in their mechanism of action. This is further supported by research showing an ablation of psychedelic effects in 5-HT_{2A}R knockout mice (Gonzalez-Maeso et al., 2007) and following the administration of selective 5-HT_{2A}R antagonists in animals and humans (Vollenweider et al., 1998; Glennon et al., 1984; Colpaert et al., 1985; Gresch et al., 2005; Fiorella et al., 1995; Smith et al., 1995). Specifically, 5-HT_{2A}Rs of layer 5 pyramidal cells are thought to be a key mediator of psychedelic activity (Muthukumaraswamy et al., 2013; Nichols, 2016; Vollenweider and Kometer, 2010).

Psychedelic drug effects show strong manifestation in human psychological functioning, but psychedelics also produce somatic effects across mammals. For instance, LSD has been shown to induce pupil dilatation, patellar hyperreflexia, temperature dysregulation, as well as increase in breath rate, heart rate, and blood pressure in humans (Isbell, 1959; Schmid et al., 2015). Likewise, 'serotonin syndrome' has been described in the literature and refers to stereotypical movements coinciding other motor symptoms in animals (e.g. Corne and Pickering, 1967; Sloviter et al., 1980; Buchborn et al., 2015). Our knowledge of the neurophysiological correlates of the human psychedelic brain state largely feeds from BOLD-fMRI and PET based research. As the given methods indirectly measure neuronal activity by extraction of blood-flow related parameters, the vasoactivity intrinsic to psychedelics deserves particular experimental scrutiny when interpreting such correlates.

In addition to their neuronal expression in the brain, 5-HT_{2A}Rs are widely expressed across the vascular system (Cohen, 1988; Ullmer et al., 1995) and there is ongoing controversy as to how a direct interaction between psychedelics and the vessels might contribute to the overall corticodynamics associated with the psychedelic state (Lewis et al., 2017). Psychedelics are likely to interfere with cerebral blood flow by targeting 5-HT receptors within the brain's microcirculation itself (Cohen et al., 1996). Despite autoregulatory shielding of brain blood flow from the capillaries of the periphery (Yang and Liu, 2017), heart rate and systemic blood pressure also have clear effects on cerebral haemodynamics (Ma et al., 2016; Kalisch et al., 2005; Terem et al., 2018).

BOLD-fMRI based brain functioning studies, thought to mainly reflect neuronal activity, might therefore partially be confounded by local and/or cardiovascular bottom-up effects (e.g., van't Ent et al., 2014; de Munck et al., 2008). Although there is general effort to control for these complex interactions in brain imaging studies on psychedelics (Müller et al., 2018; Carhart-Harris et al., 2012a), it appears essential to learn how 5-HT_{2A} activation influences the brain's supply of blood when disentangling neuro- and haemodynamic mechanisms involved in the psychedelic brain state. Cardiovascular 5-HT_{2(A)} receptors have been subject to extensive research (see Kermorgant et al., 2018; McCall and Clement, 1994; Nagatomo et al., 2004; Ramage, 2001), yet our understanding of how they regulate systemic circulation has largely been occluded by the unavailability of selective agonists.

In summary, the 5-HT_{2A}R is considered as an important drug target with potential implications of both agonists and antagonists in the treatment of various psychiatric conditions, including depression and anxiety (Quesseveur et al., 2012; Buchborn et al., 2014; Ross et al., 2016; Mascher, 1967; Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a). Despite the availability of relatively selective 5-HT_{2A}R antagonists (see PDSP Ki database; Roth et al., 2000), our understanding of the general neurophysiology of (cortical) 5-HT_{2A}Rs is limited by the lack of highly selective 5-HT_{2A} agonists. The 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action also deserves particular experimental scrutiny considering the use of BOLD-fMRI in human studies. The phenylaminergic psychedelics DOI and DOM (2,5-dimethoxy-4-iodo-/methyl-amphetamine) have been the state-of-the-art drugs for delineation of 5-HT_{2(A)} specific functioning, yet seem to have confounded affinity for 5-HT₂ and (nor-)adrenergic receptors (Ray, 2010). This makes it difficult to examine 5-HT_{2A}-specific vasoactive and psychoactive effects.

Since psychedelic phenethylamines display reasonable selectivity for the 5-HT₂ receptor but typically lack selectivity among the subtypes (Glennon et al., 1992; Roth et al., 1998), extensive medicinal chemistry efforts have focused on this scaffold in order to produce highly selective agonists. In search of agonists with higher 5-HT_{2A}R selectivity, a novel series of 48 compounds was recently developed based on the phenethylamine backbone shared by DOI-like psychedelic drugs (Hansen et al., 2014). Amongst these, extensive follow-up *in vitro* receptor binding screening demonstrated that the newly designed *N*-(2-hydroxybenzyl) substituted 4-cyano-2,5-dimethoxyphenethylamine (25CN-NBOH) was

one of the most promising candidates.

25CN-NBOH

25CN-NBOH is characterised by a high affinity for 5-HT_{2A}Rs (*in vitro* radioligand competition binding, av. K_i : ~1.72 nM), by a ≥100-fold selectivity for binding to this receptor as compared to a plethora of non-5-HT₂ targets (including other G-protein coupled receptors, ion channels, transporters, and enzymes), and robust 5-HT_{2A} selectivity over 5-HT_{2B/2C} receptors (K_i *in vitro* [averaged across studies]: ~56 nM for 5-HT_{2B} and ~83 nM for 5-HT_{2C}, respectively) (Jensen et al., 2017a; Halberstadt et al., 2016; Hansen, 2010). Therefore, 25CN-NBOH appears advantageous over other currently used 5-HT_{2A}R agonists subjected to a similarly scrutinous receptor profiling (see Ray, 2010; and PDSP K_i database, Roth et al., 2000).

The structure of 25CN-NBOH is similar to that of other *N*-benzyl substituted phenethylamines currently emerging among recreational psychedelic drug users, including the NBOMe series (Lawn et al., 2014), all of which induce potent psychedelic effects in humans. Moreover, recent anecdotal reports relating to psychedelia specifically induced by 25CN-NBOH began emerging this year. These reports are consistent with research demonstrating that *N*-benzyl substitutions dramatically increase affinity for 5-HT_{2A}R *in vitro* and *in vivo* (Blaazer et al., 2008; Halberstadt and Geyer, 2014; Nichols et al., 2008). Although most information regarding the psychedelic effects of novel *N*-benzyl substituted phenethylamines in humans is largely anecdotal in nature, accounts posted by recreational users online (for example, erowid.org and bluelight.org) strongly attest to the psychoactive properties of these substances. The confirmation of psychedelic effects of 25CN-NBOH in humans provides further support for its use in animal studies of psychedelic drug action.

5.2.3. Optogenetics for psychedelic research

Evidence suggest that psychedelics have therapeutic potential, yet very little is known about how they work in the brain mechanistically to give rise to their profound effects. Unveiling the effects of psychedelics with cell-type and circuit specificity might provide insight into the mechanisms underlying the therapeutic effects of psychedelics as well as many psychiatric disorders. A cell-type specific and circuit-centric investigation of the effects of psychedelics on brain activity holds exciting opportunities and has never been performed.

Since the advent of the game-changing field of optogenetics (Boyden et al., 2005; Li et al., 2005), it is now possible to record neuronal activity with cell-type and circuit specificity in the awake, behaving animal. The term ‘optogenetics’ refers to the combination of genetics and optics to manipulate and monitor the activity of defined cell classes at the level of neuronal circuits. There are two different types of tools depending on the optogenetic approach taken: (i) actuators, which are proteins that transduce light into neuronal signals, have been designed to manipulate neuronal circuits; and (ii) indicators, which are proteins that transduce neuronal signals into optical signals, have been designed to monitor

neuronal circuits (Song and Knopfel, 2016). Optogenetic indicators include genetically encoded calcium (GECI) and voltage (GEVI) indicators. Whereas GECIs give an indirect readout of neural activities, GEVIs directly measure changes in membrane potential with subthreshold sensitivity and are capable of detecting inhibitory electrical inputs (Knopfel, 2012). GEVI-based approaches allow the efficient detection of neural ensembles, network properties and orchestrated activities as well as the underlying circuitry (Antic et al., 2016). Therefore, using GEVI-expressing transgenic mice to conduct cell-type and circuit-centric explorations of psychedelic drug action would be advantageous and holds great potential.

5.3. OBJECTIVES AND HYPOTHESES

The present study aimed to determine the suitability of 25CN-NBOH in mice for prospective *in vivo* optical imaging studies. The head twitch response (HTR) was used as a behavioural readout of central 5-HT_{2A}R activity to determine the following factors: (i) The dose range of 25CN-NBOH required for parenteral administration to induce central 5-HT_{2A}R activation, (ii) the time-course of the central action, (iii) sensitivity to 5-HT_{2A}R antagonism and experimental familiarity, (iv) a possible involvement of 5-HT_{2C}Rs in 25CN-NBOH induced HTRs, and (v) potential development of tachyphylaxis and tolerance. 25CN-NBOH was then tested with regard to its effects on heart rate, blood flow, blood oxygenation, and respiratory rate, monitored by non-invasive pulse oximetry drawn from the animals' brain-imminent neck arteries. Since 5-HT_{2A}R-related vasoconstriction is thought to be a main effector site of serotonergic thermoregulation (e.g., Ootsuka et al., 2004), the cardiovascular effects of 25CN-NBOH were examined by varying the temperature of the heat-pad supporting the animal.

Study objective: To behaviourally characterise the effects of 25CN-NBOH for prospective *in vivo* optical imaging experiments and elucidate 5-HT_{2A}R-mediated vascular effects via neck arterial blood-flow parameters.

Hypotheses:

- 25CN-NBOH will show psychedelic activity by inducing the HTR and will demonstrate 5-HT_{2A} selectivity.
- Experimental familiarity and habituation will improve the HTR readout.
- 25CN-NBOH will produce tolerance and tachyphylaxis, consistent with similar phenethylamine psychedelics.
- 25CN-NBOH will demonstrate vasoactivity by altering parameters of neck arterial blood-flow.

5.4. METHODS

5.4.1. *Statement of contributions*

Personal contribution: In accordance with the UK Animal Scientific Procedures Act (1986), I completed the Home Office Module 1 – 4 course (Certificate number: IMP/16/011), obtained a personal licence (PIL number: I5B5A6029; Category C) and received training and competency assessments in order to carry out the regulated procedures necessary for this study (see appendices). I carried out the animal handling, husbandry and procedures (i.e. intraperitoneal drug administration, transcardial perfusions, brain extraction and slicing, head-fixation training, and schedule 1 culling) outlined in this chapter. I also carried out all of the confocal microscopy, designed and carried out the behavioural and physiological experiments, developed the head-fixation habituation protocol and conceptualised the study design of a proposed optogenetic investigation to be completed in the future. I collected, plotted and interpreted all of the data presented in this chapter. Additionally, I designed and built the observation boxes used in the behavioural experiments.

Team contribution: As a joint project, Tobias Buchborn and I contributed equally to the behavioural and physiological experiments. Tobias Buchborn carried out procedures that I had not been assessed in (i.e. subcutaneous drug administration and cervical dislocation as the primary method for schedule 1 culling). Prof Thomas Knöpfel held the project licence (PPL number: 70/7818) and provided the supervision and advice required for the conceptualisation of the experimental designs, methodology and interpretation of the findings presented here. Prof Thomas Knöpfel also provided the laboratory facilities and equipment. Chenchen Song, Peter Quicke, Samuel Barnes and the Imperial College Central Biomedical Services (CBS) staff provided the training required for my competency in the procedures outlined in this work. Chenchen Song and Gemma Oliver assisted with animal husbandry and breeding. The CBS provided the animal facilities and assisted with housing, husbandry, breeding, training and competency assessments. All members of the Knöpfel laboratory provided critical comments and encouragement.

5.4.2. *Animals*

All experimental procedures performed at Imperial College London were in accordance with the UK Animal Scientific Procedures Act (1986) under Home Office Personal and Project licenses (I5B5A6029, IA615553C; PPL 70/7818), following appropriate ethical review. Adult mice of both sexes and with mixed genetic background (stock of mainly C57BL/6JxB6CBAF1 background) were bred in-house at the Central Biomedical Services (CBS) of Imperial College London. They were housed in individually ventilated cages with a 12:12 h light/dark cycle and maintained at an ambient temperature of $21 \pm 2^\circ\text{C}$ at $55 \pm 10\%$ humidity. Mice were provided with standard rodent-chow pellets (Special Diet Services, #RM1) and water *ad libitum*.

5.4.3. Drugs

25CN-NBOH (a kind gift from Jesper L. Kristensen, University of Copenhagen) and ketanserin (Tocris Biosciences; Avonmouth, UK) were dissolved in isotonic saline, SB-242084 (Tocris Biosciences; Avonmouth, UK) in DMSO (10% in saline). Drugs were applied intraperitoneally or subcutaneously (<10 ml/kg), as indicated. Pulse oximetry and body core temperature experiments were performed under general anaesthesia using isoflurane (IsoFlo; Zoetis, UK) delivered in 100% oxygen, as detailed below.

5.4.4. Behavioural Experiments

Animals were allowed to habituate to the (observational) non-home boxes (25.5 × 12.5 × 12.5 cm [L × W × H]) for 30min. Observational boxes were shielded with red acrylic plates to minimise the animals' visual awareness of the experimenter.

Head Twitches

For quantification of HTRs, animals were treated with saline or the respective dose of 25CN-NBOH (0.5–3.0 mg/kg, i.p. (or 1.5 mg/kg, s.c.)) and observed for 20–60min. The frequency of head twitches, defined as rapid and brisk rotational movement of the head around the longitudinal axis of the animals' body (Handley and Singh, 1986) (see supplemental Movie), was counted in 2- or 5-min bins starting directly after injection. The sensitivity of 25CN-NBOH (1.5 mg/kg [vs. 3.0] mg/kg, i.p.) induced head twitches to 5-HT_{2A} and/or 5-HT_{2C} antagonism was tested by pre-treating the animals with ketanserin (0.75 mg/kg, i.p.) and SB-242084 (0.5 mg/kg, i.p.), respectively, 30min prior to agonist administration. The impact of experimental familiarity was investigated by simulation of aspects of the experimental procedure (i.e., handling, injection of saline, and exposure to the observational non-home cages for 30min before, and for 20min after saline injection) 1 day before the actual 25CN-NBOH experiment.

Tachyphylaxis, defined as in-between-session tolerance occurring at repeated short-interval application, was tested by injecting saline or 25CN-NBOH (0.75 or 1.5 mg/kg, i.p.) twice at a 1.0- or 1.5-h interval, and comparing the average HTR frequency in each 30-min observation period. For subchronic tolerance, animals were treated for 4 days with saline or 25CN-NBOH. 25CN-NBOH was injected once (1.5 mg/kg, i.p. [morning]) or twice (1.5 mg/kg, i.p. [morning] and 3.0 mg/kg, i.p. [evening of days 1–3]) per day and HTRs were counted for 20min after each morning application. The twice-per day regimen, with the increased dose (i.e., 3.0 mg/kg) in the evening, was chosen based on findings for LSD, which indicate that tolerance development is slightly facilitated by a higher frequency of application, but more strongly facilitated by a higher frequency of application with increased dosing every other application injection (Buchborn et al., 2016).

Statistics

The data from the dose-response, antagonist, and prehabilitation experiments were analysed using non-parametric Kruskal-Wallis test with Dunn's multiple post-hoc comparisons, or Mann-Whitney U-testing (as planned *a priori*). Tolerance and time-course data were subjected to a two- and three-factor

ANOVA with repeated measures on one factor, respectively, and followed up on by Bonferroni-corrected multiple comparisons. For HTRs, time-course data of interest were fitted by non-linear regression analysis, and the decay constant provided by the best-fit exponential equation ($HTR_t = HTR_0 \cdot e^{-\lambda \cdot t}$) subsequently used for determination of 25CN-NBOH's half-life ($t_{1/2} = \ln[2]/\lambda$). Calculations were carried out by SPSS or GraphPad Prism software. Statistical significance was assumed if the null hypothesis (e.g., drug has no action) could be rejected at $p \leq 0.050$ probability level.

5.4.5. *Pulse oximetry and body core temperature measurements*

Pulse oximetry was performed in anaesthetised mice using the rodent-specific MouseOx Plus collar-sensor system (STARR Life Sciences Corp., Oakmont, PA). Anaesthesia was induced in an anaesthesia chamber (VetTech Solutions, Cheshire, UK) at 2-2.5% isoflurane, and following the loss of the righting reflex maintained via two-port mask supply at 1.5-2.0% concentration. Body temperature was maintained by a feedback-loop controlled heat-pad (TR-200 Fine science tools, USA), which drew input from a thermosensor attached to the heat-pad's surface, controlling for 37 or 41°C pad temperature. The collar-sensor was placed around the shaved necks of the animals to read out oxygen saturation (% of functional arterial haemoglobin), heart rate (beats per minute [bpm]), breath rate (breaths per minute [brpm]), and pulse distention of carotid arteries (Figure 5-4 D). Pulse distention reflects changes in light absorption by haemoglobin and via Beer's law provides the length of the optical path through the blood (Hete et al., 2013). Recordings were made at 1 Hz, with a 20-minutes baseline period, followed by injection of 25CN-NBOH and saline, respectively, and a 20-minutes post-injection period. Body core temperature was monitored via a digital thermometer and rectal probe and registered at 2-minutes intervals.

Statistics

Pulse oximetry 1-Hz readouts –as aggregated in 5-min bins– and body core temperature measurements were analysed via SPSS-implemented 3-factorial repeated measures ANOVA (with *group* and *pad temperature* as between-subject factors, and *time* as within-subject factor), and analysed for main effects, interaction, and contrast, respectively. Significant interactions were followed up on by Bonferroni-corrected multiple comparisons. For cross-parameter comparability, results were normalised to their respective baseline average and depicted as -fold baseline.

5.5. RESULTS

5.5.1. Dose-response, ketanserin-sensitivity and effect of experimental familiarity

Mice received a single injection of either saline (control) or 25CN-NBOH (0.5–3.0 mg/kg, [i.p. or s.c.]) and were monitored for HTRs during the following 20min. While control animals in this observation period rarely showed any behaviours rated as HTRs [0.83 ± 0.31 (mean $\pm$ SEM)], the incidence of HTRs markedly increased for all tested doses of 25CN-NBOH. Frequencies (mean $\pm$ SEM) ranged from 13.67 ± 4.26 for the lowest dose (0.5 mg/kg, i.p.), 37.67 ± 6.04 to 40.67 ± 5.77 for the medium dose (1.5 mg/kg, i.p. vs. s.c.), and 27.60 ± 4.52 for the highest dose tested (3.0mg/kg, i.p.; Figure 5-1). Inference-statistically, however, only the 1.5-mg/kg dose significantly differed from the saline group ($X^2_{(10, N=62)}=31.49$, $p \leq 0.001$; Dunn's *post-hoc* [comparison to saline], 1.5 mg/kg i.p. or s.c., each $p \leq 0.01$). At none of the doses tested, stereotypies other than HTRs (including any symptoms of the serotonin syndrome) were noted.

The 5-HT_{2A} antagonist ketanserin (0.75 mg/kg, i.p.), when applied 30min before the agonist, near-completely prevented the 25CN-NBOH (1.5-mg/kg, i.p.) induced HTRs (mean $\pm$ SEM: 2.0 ± 1.29) ($X^2_{(4, N=30)}=14.47$, $p \leq 0.010$; Dunn's *post-hoc* [comparison to 1.5 mg/kg 25CN-NBOH w/o antagonist and to control, respectively], $p \leq 0.050$ and n.s.). The selective 5-HT_{2C} antagonist SB-242084 (0.5 mg/kg, i.p., 30min pre-treatment), did not prevent 25CN-NBOH from inducing significant HTRs (mean $\pm$ SEM: 31.50 ± 8.25 ; Dunn's *post-hoc* (comparison to saline), $p \leq 0.050$). SB-242084, although not affecting the effect of 1.5 mg/kg 25CN-NBOH, increased the animals' response to the 3.0 mg/kg dose of the agonist, leading to HTRs of significant extent (mean $\pm$ SEM: 36.20 ± 7.02 , $p \leq 0.05$; Figure 5-1 B).

Handling the animals and thoroughly habituating them to the experimental procedures (including exposure to observational boxes and saline injection 1 day before the 25CN-NBOH experiment), respectively, facilitated agonist induced HTRs; a 0.75 mg/kg sub-threshold dose was enabled to induce significant twitching (mean $\pm$ SEM: 33.0 ± 5.28 ; $p \leq 0.01$) and the response to a 1.5 mg/kg dose was significantly increased (mean $\pm$ SEM: 61.5 ± 6.3 ; $p \leq 0.050$; Figure 5-1 A).

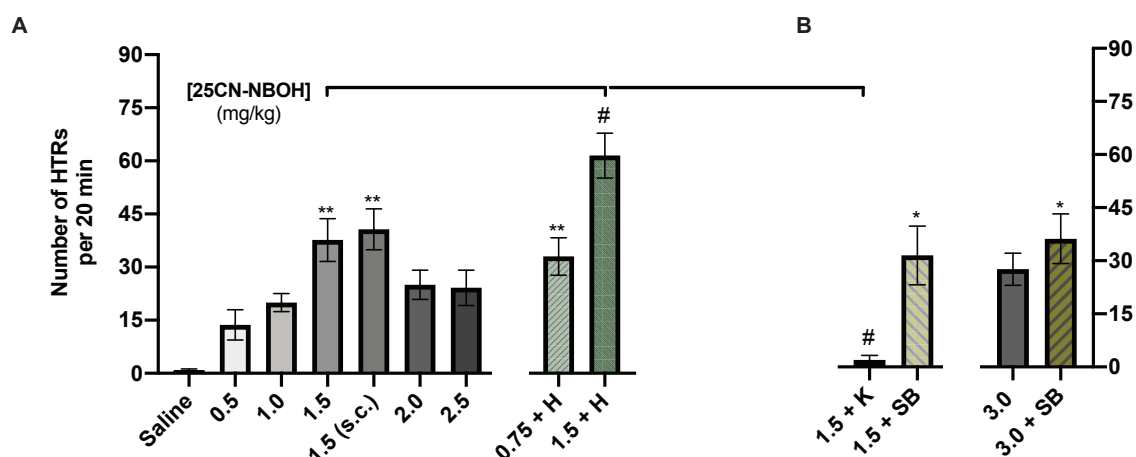


Figure 5-1. 25CN-NBOH induced head twitch response (HTR) - (as observed for the first 20min post-application). **(A) Dose-response relation** (0.5–2.5 mg/kg i.p. vs. 1.5 mg/kg s.c.) and effect of experimental habituation (H; 0.75 vs. 1.5 mg/kg 25CN-NBOH i.p. + H). **(B) Effect of the 5-HT_{2A} antagonist ketanserin** [1.5 mg/kg 25CN-NBOH + 0.75 mg/kg K (30 min pre-treatment), i.p.] and the 5-HT_{2C} antagonist SB-242084 [1.5 vs. 3.0 mg/kg NBOH + 0.5 mg/kg SB (30min pre-treatment), i.p.], respectively. Mean $\pm$ SEM; $n=4-6$ per group. Kruskal-Wallis *post-hoc* comparison to saline, * $p\leq 0.050$ and ** $p\leq 0.010$; or to agonist w/o habituation and w/o antagonist pre-treatment, respectively, # $p\leq 0.050$.

5.5.2. Time-course of head twitches

To investigate the time-course of the behavioural effect of the different 25CN-NBOH doses, we counted HTRs in 5-min bins as they occurred over 30min post-injection (Figure 5-2). Repeated measures ANOVA with Bonferroni-corrected post-hoc analysis revealed significant increases in HTR frequency as a function of *time* [$F_{(1.84, 27.66)}=57.52, p\leq 0.001$], *treatment* [$F_{(2, 15)}=30.41, p\leq 0.001$], as well as of *time x treatment* interaction [$F_{(3.68, 27.66)}=26.52, p\leq 0.001$]. All but the 0.5 and the 1.0 mg/kg doses elicited significant HTRs in the first observation window (0–5 min), where they peaked (1.5 mg/kg: $14.33 \pm 2.67, p\leq 0.001$; 2.0 mg/kg: $9.67 \pm 2.6, p\leq 0.050$; 2.5 mg/kg: $11.17 \pm 1.68, p\leq 0.01$) and then steadily decreased over time. As depicted in the inset of Figure 5-2, the temporal course of 25CN-NBOH (1.5 mg/kg, i.p.) induced HTRs is well-fitted by an exponential decay function, with the effect of the drug declining to half ($t_{1/2}$) after 11 min. The 1.0, 2.0, and 2.5 mg/kg doses decayed with similar half-lives between around 9 and 12 min.

In an additional set of experiments, we studied the time-course of the effect produced by the 1.5 mg/kg dose over a longer observation time period. To this end, 25CN-NBOH induced head twitches (i.p. vs. s.c.) were counted over 60 min at a 2-min-observation resolution. After i.p. injection, the frequency of HTRs peaked within the first 2-min bin (mean $\pm$ SEM: 6.8 ± 1.32) and then decayed with a $t_{1/2}$ of 8.56 min. For s.c. injection, the peak occurred with a slight delay in the second 2-min bin (mean $\pm$ SEM: 7.00 ± 0.71) and decayed at a $t_{1/2}$ of 13.59 min. Overall, as indicated by repeated measures ANOVA, there

were no route-dependent time-course differences. The average ($\pm$ SEM) of the three half-lives calculated for the 1.5 mg/kg dose is 11.05 ± 1.45 .

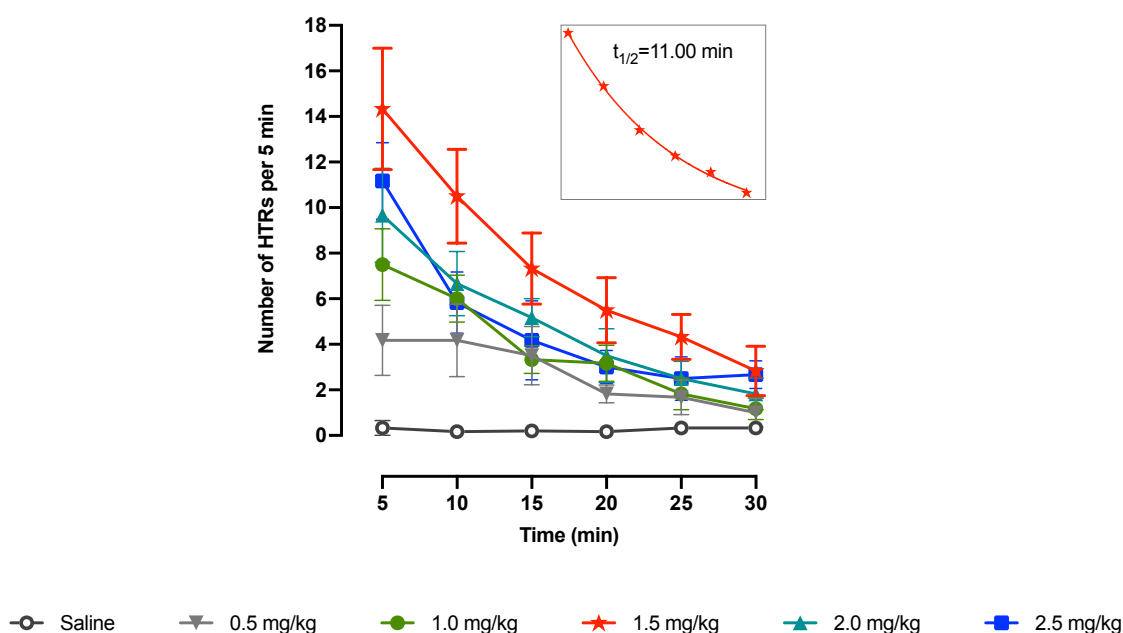


Figure 5-2. Time-course of 25CN-NBOH (0.5–2.5 mg/kg, i.p.) induced HTR (as observed for the first 30min post-application). Mean $\pm$ SEM; $n=6$ per group. The inset replots the means of the 1.5 mg/kg dose as fitted by an exponential decay function.

5.5.3. Tachyphylaxis and tolerance

In an independent set of experiments, we explored whether 25CN-NBOH induced HTRs showed signs of short-term tolerance. We therefore repeatedly applied the drug at different intervals. Similarly, as reported above, 25CN-NBOH (1.5 mg/kg, s.c.) treated mice exhibited an average of 34.67 ± 4.65 HTRs within 30 min, which was significantly more than observed in saline treated mice (mean $\pm$ SEM: 1.4 ± 0.25) ($p \leq 0.010$). Reapplying the same dose of 25CN-NBOH at 1 or 1.5 h after the first application substantially reduced the efficacy of the drug. Main effects for *time* [$F_{(1, 24)}=64.28$, $p \leq 0.001$], *group* [$F_{(1, 19)}=62.1$, $p \leq 0.001$], and *time x group* interaction [$F_{(4, 24)}=10.47$, $p \leq 0.001$] proved significant. Saline treated animals at both intervals did not show significant differences from the first saline application (not shown), 25CN-NBOH treated mice—with only $24.98 \pm 4.54\%$ (1 h) and $19.48 \pm 4.59\%$ (1.5 h) of the original responsiveness—twitched significantly less (first vs. resp. second injection, *post-hoc* each $p \leq 0.010$; Figure 4). As shown for the 1.5 h interval, a reduced but still highly significant loss of drug efficacy could likewise be demonstrated when 25CN-NBOH was injected twice at a lower dose (0.75 mg/kg, s.c.) to handling habituated mice (mean $\pm$ SEM [% of the original responsiveness]: 30.66 ± 5.86 ; first vs. second injection, $p \leq 0.010$) (Figure 5-3 A).

In addition to tachyphylaxis occurring at repeated short interval injection, a substantial loss of responsiveness to 25CN-NBOH could also be demonstrated when the drug (1.5 mg/kg, i.p.) was repeatedly applied at 1-day intervals [main effect *time*: $F_{(1.84, 27.66)}=57.51$, $p\leq 0.001$]; main effect *group* [$F_{(2, 15)}=30.41$, $p\leq 0.001$]; *time x group* interaction [$F_{(3.68, 27.66)}=26.52$, $p\leq 0.001$] (Figure 5-3 B). Unlike with the short-interval application, however, the decrease in HTRs only with the third injection of 25CN-NBOH (i.e. on the 3rd and 4th day) became significant [1x 25CN-NBOH per day: Day 1 (mean $\pm$ SEM: 37.67 ± 5.99) vs. day 3 and day 4 [56.19 and 54.87% of day 1, respectively], each $p\leq 0.010$]. When a second (higher) dose of the agonist (3.0 mg/kg, i.p.) was applied in the evening of the first 3 days, the onset of tolerance proved significant by day 2 [2x 25CN-NBOH per day: Day 1 (mean $\pm$ SEM: 61.5 ± 6.34) vs. day 2, day 3, and day 4 (34.15, 18.69, and 17.88% of day 1, respectively), each $p\leq 0.001$]. Also, the extent of tolerance was more pronounced. Whereas the HTRs exhibited by the once-per-day-application mice on day 4 still were statistically different from the saline-treated control animals (mean $\pm$ SEM: 20.67 ± 3.49 , $p\leq 0.001$), those in the twice-per day mice more closely approached the control level (mean $\pm$ SEM: 11.00 ± 1.73 , n.s; Figure 5-3 B).

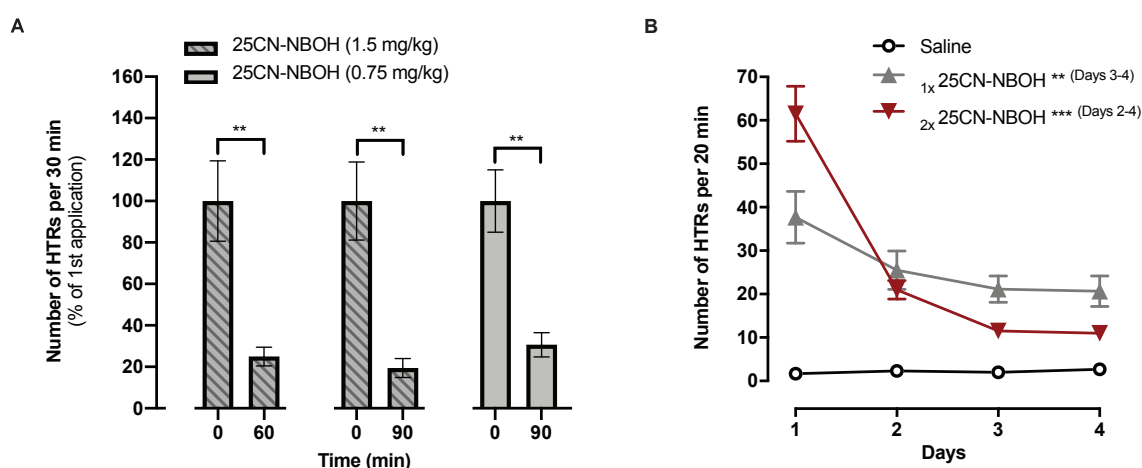


Figure 5-3. Tolerance and tachyphylaxis. (A) Tachyphylaxis of 25CN-NBOH induced head twitches (0.75 or 1.5 mg/kg, s.c.; as observed for the first 30 min post-application). Agonist was applied twice at an interval of 60 and 90min, respectively. Mean $\pm$ SEM; $n=5-6$ per group. Repeated measures ANOVA, Bonferroni-corrected *post-hoc* comparison to respective first application (0 min), ** $p\leq 0.010$. **(B) Subchronic tolerance to 25CN-NBOH induced head twitches**, with a total of four [1x 25CN-NBOH: Once per day, 1.5 mg/kg (morning)] and seven [2x 25CN-NBOH: Twice per day, 1.5 mg/kg (morning) vs. 3.0 mg/kg (evening)] i.p. injections, respectively. Head twitches were counted for the first 20min after the morning application). Percent values indicate percent of HTRs relative to respective first day. Mean $\pm$ SEM; $n=6$ per group. Repeated measures ANOVA, Bonferroni-corrected *post-hoc* comparison to respective first-day measurement, ** $p\leq 0.010$, *** $p\leq 0.001$ (brackets behind asterisks indicate interval(s) significance applied to).

5.5.4. Pulse distention

For the control (saline) animals at both 37°C and 41°C pad temperature, pulse distention remained relatively stable throughout the 40-minutes recording period (Figure 5-4; repeated measures ANOVA,

significant differences as a function of *time* ($F_{(2.52, 93.27)}=3.51$, $p=0.001$), *group* ($F_{(1, 37)}=3.51$, $p\leq 0.001$), *time x group* ($F_{(2.52, 16.37)}=16.37$, $p\leq 0.001$), as well as *time x group x temperature* ($F_{(2.52, 93.27)}=4.99$, $p=0.005$). Post-hoc analysis revealed significant group differences at any of the four post-injection measurements for both temperature conditions (saline vs. 25CN-NBOH [mean $\pm$ SEM, fold change]: 0.98 $\pm$ 0.017 vs. 1.05 $\pm$ 0.03 [25 min], $p=0.021$; 0.97 $\pm$ 0.019 vs. 1.04 $\pm$ 0.026 [30 min], $p=0.036$; 0.96 $\pm$ 0.019 vs. 1.04 $\pm$ 0.026 [35 min], $p=0.022$; 0.96 $\pm$ 0.021 vs. 1.049 $\pm$ 0.026 [40 min], $p=0.028$ for 37°C) (0.97 $\pm$ 0.01 vs. 1.05 $\pm$ 0.015 [25 min], $p=0.005$; 0.97 $\pm$ 0.012 vs. 1.15 $\pm$ 0.027 [30 min], $p\leq 0.001$; 0.96 $\pm$ 0.012 vs. 1.15 $\pm$ 0.024 [35 min], $p\leq 0.001$; 0.96 $\pm$ 0.013 vs. 1.15 $\pm$ 0.037 [40 min], $p\leq 0.001$ for 41°C). At increased pad temperature, 25CN-NBOH induced increase in arterial distension was much more pronounced (25CN-NBOH 37°C vs. 41°C: $p=0.026$ [25 min]; $p=0.031$ [30 min]; $p=0.029$ [35 min]; $p=0.036$ [40 min]). On the other hand, the saline treated control mice demonstrated no such temperature effect. Mean absolute values of arterial distension for the pre- vs. post-injection intervals (given in μm) are presented in Table 5-1 and Table 5-2.

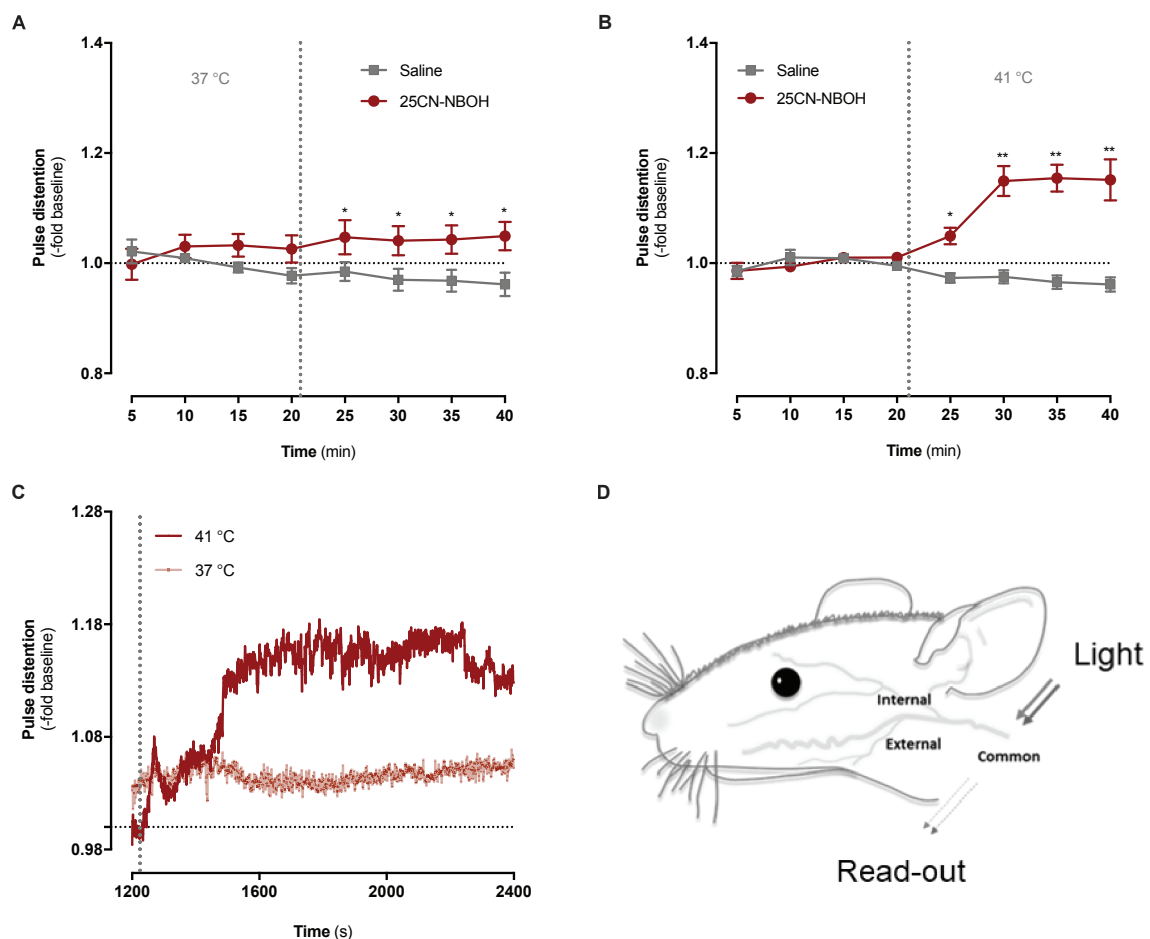


Figure 5-4. Arterial distension. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on arterial distension of isoflurane-anaesthetised mice (at (A) 37 vs. (B) 41°C pad temperature). Injection occurred following a 20-minutes baseline measurement (indicated by dotted vertical lines). Mean $\pm$ SEM (-fold change of baseline, μm); $n=8-14$ per group. Repeated measures ANOVA comparison to saline, * $p\leq 0.050$ and ** $p\leq 0.010$. (C) Temperature-dependency of 25CN-NBOH's effect on pulse distension, depicted in 1-Hz resolution (post-injection). (D) Schematic depiction of the major carotid branches of the mouse with area of neck-arterial pulse oximetry indicated by arrows.

5.5.5. Heart rate

Isoflurane-anesthetised mice overall exhibited a tendency for an increase of heart rate over time (Figure 5-5; repeated measures ANOVA, significant main effect *time*: $F_{(1.77, 65.40)}=65.76$, $p\leq 0.001$). Furthermore, a significant *time x group* interaction ($F_{(1.77, 65.40)}=4.59$, $p\leq 0.001$) and a significant *time x treatment x temperature* interaction ($F_{(1.77, 65.40)}=3.93$, $p=0.029$) indicated that the temporal changes in heart rate differed between both groups in a temperature dependent manner. Bonferroni-corrected post-hoc comparisons revealed that the significant *time x group* interaction was due to an 25CN-NBOH induced tachycardia at all four post-injection measurements for the 37°C condition (saline vs. 25CN-NBOH [mean±SEM, -fold change]: 1.05±0.018 vs. 1.12±0.018 [25 min], $p=0.041$; 1.07±0.02 vs. 1.18±0.027 [30 min], $p\leq 0.001$; 1.08±0.026 vs. 1.19±0.031 [35 min], $p=0.010$; 1.08±0.03 vs. 1.19±0.032 [40 min], $p=0.016$) and at the first post-injection measurement for the 41°C condition, respectively (saline vs. 25CN-NBOH [mean±SEM, -fold change]: 1.07±0.016 vs. 1.13±0.026 [25 min], $p=0.044$). Accordingly, temperature-dependent heart rate differences in 25CN-NBOH treated animals could be detected at the 35- and 40-minutes measurements, respectively, and as trend for the 30-minutes measurement (25CN-NBOH 37 vs. 41°C 30 min: $p=0.095$; 35 min: $p=0.015$; 40 min: $p=0.029$; 40 min: $p=0.014$). Mean absolute values for the pre- vs. post-injection intervals (given in bpm) are shown in Table 5-1 and Table 5-2.

5.5.6. Breath rate

Isoflurane-anesthetised mice overall exhibited a tendency for a decrease of breath rate over time (Figure 5-6; repeated measures ANOVA, significant main effect *time* ($F_{(2.72, 100.58)}=47.46$, $p\leq 0.001$). There was a significant main effect *group* ($F_{(1,37)}=8.27$, $p=0.007$) and a significant *time x group* interaction ($F_{(2.72, 100.58)}=3.21$, $p=0.030$) without additional temperature dependency. Following up on the *time x group* interaction, 25CN-NBOH significantly decreased breath rate at all four post-injection measurements of the 37°C condition (saline vs. 25CN-NBOH [mean±SEM, -fold change]: 0.96±0.041 vs. 0.88±0.038 [25 min], $p=0.010$; 0.91±0.041 vs. 0.78±0.053 [30 min], $p=0.014$; 0.87±0.048 vs. 0.72±0.046 [35 min], $p=0.025$; 0.87±0.051 vs. 0.7±0.048 [40 min], $p=0.010$), but only as a trend at the 25-minutes post-injection measurement of the 41°C condition (0.94±0.015 vs. 0.86±0.025 [25 min], $p=0.073$ for 41°C). Mean absolute values for the pre- vs. post-injection intervals (given in brpm) are listed in Table 5-1 and Table 5-2.

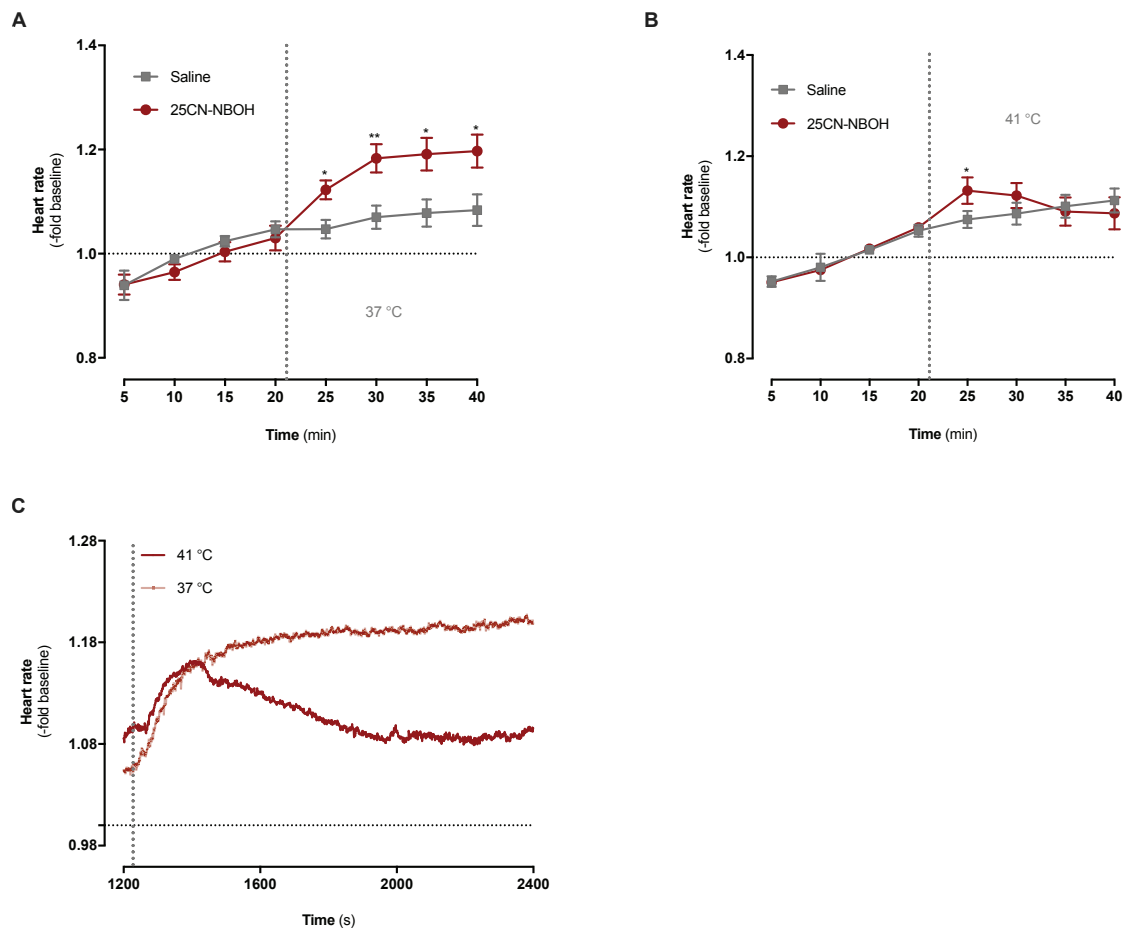


Figure 5-5. Heart rate. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on the heart rate of isoflurane-anaesthetised mice (at **(A)** 37 vs. **(B)** 41°C pad temperature). Injection occurred following a 20-minutes baseline measurement (indicated by dotted vertical lines). Mean±SEM (-fold change of baseline bpm); $n=8-14$ per group. Repeated measures ANOVA comparison to saline, * $p\leq 0.05$ and ** $p\leq 0.01$. **(C) Temperature-dependency of 25CN-NBOH's effect on heart rate**, depicted in 1-Hz resolution (post-injection).

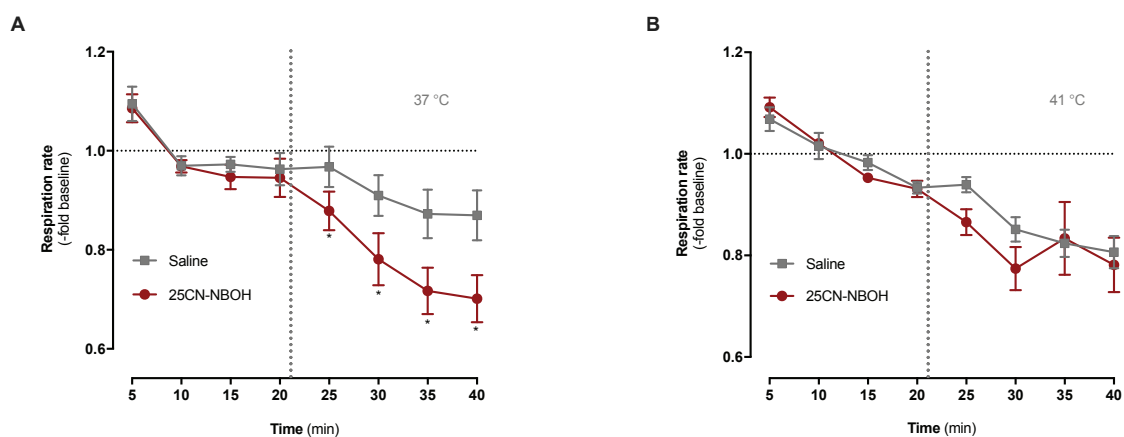


Figure 5-6. Breath rate. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on breath rate of isoflurane-anaesthetised mice (at **(A)** 37 vs. **(B)** 41°C pad temperature). Injection occurred following a 20-minutes baseline measurement (indicated by dotted vertical lines). Mean±SEM (-fold change of baseline brpm); $n=8-14$ per group. Repeated measures ANOVA comparison to saline, * $p\leq 0.050$.

5.5.7. Oxygenation

Overall, there was a slight tendency for blood oxygenation to decrease during anaesthesia, as implied by a significant main effect time in the repeated measures ANOVA ($F_{(1.81, 66.96)}=3.24$, $p=0.05$; Figure 5-7). Main effect *group* and *time x group (x temperature)* interactions were not significant, though, indicating that blood oxygenation was not affected by 25CN-NBOH nor its interaction with the pad temperature. Mean absolute values for the pre- vs. post-injection intervals (given in % saturation) are presented in Table 5-1 and Table 5-2.

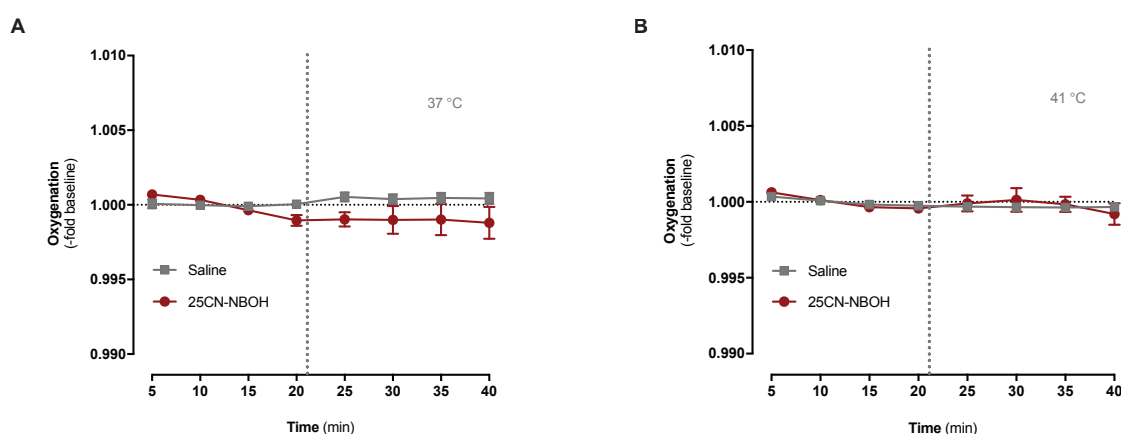


Figure 5-7. Oxygenation. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on blood oxygenation of isoflurane-anaesthetised mice (at **(A)** 37 vs. **(B)** 41°C pad temperature). Injection occurred following a 20-minutes baseline measurement (indicated by dotted vertical lines). Mean±SEM (-fold change of baseline % oxygen saturation); $n=8-14$ per group. Repeated measures ANOVA comparison to saline, ns.

5.5.8. Body core temperature

For a subpopulation of animals, body core temperature was quantified via rectal probe. Over the 40-minutes observation period, rectal temperature was noted to slightly increase across study groups (*time*, $F_{(1, 25)}=36.53$, $p<0.001$). *Time x treatment* interaction likewise turned out significant ($F_{(1, 25)}=9.13$, $p=0.006$), with a marginal contribution from pad temperature (*time x group x temperature* ($F_{(1, 25)}=1.85$, $p=0.095$)). Follow-up analysis revealed that the *time x group* interaction was driven by 25CN-NBOH favouring body warming during the last intervals of recording at 41°C (saline vs. 25CN-NBOH [mean±SEM, -fold change]: 1.006±0.002, 1.0053±0.0025, and 1.005±0.002 vs. 1.014±0.005, 1.014±0.004, and 1.02±0.005 [34, 36, and 40 min], $p<0.050$; Figure 5-8). Mean absolute values for the pre- vs. post-injection intervals (given in °C) can be extracted from Table 5-1 and Table 5-2.

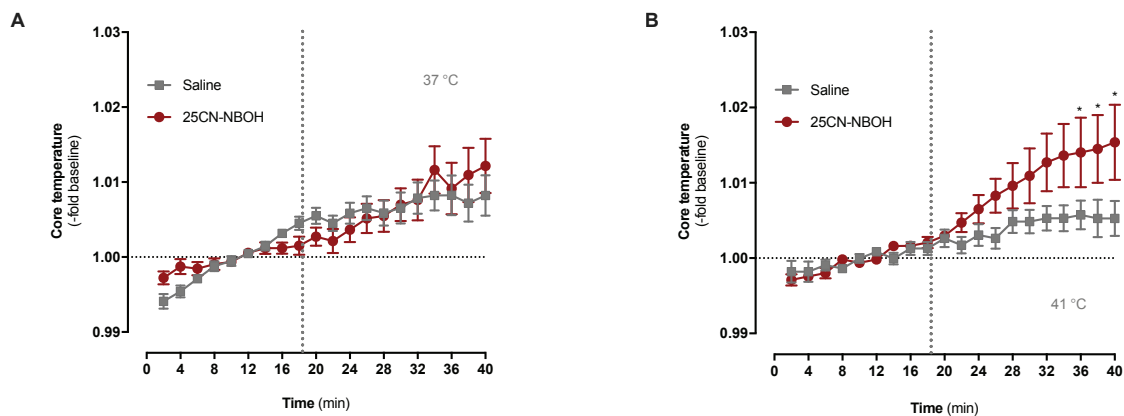


Figure 5-8. Body core temperature. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on body core temperature of isoflurane-anaesthetised mice (at **(A)** 37 vs. **(B)** 41 °C pad temperature). Injection occurred following a 20-minutes baseline measurement (indicated by dotted vertical lines). Mean±SEM (-fold change of baseline °C); $n=6-9$ per group. Repeated measures ANOVA, *post-hoc* comparison to saline, * $p \leq 0.050$ and ** $p \leq 0.010$.

Table 5-1. Autonomic functioning at 37 °C. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on autonomic functioning of isoflurane-anaesthetised mice at 37 °C pad temperature. Mean (SEM) absolute values of 20-minutes baseline vs. 20-minutes post-injection period. $N = 8-14$ per group.

37 °C	Saline		NBOH	
	Baseline	Post-injection	Baseline	Post-injection
Pulse distention (µm)	477.69 (35.90)	464.02 (37.54)	496.57 (23.83)	507.77 (24.96)
Heart rate (bpm)	459.09 (18.95)	492.50 (24.17)	430.38 (16.89)	511.05 (15.49)
Breath rate (brpm)	68.80 (5.96)	62.94 (7.11)	76.13 (4.66)	59.17 (15.49)
Oxygenation (%)	98.85 (0.15)	98.89 (0.12)	98.81 (0.14)	98.94 (0.06)
Temperature (°C)	37.06 (0.044)	37.31 (0.015)	36.85 (0.019)	37.11 (0.036)

Table 5-2. Autonomic functioning at 41 °C. Effect of 25CN-NBOH (1.5 mg/kg, s.c.) on autonomic functioning of isoflurane-anaesthetised mice at 41 °C pad temperature. Mean (SEM) absolute values of 20-minutes baseline vs. 20-minutes post-injection period. $N = 8-14$ per group.

41 °C	Saline		NBOH	
	Baseline	Post-injection	Baseline	Post-injection
Pulse distention (µm)	483.53 (37.19)	468.00 (35.08)	461.96 (30.48)	517.20 (30.21)
Heart rate (bpm)	466.12 (15.61)	508.57 (16.05)	474.24 (17.83)	522.84 (15.06)
Breath rate (brpm)	81.42 (6.82)	70.42 (6.88)	83.67 (9.82)	66.84 (8.42)
Oxygenation (%)	99.08 (0.057)	99.05 (0.056)	98.97 (0.067)	98.95 (0.066)
Temperature (°C)	37.17 (0.018)	37.33 (0.016)	37.24 (0.024)	37.65 (0.043)

5.6. DISCUSSION

The present study characterised the behavioural effects of the selective 5-HT_{2A} agonist, 25CN-NBOH. The HTR was used as a behavioural output based on evidence suggesting that the HTR engages (Gonzalez-Maeso et al., 2007; Willins and Meltzer, 1997), predicts and/or models 5-HT_{2A}R function in the cortex (e.g., Buchborn et al., 2015; Goodwin et al., 1984; Zhang and Marek, 2008). As (5-HT_{2A}ergic) vasoactivity of psychedelics might contribute to their overall brain dynamics, this study also characterised the effects of 25CN-NBOH on parameters of neck-arterial blood flow.

5.6.1. Behavioural effects

25CN-NBOH evoked marked HTRs in mice at the full range of doses tested (i.e., 0.5–3.0 mg/kg). In the context of the multiple-comparisons analysis, however, only the medium dose (1.5 mg/kg) was significant, which mirrors previous findings of the drug being most active at around 1.0–3.0 mg/kg (Halberstadt et al., 2016; Fantegrossi et al., 2015). The inverted u shape-like dose-response curve of 25CN-NBOH (with higher doses entailing a relative loss of responsiveness) might be due to increasing occupancy of 5-HT_{2C} receptors (whose activity may counteract the 5-HT_{2A}-mediated HTR induction), and/or due to 5-HT₂ receptors requiring a tuned level of occupancy (compare for DOM, DOB and DOI: Buchborn et al., 2015; Fantegrossi et al., 2010; Serafine and France, 2014). Consistent with its sensitivity to MDL100907 (Fantegrossi et al., 2015), one of the most selective 5-HT_{2A} antagonists at present (av. K_i [K_i database]: ~1.92 nM for 5-HT_{2A}; ~112 nM for 5-HT_{2C}), 25CN-NBOH (1.5 mg/kg, i.p.) induced HTRs were blocked by the 5-HT_{2A} antagonist ketanserin (av. K_i [K_i database]: ~1.6 nM for 5-HT_{2A}; ~52 nM for 5-HT_{2C}). The selective 5-HT_{2C} antagonist SB-242084 (av. K_i [K_i database]: ~2.85 for 5-HT_{2C}; ~505 nM for 5-HT_{2A}), on the other hand, did not affect HTRs evoked by a 1.5-mg/kg dose of 25CN-NBOH. Interestingly, it slightly increased the behavioural effect of the higher 3.0-mg/kg dose of the agonist. Thus, it might be suggested that low to medium doses of 25CN-NBOH primarily interact with 5-HT_{2A}R; but higher doses of 25CN-NBOH may also recruit 5-HT_{2C}Rs, thereby interfering with the actions mediated by 5-HT_{2A}Rs (compare for DOI: Fantegrossi et al., 2010). The finding that 3.0 mg/kg 25CN-NBOH given to mice reach brain concentrations that fall into the range of the agonist's in vitro affinity for 5-HT_{2C}Rs (Jensen et al., 2017b), would be in line with such an interpretation.

25CN-NBOH induced HTRs were facilitated when animals had been thoroughly habituated to the experimental procedures on the day before the drug application. The latter finding supports observations made in rats, where the presence of familiar littermates allowed hallucinogens to evoke substantially more HTRs than when observed in isolation (Buchborn et al., 2015). The dependence of 5-HT_{2A}R-related behaviour in rodents on what may be described as state-environment interaction, mirrors characteristics of human psychedelia, which is critically determined by set and setting, i.e., psychological constitution and environmental circumstances (see Hartogsohn, 2016; Carhart-Harris et al., 2018). 25CN-NBOH induced HTRs occurred within <1min after i.p. injection, reached maximal

frequencies within 2–5min, and declined to half-maximal at ~11min (compare for 25I-NBOMe and 25I-NBMD: Halberstadt and Geyer, 2014). In contrast to the i.p. route, s.c. administration—as shown for the 1.5-mg/kg dose—was associated with a slight delay in peak and somewhat longer duration to half-maximum. 25CN-NBOH, both in plasma and brain, reaches maximum concentrations within 15min, where it remains relatively stable until at least 30min post-application (Jensen et al., 2017). The fast decline in the behavioural response observed here therefore does not seem to be a consequence of fast drug clearance. One possible explanation for the apparent disconnect between 25CN-NBOH's brain concentration and behavioural output is the development of acute tolerance. Indeed, DOI which shares the phenethylamine backbone of 25CN-NBOH, internalises and desensitises 5-HT_{2A}Rs within 15–20 min *in vitro* (Porter et al., 2001; Raote et al., 2013).

To follow up on the possibility of tolerance development, this study applied 25CN-NBOH (1.5 mg/kg) twice at a 1.0- or a 1.5-h interval and found a rapid loss of responsiveness to the second injection (compare for LSD and DOI: Darmani and Gerdes, 1995; Buchborn et al., 2016). To test the hypothesis that the observed tachyphylaxis was due to substance accumulation (i.e., two single 1.5 mg/kg doses accumulating to a less active 3.0-mg/kg dose), the experiment was repeated with a 0.75-mg/kg dose (which after thorough animal habituation induced significant HTRs; see Figure 5-1 A) injected twice at a 1.5-h interval. If the two 0.75 mg/kg doses had accumulated to form a 1.5-mg/kg dose, the second dose should have induced unchanged or more HTRs than the first. However, as the 0.75-mg/kg regimen led to a similar-degree tachyphylaxis as did the 1.5-mg/kg dose, it is unlikely that drug accumulation is the underlying cause for the rapid tolerance development. Instead processes of 5-HT_{2A} regulation might play a role; indeed, DOI applied twice at a 4-h interval *in vivo*, desensitises 5-HT_{2A} mediated glutamate release in the cortex (Scruggs et al., 2003). Finally, the present study investigated subchronic tolerance as it would develop with a once-a-day application of 25CN-NBOH (1.5 mg/kg, i.p.) over 4 days. HTRs steadily decreased (e.g., compare for DOI and 2C-T-7: Smith et al., 2014), with the most pronounced loss of responsiveness exhibited in between the first days. The extent of tolerance could be substantially augmented by applying a second (higher) daily dose of 25CN-NBOH (3.0 mg/kg, i.p.) at an ~8-h interval.

5.6.2. Physiological effects

At 37°C pad temperature, 25CN-NBOH induced a slight increase in neck-arterial pulse distention, a measure of local tissue perfusion that parallels changes in mean arterial blood pressure (Hete et al., 2013; Olivera et al., 2010). Pulse oximetry is largely driven by strong-pulse arteries. Being the most dominant neck arteries with immediate pulse from the heart-imminent aortic arch (Ruberte et al., 2017), the collar-clip based pulse distention measurement most likely reflects haemodynamics of the carotid arteries. The effect of 25CN-NBOH on neck-arterial blood flow is in line with diverse reports of psychedelics increasing blood pressure (Dedeoğlu and Fisher, 1991; Dolder et al., 2017; Huang and Ho, 1972; Wolbach et al., 1962a), possibly resulting from vasoconstriction and increased total peripheral resistance (Chaouche-Teyara et al., 1994). The rise in arterial distension was more

pronounced when 25CN-NBOH was applied at elevated pad temperature. Similarly, the 25CN-NBOH induced rise in body temperature only occurred at 41, not at 37 °C. Psychedelics evoke hyperthermia (e.g., Jacob and Lafille, 1963), particularly when primed by environmental heat load (Buchborn et al., 2016). The effects of 25CN-NBOH on body core temperature and pulse distention found here to be temperature-dependent suggests a close relationship. Indeed, psychedelic-induced hyperthermia (like hypertension) is thought to engage 5-HT_{2A} related vasoconstriction in the cutaneous bed, which interferes with heat dissipation (Blessing and Seaman, 2003). Increased body temperature might drive carotid blood flow, favouring output to external over internal branches in relation to the brain's thermoregulatory needs (Hellstrom et al., 1996; Baker et al., 1982).

The present study found 25CN-NBOH-evoked tachycardia that sustained at physiological pad temperature but quickly reversed at elevated pad temperature. Mirroring this observation, psychedelics have been demonstrated to increase heart rate both in humans (DiMascio et al., 1957; Dolder et al., 2017; Strassman and Qualls, 1994) and anaesthetised animals (Knowles and Ramage, 1999; Ramage et al., 1993; Friedman et al., 1978). Reports of psychedelics increasing the heart rate of animals appear more controversial, with several reports of bradycardia (Anderson et al., 1995; Chaouche-Teyara et al., 1994; Tadepalli et al., 1975), mixed effects or lack of effects (Dabiré et al., 1989; Dedeoğlu and Fisher, 1991). The controversy in the literature might partially arise from the adrenergic pharmacodynamics of the psychedelics used (Ray, 2010). Indeed, the bradycardic effect of the first-generation selective 5-HT_{2(A)} agonist DOM shows sensitivity to 5-HT_{2A} as well as adrenergic antagonism (Tadepalli et al., 1975). Beyond unselectivity, however, conflicting results might also be reconciled with regard to two opponent counterforces downstream of 5-HT_{2A}. To this end, tachycardia might be due 5-HT_{2A} mediated sympathetic cardiac nerve output (Anderson et al., 1995; Saxena and Villalón, 1990). On the other hand, bradycardia might result from 5-HT_{2A} mediated hypertension inviting the baroreceptor reflex to cancel out the sympathetically driven chronotropy (Ramage et al., 1993; Tadepalli et al., 1975). In line with the proposed opponency, this study found that the tachycardic effect of 25CN-NBOH was reversed at elevated pad temperature and as pulse distention increased.

Apart from its effect on pulse distention and heart rate, 25CN-NBOH facilitated the development of bradypnea, which mirrors findings for DOI (Khater-Boidin et al., 1999; Rose et al., 1995) and might be due to 5-HT_{2(A)} mediated inhibition of phrenic nerve activity (Lalley, 1982). Interestingly, phrenic inhibition induced by the psychedelic drug, 5-MeO-DMT, could be overcome by ambient heat (King and Holtman, 1990). This is consistent with the rapid reversal of 25CN-NBOH's bradypneic effect at increased pad temperature. Blood oxygen saturation was not significantly altered by 25CN-NBOH. Therefore, the observed respiro-/cardiovascular effects of the drug do not seem to reflect changes in functional arterial haemoglobin availability.

5.6.3. *Conclusions*

In conclusion, this study demonstrated that the selective 5-HT_{2A} agonist 25CN-NBOH induced a dose- and time-dependent, ketanserin-sensitive increase in HTRs in mice, which was subject to short-interval and subchronic tolerance. Future research, correlating HTRs with pharmacokinetic-/dynamic adaptations at various time points during tolerance development might provide insight as to a possible mechanism. Furthermore, making use of new technologies to image cortical activities in awake behaving mice using genetically encoded, cell-class specifically targeted voltage and calcium indicators (Carandini et al., 2015; Song et al., 2017; Knöpfel et al., 2015) might take our mechanistic understanding of the underlying processes from basic receptor pharmacology to systemic circuit dynamics.

The present findings also show that 25CN-NBOH induces a temperature-dependant increase in heart rate, decrease in breath rate, and increase in arterial blood flow, as measured by pulse oximetry drawn from brain-supplying (most likely carotid) arteries. 25CN-NBOH is the most selective 5-HT₂ agonist at present, with robust preference for 5-HT_{2A} over 5-HT_{2B} and 5-HT_{2C} and lack of appreciable affinity for adrenergic receptors (Hansen, 2010; Halberstadt et al., 2016; Jensen et al., 2017a). Given the primary relevance of 5-HT_{2A}Rs for the psychedelic principle along with the immediacy of neck-arterial blood flow to the brain's metabolic demands, the present results might contribute to a more systemic understanding of how psychedelic-5-HT_{2A} interactions translates into the brain (haemo-)dynamics characteristic for psychedelia.

5.6.4. *Future work: prospective optical imaging experiments*

Previous brain slice experiments have shown that psychedelics increase the frequency of spontaneous excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents in cortical layer 5 pyramidal neurons. Therefore, it appears that psychedelics recruit glutamatergic and GABAergic neurons to produce enhanced excitatory and inhibitory recurrent network activity (Lambe and Aghajanian, 2007). In addition to the fact that layer 5 pyramidal neurons directly synapse onto inhibitory interneurons, research has shown that 5-HT_{2A}Rs are also densely expressed on interneurons (Puig et al., 2010; Celada et al., 2013; Wood et al., 2012). Rather than being directly glutamatergic, psychedelics could be modulating the excitability of neuronal masses by modulating the gain of excitatory and inhibitory populations that contain 5-HT_{2A}Rs.

Electrophysiological studies in rodents have shown that the psychedelic-induced stimulation of 5-HT_{2A}Rs results in the decoupling of pyramidal neuron discharge from the rhythmic oscillations of larger neuronal populations in the PFC (Celada et al., 2008; Wood et al., 2012). This is consistent with a MEG study in humans showing that psilocybin induces broadband desynchronisation of cortical oscillatory rhythms and reduces oscillatory power in regions corresponding to the DMN (Muthukumaraswamy et al., 2013). Given the large proportion of layer 5 pyramidal neurons and interneurons expressing 5-

HT_{2A}Rs in the PFC, the effects of psychedelics on cortical networks may derive from a depolarising action in such a large neuronal proportion that it alters the association between individual states and population measures; this net disruption in population activity thereby decreases cortical rhythmicity and oscillatory power, thus disorganising PFC network activity (Celada et al., 2013; Celada et al., 2008). The PFC seems to exert a top-down control of neuronal activity in subcortical structures via descending excitatory axons (Miller and Cohen, 2001); thus, the psychedelic-induced alterations in PFC network activity may subsequently alter the activity of other brain areas.

Using fMRI measures in humans, psilocybin has been shown to decrease cerebral blood flow, venous oxygenation and functional connectivity in the major connector hubs of the brain (e.g. thalamus, PCC and ACC) as well as decrease positive coupling between the PCC and vmPFC (Carhart-Harris et al., 2012a). In contrast, human studies using PET have shown psilocybin-induced increases in blood supply and glucose metabolism in most cortical regions (Vollenweider et al., 1997a). It is unclear whether the timing differences between these measures play a role in this controversy, as PET measures are averaged over a longer period of time relative to fMRI. In an attempt to bridge this paradox, it has been suggested that the reduced BOLD activity reflects the desynchronisation of oscillatory rhythms while the PET metabolic increases correspond with the increased excitability of layer 5 pyramidal neurons (Halberstadt, 2015). On the other hand, parvalbumin-expressing, fast spiking interneurons have high-energy consumption and are among the most metabolically demanding cells in the central nervous system, which may account for psychedelic-induced metabolic increases observed with PET imaging.

As mentioned previously, hemodynamic methods to image brain function are indirectly sensitive to neuronal activity due to their vascular nature, limiting their interpretability and quantification. Widely used methods (such as BOLD fMRI) are further complicated by their opposite sensitivity to CBF, CBV and cerebral oxidative metabolism (Buxton et al., 1998; Davis et al., 1998; Kim et al., 1999). Significant progress has been made in determining the properties of hemodynamic responses (Hillman, 2014; Kida and Hyder, 2006), yet fundamental questions remain. For instance, the relative contribution of excitatory and inhibitory neuron activity to hemodynamic responses and metabolic load is unclear, and the impact of increases in inhibitory neuron activity on vascular and metabolic responses is even less clear. Arguments for increases and decreases in hemodynamic signals have been proposed with increases in inhibitory activity (Boorman et al., 2015; Devor et al., 2008; Shmuel et al., 2006; Stefanovic et al., 2004). Unveiling the contributions of excitatory and inhibitory activity to neurovascular and neurometabolic coupling is a vital and necessary step towards understanding hemodynamic signals as well as their sensitivity to brain function and dysfunction at the neuronal level.

In vitro evidence suggests that psychedelics enhance the excitability of layer 5 pyramidal neurons (Aghajanian and Marek, 1999; Aghajanian and Marek, 1997; Marek et al., 2001), yet *in vivo* research suggests an overall net inhibition of pyramidal neuron population activity (Abi-Saab et al., 1999; Zhang et al., 2010; el Mansari and Blier, 1997; Bergqvist et al., 1999; Puig et al., 2010). As mentioned above, a paradox also exists when different neuroimaging methods are employed in human research (i.e., PET techniques suggest an excitatory effect whereas fMRI techniques suggest inhibition in cortical regions).

The growing body of preclinical evidence showing that 5-HT_{2A}R stimulation increases GABAergic transmission (Shen and Andrade, 1998; Scruggs et al., 2003; Abi-Saab et al., 1999; Zhang et al., 2010; Ashby et al., 1990; Marek and Aghajanian, 1996; el Mansari and Blier, 1997; Bergqvist et al., 1999) may help to explain the psychedelic paradox. However, a validation of how psychedelics act on pyramidal neurons and interneurons via 5-HT_{2A}Rs to alter excitatory and inhibitory neural circuitry in the intact brain has never been performed.

Optogenetic monitoring involves the use of genetic methods to target activity indicators—proteins that transduce neuronal activity into optical signals for recording—to defined cell classes. Optogenetic indicators include genetically encoded calcium (GECI) and voltage (GEVI) indicators. Whereas GECIs give an indirect readout of neural activities, GEVIs directly measure changes in membrane potential with subthreshold sensitivity and are capable of detecting inhibitory electrical inputs (Knopfel, 2012). GEVI-based approaches allow the efficient detection of neural ensembles, network properties and orchestrated activities as well as the underlying circuitry (Antic et al., 2016). Therefore, using GEVI-expressing transgenic mice enables an *in vivo* investigation of the effects in excitatory and inhibitory neuronal circuits induced by 25CN-NBOH, a psychedelic that is the most selective agonist ligand for the 5-HT_{2A}R yet discovered. A cell-type specific and circuit-centric investigation of the effects of psychedelics on brain activity holds exciting opportunities and has never been performed.

In order to design later voltage imaging experiments, the present study performed a series of behavioural pharmacology experiments using the HTR as a behavioural readout of psychedelia. A dose of 1.5 mg/kg 25CN-NBOH was found to induce the greatest HTR frequency (Figure 5-1) and is thus chosen as the most effective dose for future voltage imaging experiments. As 25CN-NBOH was found to have a very rapid onset of action and relatively short duration of action (Figure 5-2), the drug will be administered s.c. in the head-fixed animal when already in the voltage imaging setup to capture the onset of the drug effects. As 25CN-NBOH was found to induce tachyphylaxis (Figure 5-3 A), the drug will not be reapplied during voltage imaging experiments. In addition, 25CN-NBOH was found to induce tolerance (Figure 5-3 B), providing considerations for chronic imaging sessions. By taking a reverse translational approach, a deeper understanding can be obtained in transgenic mice and used to inform the findings obtained in humans. Thus, the proposed future work set out above holds great impactful potential (see Appendix E for methods of planned future optical imaging project).

6. GENERAL DISCUSSION

“We build too many walls and not enough bridges.”

– Isaac Newton

6.1. OBJECTIVES OF THIS THESIS

Our understanding of psychedelic drug action and implications for neuroscience and psychiatry has rapidly developed over recent years with the growth of psychedelic research. Yet despite impressive progress, fundamental questions remain. Accordingly, the present work outlined in this thesis set out to address some of these important knowledge gaps. For instance, a wealth of evidence indicates that psychedelics have therapeutic potential based on their ability to induce positive psychological changes that persist long-term (see Carhart-Harris and Goodwin, 2017; Rucker et al., 2018; Schenberg, 2018), and elucidation of their acute and post-acute brain effects is currently underway (e.g. Carhart-Harris et al., 2012a; Carhart-Harris et al., 2016c; Carhart-Harris et al., 2017d; Lebedev et al., 2016; Roseman et al., 2016; Vollenweider and Kometer, 2010). Yet, the potential for psychedelics to induce long-term brain changes has not been sufficiently studied. Determining post-psychedelic long-term brain effects and neurobiological mechanisms underlying positive psychological change would hold significant value – not just for the field of psychedelic science, but for the wider fields of neuroscience and mental health. To this end, the key site of action for psychedelic drugs – the 5-HT_{2A}R – is considered an important drug target with potential implications of both agonists and antagonists in the treatment of various psychiatric conditions (Quesseveur et al., 2012; Buchborn et al., 2014; Ross et al., 2016; Mascher, 1967; Carhart-Harris et al., 2017a; Carhart-Harris et al., 2016a). Yet, in addition to their expression in the brain, 5-HT_{2A}Rs are widely expressed across the vascular system (Cohen, 1988; Ullmer et al., 1995). This complicates matters as our knowledge of the neurophysiological correlates of the human psychedelic brain state largely stems from BOLD-fMRI techniques, which indirectly measure neuronal activity by extraction of blood-flow related parameters. As such, the potential 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action deserves particular experimental scrutiny as it may bring the validity of the acute brain findings into question. Taken together, these uncertainties inspired the various works that make up this thesis.

Providing further inspiration for the studies presented here, the REBUS model of psychedelic drug action was recently developed in an attempt to explain the acute and enduring effects occasioned by psychedelic drugs in humans (Carhart-Harris and Friston, 2019). The REBUS model theorises that psychedelics induce an acute state of heightened brain entropy via their 5-HT_{2A}-mediated activity (Carhart-Harris, 2018), which reflects relaxation of the precision weighting of priors that coincides with the increased weight of bottom-up signalling; this allows bottom-up information to have more influence on conscious experience and on the configuration of the hierarchy overall (Carhart-Harris and Friston, 2019). The REBUS model postulates that the effects of psychedelics on the precision weighting of high-level priors or beliefs may mediate the therapeutic efficacy of these compounds. As the REBUS model states that high-level priors may be sensitised to liberated bottom-up information flow, combining the belief relaxation process with the right intention, care provision and context may help cultivate the revision of such entrenched pathological priors. The REBUS model has the potential to greatly aid our understanding of psychedelic drug action, yet substantial research efforts are required to test the model's theories, determine its validity and refine the model as required.

6.2. FINDINGS & CONTRIBUTION TO THE FIELD

The findings of Chapter 3 (STUDY 1) suggest that the psychologically supportive administration of psilocybin remediates negative cognitive biases characteristic of severe depression. In line with the REBUS model, the presence of a pessimism bias in these patients indicates excessive precision, weight, or influence of this prior, and its alleviation indicates that the psilocybin treatment induced belief relaxation and recalibration processes. A key strength of Chapter 3 is that it presents the first study to use a behavioural instrument to more objectively measure optimism/pessimism in the context of psychedelic research. As well as being the first to address psychedelic-induced changes in biases that are not purely subjective and self-referential, this is also the first study to analyse the effects of a psychedelic compound on cognitive biases in patients with severe depression. To this end, the results presented in Chapter 3 substantially extend findings of previous research demonstrating increased optimism following psychedelic drug use, which have tended to focus on healthy participants via use of subjective measures (Barbosa et al., 2009; Carhart-Harris et al., 2016b; Griffiths et al., 2008; Griffiths et al., 2011).

Given the remediation of negative cognitive biases found in Chapter 3 and previous research showing enduring post-psychedelic increases in optimism in healthy participants (Smigielski et al., 2019; Carhart-Harris et al., 2016b), it's surprising that Chapter 4 did not replicate this well-established finding – i.e. in Chapter 4, no change in optimism was observed following psilocybin treatment measured via the LOT-R. It should be noted that Chapters 3 and 4 differed greatly in the populations studied (i.e. depressed patients vs healthy participants) and measures of cognitive bias utilised (i.e. POFLE vs LOT-R). However, one possible explanation for the discrepancy with previous research in healthy participants (Smigielski et al., 2019; Carhart-Harris et al., 2016b) is the ceiling effect, as the majority of participants in Chapter 4 scored high for optimism at baseline; this renders little chance for a significant increase after the intervention. Nevertheless, REBUS and the findings of Chapter 3 prompted key questions: (i) If psychedelics facilitate the relaxation of prior assumptions, does this result in heightened openness and receptivity to new perspectives (i.e. the precondition for psychological insight)? And if so, (ii) does psychological insight mediate the positive long-term outcomes that psychedelics can induce? Notably, insightfulness during the psilocybin experience was revealed to be predictive of long-lasting clinical improvements in a larger study of the same sample of TRD patients discussed in Chapter 3 (Carhart-Harris et al., 2017a).

The ability of psychedelics to facilitate psychological insight is a central principle of psychedelic therapy. This is largely based on the notion that psychedelic (“mind-manifesting”) drugs permit latent or unconscious aspects of the mind to rise into conscious awareness. To this end, REBUS theorises that an entropic psychedelic brain with reduced confidence in high-level priors may result in the attainment of psychological insight and novel perspectives by disinhibiting latent (lower-levels of the hierarchy) contents of the mind, thus permitting their emergence into conscious awareness. These ideas raised the following questions: (i) Is heightened brain complexity (entropy) during the acute psychedelic state

positively correlated with insight? (ii) Does insight occur only in a state-like fashion during the acute psychedelic state, or can it crystallise in the weeks following a psychedelic experience? (iii) Is the occurrence of psychological insight, either acutely or after the psychedelic session, a key mediator of long-term positive outcomes?

In support of the REBUS model, Chapter 4 presents the first study to demonstrate a direct association between insightfulness and heightened complexity of spontaneous brain activity (i.e. entropic brain) during the peak intensity of a high-dose psilocybin experience – thus potentially revealing a neural basis for this phenomenon. For the first time, this study also demonstrates that psychological insight crystallises in the days to weeks following a high-dose psychedelic experience, and that this *sustained* facilitation of insight may mediate post-psychedelic long-term positive outcomes. To this end, the findings of Chapter 4 support and extend the notion that insight, as a key component of psychedelic therapy (and now extended to include sustained facilitation of post-psychedelic insight), may contribute to enduring positive change. Relatedly, the results also show post-psilocybin changes in other psychological and behavioural constructs not previously investigated in the long-term following a single high-dose psychedelic session (e.g. sustained changes in cognitive flexibility, experiential avoidance and prosocial constructs such as compassion, gratitude, empathy and social connectedness).

The REBUS model theorises that the psychedelic brain enters an entropic “hot state” that is more sensitive to context and facilitates synaptic efficacy and plasticity (Ly et al., 2018; Carhart-Harris and Friston, 2019). In support of this, 5-HT_{2A}R signalling plays an important role in conditions of uncertainty by enhancing environmental sensitivity and adaptability, particularly in situations of crisis (Carhart-Harris and Nutt, 2017). Yet the REBUS model goes further to suggest that, when the acute psychedelic effects begin to subside, the brain returns to its usual regimen of efficient free-energy minimisation and this is reflected by a renewed sense of familiarity and assuredness (Carhart-Harris and Friston, 2019). Importantly however, the brain may not return in its entirety to how it was before due to the window of opportunity for psychedelics to induce persistent changes in the brain as a result of this period of elevated plasticity (Ly et al., 2018). Given the period of elevated plasticity (Ly et al., 2018) and sensitivity to context during the psychedelic “hot state” (Carhart-Harris and Friston, 2019) combined with evidence of positive psychological outcomes in the long-term following a psychedelic experience (see Carhart-Harris and Goodwin, 2017; Elsey, 2017; Carhart-Harris et al., 2017c; Johnson et al., 2019: for reviews), the proposition that psychedelics may induce long-term brain effects is not unlikely. Accordingly, Chapter 4 explored the following key research questions: (iii) Can psychedelics induce long-term brain effects, and if so, do they correlate with positive psychological outcomes? (iv) What is the impact of contextual factors on the quality of the acute psychedelic experience and what is the impact of experiential and post-experiential integration components on the quality of the outcomes?

Chapter 4 presents the first study to unveil sustained brain effects one month after a psychedelic experience in healthy, psychedelic naïve participants. Notably, these persistent brain effects were predicted by psychedelic peak experience phenomena and were also found to correlate with positive

and sustained psychological changes. The results of Chapter 4 highlight the importance of developing a strong therapeutic alliance in the context of psychedelic research and suggests that, in addition to the psychological effects, enduring brain effects of psilocybin may be *experience-dependent*. These findings support and extend a basic principle of the psychedelic treatment model, which posits that the quality of the acute experience mediates long-term therapeutic outcomes (now extended to include long-term *neural* outcomes). Further, the findings of Chapter 4 also support the notion that the psychedelic brain enters an entropic “hot state” that is more sensitive to context and facilitates plasticity (Ly et al., 2018; Carhart-Harris and Friston, 2019), and emphasises the importance of contextual and experiential components of psychedelic administration. Crucially, psychological insight – both acutely and as it crystallises in the days to weeks following a high-dose psilocybin experience – was identified as a mechanism that may modulate positive psychological change. As such, in addition to highlighting the importance of context and quality of experience, the results of Chapter 4 also stress the importance of providing a supported post-psychedelic integration session to facilitate further insight and positive change in the long-term. Taken together, the novel results presented here provide support for notions of the REBUS model and considerably broaden the field’s knowledge of the acute and long-term psychological and brain effects of psilocybin.

6.2.1. *Contribution to our understanding of psychedelic vasoactivity*

Science is currently gaining an initial understanding of the neural correlates of the psychedelic brain state, yet our knowledge of these acute brain effects largely feeds from blood-flow based BOLD-fMRI and PET research. This is problematic, as the abundant expression of 5-HT_{2A}Rs across the vascular system (Cohen, 1988; Ullmer et al., 1995) calls the validity of these findings into question. To this end, the final chapter of this thesis attempts to further our understanding of the 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action using the most 5-HT_{2A}-selective psychedelic yet discovered, 25CN-NBOH. To provide an alternative (direct) methodology for elucidating psychedelic effects on neural dynamics, the final chapter characterised the behavioural pharmacology of 25CN-NBOH and used these findings to design a future optogenetics study of the psychedelic brain state.

The results of Chapter 5 demonstrate temperature-dependant increases in heart rate, decreases in breath rate and increases in arterial blood flow from brain-supplying (most likely carotid) arteries following administration of 25CN-NBOH in mice. Given the primary relevance of 5-HT_{2A}Rs in psychedelic drug action along with the immediacy of neck-arterial blood flow to the brain’s metabolic demands, the results of Chapter 5 contribute to our understanding of how 5-HT_{2A}-mediated vasoactivity translates into the brain (haemo-)dynamics characteristic of psychedelia. As these findings show a 5-HT_{2A}-mediated effect of psychedelics on the vascular system, then the validity and interpretations of acute psychedelic brain findings obtained using blood-flow based techniques may be called into question. Chapter 5 therefore highlights an important caveat of using blood-flow based neuroimaging techniques to examine the acute psychedelic brain state. Accordingly, the results of Chapter 5 support the use of EEG – a *direct* measure of neuronal activity – during the acute dosing sessions in Chapter 4

(STUDY 2); with fMRI used four weeks post-dosing only, long after the drug has been cleared from the body.

Given the issues with current approaches, Chapter 5 demonstrates the suitability of 25CN-NBOH for prospective *in vivo* optical imaging studies in mice. This provides an alternative (direct) methodology that is sensitive to both neuronal and haemodynamic activity for studying the acute brain effects of psychedelics. Importantly, making use of new technologies and cell-type specific and circuit-centric approaches (Carandini et al., 2015; Song et al., 2017; Knöpfel et al., 2015) to image cortical activities in awake behaving mice might take our mechanistic understanding of the underlying processes from basic receptor pharmacology to systemic circuit dynamics. To this end, taking a reverse translational approach enables us to go beyond what is possible in humans, and may help uncover the mechanistic basis for the therapeutic effects of psychedelics as well as many neuropsychiatric disorders.

6.3. RELEVANCE & WIDER IMPACT

Psychedelics are potentially incredibly powerful tools for understanding normal brain function. For example, psychedelics offer an exciting opportunity for studying the neural substrates of consciousness and their profound ego dissolving effects informs our understanding of the neural mechanisms underlying how one forms a coherent sense of self (Vollenweider et al., 1997a; Tagliazucchi et al., 2016; Muthukumaraswamy et al., 2013; Lebedev et al., 2015; Carhart-Harris et al., 2016c). To this end, the findings of Chapter 4 elucidated a potential neural basis for insight processes. In addition to their usefulness for understanding normal brain function, psychedelics further our understanding of the serotonin system as well as the mechanisms underlying neuropsychiatric disorders. Further, psychedelics have shown promise in the treatment of various psychiatric disorders, including difficult to treat disorders where current approaches are failing – e.g. treatment-resistant depression (Carhart-Harris et al., 2016a; Carhart-Harris et al., 2017b) and addictions (Abuzzahab and Anderson, 1971; Bogenschutz et al., 2015; Krebs and Johansen, 2012). Importantly, experiential components of psychedelic therapy appear to mediate some of the positive clinical outcomes in patient populations, highlighting an important caveat in the ‘pharmaceutical medication only’ treatment approach in mental health care. This is further supported by the findings presented in Chapter 4, where peak experience phenomena during the high-dose psilocybin session predicted not only psychological outcomes, but also changes in resting-state functional connectivity one month later. Moreover, the enduring facilitation of insightfulness was found to critically mediate positive psychological outcomes. Understanding the mechanisms underlying the therapeutic effects of psychedelics has significant impactful potential in the field of mental health treatment. Notably, Chapter 4 presented potential neural correlates of enhanced well-being and social connectedness in healthy participants following a high-dose psilocybin experience.

The present findings suggest potential for psychedelic treatment in a range of disorders that are typically difficult to treat. This is promising given the failure of current approaches in the field of mental health care. The findings of Chapter 3 indicate that the psychologically supportive administration of psilocybin remediates negative cognitive biases characteristic of severe depression – giving patients a clearer, more accurate outlook on their future unfettered by excessive pessimism. Considering the REBUS model, this indicates that psilocybin treatment induced belief relaxation and recalibration processes. With this in mind, psychedelics may show great promise in the treatment of disorders characterised by entrenched pathological beliefs, such as eating disorders, OCD and addiction. Additionally, the findings of Chapter 4 indicate that psilocybin has the potential to alter prosociality in the long-term. For instance, empathy, compassion, gratitude and social connectedness were increased one month after the high-dose psilocybin session. To this end, psilocybin may show promise in the treatment of antisocial behaviours and disorders characterised by impaired social functioning – e.g. psychopathy.

In addition to improving our understanding of 5-HT_{2A}-mediated vasoactivity intrinsic to psychedelic drug action, the findings of Chapter 5 highlight an important consideration relevant to the wider field of neuropsychiatry. Namely, the issue of blood-flow based neuroimaging studies of diseased brain states, where the disorder is characterised by dysfunctional serotonergic processes. To this end, dysfunctional serotonergic transmission in neuropsychiatric disorders may produce vascular effects that confound the neuroimaging data, thus bringing the validity into question. This potentially suggests that the knowledge base on the neural mechanisms underlying such disorders (if obtained via indirect neuroimaging techniques) may be incorrect, which has serious implications for our current theories and treatment strategies.

Psychedelics have unique potential as tools to study the brain and show great promise as a treatment model for a variety of mental health disorders. Given that current treatment approaches are failing to adequately address the global crisis of mental health, the present findings are promising and significantly extend the scope of disorders that may be potentially treated with psychedelics. This thesis also highlights potentially serious implications for the use of indirect measures of neuronal functioning to assess brain states characterised by alterations in serotonergic transmission. Taken together, this thesis presents novel findings that are extremely relevant to the wider field of mental health treatment.

6.4. FUTURE WORK

The work presented here set out to address some important knowledge gaps in the field of psychedelic research. With important limitations considered¹⁹, this thesis presents novel findings that substantially contribute to the field's knowledge and understanding of psychedelic drug action. In accordance with this novelty, I encourage future research to replicate and confirm the validity of the results presented

¹⁹ Please refer to the limitations of each study discussed in the corresponding study chapters.

here. Beyond this, future research may wish to consider a large study in a patient population (such as severe depression) incorporating the following: (i) extensive study of insight to investigate its relationship with brain entropy and role in mediating positive clinical outcomes in the long-term (extending the findings of Chapter 4); (ii) an investigation of pathological beliefs among the chosen patient population, acutely during the psychedelic “hot state” and also how these potentially change long-term post-psychedelic treatment (extending the findings of Chapters 3 and 4); and (iii) an investigation of acute and long-term brain effects of psychedelics and how these relate to the clinical outcomes (extending the findings of Chapters 3 and 4). To this end, a future clinical study combining an investigation of pathological priors, psychedelic-induced psychological insight, post-treatment psychological and clinical outcomes, and acute – preferably using a direct measure of neuronal activity (based on the findings of Chapter 5, which do not support the use of blood-flow based neuroimaging techniques to measure acute psychedelic brain states due to 5-HT_{2A}-mediated vasoactivity), such as MEG or EEG – and long term neuroimaging in a patient population would considerably extend the findings of this thesis.

Further to the suggestions outlined above and in the relevant discussion sections of each Chapter, this thesis highlights constructs that may be manipulated by psychedelic treatment and encourages future clinical trials in disorders where psychedelics may have potential. The findings of Chapter 3 indicate that psilocybin may remediate negative cognitive biases in severe depression, thus inspiring future work to assess the efficacy of psilocybin treatment for other disorders characterised by pathological beliefs. Examples include eating disorders, addiction and OCD, as these disorders may rest on particularly rigid high-level priors that may be potentially amenable to revision with psychedelic therapy. Chapter 4 highlights a variety of social effects that endure long-term after a psilocybin experience. Although performed in healthy participants, these findings encourage future work to assess the efficacy of psychedelics to treat disorders characterised by impaired social functioning or antisocial behaviour – for example, Social Anxiety Disorder or psychopathy. Chapter 4 also demonstrates improved cognitive flexibility, psychological well-being and experiential avoidance long after a high-dose psilocybin session, thus inspiring future work to assess the efficacy of psychedelics on disorders characterised by dysfunction in these constructs. With this in mind, Autism Spectrum Disorder (ASD) could be interesting to explore in the context of psychedelic research – i.e., given the promising effects of psilocybin shown in Chapter 4 on several constructs relevant to deficits in ASD, including impairments in social functioning, cognitive inflexibility and experiential avoidance. Support for this stems from recent animal work, where psilocybin was found to rescue social behavioural abnormalities present in a murine model of ASD (Mollinedo-Gajate et al., 2020). Further, early studies in the 1960s reported promising results using LSD to treat autistic children, albeit with significant methodological and ethical flaws (for a critical review, see Sigafos et al., 2007).

6.5. CONCLUSIONS

Testing notions of the recently developed REBUS model of psychedelic drug action was the key backbone of the objectives presented in Chapters 3 and 4 of this thesis. Taken together, these chapters provide support for the REBUS model's theory that an entropic brain in the acute psychedelic state may encourage the attainment of psychological insight and facilitate plasticity processes, which may mediate long-term changes in deeply held prior assumptions, psychological functioning and the brain. The findings of the Chapters 3 and 4 inspire future work to combine an investigation of pathological priors, psychedelic-induced psychological insight, post-treatment psychological and clinical outcomes, and acute and long term neuroimaging in a patient population. Combining the approaches presented here would hold significant value and would considerably extend the findings of this thesis. Chapter 5 provides considerations for the suitability of indirect measures of acute psychedelic-induced neuronal activity by extraction of blood-flow related parameters. Furthermore, an alternative methodology rooted in the optogenetics approach – which is sensitive to both neuronal and haemodynamic activity – is proposed for prospective investigations of the brain effects of psychedelics (see Appendix E). By taking a reverse translational approach, unveiling the effects of psychedelics with cell-type and circuit specificity in mice might inform the mechanisms underlying the therapeutic effects of psychedelics and many neuropsychiatric disorders in humans. To this end, an optogenetic investigation of the effects of psychedelics on brain activity holds exciting opportunities.

In conclusion, this thesis provides insights on post-psilocybin changes in pathological beliefs in severely depressed patients, the acute and long-term effects of psilocybin on the brain and psychology of healthy humans, and 5-HT_{2A}-mediated vasoactivity in mice. Given the relative failure of current treatment approaches to treat psychiatric disorders, the results presented here have significant real-world relevance to society in addressing the global crisis of mental health. Notably, this thesis also highlights potentially serious implications for the use of indirect measures of neuronal functioning to assess brain states characterised by alterations in serotonergic transmission – which has considerable relevance to the wider field of neuropsychiatry, beyond the context of psychedelics. In addition to the therapeutic potential of the psychedelic treatment model, advancing our understanding of psychedelics is important for the development of new therapeutics as it increases our understanding of the mechanisms underlying their clinical efficacy. Accordingly, this thesis used a multifaceted approach – i.e. behavioural pharmacology and pulse oximetry in rodents as well as multimodal neuroimaging and a broad repertoire of psychological and behavioural assessments in humans – to comprehensively study the effects of psychedelics. The novel results presented here considerably advance our understanding of psychedelic drug action and provide inspiration for future work.

“The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability.”

– Thomas H Huxley

7. REFERENCES

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has been assessed as having satisfactorily completed accredited training for personnel working under the Animals (Scientific Procedures) Act 1986

Modules: **One, Two, Three & Four**

Species: **Rat, Mouse, Guinea Pig, Rabbit & General Principles**

Training organised by: **CBS Imperial College**

Certificate Number: **IMP/16/011** Date: **22/01/2016**

Course Organiser For the Royal Society of Biology

This is not a licence to perform procedures under the Animals (Scientific Procedures) Act 1986



Incorporated by Royal Charter
Registered Charity No: 277981

Title and Name: Ms Taylor Lyons Tel: 07738823430 PIL Details: 15B5A6C
Department: Medicine email: t.lyons15@imperial.ac.uk

SECTION BELOW SHOULD BE COMPLETED WHEN CONFIRMATION OF COMPETENCE IS REQUIRED

<u>Date</u>	<u>Species / age</u>	<u>Schedule 1 Method</u>	<u>Detail's (living / cadaver / other)</u>	<u>Training detail record</u>
08/03/16	Mice / Adult	1 st Method Rising concentration of CO ₂	Living	Taylor has successfully completed an assessment in the culling of 2 Adult mice using CO ₂ and confirmation by Cervical dislocation. Taylor was able to use the CO ₂ machine firstly checking that it was clean, that it had been vented before putting the animals into the chamber (opening the vents) after she checked there was power to the unit and enough CO ₂ to complete the cycle. She placed the cage of mice (Home cage) onto the floor of the chamber, removed the cages bottle and plastic lid and then closed the vents and lid of the chamber. After starting the cycle Taylor explained that she would stay with the animals until both cycles had been completed in case there were any
		Confirmation method Cervical dislocation		

Phil Rawson
(Assessor)

CBSF023

Version 2 Effective Date: 11/09/15

Review Date: 09/17

				problems. After the cycles Taylor removed the mice, checked for any signs of life (breathing, pinch test) and one happy the mice were dead, carried out the cervical dislocation as the confirmation method by manual means. Taylor had carried this out to a good standard where she had separated the spine and the surrounding connective tissue. She the bagged and disposed of the bodies. Taylor then opened the lower vent and cleaned the chamber ready for the next user. Taylor is now competent in culling mice using CO2 followed by cervical dislocation as the confirmation method.	
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Building and room number if Schedule 1 takes place outside the CBS facility:

Review Date (3 years from date of competency): March 2019

Assessor - Name and Position within CBS: Phil Rawson Senior tech/ Assessor

SECTION BELOW SHOULD BE COMPLETED ONLY WHEN TRAINING IS REQUIRED (NEW TECHNIQUE).

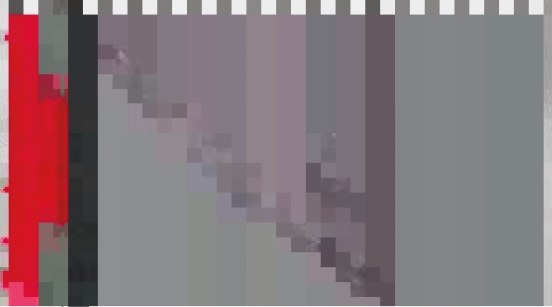
As supervisor, I am responsible for the training and supervision up and until competency is confirmed

Title/Name: Prof Knöpfel
Department: Medicine

Tel: 2016
e-mail: tknopfel@knopf-elab.net

Thomas Knöpfel

Signed by Supervisor **Date:** 14. March

<u>Date</u>	<u>Species / age</u>	<u>Schedule 1 Method</u>	<u>Detail's (living / cadaver / other)</u>	14 <u>Training detail record</u>	
07.03.2016	Mouse Adult	1 st Method Rising concentration of CO2 Confirmation method Cervical Dislocation	Living	Taylor prepared the CO2 station, checking the cylinder and power supply. Ensuring the chamber was drained of any excess CO2 from the previous user. The animals were placed into the chamber. The machine was then put into an 8 minute cycle. The animals were then left for a further 2 minutes. Once completed the animals were checked for signs of life via pedal reflex for front and hind limbs. Cervical dislocation was then performed. This was done confidently and correctly. I am happy for Taylor to be assessed for this method of schedule 1. The cylinder was turned off and the chamber cleaned and drained.	
		1 st Method Confirmation method			

Once training has been completed and assessed as competent this record needs to be uploaded to your A-tune profile and techniques added. A copy kept for your personal training records. Please be aware training is ongoing and you need to ensure that you are always using the most relevant and refined method. Any changes to your work please ask for re assessment.

APPENDIX D: Record of training and assessments – competent at performing intraperitoneal injections (IP drug administration) and transcardial perfusions in mice.

TRAINING RECORD

Name: Taylor Lyons

One sheet per technique and per species / age or weight. Please ensure all details are completed.

Procedure to be performed. IP mouse

COMPETENCY LEVEL	Working Under Supervision	Competence Assessed	Competence as Trainer
Date	Trainer Name & Signature	Trainer Name & Signature	Trainer Name & Signature
1. 23/09/2016	Click here to enter name	Savvas Piperelis DVM, PhD ,MRCVS	Click here to enter name
Taylor collected the mice that required an IP injection. Prepared the needle and syringe using the most refined size in accordance with recommendations (smaller than 25g) and suitable volume in accordance with LASA or under PPL authority is part of the project. Collected the mouse that was to be injected and placed it on a non-slip surface to restrain. Once happy with the restraint collected the needle / syringe and inserted it at a good position and angle in the lower abdominal quadrant and injected it at a good speed not too fast or slow and once injection complete returned the mouse to the cage if empty or to a separate cage if other animals are to remain in the home cage, then disposed of the needle safely. STATUS: competent to perform this procedure in live animals			
2. Click here to enter a date.	Click here to enter name	Click here to enter name	Click here to enter name
Click here to enter details of training performed /what type of training, i.e.: video, live or cadaver.			

CBSF.4.011A

Version 4 Effective Date: 15/07/16

Review Date: 07/18

TRAINING RECORD

Name: Taylor Lyons

One sheet per technique and per species / age or weight. Please ensure all details are completed.

Procedure to be performed. Perfusion fixation in the mouse under injectable anaesthesia

COMPETENCY LEVEL	Working Under Supervision	Competence Assessed	Competence as Trainer
Date	Trainer Name & Signature	Trainer Name & Signature	Trainer Name & Signature
1. 23/09/2016	Click here to enter name	Savvas Piperelis DVM, PhD ,MRCVS	Click here to enter name
Today, I assessed Taylor performing this technique in the live animal. She induced anaesthesia with an ip injection of pentobarbital. She observed the mouse until the pedal withdrawal reflex was lost before proceeding with the perfusion. She accessed the heart quickly again demonstrating very good tissue handling and experience in this procedure. She was careful to refine the procedure as much as possible, for example ensuring animals were not kept in the same room even when work is carried out under the hood. Taylor achieved good perfusions using his technique. STATUS: She is competent to perform trans cardiac perfusions under injectable terminal general anaesthesia in adult mice.			
2. Click here to enter a date.	Click here to enter name	Click here to enter name	Click here to enter name
Click here to enter details of training performed /what type of training, i.e.: video, live or cadaver.			

CBSF.4.001

Version 3 Effective Date: 12/07/16

Review Date: 07/18

APPENDIX E: Prospective optical imaging of acute and longer-term effects of 25CN-NBOH

Design of the Yellow-Red Chimeric Butterfly Voltage Sensitive Fluorescent Protein and DNA constructs

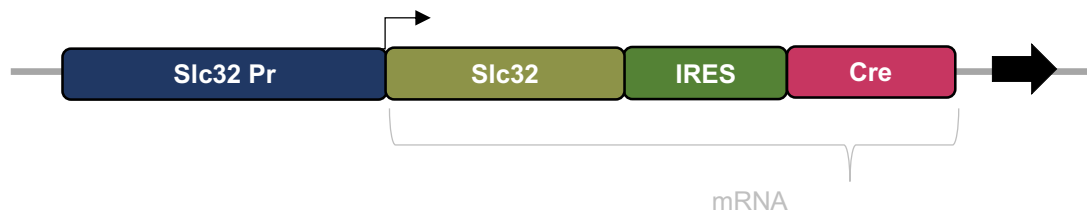
Chimeric Butterfly Voltage Sensitive Fluorescent Protein Yellow-Red (ChiVSFP Butterfly YR) is a GEVI design with a fluorescence resonance energy transfer (FRET) pair of fluorescent proteins (mCitrine/mKate2; YR) that is based on a combination of previously published VSFPs, Chimera C5 and VSFP-Butterfly 1.2 (Mishina et al., 2014). To generate ChiVSFP Butterfly YR, the voltage-sensing domain (VSD) of *Ciona intestinalis* voltage-sensor-containing phosphatase sequence was replaced with the sequence of the S4 transmembrane segment of the Kv3.1 potassium channel VSD (Mishina et al., 2014). A single mutation (K234R of mKate) was also introduced by site-directed mutagenesis for reduced intracellular aggregation and increased brightness (Mishina et al., 2014).

Plasmid encoding the DNA sequence for ChiVSFP Butterfly YR (pCAG Chimeric_Butterfly_YR_1.0) was obtained from Thomas Knöpfel (Addgene plasmid #59801; Mishina et al., 2014). The Knöpfel laboratory generated the tetO-ChiVSFP transgenic mice by subcloning the coding sequences for ChiVSFP Butterfly YR into pCAG vectors using NheI and AflIII restriction endonucleases, thus placing ChiVSFP's expression under the control of the tetracycline response element (TRE, tetO; Mishina et al., 2014). All constructs were verified by DNA sequencing analysis.

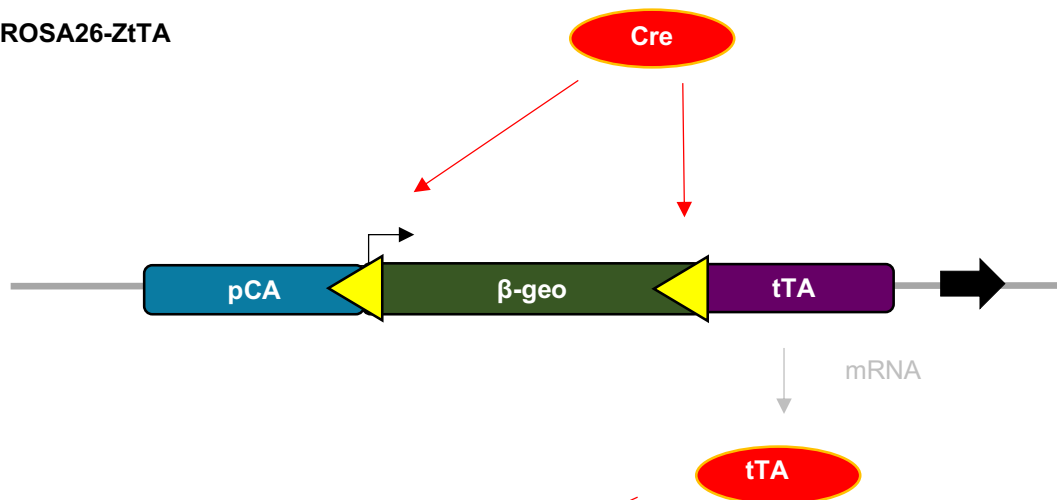
Generation of the transgenic mouse line expressing the ChiVSFP Butterfly YR in GABAergic interneurons

Three genetically modified mouse strains were crossed to generate triple transgenic mice expressing the ChiVSFP Butterfly YR in GABAergic interneurons: (i) VGAT-IRES-Cre knock-in mice (Jax Stock #016962) which have Cre-recombinase expression directed to inhibitory GABAergic neurons, (ii) ROSA26-ZtTA knock-in mice (Jax Stock #012266), which harbour a conditional mutation of the Gt(ROSA)26Sor locus with a loxP-flanked stop cassette preventing transcription of the downstream tetracycline-controlled transactivator protein (tTA), and (iii) TetO-ChiVSFP transgenic mice (generated and provided by Cris Niell, University of Oregon), which express the ChiVSFP Butterfly YR under the control of a tetracycline-responsive promoter element (TRE or tetO). The Cre-mediated recombination events result in the deletion of the floxed sequences and the subsequent expression of tTA in GABAergic interneurons. The resulting tTA protein is able to bind to TRE, which then drives the expression of the ChiVSFP in the plasma membrane of GABAergic interneurons (Figure 8-1). Following this genetic strategy, offspring that express the ChiVSFP Butterfly YR only in cortical inhibitory interneurons can be generated (VGAT;ZtTA;ChiVSFP).

(1) VGAT-IRES-Cre



(2) ROSA26-ZtTA



(3) TetO-ChiVSFP

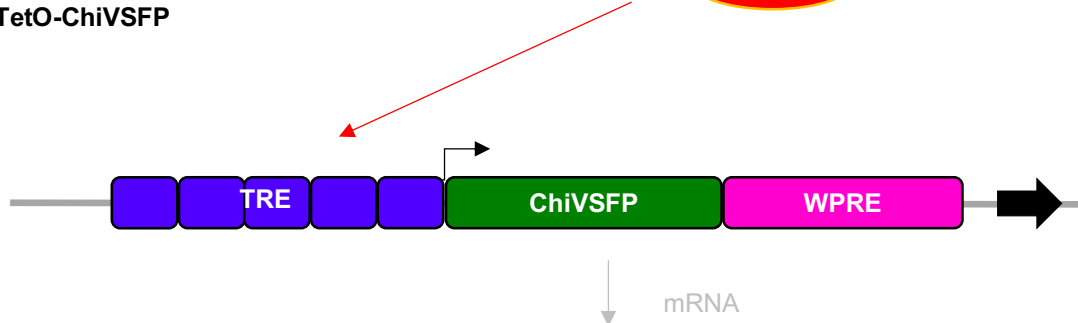


Figure 8-1 Genetic strategy. | Expression cassettes of the three mouse strains that were crossed to generate of triple transgenic mice expressing ChiVSFP in GABAergic interneurons. Abbreviations: β -geo, beta-geo transgene; Cre, cre recombinase; IRES, Internal Ribosome Entry Site; mRNA, messenger ribonucleic acid; pCA, chicken beta-actin promoter with cytomegalovirus enhancer; Slc32, solute carrier family 32; TRE, tetracycline response element; tTA, Tetracycline-responsive transactivator; VGAT, Vesicular GABA transporter; WPRE, Woodchuck Posttranscriptional Regulatory Element.

Transcardial perfusion, brain extraction, slice preparation and confocal microscopy

To validate the expression of ChiVSFP Butterfly YR in GABAergic interneurons, VGAT;ZiTA;ChiVSFP mice were terminally anaesthetised with pentobarbital sodium and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 118, NaHCO₃ 25, NaH₂PO₄ 1, KCl 3, MgCl₂ 1, CaCl₂ 2 and glucose 10) followed by 2% paraformaldehyde in 0.1 M PB (pH 8.0). Brains were extraction and post-fixed in the same fixative for 1 h, then transferred to 0.1 M PBS and stored at 4°C. The cortex was sectioned into 300 µm coronal slices with a Vibratome (VT 1000S, Leica). The native fluorescence expression was examined using a confocal laser-scanning microscope (TCS SP5 II, Leica Microsystems) and processed using Leica Application Suite Advanced Fluorescence (LAS AF) software.

Development of a behavioural habituation protocol for in vivo optimal imaging experiments

Prior to *in vivo* optical imaging in awake mice, training should be completed so that the mice become habituated to remaining still in the imaging setup, head-fixation, the experimenter's hands, sounds in the experimental room, and other factors. To this end, mice were handled prior to head-fixation habituation, then recovered from decreasing levels of light sedation under head-fixation for up to four sessions without sensory stimulation. Animals were then head-fixed awake for increasing durations for up to six additional habituation sessions without sensory stimulation. These procedures reduce experimental stress in the mice due to familiarity with the procedures, thus promoting animal welfare and enabling the collection of high-quality optical imaging data via reduced motion and other artefacts.

Habituation typically took place on successive days (with the exception of weekends) in mice that had already been fitted with a metal head-plate for head-fixation via cranial window implantation surgery. All mice were allowed to recover for at least 7 days after surgery prior to behavioural habituation. The steps outlined below should be adhered to on each habituation day.

Day 1. Acclimatisation to the experimenter's hands and experimental room. Begin by allowing the mice to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle each animal for 5 – 10 min to enable acclimatisation to the experimenter.

Day 2. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Administer pentobarbital (40 mg/kg, i.p.) and wait for the drug effects to commence (~15 min). Ensure the room is dark and head-fix the mouse in the optical imaging setup for 40 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Day 3. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Administer pentobarbital (35 mg/kg, i.p.) and wait for the drug effects to commence (~15 min). Ensure the room is dark and head-fix the mouse in the optical imaging setup for 45 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Day 4. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Administer pentobarbital (30 mg/kg, i.p.) and wait for the drug effects to commence (~15 min). Ensure the room is dark and head-fix the mouse in the optical imaging setup for 50 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Day 5. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Administer pentobarbital (25 mg/kg, i.p.) and wait for the drug effects to commence (~15 min). Ensure the room is dark and head-fix the mouse in the optical imaging setup for 55 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Day 6. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Do not administer pentobarbital. Ensure the room is dark and head-fix the mouse in the optical imaging setup for 60 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Days 7-11. Head-fixation training. Begin by allowing the mouse to acclimatise to the experimental room for 20 min. Following acclimatisation to the environment, gently handle the mouse for 5 min to enable acclimatisation to the experimenter. Do not administer pentobarbital. Ensure the room is dark and head-fix the mouse in the optical imaging setup for 60 min. During head-fixation, periodically check for the well-being of the animal and comfort as required. Repeat this procedure for subsequent mice if habituation is required for more than one animal.

Days 9-11 (optional). If planned voltage imaging experiments involve s.c. drug application within the imaging set-up, implement s.c. saline injections in addition to the steps described above for days 7-11.

Study design for prospective optical imaging project

Transgenic mice ($N=12$; $n=4$ per group) will have ChiVSFP Butterfly YR targeted to (i) pyramidal neurons in all cortical layers ($N=4$), (ii) interneurons in all cortical layers ($N=4$), and (iii) pyramidal neurons in cortical layer 5. The electrical activity will be measured (Figure 8-2) in these specific circuitries of cells before, during and after 25CN-NBOH (1.5 mg/kg, s.c., based on the results of dose-response experiments described in later sections) in the same mice for each condition. Resting-state brain activity will be functionally imaged and chronic imaging (7 days) will take place to elucidate any possible enduring effects (Figure 8-2).

Voltage imaging: An epi-illumination system will be used to monitor the FRET between the mCitrine-mKate2 fluorophore pair (Akemann et al., 2010). The mCitrine will be excited with a blue LED light or with a tungsten-halogen lamp passed through a bandpass filter. A dichroic mirror will divert the excitation light onto the cortex of the mouse. The emitted fluorescence from mCitrine (the donor) will be reflected by a second dichroic mirror, passed through an emission filter, and collected by a charged-coupled device (CCD) or scientific complementary metal oxide semiconductor (sCMOS) camera. The emitted fluorescence from mKate2 (the acceptor) will be transmitted by the second dichroic mirror, passed through an emission filter, and collected by a second CCD or sCMOS camera. Principal component analysis (PCA) of the mCitrine and mKate2 intensities will then be used to extract voltage and hemodynamic signals (Akemann et al., 2012; Knopfel, 2012).

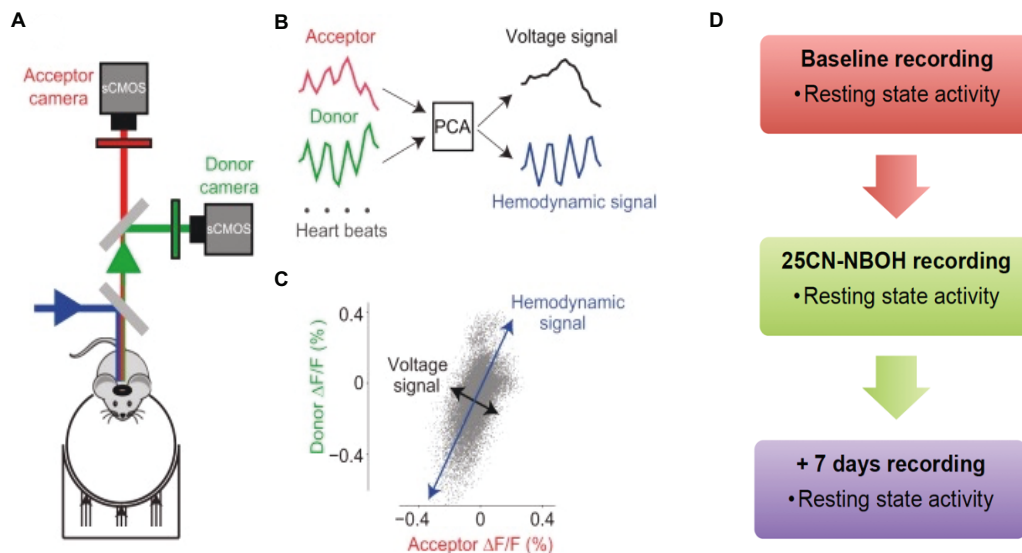


Figure 8-2. Prospective optical imaging. (A–C) Analysis of circuit dynamics at the macroscopic level, reproduced from Carandini et al. (2015). (A) Experimental setup for *in vivo* voltage imaging using the FRET-based ChiVSFP Butterfly YR in mice. The fluorescence emission from the ChiVSFP Butterfly YR is split by a dichroic mirror into donor (mCitrine) and acceptor (mKate2) colour channels and imaged by two synchronized CCD or sCMOS cameras. (B) PCA of the mCitrine-mKate2 intensities will then be used to extract voltage and hemodynamic signals, (C) by projecting the donor and acceptor intensities onto the two principal axes of variability (hemodynamic signals, blue; voltage signals, black). Dots represent the relative fluorescence ($\Delta F/F$) of different

pixels at different moments in time. **(D) Schematic diagram of the *in vivo* voltage imaging experiments.** The electrical resting state activity will be measured in cortical pyramidal neurons before, during and after 25CN-NBOH in the same mice for each condition. (A-C) Copyright information: Creative Commons Attribution 4.0 International License (CC-BY). Abbreviations: PCA, principal component analysis; sCMOS, scientific complementary metal oxide semiconductor camera