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" We can think of the world as made up of things. Of substances. Of entities. Of something that is. Or we can think of it as made up of events. Of happenings. Of processes. Of something that occurs. Something that does not last, and that undergoes continual transformation, that is not permanent in time. "

Carlo Rovelli, The Order of Time, 2017.

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Biomarkers of Mood Disorders and Ketamine's Antidepressant Effect:
a neuropsychopharmacological approach to test current hypotheses of depression

Abstract

Mood disorders are rising globally amid radical changes in the ecosystem, economic uncertainties, and social inequalities. These psychiatric conditions, characterized by dysfunctional emotional responses to external stimuli, profoundly impact individuals' daily lives. Among them, depressive disorders stand out as a prevalent and debilitating condition, ranking second in terms of disease burden worldwide. For decades, the development of new pharmacological interventions to address the mental health epidemic had mainly produced insignificant variations of old drugs with limited efficacy. By the turn of the millennium, the resurgence of scientific interest in hallucinogenic psychoactive compounds represents one of the most promising avenues for research within the field of psychiatry. This development was marked by the discovery of ketamine's rapid and long-lasting antidepressant properties. However, the aetiology of depressive disorders and the mechanisms underlying ketamine efficacy remain poorly understood. Current models suggest that the manifestation of depressive symptoms stems from neurophysiological alterations. Key theories include the "serotonin (5-HT) deficiency", "excitatory/inhibitory imbalance", and "neural atrophy" hypotheses. Psychoactive substances like ketamine, serving both as interventions and tools to perturb brain function, offer an ideal neuropsychopharmacological model to examine these hypotheses and explore novel therapeutic mechanisms in humans. The present thesis presents three original research studies utilizing psychoactive compounds in conjunction with state-of-the-art neuroimaging methods to test current neurobiological theories of the aetiology of depression. Furthermore, it incorporates a narrative review summarizing available evidence on the acute and subacute mechanisms of action of ketamine. In the first study, a direct assessment of the "5-HT deficiency" theory of depression was achieved using an amphetamine challenge to probe the state of the 5-HT system imaged through the Positron Emission Tomography (PET) radioligand [^{11}C]Cimbi-36 in patients with depression and healthy subjects. The results yielded compelling evidence indicating a reduced 5-HT release capacity in individuals with depression compared to healthy controls. Moreover, the study uncovered an intriguing difference in baseline 5-HT type 2A (5-HT2A) receptor availability between depressed patients and control subjects. Within the patient group, higher 5-HT2A receptor availability was associated with lower connectedness, a psychological trait of an individual's connection with themselves, others, and the environment. In the second study, the acute neurophysiological effects of the novel rapid-acting antidepressant ketamine were investigated using a portable EEG apparatus in a population of hospitalized patients with bipolar depression. The administration of ketamine resulted in notable alterations in rhythmic and arrhythmic components of the EEG signal, suggesting an underlying shift in cortical excitability in accordance with the "excitatory/inhibitory imbalance" hypothesis of depression. Remarkedly, the magnitude of the modulation of EEG parameters induced by ketamine, specifically of the arrhythmic components, was found to differentiate between early and late responders to ketamine therapy. The third study examined the neuroplastic effects of ketamine using the PET tracer [^{11}C]-UCBJ to image synaptic density in healthy individuals. Contrary to initial expectations, robust evidence for an increase in neuroplasticity was not observed. However, a trend toward an increase was reported, which exhibited a correlation with acute and subacute psychological outcomes. Given the considerable body of pre-clinical evidence supporting the neuroplasticity-promoting effects of ketamine, the absence of significant results in the study may be attributed to methodological limitations inherent to the applied technique or dosing regimen and individual variability in drug response. Overall, the multifaceted findings obtained from the various studies, which implicate different neurotransmitter systems at a brain-wide level, indicate that depression is characterized by alterations across numerous brain regions and circuits. This highlights the need for a non-reductionist and multidisciplinary approach to studying brain function and dysfunction. Notably, consistent results across the three studies underscore the crucial role of variability and individual predisposition in response to psychoactive drugs. This variability manifests in various ways: from differences in the state of the 5-HT system between healthy individuals and patients, and within depression endophenotypes, to the distinct neurophysiological response to ketamine treatment observed between early and late responders, and the

relative insensitivity to neuroplastic changes of ketamine in healthy subjects. The concept of individual variability adds another layer to the well-recognized factors of "set" and "setting" in influencing therapeutic outcomes of psychoactive medications. If the neurophysiological and therapeutic trajectories triggered by psychoactive medications vary based on individual brain architecture, mental state, and physical and interpersonal environment (both current and past), then a purely biological explanation of depression and antidepressant response is inadequate. Achieving a comprehensive understanding of mental well-being requires a unified framework that integrates biological, psychological, and environmental factors — a bio-psycho-social model.

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U.S.	United States	3
MDE	Major Depressive Episode	4
MDD	Major Depressive Disorder	4
BP	Bipolar Disorder	4
TR-MDD	treatment-resistant MDD	4
TR-BP	treatment-resistant BP	4
NIBS	non-invasive brain stimulation	4
CNS	central nervous system	5
TCA	tricyclic antidepressant	5
MAOI	monoamine oxidase inhibitor	5
5-HT	serotonin	5
SERT	serotonin transporter	5
MAO	monoamine oxidase	5
GPC	G protein-coupled	5
SSRI	serotonin reuptake inhibitors	5
LSD	lysergic acid diethylamide	6
N,N-DMT	N,N-Dimethyltryptamine	6
MDMA	Methylenedioxymethamphetamine	6
PCP	phencyclidine	6
i.v.	intravenous	6
FDA	food and drugs administration	6
NMDA	N-methyl-D-aspartate	7
GABA	γ-aminobutyric acid	7
MEG	magnetoencephalography	10
EEG	electroencephalography	10
MRI	magnetic resonance imaging	10
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fMRI	functional MRI	11
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CBF	cerebral blood flow	11
d-amphetamine	dextro-amphetamine	14
Tph2	tryptophan hydroxylase 2	14
STOP	stable tubule only polypeptide	14
5-HT2A	5-HT type 2A	14
PD	Parkinson's Disease	15
MDPD	MDD due to PD	15
HC	healthy controls	15
MINI	Mini-International Neuropsychiatric Interview	15
HAM-D	Hamilton Depression Rating Scale	17
BDI	Beck Depression Inventory	17
SHAPS	Snaith-Hamilton Pleasure Scale	17
SSAI	State Anxiety inventory	17
RS	Rumination scale	17
WEMWBS	Warwick-Edenborough Mental Wellbeing Scale	17
WCS	Watts Connectedness scale	17
VAS	visual analogue scale	17
HPLC	high-performance liquid chromatography	17

MP-RAGE	magnetization prepared rapid gradient echo	17
MNI152	standard stereotactic space	17
ROIs	regions of interest	17
CIC	Imperial College London Clinical Imaging Centre	17
VT	volumes of distribution	17
MA1	multilinear analysis-1	18
BP_{ND}	non-displaceable binding potential	18
ANOVA	Analysis of Variance	18
ANCOVA	Analysis of Covariance	18
d	Cohen's d	18
BF	Bayes Factor	18
FDR	false discovery rate	18
VMAT2	vesicular monoamine transporter type 2	26
5-HIAA	main metabolite 5-Hydroxyindoleacetic acid	27
PTSD	post-traumatic stress disorder	31
HK	hydroxyketamine	31
HNK	hydroxynorketamine	31
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	31
HCN	hyperpolarization-activated cyclic-nucleotide-gated	31
BDNF	brain-derived neurotrophic factor	32
mTOR	Mammalian target of rapamycin	32
TrkB	Tropomyosin receptor kinase	32
PFC	pre-frontal cortex	32
Drd1	dopamine receptor D1	33
ACC	anterior cingulate cortex	33
DMN	default-mode network	33
CEN	central executive network	33
NSPC	neural stem and progenitor cells	36
DG	dentate gyrus	36
VTA	ventral tegmental area	37
LTP	long term potentiation	37
LTD	Long term depression	37
EPSP	evoked excitatory post-synaptic potential	38
CA3-CA1 synapses	CA3-Schaffer collateral pathway to CA1-stratum radiatum	38
HFS	High-frequency stimulation	38
NRG1	neurotrophic factor Neuregulin-1	38
IPSC	inhibitory post-synaptic current	38
NAc	nucleus accumbens	39
DCM	dynamic causal modelling	39
ERP	evoked-response potential	39
MMN	mismatch negativity	39
SN	salience network	39
IEGs	immediate-early genes	40
Arc	activity-regulated cytoskeleton-associated protein	40
Homer1a	Homer protein homolog 1	40

ERK	extracellular-signal regulated kinase	40
PSD	post-synaptic density proteins	40
HDAC5	histone deacetylase 5	40
eEF2	eukaryotic translation elongation factor 2	40
CADSS	Clinician-Administered Dissociative States Scale	44
BPRS	Brief Psychotic Rating Scale	45
ASCQ	Altered States of Consciousness Questionnaire	45
FFT	Fast Fourier Transform	45
ING	interneuron network gamma	46
PING	pyramidal interneuron network gamma	46
PV	parvalbumin-expressing	46
PLE	power-law exponent	47
LZc	Lempel-Ziv complexity	47
TMS	transcranial magnetic brain stimulation	48
MADRS	Montgomery-Åsberg Depression Rating Scale	48
OP	eyes open	50
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T0	time of recruitment	50
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ICA	independent component analysis	50
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IRASA	Irregularly Resampled Auto Spectral Analysis	51
PLEhf	PLE high-frequency	51
PLElf	PLE low-frequency	51
CSER	Complexity via State-space Entropy Rate	51
PLEbeta	PLE high beta	57
DTI	diffusion tensor imaging	71
SV2A	synaptic vesicle protein 2A	71
AD	Alzheimer's disease	72
BPRS	Brief Psychiatric Rating Scale	73
MOAA/S	Modified Observer's Assessment of Alertness/Sedation	73
STAI Y-2	State-Trait Anxiety inventory for Adults Y-2	73
POMS	Profile of Mood States Short Form	73
MODTAS	Modified Tellegen Absorption Questionnaire	73
MPFI	Multidimensional Psychological Flexibility Inventory	73
FFMQ	Five Facets of Mindfulness Questionnaire	73

BEAQ	the Brief Experiential Avoidance Questionnaire	73
5D-ASCQ	5-Dimensional ASCQ	74
MEQ	Mystical Experience Questionnaire	74
CEQ	Challenging Experiences Questionnaire	74
PSI	Psychotomimetic States Inventory	74
EBI	Emotional Breakthrough Inventory	74
EDI	Ego Dissolution Inventory	74
POB	plasma-over-blood	74
BP	binding potential	74
FP	free-fraction corrected	74
CS	centrum semiovale	74
tDCS	transcranial direct current stimulation	88
tACS	alternating current stimulation	88
tRNS	random noise stimulation	88
TI	temporal interference	88

1. Chapter 1: Introduction

1.1. History of mental health disorders

The study of abnormal behaviours has long been a focus of societal interest, leading to varied attempts aimed at understanding and addressing them or exerting control over them. Societies across different epochs have developed diverse approaches, ranging from ancient rituals to contemporary therapeutic interventions, in response to behaviours deemed deviant. Arguably, the classification of behaviour as normal or abnormal is contingent upon contextual factors and evolves over time and across cultures. In modern Western societies, abnormal behaviour is usually evaluated based on its potential threat to an individual or their interpersonal relationships within the community (i.e., functionalist view). Those illnesses characterized by behavioural abnormalities lacking evident physiological origins are commonly referred to as mental disorders. Throughout history, three main theories have emerged regarding the aetiology of mental illnesses: supernatural, psychological, and biological (Figure 1) [1]. Supernatural theories attribute abnormal behaviours to phenomena such as possession by evil spirits, divine displeasure, celestial events, curses, and moral transgressions. Psychological theories emphasize the role of traumatic experiences, maladaptive learning, impaired cognition, subconscious mechanisms, and perceptual alterations in the development of mental disorders. Biological theories focus on disruptions in organism functioning stemming from illness, genetic factors, or brain dysfunction. The understanding of the causes of mental illness is pivotal in shaping societal attitudes and responses towards affected individuals, as it influences the provision of care and treatment for mental health conditions.

One of the earliest examples of supernatural explanations for mental illness is the archaic practice of trephination. Archaeological evidence, including prehistoric skulls and cave art dating back to approximately 6500 BC, reveals instances of surgical drilling of holes in skulls. This procedure was undertaken to address head injuries, epilepsy, and to ostensibly release evil spirits believed to be trapped within the cranium [2]. Interestingly, during the 1960s and 1970s, the practice resurfaced among certain proponents of the counterculture movement who saw it as a means to achieve higher states of consciousness [3]. In indigenous cultures, shamanic rituals traceable as far back as 5700 years BC, represent one of the oldest and still prevalent mental and spiritual healing practices. These rituals typically entail the shaman or healer consuming psychoactive plants to facilitate communication with spiritual dimensions, enabling the identification of the causes of illness and appropriate remedies [4]. Around 2700 BC, Chinese medicine introduced the concept of complementary positive and negative bodily forces, known as "yin and yang" attributing mental (and physical) illness to an imbalance between these forces. Consequently, achieving a harmonious life conducive to the proper balance of yin and yang, along with the smooth flow of vital energy, was deemed essential for maintaining mental health [5]. Interestingly, this perspective resonates with contemporary Western medicine's growing recognition of the significance of holistic approaches to mental well-being. Throughout classical antiquity, supernatural explanations such as demonic possession or divine retribution were commonly invoked to account for behaviours deemed abnormal and beyond the individual's control. Healing practices often involved temple attendance for religious ceremonies, exorcisms, and incantations aimed at invoking divine intervention [1].

Theories of mental health conditions rooted in physiology trace back to Mesopotamian and Egyptian papyri from 1900 BC, describing women suffering from mental distress resulting from a wandering uterus, defined as hysteria by the Greeks [6]. Classic Greek physicians notably rejected supernatural attributions for mental disorders. It was circa 400 BC that Hippocrates endeavoured to delineate a clear demarcation between superstition, religion, and medicine. He proposed a systematic framework positing that imbalances in the essential bodily fluids, known as "humours", underlie both physical and mental ailments. Hippocrates further categorized mental illness into four distinct types: epilepsy, mania, melancholia, and brain fever. The concept of humourism persisted as a prevalent physical theory of mental health conditions until the 19th century. Notably, the Greek physician Galen (AD 130-201) introduced the first notion of psychological explanations for mental illness, acknowledging mental stress as a potential causal factor for behavioural abnormalities [1].

By the late Middle Ages, Christianity was the dominating religion and supernatural theories of mental disorders again dominated Europe, fuelled by natural disasters like plagues and famines that were

interpreted as brought about by the Devil. Superstition, astrology, and alchemy took hold, and common treatments for mental illnesses included prayer rites, relic touching, confessions, and atonement. In the 13th century, individuals afflicted by mental disorders, particularly women who were often labelled as witches, faced persecution from the Church. This persecution stemmed from the belief that these individuals were possessed by the Devil and involved in practices associated with black magic [1].

It was not until the 16th century that governmental institutions started housing and confining individuals deemed mentally ill, as well as those classified as poor, homeless, unemployed, or criminal, in isolated structures called asylums. Many of these individuals were involuntarily institutionalized and subjected to squalid living conditions, often restrained and exhibited to the public for entertainment purposes. Somatogenic views of mental disorders predominated, leading to treatments such as restraints, invasive electro-convulsive shock therapy, and lobotomies, practices that persisted until the late 1970s. Protests against the deplorable conditions in mental asylums began to emerge in the 18th century, spurring a shift towards a more humanitarian perspective on mental illness. In 1785, Italian physician Vincenzo Chiarughi initiated reforms at St. Boniface Hospital in Florence, Italy, by removing patients' chains and advocating for improved hygiene, recreational activities, and vocational training [7]. Similar reforms were introduced in France, England, and other nations during the same period. However, psychiatry during the late 18th and 19th centuries grappled with the tension between physical and psychological explanations of mental illness. Sigmund Freud played a pivotal role in resolving this dispute in favour of psychological explanations. Through techniques such as hypnosis and dream analysis, Freud pioneered the cathartic method, laying the groundwork for psychoanalysis in the first half of the 20th century, termed the century of neurosis. Psychoanalysis emerged as the dominant treatment modality for mental illness during this period, spawning various schools of psychotherapy, including behavioural, cognitive, cognitive-behavioural, and psychodynamic approaches [1]. The post-World War II pharmaceutical revolution heralded the advent of medications for mental illness, giving rise to the field of psychopharmacology. The serendipitous discovery of effective medications for mental disorders led to a resurgence of physical theories of mental health rooted in neurobiology and pharmacology. This convergence of pharmacological and neurobiological advancements revolutionized the understanding and treatment of mental illness in the latter half of the 20th century [8].

These developments were paralleled by progress in diagnostic methodologies. While the concept of diagnoses dates back to ancient Greek times, it wasn't until 1883 that German psychiatrist Emil Kräpelin considered the godfather of modern psychiatry, introduced a comprehensive framework for categorizing psychological disorders [9]. Kräpelin's system focused on identifying patterns of symptoms, or syndromes, indicative of an underlying physiological basis for the disorder. Various clinicians had proposed their own classification systems before Kräpelin's work, and the need for a unified, standardized approach paved the way for the American Psychiatric Association's publication of the first Diagnostic and Statistical Manual (DSM) in 1952 [10]. The DSM represented a significant milestone in the field of mental health, providing a common language and framework for diagnosing psychological disorders, thereby facilitating improved communication among practitioners and enhancing the reliability and validity of clinical research.

The DSM has undergone several revisions since its inception, occurring in 1968, 1980, 1987, 1994, 2000, and 2013. The 1980 version, known as DSM-III, introduced a significant change by implementing a multiaxial classification system. This system aimed to provide a comprehensive assessment of the individual, considering not only specific problem behaviours but also broader aspects of their functioning, giving origin to the functionalist view of mental health of modern times [10]. While the DSM has provided a necessary shared language for clinicians it is not without limitations. The DSM is based on clinical and research findings from Western culture, primarily the United States (U.S.). It is also a medicalized categorical classification system that assumes disordered behaviours do not differ in degree but in kind, as opposed to a dimensional classification system that would put mental disorders along a continuum. Critically, the DSM relies solely on symptom assessment by clinicians and does not integrate data from various biological levels of analysis, such as neurophysiological data, to explore fundamental dimensions of functioning across

clinical populations [11]. In summary, despite their long history and widespread occurrence, the understanding of mental disorders still exists within a limited conceptual framework.

1.2. Unipolar and bipolar depression

Emil Kraepelin's initial formulation of mental disorders delineated two primary categories: "manic-depression" and "dementia praecox" which align with the modern classification of "mood disorders" and "psychotic disorders" conditions, respectively [12]. In modern Western societies, mood disorders are experiencing a significant and concerning trend of global increase in the face of a rapidly changing socioeconomic landscape, characterized by urbanization, economic uncertainty, and social inequality [13]. Mood disorders manifest as clinically significant disturbances in emotional response to external stimuli, often leading to maladaptive behaviours. This spectrum of disorders profoundly affects individuals' emotional states, cognitive functions, and daily functioning. Among those, depressive disorder, commonly referred to as depression, emerges as one of the most prevalent and debilitating mental health conditions globally. Approximately 5% of adults globally experience depression, with higher prevalence rates among specific demographic groups, such as women and the elderly [14]. The burden of depression is further compounded by its association with comorbidities, such as cardiovascular diseases, and a significant risk of suicide, which ranks as the fourth leading cause of death among adolescents and young adults. Significant treatment gaps exist in the population, particularly in low- and middle-income countries, where over 75 % of affected individuals receive no treatment [15]. Barriers to effective care include insufficient investment in mental health infrastructure, a shortage of trained healthcare professionals, and pervasive social stigma surrounding mental health disorders. Furthermore, even among those who receive standard-of-care antidepressant treatment, only 30% achieve complete remission. These factors, together with the ever-increasing number of diagnoses of clinically relevant symptoms of depression and increasing requests for treatment, make depressive disorder the 2nd in terms of disease burden worldwide according to the Global Burden of Disease Study based on years lived with disability [14]. Given the immense disease burden associated with depression and the limitations of current treatment approaches, there is an urgent need for a deeper understanding of its underlying aetiology.

During a depressive episode, individuals typically experience a constellation of symptoms characterized by a depressed mood, which may manifest as feelings of sadness, irritability, or emptiness, along with a loss of interest or pleasure in previously enjoyable activities, known as anhedonia. Additionally, individuals may struggle with poor concentration, excessive guilt or feelings of worthlessness, a pervasive sense of hopelessness about the future, and recurrent thoughts of death or suicide. Sleep disturbances, changes in appetite or weight, fatigue, and diminished energy levels are also common features of depression [10]. The severity of a depressive episode can vary, ranging from mild to moderate or severe, depending on the number and intensity of symptoms and their impact on the individual's daily functioning. According to the last edition of the DSM (i.e., DSM-V) a Major Depressive Episode (MDE) is distinguished from normal mood fluctuations by the presence of five or more symptoms persisting most of the day, nearly every day, for at least two weeks [10]. Notably, one of these symptoms must include either low mood or anhedonia. Various patterns of depressive episodes are recognized, including single MDE disorder, which denotes the individual's first and only episode; Major Depressive Disorder (MDD), characterized by a history of at least two MDEs; and Bipolar Disorder (BP), where depressive episodes alternate with periods of manic symptoms. Manic symptoms may encompass feelings of euphoria or irritability, increased activity or energy, heightened talkativeness, racing thoughts, inflated self-esteem, reduced need for sleep, distractibility, and impulsive or reckless behaviour. Individuals who do not achieve remission after at least two courses of treatment, particularly those with MDD and BP, may be classified as having treatment-resistant MDD (TR-MDD) or treatment-resistant BP (TR-BP), respectively, signifying the severity and resistance to conventional therapeutic interventions [10].

Currently approved and recognized treatments for depression include psychotherapy, non-invasive brain stimulation (NIBS) techniques, and pharmacological compounds. In contemporary psychiatric practice, pharmacotherapy, often involving a combination of multiple psychoactive drugs, remains the cornerstone in the management of depressive disorders, particularly for severe cases [16]. The term "psychoactive" will

be employed throughout this thesis to denote drugs that exert their effects on the central nervous system (CNS) and induce psychological responses. It is important to acknowledge that various categorizations based on different levels of analysis, including chemical, pharmacological, neural, phenomenological, or behavioural exist within the realm of psychoactive compounds. When classified based on their behavioural effects, psychoactive compounds can be divided into antiepileptics, antipsychotics, antidepressants, mood stabilizers, stimulants, sedatives, and hallucinogens [17]. These medications target various neurotransmitter systems within the CNS, aiming to alleviate specific symptoms of neuropsychiatric conditions. The clinical success of pharmacotherapy for mental disorders has significantly contributed to the emergence of brain-based somatogenic theories of mental health conditions, including depression.

1.3. Neurobiological theories of depression

To comprehend the genesis of neurobiological theories of depression, it is relevant to consider the history of antidepressant medications (Figure 1). While psychoactive drugs have been used for millennia to alter mental states and ease suffering, before the 1950s, no official antidepressant medications were available [8]. Investigation into anti-histaminergic agents led to the identification of the first antipsychotics, which through chemical modification resulted in the discovery of the first tricyclic antidepressant (TCA) [18]. Concurrently, the exploration of monoamine oxidase inhibitors (MAOIs) for tuberculosis treatment serendipitously unveiled their antidepressant properties [19]. TCAs and MAOIs constituted the first generation of pharmaceuticals defined and commercialized under the name of antidepressants. Research on the mechanism of action of TCAs and MAOIs showed their modulatory activity on neurotransmitter levels, such as serotonin (5-HT) [20]. This discovery provided the first link between monoaminergic systems and depression, contributing to the formulation of influential neurobiological theories of depression, such as the "5-HT deficiency" hypothesis (Figure 1) [8].

The "5-HT deficiency" hypothesis of depression states that depressive symptoms arise from insufficient levels of synaptic 5-HT in the brain. Endogenous 5-HT plays a pivotal role in various physiological functions, serving both as a hormone and as a neurotransmitter across the animal kingdom. In humans, 5-HT is distributed throughout the body, found both peripherally and centrally in the nervous system. In the brain, serotonergic neurons are primarily located in the brainstem. Notably, the brainstem contains only a small fraction (1-2%) of the total 5-HT present in the entire organism, which is mainly concentrated in the etheric system [21]. In the brainstem, 5-HT is synthesized from tryptophan, an essential amino acid obtained from dietary sources, as exogenous serotonin cannot cross the blood-brain barrier. This synthesis occurs within specific neurons clustered in the raphe nuclei of the brainstem [22]. Axons originating from these neurons innervate virtually the entire brain, projecting their terminals to either the fore-brain (upper raphe nuclei) or to the spinal cord (lower raphe nuclei) [23]. Upon release into the synaptic cleft, 5-HT interacts with specific receptors located either pre- or post-synaptically. Subsequently, 5-HT is recaptured into serotonergic neurons via the presynaptic serotonin transporter (SERT) and either stored for future release or metabolized by the monoamine oxidase (MAO) enzymes. Once released, 5-HT exerts its physiological effects by binding to various types of cell membrane receptors. Currently, research has identified 14 different 5-HT receptor types, classified into seven subfamilies based on their primary structure and functional properties [21]. Except 5-HT3, which belongs to the ion channel receptor superfamily, the majority of 5-HT receptors are G protein-coupled (GPC) receptors [24]. The involvement of 5-HT in depression converges from multiple lines of research and will be reviewed in detail in the next chapter.

The postulation of the “5-HT deficiency” hypothesis of depression paved the way for the emergence of a novel generation of compounds, manifested in the late 1970s as selective serotonin reuptake inhibitors (SSRIs). For the first time in psychopharmacology, the development of SSRIs adhered to a deliberate strategy and a rational design methodology, to make drugs specifically targeting the presynaptic SERT [20]. By the 1990s, SSRIs had become the most extensively prescribed pharmaceuticals in the U.S., ranking as the second highest-selling drug globally. The incidence of visits to general practitioners for depression doubled in the period between 1988 and 1994, paralleled by a surge in antidepressant prescriptions, a period denoted as the "Prozac Boom" [8,25]. Despite dominating the antidepressant market in the 1990s and still holding the status of the most prescribed antidepressants, SSRIs have not universally met expectations [8].

The efficacy of SSRIs is limited, proving ineffective for a substantial portion of patients, particularly those with severe cases of depression. Literature examining remission rates post-treatment reports results ranging from 30% to 45% of patients [26,27]. Additionally, SSRIs frequently induce side effects, impacting sleep, appetite, and libido, and leading to vomiting, nausea, irritability, anxiety, insomnia, and headaches. Chronic intake is necessary to achieve therapeutic benefits, with an average onset latency of 2-4 weeks. Notably, patients often report an emotionally numbing effect attributed to SSRIs, known as "emotional blunting" [28,29]. Efforts to mitigate the adverse effects of early SSRIs led to the emergence of other antidepressants in the 1990s, including selective noradrenaline reuptake inhibitors, selective 5-HT receptor antagonists, and α2-adrenoreceptor blockers. However, these new agents exhibited varying tolerance rates and side effects without substantially improving efficacy compared to SSRIs [8]. The ineffectiveness of antidepressants has sparked considerable debate in recent decades, with the pharmaceutical industry witnessing insignificant variations of old drugs and major companies withdrawing from brain target research, threatening research progress [30]. By the turn of the millennium, the scientific re-exploration of the therapeutic potential of various illicit hallucinogenic substances is progressively fuelling a revolution in mental health research [31]. Particularly prominent among those drugs are the classic psychedelics (from the Greek words psyché "soul, mind" and dēleín "to manifest"), such as psilocybin, lysergic acid diethylamide (LSD), mescaline, and N,N-Dimethyltryptamine (N,N-DMT), as well as non-classic psychedelics, like the empathogen 3,4-Methylenedioxymethamphetamine (MDMA), and the dissociative ketamine [30].

Already during the 1920s, the clinical use of classic psychedelics, particularly mescaline, was being explored for various psychiatric conditions. However, the actual surge of scientific interest in psychedelics occurred after the discovery of LSD's psychoactive properties by the Swiss chemist Albert Hoffmann in 1943. In the period between the 1950s and 1960s, the therapeutic potential of psychedelics for mood disorders and addictions was actively researched with highly promising results [4]. These compounds were synthesized chemically and made available to psychiatrists and psychopharmacologists for study throughout America and Europe. Research into these compounds also contributed to the understanding of their chemical properties and their effects on brain functioning and cognition. For example, studies on LSD elucidated its structural similarity with 5-HT, advancing the scientific understanding of the role of the serotonergic system in brain functioning and cognition [32]. However, as the recreational use of these compounds became associated with the counterculture movement of the 1970s, a societal backlash ensued. In the U.S., the War on Drugs led to a ban on research into psychedelics, effectively halting scientific inquiry into their therapeutic potential (Figure 1) [30]. This ban was later adopted by other countries, further restricting research on numerous psychoactive compounds. Notably, ketamine was one of the first hallucinogens whose potential as a treatment for mental illness was re-investigated by modern trials.

Ketamine was synthesized as a replacement for the anaesthetic phencyclidine (PCP) at Parke, Davis & Co. The incidence of prolonged reminiscent of psychosis induced by PCP made the compound unsuitable for clinical use, leading to the discovery of the analogue ketamine and its approval for human research in 1965 [33]. The first human was given ketamine in an intravenous (i.v.) subanaesthetic dose by Dr. Edward Domino. He observed that ketamine's pharmacodynamics exhibited a progressive increase from no effect to a conscious yet "spaced out" state, culminating in general anaesthesia. Compared to PCP, ketamine displayed less pronounced and shorter psychotomimetic effects while preserving autonomic respiratory functions [34]. In an initial clinical study involving 130 patients undergoing surgical procedures, ketamine successfully induced anaesthesia, yet accompanied by a distinctive altered state of consciousness, characterized by a perceived disconnection from the body and the environment and the occurrence of dream-like experiences. To get the Food and Drugs Administration (FDA) approval for clinical use during the period of the War on Drugs, it was decided to call the compound a "dissociative anaesthetic", which proved to be a successful measure [33]. Outside the scientific circles, the psychoactive properties of ketamine led to the diffusion of the drug within the "rave culture", with a progressive increase in ketamine use as a recreational drug, a drug of abuse, and a psychedelic-like compound. First documented on the West Coast of the U.S. in the early 1970s, ketamine's recreational popularity continued to rise, with common street names including "K", "Special K", "Vitamin K", and "Kiddy Smack" [35]. A major concern of ketamine's non-medical use was its potential for abuse and addiction, with documented neurotoxic and renal, urinary,

hepatic, and intestinal damages associated with chronic long-term use of the drug [35,36]. Consequently, ketamine was included in an extensive list of drugs of abuse, attaining Schedule 3 status [33]. However, in contrast to other hallucinogenic compounds, such as MDMA and the classic psychedelics, scientific research on ketamine persisted, legitimized by the previous approval by the FDA and the lower schedule status. In the 1980s, the use of ketamine in different kinds of anaesthesia as well as its subanaesthetic dissociative effect were well confirmed and established [8]. Outside of the U.S., the potential of the dissociative state induced by subanaesthetic use of ketamine to treat psychiatric and psychological conditions was also being considered, building on previous evidence collected on the classic psychedelics [37]. Notably, in Iran, ketamine was reported as an effective adjunct to psychotherapy for conditions including depression, anxiety, obsessive-compulsive neurosis, conversion reaction, and hypochondriasis [38]. Argentina explored ketamine as an adjunct for psychotherapy to facilitate the regression of depression, while in Mexico, ketamine was examined in group settings as part of psychedelic-inspired psychotherapy sessions for patients with neurosis and personality disorders [39]. From 1985, ketamine-assisted psychedelic therapy was used in Russia for a range of neurotic and personality disorders with particularly impressive results in the treatment of alcoholism [40]. In parallel, animal research with ketamine reported antidepressant-like effects in a range of pre-clinical models [41]. The glutamatergic system was also discovered, with the recognition of the role of glutamate and its target, the N-methyl-D-aspartate (NMDA) receptor, in mediating learning, and memory [42]. Consequently, ketamine was under the spotlight since the main mechanism of action behind its psychoactive effects was found to be the modulation of glutamatergic neurotransmission via antagonism at the NDMA receptors [43]. While the classic psychedelics and MDMA are mainly serotonergic drugs, akin to the SSRIs, the glutamatergic action of ketamine offered a new target for antidepressant effects. In the 1990s, studies demonstrated that the injection of NMDA receptor modulators induced antidepressant-like effects in mice and stress exposure increased glutamate release in key brain areas for behaviour and mood regulation. Furthermore, treatment with traditional monoaminergic antidepressant drugs induced adaptive changes in NMDA receptors in brain regions crucial for mood disorders [41]. In this way, mechanistic models of depression shifted from a monoaminergic basis towards a hypothesis based on glutamate and cortical excitability [44].

Glutamate and γ-aminobutyric acid (GABA) play a fundamental role in brain function and information processing, especially in the neocortex [45]. Glutamate serves as the primary excitatory neurotransmitter in the CNS, promoting postsynaptic depolarization and consequent increases in neural firing upon binding to NMDA receptors. Conversely, GABA functions as the primary inhibitory neurotransmitter in the adult nervous system, inducing postsynaptic hyperpolarization and reductions in neural firing when binding to GABA receptors. The chemical balance between excitation (i.e., glutamate) and inhibition (i.e., GABA) in the brain is crucial for shaping cortical excitability and functioning [46]. Indeed, cortical networks of neurons are synchronized through reciprocal connections between excitatory and inhibitory neurons, producing specific frequencies of brain activity associated with specific brain functions and mental states. The firing frequency of neural populations is determined by the control exerted by inhibitory neurons on the firing of excitatory neurons [47]. According to the "excitatory/inhibitory imbalance" hypothesis, conditions such as depression may arise from abnormal circuit information processing due to chemical imbalances in glutamatergic and GABAergic modulation within mood-regulating brain regions (Figure 1) [46]. The "excitatory/inhibitory imbalance" hypothesis of depression and its implications for the mechanism of action of ketamine will be discussed in Chapter 4.

In recent years, research into the mechanisms underlying the action of various antidepressant treatments, including ketamine, classic psychedelics, SSRIs, and NIBS, has revealed a shared process of the antidepressant response [48]. Specifically, there is growing evidence implicating the modulation of neuroplasticity — the ability of neurons to undergo structural and functional changes in response to external stimuli — as a key therapeutic target of many antidepressant interventions [49]. Modern definitions of neuroplasticity generally distinguish between structural and functional plasticity. While not mutually exclusive, the first refers to the shaping of the nervous system, while the latter describes changes in the communication between synapses [49]. In evolutionary terms, neuroplasticity can lead to gain or loss of functions which can be viewed as either adaptive or maladaptive. Additionally, many neuronal structures

and functions have a limited time window to optimally develop. These windows are known as critical periods of plasticity, and the majority tend to close before adulthood [50]. Therefore, the context and timing of neuroplasticity alterations are critical factors in determining their significance. Nonetheless, a compromised capacity to undergo adaptive neuroplastic changes has been consistently associated with various neuropsychiatric pathologies, including depression [51]. This recent body of work contributed to the proposition of the "neural atrophy" hypothesis of depression, which posits that reduced neuroplasticity contributes to depressive symptoms (Figure 1) [51]. The "neural atrophy" hypothesis of depression will be the focus of chapter 5 of the dissertation.

It is worth noting that other neurobiological theories of depression exist. For instance, the author of this thesis recently published a review about neuomarkers of circadian rhythms in depression [52]. Also, several circuit-based and functional connectivity models of depression have been proposed [53]. Nonetheless, the "5-HT deficiency", the "excitatory/inhibitory imbalance", and the "neural atrophy" hypotheses of depression are probably the most influential neurobiological theories of depression, as they account for general principles of brain functioning with a close relationship with antidepressant responses. Despite their popularity, these hypotheses and their underlying mechanisms are mostly based on animal research. A direct assessment of such theories in the living human brain is still lacking. However, recent advancements in neuroimaging techniques allow the non-invasive investigation of novel neurobiological phenomena in humans.

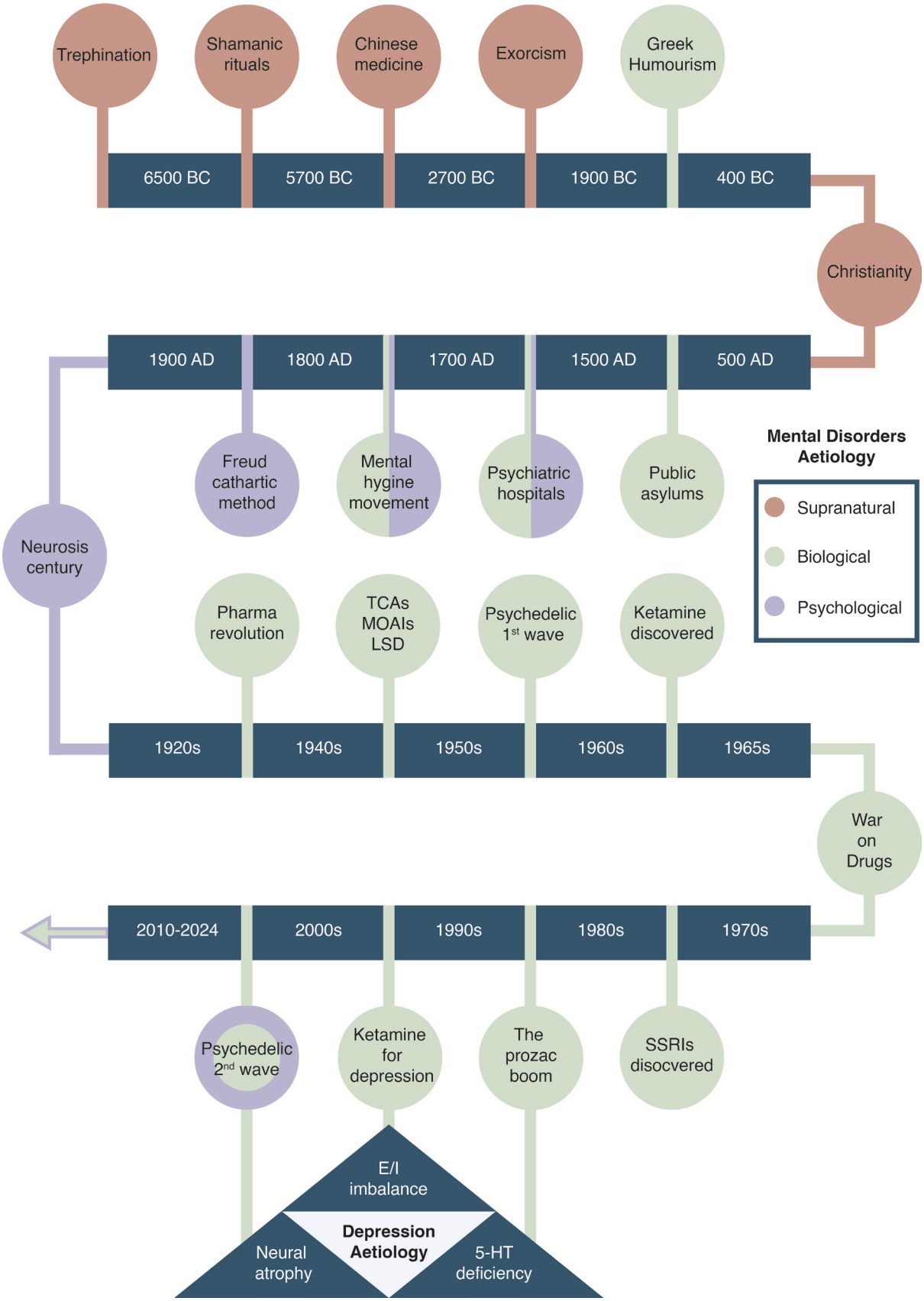

Figure 1: History of aetiological theories of mental disorders, depression, and antidepressants.

1.4. Neuropsychopharmacology

Neuropsychopharmacology is a relatively new and exciting area of research studying the effects of psychoactive drugs on the brain, mind, and behaviour. This branch of psychiatry arises from the integration of contemporary neuroscientific methodologies and theories with psychopharmacology. Its primary objective is to explore the neural mechanisms underlying the therapeutic effects of mind-altering drugs that act on the CNS in the context of specific mental health conditions [54]. Research within this domain encompasses the study of neuropathological mechanisms, pharmacodynamics, brain functioning, psychological processes, and altered states of consciousness. In comparison to psychopharmacology, neuropsychopharmacology addresses issues on the mechanism (i.e., the "How") and function (i.e., the "Why") of psychoactive medications. Furthermore, it broadens its scope to encompass both clinical and fundamental aspects of neural and mental functioning. Human neuropsychopharmacology makes use of psychoactive drugs and modern non-invasive neuroimaging techniques to study the mechanism of action of medications as well as the aetiology of psychiatric conditions. In this context, psychoactive drugs are used both as treatments and as perturbational tools to probe the state of the brain, revealing its dynamics in health and pathology. This modern approach to psychiatry allows for the causal and mechanist assessment of brain functioning.

To date, the non-invasive investigation of brain modifications induced by psychoactive compounds is based on 3 main neuroimaging techniques: magneto-(MEG) and electro-encephalography (EEG) [55], magnetic resonance imaging (MRI) [56], and positron emission tomography (PET) [57]. These techniques have specific advantages and disadvantages, capturing brain functioning at different spatial and temporal scales.

1.5. Electroencephalography (EEG) and magnetoencephalography (MEG)

EEG and MEG are widely used techniques to measure the acute neurophysiological changes associated with psychoactive drugs non-invasively in humans, an approach known as pharmaco-EEG/MEG. While EEG measures the electrical activity of the brain via electrodes (i.e., channels) positioned on the scalp, MEG measures the magnetic field generated by the electrical activity of neurons using superconducting quantum interference devices [58,59]. The primary contributors to the scalp EEG/MEG signal are the potentials produced in the longitudinally oriented dendrites of cortical excitatory neurons. The emergence of a MEG/EEG signal of sufficient magnitude for external detection necessitates the synchronized activity of substantial populations of pyramidal neurons. This synchronicity is paced by the activity of specific populations of GABAergic inhibitory interneurons which regulate the irregular firing of excitatory cells [60,61]. When this firing is synchronized across a sufficiently large population of neurons, the resulting signal becomes detectable at the scalp level. Consequently, the signals captured by EEG/MEG reflect, in part, the synchronicity of the brain's dynamic activity [62]. Both EEG and MEG exhibit limited sensitivity to the activity of deep neural sources, such as subcortical structures, and have poor spatial resolution, a few millimetres for MEG and a few centimetres for EEG [58]. Those are the major limitations of EEG/MEG as compared to other non-invasive methods such as MRI and PET. However, EEG/MEG methods have a higher temporal resolution, being able to capture neural activity at the millisecond resolution [63]. Diverse configurations of EEG electrodes exist, ranging from high-density EEG with up to 256 channels to portable single headbands. The density of electrodes positively correlates with signal resolution and quality, but inversely affects portability, cost, and adaptability to various contexts and conditions. On the other hand, MEG systems are currently unwieldy and expensive, and only a limited number of sites possess them [64].

1.6. Magnetic resonance imaging (MRI)

MRI is a technique that allows the simultaneous acquisition of structural and functional brain data. In particular, Structural MRI offers high-resolution imaging of brain anatomy, allowing for precise delineation of grey and white matter structures, as well as the detection of abnormalities or lesions. This technique facilitates the mapping of structural alterations associated with neuropsychiatric disorders and the monitoring of changes throughout treatment interventions. Also, structural MRI is often used for the generation of individual-based brain maps in support of other techniques, such as PET [65]. Functional MRI

(fMRI) captures changes in brain activity associated with cognitive tasks, emotional processing, or pharmacological manipulations. By measuring changes in blood oxygenation levels (i.e., BOLD signal), fMRI detects regional alterations in cerebral blood flow and neural activity, providing insights into the functional organization of the brain [66]. By comparing brain activity patterns under different experimental conditions, the neural mechanisms underlying drug effects, therapeutic responses, and pathological traits can be investigated. Despite its advantages, fMRI has limitations. While offering superior spatial resolution compared to other neuroimaging techniques like EEG/MEG and PET, fMRI lacks the temporal resolution necessary to capture rapid neural events [67]. Additionally, MRI's reliance on strong magnetic fields and specialized equipment presents logistical challenges and limits its accessibility in certain research settings.

1.7. Positron Emission Tomography (PET)

PET enables the quantitative assessment of neuroreceptor binding, neurotransmitter release, and metabolic activity in the living brain, providing fundamental insights into the neurochemical correlates of psychiatric disorders and the mechanisms of drug action [57]. PET utilizes radiolabelled compounds, known as tracers, which emit positrons upon decay. These positrons collide with electrons, resulting in the emission of gamma rays that are detected by PET scanners. By administering specific radiotracers targeting neurotransmitter systems or metabolic processes, PET can map regional brain activity and receptor occupancy, offering insights into the neurochemical alterations induced by pharmacological interventions. Further, PET imaging allows for the visualization and quantification of specific neuroreceptors and molecular targets, enabling the investigation of alterations in receptor density or binding affinities associated with psychiatric disorders [68]. Additionally, PET can measure changes in regional cerebral blood flow (CBF) and glucose metabolism, providing indirect indices of neuronal activity and energy utilization in the brain [57]. Despite its strengths, PET imaging has limited spatial resolution and requires radioactive tracers, which impose logistical challenges and safety considerations. Furthermore, PET's temporal and spatial resolution is lower compared to other neuroimaging modalities such as EEG and fMRI, limiting its ability to capture transient neural dynamics. Lastly, conducting PET studies is more costly and more invasive compared to other techniques, limiting the statistical power and sample sizes of PET studies [67]. Nonetheless, ongoing advancements in PET technology, including the development of novel radiotracers, tracer signal optimization methods (such as arterial spin labelling), and image analysis methods, continue to enhance its utility in neuropsychopharmacological research.

Collectively, these main techniques and their variations offer the exciting possibility of directly assessing brain-based theories of depression and novel mechanisms of action of antidepressants. The present thesis encompasses 3 original research studies utilizing psychoactive compounds in conjunction with state-of-the-art neuroimaging methods to test contemporary neurobiological theories of the aetiology of depression. Additionally, the mechanism of action of the rapid-acting antidepressant ketamine will be explored through multimodal imaging approaches in both healthy individuals and patient populations.

In brief, Chapter 2 will present the result of an experiment using an amphetamine challenge in combination with PET/MRI imaging of the 5-HT system in both healthy subjects and individuals with depression. The study represents the first direct assessment of the "5-HT deficiency" hypothesis of depression in the living human brain. In Chapter 3, the novel antidepressant ketamine will be introduced, with a comprehensive review of its acute and subacute neural effects. Chapter 4 will showcase the results of a study investigating the acute neurophenomenological and therapeutic effects of ketamine administration in patients with bipolar depression, utilizing EEG recording in a real-world hospital setting. The findings of this study will be discussed within the context of the "excitatory/inhibitory imbalance" theory of depression. Finally, Chapter 5 will present the results of a study examining the neuroplastic effects of ketamine using PET/MRI imaging of synaptic density in healthy individuals. This study holds significant implications for the "neural atrophy" theory of depression. The thesis will conclude by discussing the implications of the results for the understanding of the aetiology of mental disorders. Furthermore, a novel framework centred around the concept of individual and subjective variability in drug response will be proposed and discussed, drawing upon observations common to the 3 experiments.

2. Chapter 2: Brain serotonin (5-HT) release is reduced in patients with depression

The following chapter will present the results of a study measuring the difference in 5-HT release between patients with depression and healthy controls using a dextro(d)-amphetamine challenge in combination with the PET tracer [^{11}C]Cimbi-36.

2.1. Introduction

As discussed in the previous chapter, the "5-HT deficiency" hypothesis is one of the oldest, yet most influential, neurobiological theories of depression. The serotonergic-releasing action of most of the approved antidepressants (TCAs, MAOIs, and SSRIs) provided the foundation for the hypothesis [69]. The finding that a pharmacological blockade of 5-HT synthesis, by the tryptophan hydroxylase inhibitor p-chlorophenylalanine, reverses the antidepressant effects of both MAOIs and TCAs provided further support for the involvement of 5-HT in the pathophysiology of depression [70,71]. Moreover, experimental reduction in brain 5-HT levels by dietary depletion of its amino acid precursor tryptophan, was found to trigger relapse in individuals with depression who have been successfully treated with an SSRI [72,73], and monoamine depletion was found to correlate with decreased mood both in individuals with a family history of MDD and in drug-free patients in remission [74]. These human findings are consistent with several observations in pre-clinical models. Notably, a murine knock-in of a specific tryptophan hydroxylase 2 (Tph2) mutation, previously found to be linked with unipolar MDD in humans [75], results in Tph2 deficiency and subsequent reduced 5-HT synthesis, leading to marked decreases in brain 5-HT levels and consistent increases in depression-like, anxiety-like and aggressive behaviours [76]. Similarly, mice lacking the stable tubule only polypeptide (STOP), a key regulator of normal 5-HT axonal growth, have reduced levels of 5-HT and SERT in cortical projection areas and show increased helplessness and depression-like behaviours [77]. Furthermore, loss of the adhesion protein αC2 isoform in 5-HT neurons leads to abnormal projection of serotonergic axons, associated with increased depression-like behaviours [78]. Despite these findings, a recent umbrella review by Moncrieff et al. (2022) suggested that the evidence linking 5-HT dysregulation to depressive symptoms is inconclusive [79]. This observation stems from the lack of direct assessments of brain 5-HT in human studies investigating the hypo-serotonergic state of depression.

A more direct assessment of cerebral 5-HT levels in the human brain would be important to understand its role in depressive disorders, and could potentially be used as a predictor of treatment response to serotonergic antidepressants. Recently, the novel 5-HT type 2A (5-HT2A) receptor agonist PET radioligand, [^{11}C]Cimbi-36, was found to be sensitive to pharmacological challenges leading to 5-HT release in the pig [80] and non-human primate brains [81]. Acute SSRI administration in healthy controls does not elicit changes in neocortical 5-HT levels sufficient to be detected with [^{11}C]Cimbi-36 PET [82]. However, a comparison of tissue increases in 5-HT concentration, as measured by microdialysis in the pig brain, with the change in [^{11}C]Cimbi-36 binding, found an 11-fold increase in extracellular 5-HT following the administration of d-fenfluramine, a relatively selective 5-HT releaser, which resulted in a 44% reduction in [^{11}C]Cimbi-36 binding [80]. This indicates that [^{11}C]Cimbi-36 may have similar sensitivity to 5-HT release as the established paradigms assessing dopamine release using dopamine D2/3 receptor radioligands, such as [^{11}C]raclopride and [^{11}C]PHNO [83]. In humans, Erritoze et al. (2017) [84] found significant reductions in [^{11}C]Cimbi-36 binding in the frontal cortex of healthy volunteers following administration of the monoamine-releasing agent d-amphetamine, which is also a potent 5-HT releaser [85–87]. They conceptualized the change in [^{11}C]Cimbi-36 binding following the d-amphetamine challenge as an index of 5-HT release capacity and validated the method for the assessment of the 5-HT system in the living human brain. The combination of [^{11}C]Cimbi-36 with the d-amphetamine challenge offers the unprecedented possibility of directly testing the "5-HT deficiency" hypothesis in patients with depression.

Interestingly, the activation of cortical 5-HT2A receptors (the target of [^{11}C]Cimbi-36) by psychedelics is widely acknowledged to underlie the alterations in consciousness induced by these compounds. Moreover, this receptor activation is implicated in triggering the sustained improvements in depressive symptoms observed in clinical trials, persisting for months following a single administration [4]. Classic psychedelics exhibit the unique characteristic of positively influencing a range of psychological traits, that are often impaired in depression, and where traditional antidepressants like SSRIs may be ineffective or even detrimental [88]. These beneficial effects encompass improvements in well-being [89], emotional

responsiveness [90], cognitive flexibility [91], experiential avoidance [92], mindfulness [93,94], openness [93,95], insightfulness [96], and connectedness [97]. To date, the potential linkage between the status of these psychological traits and the state of the 5-HT system remains unknown. Thus, the characterization of [11C]Cimbi-36 binding profile in depression might offer valuable insight into the relationship between 5-HT2A receptor binding and the psychopathology of depression.

2.2. Study aim

The primary objective of this investigation was to assess the "5-HT deficiency" hypothesis of depression. Specifically, the study compared the release of 5-HT, along with baseline 5-HT2A receptor binding, in a cohort of individuals with MDE, against a control group of healthy individuals. The central hypothesis posited that the 5-HT release capacity in the frontal cortex of healthy subjects would be significantly higher as compared to individuals with MDE. The main findings of this study were reported in a recent publication [98]. Additionally, an ancillary analysis was conducted on the published data to investigate the relationship between measures of the 5-HT system and psychometric evaluations of depressive symptomatology. This supplementary analysis also explored measures of well-being and connectedness within the MDE group.

2.3. Materials and Methods

2.3.1. Patient population

Seventeen antidepressant-free adult patients undergoing MDE were recruited from specialized clinics at Oxford University and Kings College London. Twelve patients had MDE in the context of an MDD (for subgroup analyses referred to as MDD; mean age: 40 ± 11 years; 3 female). Five patients had the diagnosis of MDD due to Parkinson's Disease (PD), diagnosed according to UK PDS Brain Bank Criteria, and were for subgroup analyses referred to as MDPD (mean age: 55 ± 10 years; all male). Twenty healthy controls (HC) (mean age: 32 ± 9 years, 3 females) were recruited by advertisements and word of mouth. All participants received general medical and psychiatric screening, with the MINI (Mini-International Neuropsychiatric Interview) used to confirm a diagnosis of a major depressive episode and to screen for any co-morbid psychiatric conditions. None of HC had a history of present or past use of psychoactive medications including SSRIs, while 6 of the patients (all in the MDD subgroup) had previously received treatment with antidepressant medication but were medication-free for at least 6 months (ranging from 6 months to >10 years). The remaining 6 patients with MDD and the 5 with MDPD were antidepressant-naïve. Of the MDPD subgroups, 2 patients were taking dopaminergic medication. Patient demographics are summarised in Table 1.

Demographics and Clinical	**HC**	**MDE**	**MDE vs HC (p-value)**	**MDD**	**MDPD**
Number of subjects	n=20	n=17	-	n=12	n=5
Age (years)	32 ± 9	44 ± 13	0.002	40 ± 11	55 ± 10
Gender (F/M)	3F/17M	3F/14M	-	3F/9M	0F/5M
Diagnoses	N/A	MDD & MDPD	-	MDD	MDPD
HAM-D inclusion	N/A	21 ± 4 (16-30)	-	21 ± 4	20 ± 3
BDI baseline	N/A	24 ± 8 (12-40)	-	27 ± 8 (15-40)	16 ± 4 (12-20)
Previous AD treatment	-	n=6	-	n=6	n=0
PET scan 1	**Mean ± SD**	**Mean ± SD**	**p-value**	**Mean ± SD**	**Mean ± SD**
Injected dose (MBq)	162 ± 56	155 ± 44	0.671	148 ± 39	171 ± 56
Injected mass of [^{11}C]Cimbi-36 (ng/kg)	1.32 ± 0.14	1.29 ± 0.08	0.465	1.28 ± 0.05	1.33 ± 0.12
PET scan 2	**Mean ± SD**	**Mean ± SD**	**p-value**	**Mean ± SD**	**Mean ± SD**
Injected dose (MBq)	184 ± 60	170 ± 51	0.474	156 ± 46	206 ± 47
Injected mass of [^{11}C]Cimbi-36 (ng/kg)	1.30 ± 0.16	1.22 ± 0.15	0.115	1.22 ± 0.13	1.23 ± 0.19
PET scan 1 vs 2	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
Injected dose (MBq)	0.100	0.072	-	0.402	0.097
Injected mass of [^{11}C]Cimbi-36 (ng/kg)	0.506	0.099	-	0.219	0.341
Mid PET scan 2	**Mean ± SD**	**Mean ± SD**	**p-value**	**Mean ± SD**	**Mean ± SD**
Plasma Amph levels mid PET2 (ng/ml)	68 ± 19	77 ± 12	0.111	78 ± 12	74 ± 11
Drug intensity mid PET2 (VAS, %)	29 ± 17	35 ± 28	0.431	38 ± 27	28 ± 31

Table 1: Demographics, clinical and scan parameters.

2.3.2. Study design

After recruitment, participants received general medical and psychiatric screening. Patients with MDE completed psychometric measures of depressive symptomatology, well-being and connectedness at

baseline. All participants received two PET scans with [11C]Cimbi-36, before (i.e., Scan 1) and 3 hours after (i.e., Scan 2) a single oral dose of d-amphetamine (0.5 mg/kg). Self-rating of the acute d-amphetamine-induced subjective effect was also acquired immediately before and after the post-dose PET scan, and the average rating between the two assessments was used for analysis.

2.3.3. Psychometric measures

At baseline, the following psychometric measures of depressive symptomatology were collected at baseline: the Hamilton Depression Rating Scale (HAM-D) [99], the Beck Depression Inventory (BDI) [100], the Snaith-Hamilton Pleasure Scale (SHAPS) [101], the State Anxiety Inventory (SSAI) [102], the Rumination scale (RS)[103]. Also, the Warwick-Edenborough Mental Wellbeing Scale (WEMWBS) [104], and the Watts Connectedness scale (WCS) [97] were measured at baseline. Self-rating of the acute d-amphetamine-induced subjective effect was obtained for 31 out of the 37 subjects ratings using a visual analogue scale (VAS) of "I feel a drug effect" ranging from "not at all" to "extremely", whereas for 6 HC subjects a VAS score was estimated from an average of a Likert rating of least (=1) to most high (=5).

2.3.4. d-amphetamine plasma measurement

Blood samples for d-amphetamine levels were acquired immediately before and after Scan 2 for each individual (i.e. at 3 and 4.5 hours post-d-amphetamine administration), and an average of the 3 and 4.5 hours plasma level concentrations was used as a parameter to examine the relationship between d-amphetamine dose and PET outcome parameters.

2.3.5. PET acquisition and imaging

The [11C]Cimbi-36 tracer was synthesized onsite and administered as a bolus over 20 seconds in a volume of 20 mL at the start of the PET scans, all acquired on Siemens PET/CT scanners (two similar scanners used: Hi-Rez Biograph 6 and Biograph 6 TruePoint with TrueV, Siemens Healthcare, Erlangen, Germany). Whole blood activity was measured using a continuous automatic blood sampling system (Allogg AB, Marlefred, Sweden) acquired at a rate of 5 mL/min. Discrete blood samples were used to determine the fraction of plasma radioactivity constituted by unchanged parent radioligand using high-performance liquid chromatography (HPLC) analysis. For each ligand, the plasma free fraction was measured by ultrafiltration in triplicate using an arterial blood sample taken before tracer injection. Structural MRI data were acquired over 30 minutes on the same day as the PET scan, using 3T (Magnetom Trio and Verio, Siemens Healthcare Sector, Erlangen, Germany) with a 32-receiver channel head matrix coil, in the sagittal plane, utilising a 3D magnetization prepared rapid gradient echo (MP-RAGE) scan with the following parameters: repetition time = 2300 milliseconds, echo delay time = 2.98 ms, flip angle = 9°, isotropic voxels = 1.0 mm x 1.0 mm x 1.0 mm, 160 slices, total scanning time = 5 minutes, 3 seconds. Corrections were applied for attenuation, randoms and scatter, and subject motion.

The PET data were acquired over 90 minutes with associated arterial blood sampling to provide an input function. All image data were analysed using Invicro London's in-house PET data quantification tool, MIAKATTM (version 4.3.15, http://www.miakat.org). MIAKATTM is implemented using MATLAB (version R2016a; Mathworks Inc., Natick, MA, U.S.), and makes use of SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) functions for image segmentation and registration. Each participant's MRI image underwent grey matter segmentation and was then registered to an anatomical template image in standard stereotactic space (i.e., MNI152). Dynamic PET images, registered to the MRI scans of participants, were corrected for any motion. An automated definition of regions of interest (ROIs) was performed on the MNI152 space based on the Imperial College London Clinical Imaging Centre (CIC) atlas [105]. The PET images were grey matter masked using the subject's own MRI. The region-of-interest (ROI) time-activity data were sampled from the frontal cortex, a large region with high 5-HT2A receptor density that was determined a priori as the main ROI. Exploratory analysis was conducted for the parietal, temporal, and occipital cortices. The ventrolateral cerebellum was used as a reference region to estimate the [11C]Cimbi-36 non-displaceable binding [106].

Regional total volumes of distribution (VT) were derived from kinetic analysis using the multilinear analysis-1 (MA1) method. The ROI non-displaceable binding potential (BP_{ND}) was calculated by correcting the ROI VT for the VT of the reference region (i.e., cerebellum), according to

Equation 1. The 5-HT release capacity was quantified by dividing the ROI BP_{ND} at Scan 2 by ROI BP_{ND} at Scan 1 to obtain ΔBP_{ND} (Equation 2). The frontal cortex baseline BP_{ND}, reflecting 5-HT2A receptor availability, and the ΔBP_{ND} , reflecting 5-HT release capacity, were the main PET outcomes of the study.

Equation 1: [11C]Cimbi-36 Reference region correction

$$BP_{ND} = \frac{VT\ (ROI)}{VT(Cerebellum)} - 1$$

Equation 2: [11C]Cimbi-36 BP_{ND} difference

$$\Delta BP_{ND} = 1 - \frac{Scan\ 2\ BP_{ND}\ (ROI)}{Scan\ 1\ BP_{ND}\ (ROI)}$$

2.3.6. Statistical analyses

Prior to statistical inference, the distribution of the [11C]Cimbi-36 BP_{ND} and ΔBP_{ND} data was inspected and tested for meeting parametric statistical assumptions. Shapiro-Wilk normality test (W), as well as skewness and kurtosis analyses, were conducted on the distribution of [11C]Cimbi-36 BP_{ND} and ΔBP_{ND} data for both groups. Where non-normality of the data was detected, analysis of the quantile distribution was performed to detect possible outliers using Tukey's rule [107]. Outliers were defined as data points laying 1.5 times the interquartile range above the third quartile (75% of the distribution) or below the first quartile (25% of the distribution). One participant in the patient group was identified as an outlier for ΔBP_{ND} in the frontal, occipital, temporal and parietal cortices, and was therefore not included in all the parametric statistics involving ΔBP_{ND}.

Two-sided Student's t-test was used to compare the between-group differences (i.e., HC vs MDE) in demographic variables, while two-sided Analysis of Variance (ANOVA) with post-hoc Tukey correction for multiple comparisons was used to compare the three subgroups (i.e., HC, MDPD, MDD). Based on a statistically significant difference in age between HC and MDE groups (see Table 1: Demographics, clinical and scan parameters. and Results), age was included as a covariate when comparing baseline [11C]Cimbi-36 BP_{ND} (two-sided) and ΔBP_{ND} (one-sided) between groups using Analysis of Covariance (ANCOVA). One-sided ANCOVA was used for group comparison of ΔBP_{ND} because of our a priori hypothesis of reduction in 5-HT release capacity in patients with depression. Statistical significance was defined at $\alpha < 0.05$. For all models, the effect size was estimated using Cohen's d (d) effect size [108]. Also, the confidence of the result was estimated by computing the Bayes Factor (BF) [109]. As a supplementary analysis, the ΔBP_{ND} of the frontal cortex was analysed with the inclusion of the above-mentioned outlier using non-parametric statistics. One-sided Mann-Withney-Wilcoxon test and bootstrapping statistics were conducted on the ΔBP_{ND} comparison between patients and controls. In addition, one-sided ANCOVA was undertaken for the between-group difference of post-dose BP_{ND}, using baseline BP_{ND} and age as covariates.

A two-sided Paired Student t-test was used to evaluate the change in baseline versus post-d-amphetamine [11C]Cimbi-36 BP_{ND}. The relationships between baseline BP_{ND} and ΔBP_{ND} with psychometric measures (HAM-D, BDI, SHAPS, RS, SSAI, WEMWBS, BFI, WCS), subjective drug effects (VAS), and plasma d-amphetamine levels were tested using Pearson's correlation tests. The correlation between baseline BP_{ND} and ΔBP_{ND} with psychometric measures was corrected for age using partial Persons's correlation, due to the observed association between baseline ΔBP_{ND} and age. To account for false discovery rate (FDR) inflation due to multiple comparisons, the p-values resulting from Persons's tests were adjusted independently using the Benjamini-Hochberg adjustment [110]. The results of the FDR correction are reported as "p adj. " in the main text.

2.4. Results

2.4.1. Demographic, clinical and PET scan characteristics

Gender distribution was similar across the two groups (MDE vs HC), with 3 female MDE and HC included in the study (Table 1). The mean age was higher in the MDE group (Mean: 44 ± 13 years) as compared to the HC group (Mean: 32 ± 9 years; p = 0.002), mainly driven by higher ages in the MDPD group (Mean: 55 ± 10 years) as compared to both the HC (Mean: 32 ± 9 year; p < 0.001) and MDD (Mean: 55 ± 10 vs 40 ± 11; p = 0.017) groups, see Table 1. Average HAM-D scores in the MDE group at baseline were 21 ± 4 (range: 16-30) indicating mild/moderate to severe depression. There was no difference in average [^{11}C]Cimbi-36 mass or radioactive dose injected between the baseline (i.e., Scan 1) and post-d-amphetamine scan (i.e., Scan 2) conditions for any of the groups, and no group difference between MDE vs HC (Table 1). Consistent with previous imaging studies with d-amphetamine as a pharmacological challenge [111,112], an oral dose of 0.5mg/kg of d-amphetamine was well tolerated, and no significant adverse effects were reported. The d-amphetamine plasma concentrations were consistent with previous studies using oral d-amphetamine as a challenge [83,113], with no group difference in the average plasma concentration (HC: 68 ± 19 μg/L, MDE: 77 ± 12 μg/L, ns).

2.4.2. PET metrics

Post-d-amphetamine reductions were observed in the reference region (i.e., the cerebellum) [^{11}C]Cimbi-36 VT for both groups: HC (Mean change: 5.67 ± 9.21 %; p=0.015; d = - 0.97) and MDE group (Mean change: 4.84 ± 8.30 %; p=0.035; d = -0.61). Regional VT data are listed and described in Table 2. In light of the cerebellum serving as the reference region used in the calculation of the BP_{ND}, changes in the cerebellar VT should be assessed carefully to estimate their impact on the findings. However, the cerebellar VT reductions were not statistically different between the two groups.

HC (N=20)	**V_T Scan 1 ± SD**	**V_T Scan 2 ± SD**	**Mean ΔV_T (%) ± SD**	**BP_{ND} Scan 1 ± SD**	**BP_{ND} Scan 2 ± SD**	**Mean ΔBP_{ND} (%)**
Cerebellum	13.51 ± 1.64	12.72 ± 1.83*** d = - 0.97	5.7 ± 9.2			
Frontal Ctx	27.53 ± 5.51	23.62 ± 4.02*** d = - 1.11	13.1 ± 10.1	1.04 ± 0.31	0.87 ± 0.24*** d= - 0.94	15.0 ± 14.4
Parietal Ctx	26.52 ± 5.22	23.00 ± 3.71*** d = - 0.99	12.0 ± 11.5	0.96 ± 0.28	0.82 ± 0.22** d = - 0.74	12.2 ± 19.1
Occipital Ctx	27.53 ± 4.78	24.23 ± 3.99*** d = - 0.94	11.1 ± 11.4	1.04 ± 0.23	0.91 ± 0.21** d = - 0.75	10.6 ± 15.2
Temporal Ctx	28.23 ± 4.94	24.51 ± 4.07*** d = - 0.97	12.2 ± 11.5	1.09 ± 0.24	0.93 ± 1.20** d = - 0.79	12.6 ± 15.4
MDE (All, N=17)	**V_T Scan 1 ± SD**	**V_T Scan 2 ± SD**	**Mean ΔV_T (%) ± SD**	**BP_{ND} Scan 1 ± SD**	**BP_{ND} Scan 2 ± SD**	**Mean ΔBP_{ND} (%) (No outlier, N = 16)**
Cerebellum	14.45 ± 1.96	13.75 ± 2.14* d = - 0.61	4.8 ± 8.3			

Frontal Ctx	28.43 ± 4.79	26.43 ± 5.10* d = - 0.61	6.7 ± 11.8	0.97 ± 0.25	0.92 ± 0.22 ns d = - 0.30	6.5 ± 13.1
Parietal Ctx	27.65 ± 4.54	25.72 ± 4.68* d = - 0.67	6.7 ± 10.4	0.92 ± 0.23	0.87 ± 0.16 ns d = - 0.33	4.9 ± 14.9
Occipital Ctx	28.42 ± 4.41	26.22 ± 4.56* d = - 0.68	7.3 ± 11.5	0.97 ± 0.21	0.91 ± 0.15 ns d = - 0.38	7.0 ± 15.5
Temporal Ctx	30.69 ± 4.82	27.98 ± 4.84* d = - 0.61	7.9 ± 15.6	1.13 ± 0.24	1.04 ± 0.21 ns d = - 0.39	10.9 ± 12.2
MDD (All, N= 12)	**V_T Scan 1 ± SD**	**V_T Scan 2 ± SD**	**Mean ΔV_T (%) ± SD**	**BP_{ND} Scan 1 ± SD**	**BP_{ND} Scan 2 ± SD**	**Mean ΔBP_{ND} (%) (No outlier, N = 11)**
Cerebellum	14.35 ± 2.29	13.94 ± 2.33 ns d = - 0.44	2.7 ± 7.3			
Frontal Ctx	29.03 ± 5.35	27.56 ± 5.26 ns d = - 0.53	4.7 ± 10.5	1.03 ± 0.22	0.98 ± 0.21 ns d = - 0.26	7.7 ± 12.6
Parietal Ctx	28.17 ± 5.11	26.61 ± 4.93* d = - 0.66	5.2 ± 8.6	0.97 ± 0.19	0.91 ± 0.15 ns d = - 0.40	7.6 ± 10.5
Occipital Ctx	28.61 ± 5.07	26.95 ± 4.85 ns d = - 0.60	5.4 ± 10.3	1.00 ± 0.19	0.94 ± 0.16 ns d = - 0.38	8.6 ± 12.3
Temporal Ctx	30.87 ± 5.59	290.3 ± 5.10 ns d = - 0.44	4.9 ± 15.74	1.16 ± 0.24	1.09 ± 0.22 ns d = - 0.27	10.5 ± 12.5
MDPD (N=5)	**V_T Scan 1 ± SD**	**V_T Scan 2 ± SD**	**Mean ΔV_T (%) ± SD**	**BP_{ND} Scan 1 ± SD**	**BP_{ND} Scan 2 ± SD**	**Mean ΔBP_{ND} (%)**
Cerebellum	14.70 ± 0.84	13.30 ± 1.75 ns d = - 0.99	9.7 ± 9.4			
Frontal Ctx	27.00 ± 3.04	23.73 ± 3.90 ns d = - 0.76	11.6 ± 14.4	0.85 ± 0.29	0.79 ± 0.18 ns d = - 0.39	3.9 ± 15.4
Parietal Ctx	26.39 ± 2.78	23.57 ± 3.55 ns d = - 0.69	10.1 ± 14.2	0.81 ± 0.28	0.77 ± 0.15 ns d = - 0.18	-0.9 ± 22.3
Occipital Ctx	27.95 ± 2.60	24.47 ± 3.61 ns d = - 0.83	11.9 ± 14.1	0.91 ± 0.27	0.84 ± 0.12 ns d = - 0.35	3.5 ± 22.5
Temporal Ctx	30.25 ± 2.73	25.46 ± 3.27 ns d = - 0.99	15.1 ± 14.3	1.07 ± 0.28	0.92 ± 0.12 ns d = - 0.75	11.7 ± 13.0

*Table 2: PET scan outcomes before and after d-amphetamine challenge for all groups/subgroups. Statistical analyses in the table were conducted with two-sided paired t-tests to compare PET-1 vs PET-2 V_T and PET-1 vs PET-2 BP_{ND}. Statistical significance levels: *) $p \leq 0.05$; **) $p \leq 0.01$; ***) $p \leq 0.001$; and ns meaning not significant. Cohen's d effect sizes were reported for two-sided paired t-tests noted with the letter d. Mean ΔV_T and mean ΔBP_{ND} were both calculated as the average of individual subjects' Δ values and not by using the group averages for PET-1 and PET-2. Where stated, the Mean ΔBP_{ND} values are reported without the detected outlier (for outlier detection see Materials and Methods section).*

2.4.3. Baseline 5-HT2A receptor availability

At Scan 1, the regional [^{11}C]Cimbi-36 BP_{ND} distribution for both groups was consistent with previous reports for healthy individuals, with high binding across cortical areas [114,115]. There was a negative correlation between age and baseline BP_{ND} for all groups in almost all the analysed regions (data not shown). Thus, age was inserted as a covariate in all analyses involving BP_{ND}. After correcting for age, there was a significant between-group difference in Scan 1 BP_{ND} (reflecting 5-HT2A receptor availability) in the temporal cortex, where the MDE group (Mean: 1.13 ± 0.24) showed higher baseline BP_{ND} compared to the HCs (HC: 1.09 ± 0.24; p= 0.016; d = 0.185; BF = 0.361), with other ROIs following a similar trend but not reaching statistical significance. All regional baseline BP_{ND} values are listed in Table 2.

2.4.4. 5-HT release capacity

There was no significant relationship between BP_{ND} at baseline and the magnitude of ΔBP_{ND} in any of the analysed ROIs and groups. No significant association between age and d-amphetamine plasma concentration with ΔBP_{ND} was detected in either of the two groups or for any of the ROIs (data not shown).

On average, HC had higher frontal cortex [^{11}C]Cimbi-36 BP_{ND} at baseline (BP_{ND} Scan 1: 1.04 ± 0.31) as compared to post-d-amphetamine administration (BP_{ND} Scan 2: 0.87 ± 0.24), and the reduction was statistically significant ($p < 0.001$; d = -0.944; BF = 71.794; Figure 2 A) as assessed with paired t-test. In the MDE group there was no statistically significant difference in BP_{ND} between baseline (BP_{ND} Scan 1: 0.97 ± 0.25) and post d-amphetamine (BP_{ND} Scan 2: 0.92 ± 0.22) scans (p = 0.230; d = -0.303; BF = 0.484; Figure 1 B). A similar pattern was seen in the patient subgroups (Figure 2 C-D) and across other ROI (Table 2). The [^{11}C]Cimbi-36 ΔBP_{ND} (reflecting 5-HT release capacity) was used as the main metric to test whether the within-group reduction of BP_{ND} following d-amphetamine administration differed between HC and MDE. The distribution of the ΔBP_{ND} within the frontal cortex was not normally distributed (W = 0.940; p = 0.046). One MDD patient who showed about 50% lower ΔBP_{ND} was excluded from the analysis after formal evaluation to preserve a normal distribution of the data (W = 0.990; p = 0.970). Without the outlier, a comparison of the frontal cortex ΔBP_{ND} between the HC and MDE group revealed a significantly lower ΔBP_{ND} in patients (HC: 15.0 ± 14.4 % vs MDE: 6.5 ± 13.1 %; p= 0.041; d = -0.611; BF = 1.131; Figure 2 E). A comparison of each patient subgroup to the HC indicated a similar trend, with a significant difference between HC and MDD groups (HC: 15.0 ± 14.4 % vs MDD: 7.7 ± 12.6 %; p= 0.043; d = -0.535; BF = 0.723) but not between HC and MDPD groups (HC: 15.0 ± 14.4 % vs MDPD: 3.9 ± 15.4; p= 0.099; d = -0.741; BF = 0.902), possibly due to the small sample size of the MDPD subgroup (N=5). Between-group ΔBP_{ND} differences in the other cortical ROIs did not reach statistical significance (see Table 2 for all [^{11}C]Cimbi-36 ΔBP_{ND}).

As complementary analyses, a non-parametric approach was used to interpret the data with the inclusion of the outlier. First, a one-sided Mann-Withney-Wilcoxon test was performed to compare the between-group difference of ΔBP_{ND} in the frontal cortex. The test resulted as borderline significant (HC, 15 ± 14.4 % vs MDE, 3 ± 18 %; p = 0.055). Bootstrap analysis was run on the difference in frontal ΔBP_{ND} between the MDE and HC groups. The 95% confidence interval of the obtained distribution of the difference in frontal ΔBP_{ND} between the MDE and HC groups was within 1.76 (2.5th centile) and 23.03 (97.5th centile). Given that the interval does not include 0, there is 95% confidence in having a statistically significant difference between the two groups (approx. p-value = 0.011; Figure 2 F). Thus, there is evidence to reject the null of equal mean ΔBP_{ND} between the two groups.

In addition to non-parametric statistics, an analysis of covariance was carried out on the full BP_{ND} data without using the ΔBP_{ND} parameter. When considering the distribution of the pre d-amphetamine BP_{ND} ($W = 0.972$; $p = 0.454$) and post-d-amphetamine BP_{ND} ($W = 0.984$; $p = 0.858$) in the frontal cortex of the MDE group, no violation of parametric assumption occurred. To corroborate the parametric and non-parametric analysis performed on the ΔBP_{ND}, a one-way ANCOVA was conducted on the between-groups difference in post-d-amphetamine BP_{ND}, using the pre-d-amphetamine BP_{ND} and age as covariates. Again, the reduction of BP_{ND} was significantly higher for the HC group as compared to the MDE group when controlling for age and baseline BP_{ND} ($p < 0.020$ in the frontal, temporal, and parietal cortices).

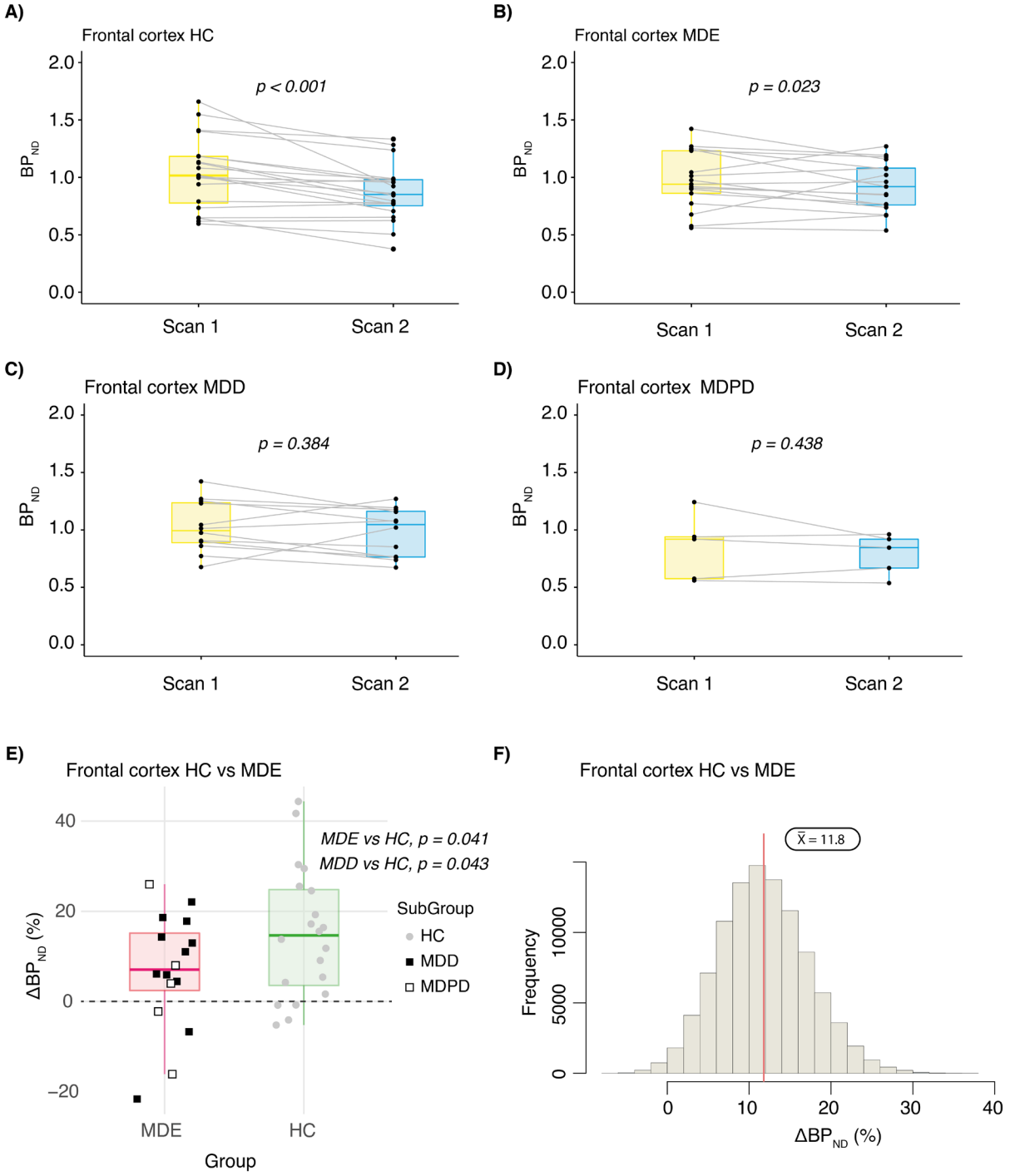

Figure 2: Frontal cortex [^{11}C]Cimbi-36 BP_{ND} and ⊿BP_{ND}. A) The difference in frontal cortex BP_{ND} in the HC group. B) The difference in frontal cortex BP_{ND} in the MDE group. C) The difference in frontal cortex BP_{ND} in the MDD subgroup. D) The difference in frontal cortex BP_{ND} in the MDPD subgroup. E) The difference in frontal cortex ⊿BP_{ND} between the HC group and MDE group (and MDD subgroup) using parametric statistics (no outlier). F) The difference in frontal cortex ⊿BP_{ND} between the HC group and MDE group using non-parametric statistics (outlier). Significance levels for the interaction effects were defined with a = 0.05 (see materials and methods for details).

2.4.5. Drug effect measures

No significant group differences were found in subjective drug effect during the post d-amphetamine PET scan. A negative correlation between the reported drug effect and ΔBP_{ND} was detected in the HC group (R=-0.49, p=0.03) but not in the MDE group. There was no relationship between plasma concentration of d-amphetamine and drug effects.

2.4.6. Relationship between PET and psychometric measures

There were no statistically significant associations between measures of depressive symptomatology (HAM-D, BDI, SHAPS, RS, SSAI) and baseline BP_{ND} or ΔBP_{ND} within the MDE group in any of the cortical ROIs. After correcting for age, there was a statistically significant correlation between baseline [^{11}C]Cimbi-36 BP_{ND} and scores on the WCS in all analysed ROIs: frontal cortex ($R = -0.86$; $p = 0.003$; Figure *3* A), parietal cortex ($R = -0.80$; $p = 0.010$; Figure *3* B), occipital cortex ($R = -0.79$, $p = 0.012$; Figure *3* C), temporal cortex ($R = -0.75$, $p = 0.020$; Figure *3* D). Only the correlation in the frontal cortex (p adj.= 0.048) survived correction for multiple comparisons. No correlations were found between WCS and ΔBP_{ND} and between measures of personality and well-being with measures of baseline BP_{ND} or ΔBP_{ND}.

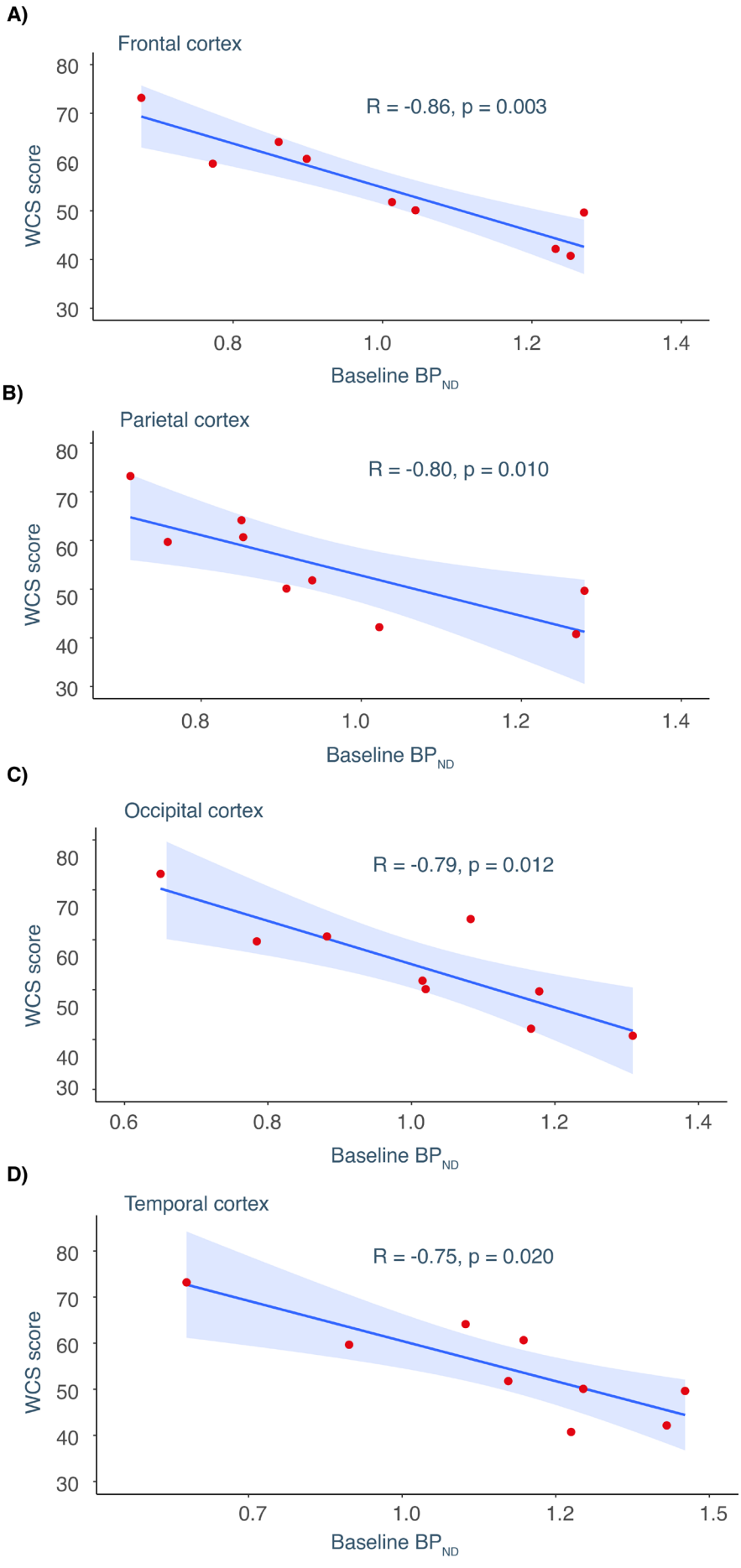
A)
Frontal cortex
R = -0.86, p = 0.003
WCS score
Baseline BP ND
B)
Parietal cortex
R = -0.80, p = 0.010
WCS score
Baseline BP ND
C)
Occipital cortex
R = -0.79, p = 0.012
WCS score
Baseline BP ND
D)
Temporal cortex
R = -0.75, p = 0.020
WCS score
Baseline BP ND

Figure 3: Correlation between connectedness and 5-HT2A receptor availability in the MDD subgroup. A) The correlation between WCS scores and frontal cortex baseline [^{11}C]Cimbi-36 BP_{ND}. B) The correlation between WCS scores and parietal cortex baseline [^{11}C]Cimbi-36 BP_{ND}. C) The correlation between WCS scores and occipital cortex baseline [^{11}C]Cimbi-36 BP_{ND}. D) The correlation between WCS scores and temporal cortex baseline [^{11}C]Cimbi-36 BP_{ND}. R coefficients and p values are derived from partial Pearson's correlation tests (corrected for age). The total number of data points is N = 9 due to missing data for 2 participants in the MDD subgroup. Significance levels were defined with a = 0.05 (see materials and methods for details).

2.5. Discussion

A reduced 5-HT release capacity was observed in the frontal cortex of depressed patients compared to healthy individuals, by a direct examination of 5-HT release using [^{11}C]Cimbi-36 PET combined with oral d-amphetamine. The difference can be interpreted as reduced synaptic 5-HT affecting the depressed patient group; the first direct evidence in support of the "5-HT deficiency" hypothesis of depression. While d-amphetamine is usually considered to be an agent leading to the release of dopamine and noradrenaline, it also potently releases 5-HT [85–87,116]. D-amphetamine has negligible affinity for the 5-HT2A receptor (Ki > 10.000 nM [117,118], whereas [^{11}C]Cimbi-36 has >1000-fold selectivity for the 5-HT2A receptor over dopaminergic and noradrenergic targets [119]). Therefore, an interaction between either d-amphetamine and [^{11}C]Cimbi-36 at the 5-HT2A receptor, or between monoamines other than 5-HT and [^{11}C]Cimbi-36 at dopaminergic or noradrenergic targets, cannot explain the decrease in [^{11}C]Cimbi-36 BP_{ND} after a d-amphetamine challenge. Further, the change in [^{11}C]Cimbi-36 BP_{ND} is proportional to the magnitude of change in 5-HT as measured by microdialysis [80]. Following this, the observed d-amphetamine-induced reduction of [^{11}C]Cimbi-36 BP_{ND} was caused by an increased extracellular 5-HT. It is not clear whether the change in [^{11}C]Cimbi-36 BP_{ND} is dependent on direct competition of 5-HT at the 5-HT2A receptor, increased internalization of the 5-HT2A receptor following increased agonist stimulation by 5-HT (analogous to the internalization of the dopamine D2 receptor invoked to explain changes in the BP_{ND} of [^{11}C]raclopride and [^{11}C]PHNO), or a combination of the two. However, as the magnitude of both direct competition and receptor internalization is ultimately dependent on the magnitude of the 5-HT concentration, the exact mechanistic explanation is not critical for the conclusion that the magnitude of [^{11}C]Cimbi-36 ΔBP_{ND} is proportional to the magnitude of the intra-synaptic 5-HT concentration.

Depression is extensively linked to reduced brain 5-HT levels. The rate-limiting enzyme in the brain 5-HT synthesis, is Tph2 [120,121]. While genotype and haplotype studies indicate a link between Tph2 and affective disorders [75,122,123], post-mortem brain sections from medication-free depressed individuals who committed suicide reveal higher Tph2 mRNA levels and higher Tph2 binding [124,125], explained as a homeostatic compensation within the cell bodies of the raphe nuclei in response to deficient 5-HT neurotransmission in the serotonergic projection areas. Reduced synaptic 5-HT concentration may result from increased 5-HT catabolism. Intraneuronal 5-HT catabolism is primarily dependent on MAO-A, and higher MAO-A expression has been detected post-mortem in the brains of patients with MDD [126]. PET studies using the MAO-A selective radioligand [^{11}C]harmine have also reported higher MAO-A expression in depressed patients in two separate studies [127,128], as well as a positive relationship between depressive symptoms and MAO-A expression in smokers [129]. A large proportion of synaptic 5-HT is re-absorbed into the pre-synaptic terminal via the SERT, and intraneuronally is packed into synaptic vesicles via the vesicular monoamine transporter type 2 (VMAT2). A dysfunction of either SERT or VMAT2 could lead to increased loss of synaptic or neuronal 5-HT, and ultimately to 5-HT depletion. The interpretation of SERT expression in depression is complicated by the fact that both increases (interpreted as a cause of reduced synaptic 5-HT) and decreases (interpreted as compensatory changes in response to 5-HT deficits caused by other factors) are plausible. Postmortem studies have detected reduced SERT availability in the frontal cortex of depressed patients [130,131], a region with known impaired functioning in depression [132,133]. One PET study, using the SERT radioligand [^{11}C]DASB, reported a reduced SERT ratio between dorsal raphe and ventral striatum in medication-free depressed patients [134]. Interestingly, this reduction was not seen when comparing remitted depressed patients with healthy subjects [135]. However, SERT imaging studies in depression overall show inconsistent results, with reports of both elevated and reduced SERT densities

among depressed unmedicated patients (for review see [136]). Similarly, PET studies in patients with MDPD have shown both positive correlations between depressive symptoms (and not with motor symptoms) and SERT binding in the median raphe nuclei, hypothalamus, limbic structures, posterior cingulate cortex [137] and frontal cortex [138] and a lack of relationship between depressive symptoms and SERT availability [139]. Human studies of VMAT2 status in depression are scarce but murine models of VMAT2 deficit produce an increased incidence of depressive-like phenotypes [140,141], and behavioural effects of conditional VMAT2 knock-out in the raphe nuclei, could be reversed by a repletion of cellular 5-HT stores [142].

The present study included patients with MDD as well as ones with MDPD. The obtained results were broadly consistent in the two patient subgroups, although the small number of MDPD patients (N=5) has limited the possibility of statistically significant effects in this subgroup. Although PD is commonly thought of as a disease of dopamine insufficiency, considerable evidence exists to implicate 5-HT deficits in its pathology. Midbrain serotonergic neurons reside in the raphe nuclei and project to cortical areas and support cortical functions impaired in patients with PD. Lewy body and Lewy neurite deposition within the raphe nuclei occurs during the early stages of the disease, resulting in a reduction of 5-HT neurons [143]. Consistent with this notion, plasma and cerebrospinal fluid concentrations of 5-HT and its main metabolite 5-Hydroxyindoleacetic acid (5-HIAA) have been reported to be lower in patients with MDPD than in non-depressed patients [144,145], with plasma levels of 5-HIAA showing a negative correlation with the severity of depressive symptoms [146]. Chronic reductions in synaptic 5-HT concentrations due to the pathology of raphe serotonergic neurons are expected to lead to homeostatic changes in 5-HT receptors and transporters. The SERT and the pre-synaptic autoreceptor 5-HT1B both function to regulate synaptic 5-HT concentrations, and the reductions seen in the expression of these proteins in the 5-HT projection areas of non-depressed patients with PD in post-mortem [147], as well as in-vivo PET imaging studies [148–151], are consistent with compensatory changes aiming to increase synaptic 5-HT concentrations. Hence, an impairment of the 5-HT system may be causally related to the high incidence of depression in PD [152], further supported by the efficacy of SSRI treatment of depressive symptoms in these patients [153]. Thus the strength of evidence for 5-HT deficits in MDPD is similar to that in patients with MDD, and is consistent with the findings of the present study.

The evaluation of serotonergic markers (i.e., availability of 5-HT receptors) in patients with depressive symptoms is complicated by the predominantly cross-sectional nature of such studies. Both increased and decreased expression of 5-HT receptors can be accommodated depending on whether these are seen as causing reduced 5-HT concentration, or as a homeostatic mechanism designed to compensate for 5-HT deficits caused by other pathological mechanisms. For example, the imaging of the raphe somatodendritic autoreceptor 5-HT1A, which modulates the firing rate of 5-HT neurons, in depressed patients demonstrates reduced [154], unchanged [155] or increased [156] expression. Similarly, a PET study using [^{18}F]MPPF, that examined the post-synaptic 5-HT1A receptors, found lower expression in the limbic regions of MDPD patients than in non-depressed patients, not clearly consistent with the notion of reduced synaptic 5-HT [157]. To date, human imaging studies examining the status of 5-HT2A receptors in depression have used antagonist radioligands, such as [^{18}F]altanserin, [^{18}F]setoperone, [^{18}F]FESP or [^{11}C]MDL100,907. These studies report a mix of increases [158,159], decreases [160–164], and no changes [165] in 5-HT2A receptor expression in depressed patients. While the majority of these studies (5 out of 8) show decreased 5-HT2A receptor binding in depression, others reported a positive association between 5-HT2A receptor binding and dysfunctional attitude [159], neuroticism [166,167], sleep deprivation [168], and pessimism [169] that all point towards high 5-HT2A receptor binding being a vulnerability factor for mood disorders. Down-regulation of 5-HT2A receptors induced by SSRIs [163,170] could theoretically explain some of this discrepancy by masking elevated 5-HT2A receptors binding among the medicated patients in these studies. However, the two studies conducted in medication-naïve patients speak against this, as they both report reduced 5-HT2A receptor binding [163,164]. The present study is the first to evaluate the expression of high-affinity (or GCP-coupled) 5-HT2A receptor in depression, as it made use of an agonist 5-HT2A receptor PET ligand. High-affinity 5-HT2A receptors may be more relevant than the total number of 5-HT2A receptors, as the endogenous neurotransmitter, 5-HT, will interact only with the high-affinity 5-HT2A receptors [171]. In the present study, a higher baseline 5-HT2A receptor binding in depressed patients

compared to HCs was observed in the temporal cortex (corrected for age), with other ROIs showing a similar trend but not reaching statistical significance, and consistent with a homeostatic adaptation to lower synaptic 5-HT. No relationship was found between the severity of depression and the magnitude of induced 5-HT release. Yet, an interesting negative correlation was found between 5-HT2A receptor availability at baseline and the psychological trait measure of connectedness. In particular, higher levels of baseline 5-HT2A receptors were associated with lower scores on the WCS. Connectedness is a psychological construct indexing a trait-level disposition toward feeling connected to oneself, others, and the wider world [97]. Social connectedness, a related construct, has shown a protective effect on depressive symptoms among adults in the general population [172]. Also, increases in connectedness, as measured via the WCS, have been shown to be consistently induced by classic psychedelics alongside their antidepressant effects. Together with the finding of a higher baseline 5-HT2A receptor availability in depressed patients, the negative correlation between 5-HT2A receptor availability and connectedness seems to support the notion of an over-expression of 5-HT2A receptors being associated with traits of depressive symptomatology. This is relevant in light of models linking chronic stress with upregulated 5-HT2A receptors [173,174].

Based on the above, it is logical to assume that a higher level of high-affinity 5-HT2A receptors would predict higher responses to receptor agonists, such as classic psychedelics, leading to higher acute subjective effects and therapeutic responses. The occupancy of 5-HT2A receptors has been consistently found to predict the intensity of psychedelic subjective effects [175], which are completely blocked by the administration of 5-HT2A antagonists [176]. However, one study found a negative correlation between baseline 5-HT2A receptor availability and the occurrence of psychedelic-induced mystical experiences, a known mediator of long-term therapeutic response [177]. Yet, similar data are lacking in patient populations. While important for future studies' hypothesis formulation, the results on baseline 5-HT2A receptor availability in the present study can't be interpreted conclusively, also due to the limited size of the statistical effect and study sample. Future large-scale studies are needed to elucidate the association between 5-HT2A receptor availability, depressive symptomatology, and response to antidepressants.

Additional limitations of the present study should be mentioned. In line with the majority of studies using a d-amphetamine challenge, a fixed-order design was used due to the complexity of conducting such as study in a randomized manner. Further, diurnal variability in 5-HT2A receptor expression or 5-HT levels may have increased the variability of [^{11}C]Cimbi-36 ΔBP_{ND}. However, no evidence for such effects for [^{11}C]Cimbi-36 exists. Test-retest data in 8 healthy human volunteers, obtained with test and retest scans conducted at different times of the day (with a duration of between 3 hours and several weeks between the two scans) do not suggest any carry-over or diurnal effect [114]. No evidence for such effects, if relevant, being different in depressed patients and healthy individuals is available either. Another factor to be considered is the possibility of increased movement by depressed subjects (and in particular the ones with MDPD) during the PET scan, leading to increased variability in the outcome parameters. All subjects were monitored for movement by the research-trained PET technologists and no reports of greater movement in patients compared to healthy volunteers were observed. In addition, the variability of the baseline [^{11}C]Cimbi-36 BP_{ND} was similar across all the groups, and lower variability in BP_{ND} was seen in all the patient groups post-amphetamine compared to baseline, arguing against increased movement in patients compared to healthy controls, or greater movement post-amphetamine. A significant reduction in cerebellar VT was seen post-amphetamine in all groups. Changes in peripheral metabolism and delivery of [^{11}C]Cimbi-36 to the brain, are the most likely causes of the changes seen. As the cerebellum is a reference region used to estimate regional BP_{ND}, a reduction in cerebellar binding could lead to an underestimation of [^{11}C]Cimbi-36 ΔBP_{ND}, and hence 5-HT release. However, irrespective of the reasons for such changes, the magnitude of reductions in cerebellar VT was similar in HC and patients with MDE, and cannot explain the differences in [^{11}C]Cimbi-36 ΔBP_{ND} observed between the groups. One subject from the patients group was excluded from the analysis of ΔBP_{ND} as an outlier, causing a reduction in inferential power. The inclusion of that data point would have maximized the difference in ΔBP_{ND} between MDE and HC groups in the direction of the main finding. To test the robustness of the findings, non-parametric statistical analysis, as well as an alternative analytical approach with the outlier included was performed, which confirmed a significant difference in ΔBP_{ND} between the two groups, with the direction of the effect being independent of

deviation from the within-group norm. Nonetheless, it is important to acknowledge the small effect size and limited confidence of the finding, as quantified via Cohen's d and BF metrics, respectively.

In conclusion, the first direct assessment in the human brain demonstrates a reduction in 5-HT release in patients with depression compared to healthy individuals. The study provides the most direct evidence to date in support of the "5-HT deficiency" hypothesis of depression. The ability to directly test 5-HT release capacity allows the examination of the mechanisms of action of various antidepressant medications, serving as an invaluable tool for neuropsychopharmacology. An evaluation of the relationship between the magnitude of 5-HT release in response to serotonergic pharmacological treatments, such as SSRIs and classic psychedelics, or with different pharmacological profiles, such as SSNIs and ketamine, can help elucidate the underlying mechanisms of drug resistance and individual variability in drug responses. Patients with 5-HT release capacity in the normal range may not demonstrate a beneficial response to 5-HT-elevating medication but may respond to noradrenergic, dopaminergic, or glutamatergic treatments. In this regard, the observed difference in baseline 5-HT2A receptor availability in depressed patients, and the association of elevated 5-HT2A receptor with low connectedness, suggest that individual variability in the psychophysiological phenotypes might predict different drug responses, both in terms of neural and therapeutic trajectories. While intriguing, future studies with bigger sample sizes are needed to confirm such observations.

3. Chapter 3: The mechanism of action of ketamine

" This pharmakon, this medicine, this philter, which acts as both remedy and poison, already introduces itself into the body of discourse with all its ambivalence. The pharmakon would be – alternately or simultaneously – beneficent or maleficent. "

Jacques Derrida, Plato's Pharmacy, 1981.

The following chapter will present a narrative review of the available evidence about the mechanisms of action of the novel rapid-acting antidepressant ketamine.

3.1. Introduction

In the early 2000s, Berman et al. conducted a groundbreaking study that demonstrated the efficacy of a single continuous injection of a subanaesthetic dose of ketamine (0.5 mg/kg) in producing rapid antidepressant effects in MDD patients. Notably, the study revealed a significant antidepressant effect of ketamine as early as 4 hours post-infusion, progressively increasing up to 72 hours [178]. Using similar doses, subsequent research has consistently confirmed the notable efficacy of ketamine in unipolar and bipolar depression, with antidepressant effects peaking after 24 hours and lasting for up to a week after a single dose, with additional doses extending the effects up to 4 weeks post-treatment [179]. The unique combination of rapid onset and prolonged duration characterizes the novel and attractive attributes of ketamine as an antidepressant. Furthermore, its effectiveness in cases of TR-MDD, notably in diminishing symptoms of suicidal ideation and anhedonia, positions ketamine as the most efficacious antidepressant presently available, a conclusion substantiated by a recent comparative meta-analysis [180]. Ketamine efficacy in mood regulation extends to various mental health conditions, including addiction and post-traumatic stress disorder (PTSD), and diverse dose ranges, formulations and routes of administration are available [37,179,181]. Despite its high safety, tolerability, and efficacy as an antidepressant, concerns persist regarding the potential for abuse and neurotoxic effects with chronic intake [182]. Nevertheless, the substantial body of evidence supporting the therapeutic potential of ketamine prompted the U.S. FDA in 2019, followed by the European Medicines Agency, to approve ketamine for the treatment of TR-MDD [183].

While ketamine is currently diffusing into psychiatric practice for diverse patient populations, its mechanism of action remains poorly understood. In the following paragraph, the available pre-clinical and clinical evidence on the acute neural effects of ketamine will be summarised.

3.2. Acute neural effects of ketamine

3.2.1. Pharmacology

Chemically categorized as an arylcyclohexylamine, ketamine exists in two enantiomers: S(+)-ketamine and R(-)-ketamine [184]. At comparable concentrations, S(+)-ketamine has a higher affinity for NMDA receptors and has stronger analgesic and anaesthetic effects, while R(-)-ketamine is believed to have stronger NMDA receptor-independent therapeutic effects [185]. In psychiatric research and practice, ketamine is administered either i.v. as a racemic mixture of both enantiomers (still the most common formulation in clinical trials) or intranasally as S(+)-ketamine alone, the formulation approved by the FDA. Ketamine has a tight dose-response curve, with dosage determining the transition from analgesic, to dissociative/psychedelic-like, into anaesthetic effects. Antidepressant doses of ketamine range from 0.5 to 1 mg/kg, and are associated with the emergence of the dissociative/psychedelic-like effects. When injected as of racemic mixture over 40 minutes, 0.5 mg/kg of ketamine leads to a peak plasma concentration of approximately 185 ng/ml, with an elimination half-life of 2-3 hours, and subjective effects lasting around 1-2 hours [186,187]. In the body, ketamine is rapidly metabolized by the cytochromes P450 system to produce the major metabolite norketamine and the minor metabolite hydroxyketamine (HK). Norketamine and HK are further metabolised into hydroxynorketamine (HNK) metabolites [187]. Following a subanaesthetic antidepressant dose of ketamine, clinically significant plasma concentrations of ketamine and its metabolites have been shown to be present during the first 4 hours after the infusion, as well as 1-day post-infusion, in individuals with TR-MDD [186,188].

Acutely, ketamine exhibits affinity for various binding sites in the brain, including glutamatergic, opioid, monoaminergic, cholinergic, nicotinic, and muscarinic receptors. However, the antidepressants and dissociative effects of ketamine are mainly linked to its acute glutamatergic mechanism of action, as a non-competitive antagonist of the NMDA receptor [185]. Even though other targets have also been proposed, like the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and the hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels [185,189]. The NMDA receptors are

glutamate-gated calcium-permeable ion channels typically forming heterotetrameric complexes consisting of two glycine-bound GluN1 subunits and two glutamate-bound GluN2 (2A–2D) subunits. The GluN subunits possess four transmembrane segments (M1 to M4), with the M2 segment representing the ionic channel of the receptor. Ketamine binds to the intrachannel PCP site of the NMDA receptor, specifically on the GluN2 subunit (Figure 4)[184]. Recently identified key residues in the human NMDA receptor binding site include Asparagine 616 on the GluN1 subunit, and Leucine 642 and Leucine 643 on the GluN2A and GluN2B subunits, respectively [190]. Calcium influx requires the voltage-dependent removal of Mg^{2+}, together with the simultaneous binding of glycine and glutamate at the NDMA receptor. Under physiological conditions, the presence of Mg^{2+} in the NMDA receptors prevents ketamine from accessing the binding site. However, AMPA-dependent removal of Mg^{2+} ketamine binding results in a reduction of channel opening time and a decrease in the amplification of the response to repeated stimulation. A hysteresis effect has also been demonstrated, where prior openings of the NMDA channel correlate with higher ketamine antagonism. Additionally, ketamine acts as an allosteric antagonist of the NMDA receptor [184,185].

The systemic administration of a subanaesthetic dose of ketamine has been consistently associated with an increase in frontocortical extracellular glutamate release, as evidenced by numerous studies in both animal models and humans, correlating with the drug's dissociative/psychedelic-like and antidepressant effects [191–195], although not confirmed by other studies [196,197]. This response seems paradoxical in light of the NMDA receptor antagonistic properties of ketamine, and different mechanistic theories exist on how NMDA receptor antagonism might result in a glutamatergic surge.

3.2.2. Animal research

The leading theory coming from pre-clinical research is the "disinhibition" model, which proposes that at subanaesthetic doses ketamine preferentially inhibits NMDA receptors expressed on GABA interneurons, leading to pyramidal cell disinhibition and an enhancement of excitatory glutamatergic neurotransmission. The rise in glutamate brought on by ketamine activates postsynaptic AMPA receptors, releasing the brain-derived neurotrophic factor (BDNF) which activates the mammalian target of rapamycin (mTOR) via agonism to the tropomyosin receptor kinase B (TrkB). In this manner, an autoregulatory feedback loop is initiated, whereby activation of mTOR increases the production of BDNF through a series of post-synaptic kinases (Figure 4). By increasing the dose towards anaesthesia, ketamine concentration would increase enough to inhibit NMDA receptors on excitatory neurons, resulting in brain-wide inhibition and cortical reduction of glutamate [185]. Inhibitory interneurons are characterized by higher firing rates as compared to pyramidal neurons, leading to higher basal depolarization of the membrane and higher accessibility of NMDA receptors for ketamine, due to increased relief of Mg^{2+} blockage [198]. In vitro evidence showed that ketamine has a higher affinity for NMDA receptors with GluN2D subunits, which are enriched in cortical inhibitory interneurons [199]. In rodents, it was found that ketamine blocks NMDA receptors on GABA interneurons to cause glutamate efflux and indirect activation of excitatory synapses in the pre-frontal cortex (PFC). Selective knockdown of GluN2B subunits on GABAergic but not glutamatergic neurons in PFC blocks the antidepressant-like effects of ketamine [193,200]. In particular, It has been shown that ketamine increases pyramidal neuron activity and cortical excitatory/inhibitory ratio via inhibition of various populations of fast-spiking interneurons [201,202]. In mice, a recent study found that the dissociative state induced by ketamine is generated by suppression of highly active excitatory neurons and activation of previously silent excitatory neurons during wakefulness, with an increase in overall activity levels. The switch occurs across cortical layers and regions, is induced by both systemic and cortical application of ketamine, and is mediated by suppression of different types of fast-firing interneuron activity via inhibition of NMDA receptors and HCN channels, and activation of AMPA receptors in the newly activated neurons [202]. This builds on previous evidence where it was shown that ketamine-induced dissociation in mice is strongly associated with an HCN-dependent 1-3-Hz rhythm in layer 5 neurons of the retrosplenial cortex [203]. However, other studies reported that subanaesthetic doses of ketamine can suppress overall dendritic NMDA receptor-mediated burst firing in PFC pyramidal neurons in vitro and in vivo, challenging the disinhibition hypothesis [204,205]. Also, evidence exists on the role of GABAergic interneuron activity in

mediating antidepressant effects [185]. Further, animal work has identified several alternative neurophysiological underpinnings of ketamine's acute mechanism of action. Those include but are not limited to, ketamine-mediated inhibition of activity-independent spontaneous NMDA receptor-mediated transmission [206], inhibition of extra-synaptic GluN2B-containing NMDA receptors [207], inhibition of NMDA receptor-dependent bursting activity of neurons in the lateral habenula [208], and stimulation of a specific subpopulation of dopamine receptor D1 (Drd1)-expressing pyramidal neurons within the PFC [193,209,210].

3.2.3. Human research

In humans, the acute effects of subanaesthetic doses of ketamine and their relationship with subjective effects and clinical outcomes have been investigated by numerous studies with various resting-state neuroimaging techniques. Using PET in healthy subjects it was shown that subanaesthetic doses of S(+)-ketamine increased cerebral metabolic rates of glucose in the frontal cortex, correlating with ego-disintegration and hallucinatory phenomena. Comparable doses of R(-)-ketamine tended to decrease cerebral metabolic rates of glucose across brain regions without producing a dissociative state [211]. Others have found that ketamine induced a global increase in CBF, accompanied by decreased regional oxygen extraction and increased cerebral glucose metabolism [212]. In patients with depression, a few studies reported a global ketamine-induced increase in regional CBF accompanied by changes in regional oxygen extraction and cerebral glucose metabolism. Increased metabolism in the hippocampus and dorsal anterior cingulate cortex (ACC) correlated with decreased symptoms of anhedonia, whereas decreased suicidal ideation scores were associated with decreased metabolism in the infralimbic cortex [213,214]. Analysis of BOLD signal from fMRI studies in healthy subjects showed that ketamine increases activity in the thalamus, hippocampus, middle and posterior cingulate, insula, cortical temporal regions, and ventrolateral PFC, while it decreases activity in the subgenual ACC and orbitofrontal cortex, and ventromedial PFC, which correlated with dissociative effects [215–221]. In patients with depression, acute modulations of BOLD activity within the subgenual and dorsal zones of the ACC have been specifically implicated in ketamine's anti-anhedonia effects [222]. The use of fMRI to measure brain connectivity during ketamine has provided evidence that ketamine administration reduces within-network functional connectivity and increases between-network connectivity. A few studies reported that ketamine produces an acute increase in global functional connectivity in every voxel in the brain, indicating overall hyperconnectivity in healthy individuals [223,224]. In patients with TR-MDD, it was found that ketamine reduces global brain connectivity of the PFC compared to healthy volunteers at baseline, but increases global brain connectivity in the posterior cingulate, precuneus, lingual gyrus, and cerebellum. Furthermore, ketamine responders had increased connectivity in the lateral PFC, caudate, and insula compared to non-responders [225]. It was shown that ketamine reduces connectivity within the default-mode network (DMN) and increases functional connectivity between the central executive network (CEN) and the rest of the brain in healthy individuals [226,227]. Importantly, the DMN is involved in narrative self-experience, mind-wondering, and rumination, and its connectivity is increased in depression [228]. A study found that reduced connectivity between the pregenual ACC and posterior cingulate cortex was associated with increased dissociation during infusion, and reduced activation in the left superior temporal cortex was associated with impaired self-monitoring, which is exacerbated in depression [229]. In task-based fMRI studies, ketamine was found to acutely impair performance on executive functions and working memory. The most studied cognitive domains in task-based neuroimaging studies of ketamine are executive functions, working memory, and emotional processing. In cognitive tasks, ketamine has been found to impair performance, associated with altered activity within both task-relevant and task-irrelevant regions, especially within the DMN [230]. In emotional processing tasks, ketamine attenuated activation of the amygdala-hippocampal complex specifically in response to negative pictures compared to neutral or positive pictures, opposite to what is observed in depression. Furthermore, increased intensity of the acute alteration of consciousness induced by ketamine predicted the reduction in neuronal responsiveness to negative (but not neutral or positive) pictures [231]. This is clinically relevant as several (but not all [232]) studies have found that individuals with MDD demonstrate attentional biases toward negative stimuli and away from positive stimuli [233–236]. The acute subjective effects induced by

ketamine as well as the effects on neurophysiological imaging techniques, such as EEG and MEG, will be reviewed in the next chapter.

3.2.4. Discussion

To summarise, ketamine produces an acute complex cellular, circuit and brain-wide modulatory effect, associated with the emergence of perceptual alterations and antidepressant responses (Figure 4). Current literature suggests that the glutamatergic action of ketamine is responsible for the subjective and therapeutic effect of the drug. In particular, ketamine antagonism at NMDA receptors expressed on glutamatergic and GABAergic neurons would result in the alteration of cortical excitatory/inhibitory balance, producing large-scale alterations of functional connectivity. This modulation seems to be characterized by a reduction of within-network integrity and increased between-network communication, especially in high-order functional networks involved with cognition and emotional regulation, with important implications for clinical manifestations of depression.

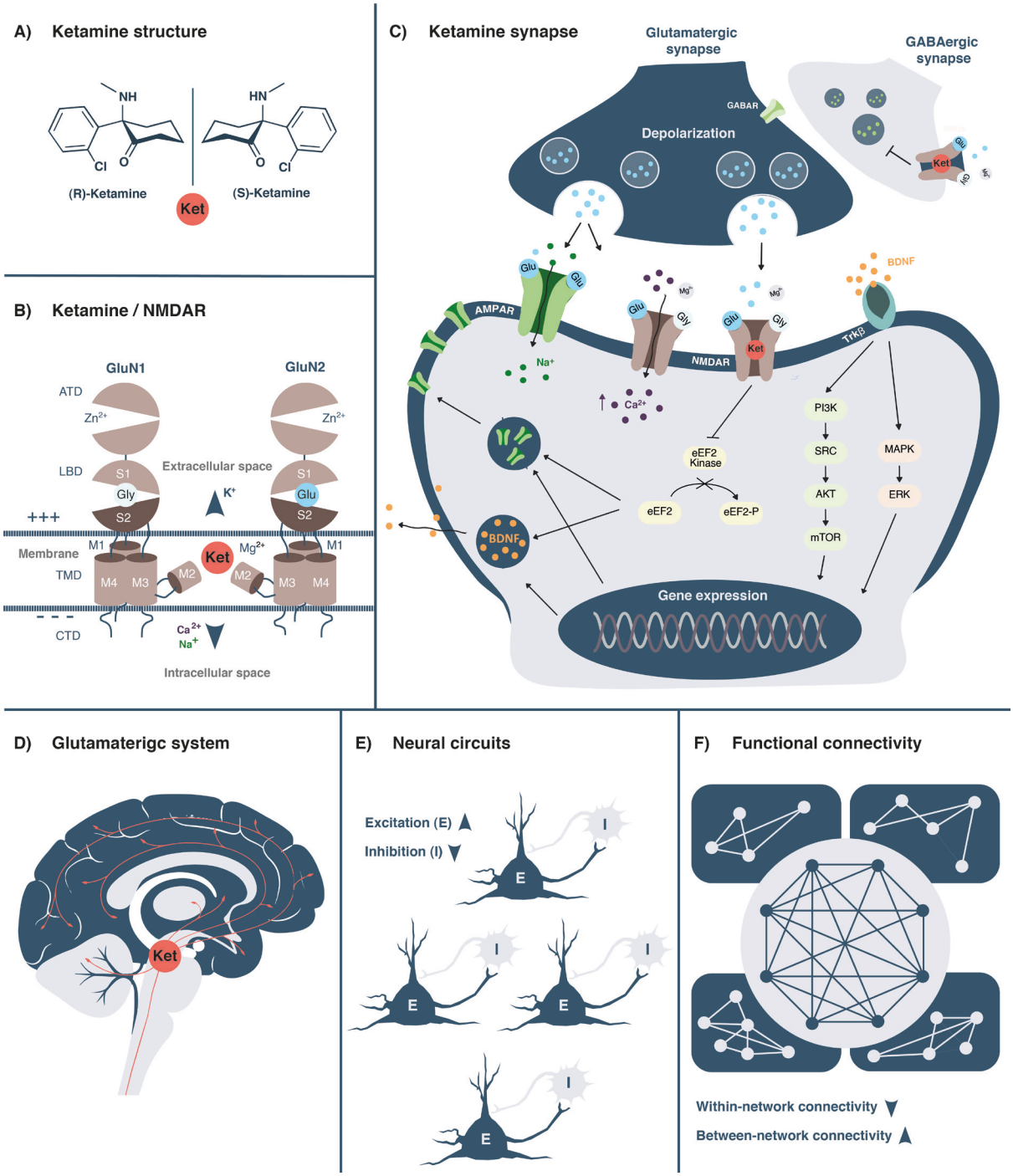

Figure 4: The acute effects of ketamine. A) The chemical structure of ketamine with its 2 enantiomers. B) An illustration of the NMDA receptor with its subunits and the binding site of ketamine. C) The synaptic effects of ketamine antagonism at the NMDA receptor. D) The diffused effect of ketamine on the brain's glutamatergic system. E) The neural circuit alterations induced by ketamine. In particular, the shift of the excitatory/inhibitory balance as a result of ketamine antagonism at the NDMA receptors expressed on GABAergic interneurons. F) A diagram of the brain-wide effects of ketamine on functional connectivity. In particular, the reduction in within-network functional connectivity and the increase in between-network functional connectivity. Abbreviations: Ket = ketamine; NMDAR = N-methyl-D-aspartate receptor; AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GABAR = γ-Aminobutyric acid receptor; BDNF = Brain-derived neurotrophic factor; TrkB = Tropomyosin receptor kinase B; ATD = amino terminal domain; LBD = ligand-binding domain; TMD = transmembrane domains; CTD = intracellular carboxyl terminal domain; Glu = glutamate; Gly = glycine; Zn^{2+} = zinc ions; Mg^{2+} = magnesium ions; Na^{+} = sodium ions; Ca^{2+} = calcium ions; K^{+} = potassium ions; eEF2 = eukaryotic translation elongation factor 2; PI3K = Phosphoinositide 3-kinases; SRC = Proto-oncogene tyrosine-protein

kinase Src; AKT = Protein kinase B; mTOR = Mammalian target of rapamycin; MAPK = mitogen-activated protein kinases; ERK = Extracellular-signal regulated kinase.

3.3. The subacute neural effects of ketamine

Beyond the acute effects of ketamine, the persistence of the antidepressant response for days after the elimination of the drug from the body suggests the presence of significant subacute neurophysiological modifications produced by the drug [237–239]. Based on recent evidence, a framework is emerging around the idea that ketamine may promote a post-acute heightened state of neuroplasticity which provides a window of opportunity for therapeutical intervention [240–242]. Neuroplasticity is gaining traction both as a potential biomarker of neuropsychiatric illnesses and as a target for therapeutics. In conditions such as depression, clinical and preclinical studies have reported structural and cellular alterations, specifically neuronal loss and synaptic dysfunction, in cortico-limbic brain regions controlling mood and cognition [243–245].

In support of this view, a wealth of pre-clinical research has shown that ketamine is able to rapidly and persistently induce neuroplastic changes, which often parallel improvements of mood-related symptoms (reviewed in the following section). It must be noted that ketamine is not the only plasticity-enhancing pharmacological agent, nor is a heightened state of plasticity an inherently good thing. For example, plasticity within reward circuity has been identified as a mechanism of addiction to compounds such as cocaine [246,247]. Also, traditional antidepressants, like SSRIs, have been demonstrated to modify neuroplasticity when exerting their therapeutic effects [240,248]. Nevertheless, it appears that a mechanistically distinct ability to promote neuroplasticity that is unique in both intensity and kind may account for the difference in effect sizes and speed of response to ketamine compared with currently available mental health medications.

The available evidence on subacute structural and functional neuroplastic effects induced by subanaesthetic doses of ketamine will now be reviewed and discussed.

3.3.1. Structural neuroplasticity

Structural plasticity encompasses all those processes that regulate the morphological re-modelling of the brain. This phenomenon includes the formation of new neurons (i.e., neurogenesis) or their death (i.e., neuronal apoptosis), the increases in neuronal structural complexity (i.e., neuritogenesis) or its decrease (i.e., neuronal atrophy), the increases in the number of connections with other neurons (i.e., synaptogenesis) or its decrease (i.e., synaptic pruning) [249,250]. Neuritogenesis is a process whereby the neurons undergo a remodelling of their appendixes, resulting in the formation of new dendrites (dendritogenesis), new spines (spinogenesis), or axonal elongation. Synaptogenesis refers to the formation of new connections between neurons and their maturation into functional synapses [249].

3.3.1.1. Animal research

In vitro, treatment of animal-derived brain tissue with ketamine has been found to affect structural plasticity. Various concentrations of ketamine promoted the proliferation phase of cultivated rat neural stem and progenitor cells (NSPCs) 3 weeks after exposure. However, the high concentrations, translating to anaesthetic doses in humans, inhibited the proliferative phase of neurogenesis 24 hours after treatment [251]. Different studies have shown that rat-derived neurons exposed to medium concentrations of ketamine, corresponding to dissociative subanaesthetic doses in humans, increased spinogenesis, dendritogenesis, dendritic arbour complexity, and soma size after 72 hours, with lowest concentrations and short periods of exposure having the highest efficacy [252–255]. In vivo, administration of a single medium dose of ketamine has been shown to promote structural plasticity in a large number of studies. Accelerated progenitor cell differentiation into newborn neurons and maturation of new neurons in the dentate gyrus (DG) of mice was seen 24 and 48 hours after ketamine administration. Interestingly, ketamine promotion of late neurogenesis and activity of DG adult-born immature granule neurons was associated with the long-term antidepressant-like effects of the drug [256–258]. A few studies have reported that ketamine increased spine density in the PFC at 24 hours following administration, an effect associated with the emergence of an

antidepressant-like response, that lasted up to 2 weeks after a single subanaesthetic dose [259–261]. Another study found an increase in the total density of dendritic spines in rat cerebral cortex 6 hours after injection of ketamine [255]. Recently, dual laser two-photon glutamate uncaging and imaging were used to resolve the temporal dynamics of the ketamine-induced spinogenesis in the mPFC layer 5 pyramidal neurons. Administration of a single medium dose of ketamine to mice enhanced glutamate evoked spinogenesis 2 and 4 hours after treatment, temporally matching the emergence of ketamine's therapeutic effects. However, 12 hours after ketamine was administered, evoked spinogenesis decreased back to baseline levels. In parallel, the increase in dendritic spine density was delayed until 12 hours after treatment and lasted for 72 hours post-treatment. This temporal precedence of ketamine-associated potentiation of evoked spinogenesis suggests that changes in the potential for activity-dependent plasticity may contribute to slower accumulating increases in spine density after ketamine treatment. in an uncontrollable stress paradigm, the probability of glutamate evoking spinogenesis decreased relative to the baseline, with ketamine treatment restoring the baseline potential for plasticity [210]. Interestingly, those effects were observed in Drd1-expressing mPFC neurons and were dependent upon the activity of DA neurons projecting from the ventral tegmental area (VTA) [210]. In rodent models of depression, a single medium dose of ketamine rescued the model's depressive-like behaviour and PFC spine density at 3, 12, and 24 hours post-injection. These effects were not due to modification of elimination rates but to an increase in spine formation rates [245,262]. A similar result was obtained with S-ketamine, which induced spinogenesis in the hippocampus 1 hour post-injection, and increased excitatory synaptogenesis 1 to 7 days after treatment. However, the effects observed in models of depression were not observed in the non-vulnerable rat line [263]. One study found no increase in spine density in the hippocampus 3 hours post-injection of a medium dose of ketamine [264]. Importantly, prolonged exposure to high anaesthetic doses of ketamine, administration during development, and/or chronic administration of the drug have been found to induce neuronal degeneration and reduce neurogenesis and cortical spine density [260,265–267]. However, one study showed that pre-treatment with a single subanaesthetic dose of ketamine before prolonged exposure to an anaesthetic dose was able to prevent neuronal cell death [266].

3.3.1.2. Human

In human studies, direct evidence for the effect of ketamine on structural plasticity is still very limited. The few available studies on the topic will be discussed in Chapter 5 of this thesis.

3.3.2. Functional neuroplasticity

Functional plasticity is often subdivided into activity-dependent (or homosynaptic plasticity) and activity-independent (or heterosynaptic plasticity) [268]. Activity-dependent plasticity is a type of plasticity that depends on positive feedback loops and includes phenomena such as short-term plasticity and long-term plasticity (i.e., Hebbian plasticity). Short-term plasticity includes processes happening in the timeframe of milliseconds to minutes and refers to the phenomenon in which synaptic efficacy changes over time in a way that reflects the history of presynaptic activity. Examples of this type include short-term synaptic facilitation, depression, and habituation [269]. Long-term plasticity refers to changes in synaptic strength and efficacy observed in the timeframe of minutes to hours and is thought to be the biological process underlying classical forms of learning and memory. Based on the strength, frequency, and number of pre-synaptic inputs, a synapse can undergo long-term potentiation (LTP) or depression (LTD) [268]. Activity-independent types of plasticity act at wide timescales and include different forms of homeostatic plasticity such as synaptic scaling and spontaneous neurotransmitter release. Homeostatic plasticity refers to the ability of neurons to adjust synaptic or intrinsic excitability via a negative feedback loop to keep firing rates relatively constant within a network [242]. When either activity-dependent or independent forms of plasticity are directly enhanced by a pharmacological intervention the result can be termed functional hyper-plasticity, while an intervention that changes the threshold to undergo the various forms of functional synaptic plasticity is referred to as functional meta-plasticity [270].

3.3.2.1. Animal research

In vitro, rat visual cortex neurons exposed to high concentrations of ketamine showed increased excitability, as indexed via increased amplitude of the baseline response to single-pulse stimulation-evoked excitatory post-synaptic potential (EPSP), but reduced LTP. Following the washout of ketamine for 30 minutes, the reduction in induced LTP was normalized [271]. In hippocampal slices, stimulation of the CA3-Schaffer collateral pathway to CA1-stratum radiatum (CA3-CA1 synapses) during exposure to high and low concentrations of ketamine increased excitability only at specific low stimulation frequencies [272] while others found dose-dependent inhibition of CA1-evoked EPSPs [273] or no effect at low concentrations [274]. High-frequency stimulation (HFS)-induced LTP during ketamine treatment was found to be either impaired [274] or unaffected at low concentrations [273], and inhibited at high concentrations [206]. In one study, a low concentration of ketamine did not affect HFS-induced LTP but acutely reduced low-frequency stimulation (LFS)-induced LTD. Just after exposure to low concentration of ketamine, there was enhanced excitability for at least 2 hours [273], and 1 hour following drug washout [206]. When HFS was administered 1 hour after ketamine washout, LTP was increased by low concentrations of ketamine. When HFS was administered 2 to 4 hours after ketamine washout, however, LTP was inhibited [273]. Several studies investigated the functional neuroplastic effect of in vivo administration of a single subanaesthetic dose of ketamine at different time points. One study found that ketamine increased neuronal excitability measured via single-pulse evoked EPSCs in the CA3-CA1 synapse of rat hippocampal slices compared to control 24 hours after drug exposure [275]. In a depression-like mice model, ketamine reversed the deficit in cortical excitability as indexed via single-pulse-induced NMDA-dependent EPSCs [116]. Short-term plasticity indexed via paired-pulse-induced facilitation in CA3-CA1 synapses was found to be unaltered at 3 hours post-drug administration in the wild-type [264] and depression-like mice model, but increased at 24 hours in the depression model [276]. In wild-type rodents, response to stimulation-induced LTP in CA3-CA1 synapses after ketamine was found to be increased at 3 hours with theta-burst stimulation but not HFS [264,276], and increased at 24 hours with combined theta-burst/HFS [275] or HFS, and increased at 72 hours with HFS [274]. In models of depression, the deficit in response to stimulation-induced LTP was found to be reversed by ketamine at 3 and 24 hours with HFS [116,276]. The neurophysiological effects observed in the depressed model were associated with antidepressant-like response and improvement of hippocampal-dependent long-term spatial memory and contextual fear conditioning [116,276].

Using monocular deprivation paradigms it was shown that medium doses of ketamine accelerate functional recovery of adult mice's visual cortex, reopening the critical window of developmental plasticity, either after a single dose [277,278] or repeated exposure [278,279]. The effect of ketamine on ocular dominance has been proposed to be mediated by allosteric binding to TrkB, with a mechanism similar to what has been reported for classic psychedelics and SSRIs [279,280]. Further, it was shown that ketamine disrupts the interaction between TrkB and PTPσ, a tyrosine phosphatase that acts as a receptor for components of the perineuronal nets surrounding inhibitory interneurons, a mechanism which might allow for the re-opening of functional critical windows of plasticity [278]. In an adult mice model of amblyopia, ketamine injection following 2 weeks of monocular deprivation promoted functional recovery of deprived eye performance in the visual water maze task. These effects were found to be dependent on the expression of the tyrosine kinase receptor ErbB4, the target of the neurotrophic factor Neuregulin-1 (NRG1), expressed on V1 inhibitory interneurons. It was found that via downregulation of this NRG1/ErbB4 pathway on inhibitory neurons, ketamine treatment decreases inhibitory inputs to L2/3 excitatory neurons at 1, 24, 48, 72 hours, and 1 week as measured via inhibitory post-synaptic current (IPSC) amplitudes following electrical stimulation in V1 visual cortex. This effect was paralleled by a reduction of the excitatory inputs to inhibitory neurons, but no changes to the excitatory inputs to V1 excitatory neurons. As a result of this cortical disinhibition, the activity of excitatory neurons, measured via two-photon calcium imaging, significantly increased at 24, 48, and 72 hours following ketamine and returned to baseline after 1 week. Also, ketamine treatment increases the visually evoked potential amplitudes in V1 at 24 and 48 hours after treatment [277]. In a recent study, Nardou et al. (2023) showed that a single subanaesthetic dose of ketamine re-opens the critical period for social reward learning in adult mice, as measured via the social reward conditioned place preference assay, measured at 48 hours after administration. This behavioural effect was found to be independent of

5-HT2ARs-mediated signalling and was paralleled by meta-plastic changes in the nucleus accumbens (NAc), where ketamine was found to potentiate the LTD induced by exposure to oxytocin. No changes in baseline EPSC amplitude or frequency following ketamine treatment were detected in the NAc or medial PFC [281]. High doses of ketamine either enhanced [274] or unaltered HFS-induced LTP at 24 hours [282] post-injection in CA3-CA1 synapses of wild-type mice. No changes in neuronal excitability were detected after a high anaesthetic dose of ketamine with single-pulse-evoked EPSP in the dorsal raphe nucleus at 24 hours [283]. Also, neonatal exposure to high doses of ketamine induced an impairment of LTP evoked by HFS in the DG after 10 weeks, with no difference in single-pulse-evoked EPSCs [284].

3.3.2.2. Human research

In humans, Sumner et al. used the visual LTP paradigm during EEG in combination with dynamic causal modelling (DCM) to study the effect of a single injection of a medium dose of ketamine in patients with TR-MDD measured 3-4 hours after drug administration. They found that the P2 component of the evoked-response potential (ERP) was significantly more positive in the late post-tetanus block after ketamine compared with the active placebo, showing higher meta-plasticity induced by ketamine. Also, during the LTP task, ketamine increased the modulation of forward connections from the left middle occipital gyrus to the left inferior temporal cortex and the left and right superior parietal cortex. Backward connections were decreased from the left inferior temporal cortex to the left middle occipital gyrus, and from the right superior parietal cortex to the right middle occipital gyrus. Backward connections were increased from the right superior parietal cortex to the right middle occipital gyrus and intrinsic connections in the left middle occipital gyrus were decreased [285]. In a sister study, a mismatch negativity (MMN) task was administered 3-4 hours post ketamine injection to measure repetition suppression of sensory-evoked EEG components. Ketamine was found to increase the negativity of the MMN response to deviant tones, indicating increased sensitivity to prediction error. Also, the source-level results demonstrate a significant main effect of ketamine on the strength of activation in the inferior temporal cortex in response to deviant tones, which showed higher activation post-ketamine [286]. Another line of research investigated the effects of ketamine in TR-MDD at 6.5 hours with MEG during a tactile stimulation to measure stimulus-evoked somatosensory cortical excitability. This type of stimulation elicited an evoked response approximately after the stimulus with a spectral peak in the gamma band over the contralateral hemisphere. Responders showed significantly increased somatosensory cortex gamma band response after ketamine relative to baseline while non-responders showed no change [287] (Those results were followed up by a replication study [288]). Another study used MEG and DCM with the same paradigm in both TR-MDD patients and healthy controls at 6 to 9 hours after a single medium dose of ketamine or placebo. It was found that the NMDA-mediated backward connectivity from the right frontal cortex to the right primary somatosensory cortex after ketamine was higher for patients compared with ketamine-treated controls. Also, there was an increase in NMDA-mediated connectivity in the forward connection from the right somatosensory cortex to the right frontal cortex for healthy subjects following ketamine administration compared to the placebo, but not baseline, within the same group. These results were interpreted as an effect of ketamine-induced NMDA antagonism leading to short-term sensitization of postsynaptic mechanisms, affecting forward and backward NMDA connectivity separately for both MDD subjects and healthy controls [289].

Using fMRI, it was shown that the acute alterations in brain activity and connectivity within- and between-neural networks induced by ketamine persist sub-acutely. In healthy subjects, the acute reduction in the integrity of the DMN induced by a single administration of ketamine was found to persist 1 day following exposure to the drug [290] Also, ketamine was found to reduce connectivity within the salience network (SN), involved in bodily self-experience, and suppression of the anti-correlation between the DMN and other networks 24 hours after the infusion [291]. In depressed individuals, previous research has found that those who responded to ketamine showed increased global brain connectivity in the lateral PFC, caudate and insular cortex 24 hours after the infusion and that global brain connectivity in the PFC positively predicted depression improvement [225,292]. However, a subsequent study failed to replicate this finding 48 hours after the dosing session in a different sample [293].

3.3.3. Molecular markers of plasticity

The changes in structural and functional plasticity induced by ketamine require the synthesis of specific molecular constituents of the neuron. Thus, modification in the expression profile of the genes coding for such proteins is necessary for neuroplastic changes to endure. Neuronal activity- and plasticity-relates genes, coding for related proteins, are modulated by ketamine in the minutes and days following drug exposure. The most studied class of activity-dependent genes are the immediate-early genes (IEGs), whose activation and transcription begin within minutes after neuronal stimulation. Those include factors such as the activity-regulated cytoskeleton-associated protein (Arc), protein c-Fos, and the Homer protein homolog 1 (Homer1a) [294]. Plasticity-related genes encode proteins whose expression was found to be required for the functional and structural remodelling of neurons, including factors such as BDNF, extracellular-signal-regulated kinase (ERK), and post-synaptic density proteins (PSD) [295]. Moreover, epigenetic changes that alter the chromatin status and gene accessibility by transcription factors also play an important role in neuroplastic changes induced by exogenous compounds. Those include histone modifications such as those performed by histone deacetylase 5 (HDAC5) [296].

3.3.3.1. Animal research

In vitro, exposure of primary cortical neurons to low and medium concentrations of ketamine resulted in significant increases in BDNF release acutely and after 1 hour [297,298]. Ketamine also produces dose-dependent and time-dependent epigenetic changes, such as the stimulation of HDAC5 phosphorylation and its nuclear export in rat-derived hippocampal neurons. Peak phosphorylation was achieved with medium concentrations at 3 to 6 hours following treatment with a return to baseline after 24 hours. Histone modification was followed by enhanced transcription of the myocyte enhancer factor 2, and subsequent activation of its target genes regulating neuronal structural and functional plasticity [299]. In vivo, significant increases in BDNF protein levels have been found in the hippocampus and PFC following administration of subanaesthetic doses of ketamine in mice immediately and at 1 and 30 days, with no changes following high doses [300,301]. The antidepressant-like effects produced by ketamine did not occur in BDNF and eukaryotic translation elongation factor 2 (eEF2) knock-out mice [206,302] and synaptogenesis increase was not seen in the mPFC of a mouse model of the BDNF Val66Met low-functioning polymorphism [303]. In rats, changes in the expression of Arc gene expression and protein levels of Arc, CREB, phospho-CREB, ERK, and phospho-ERK were detected in the hippocampus 10 min and 24 hours after a single dose of ketamine [304]. Another study found upregulation of Arc, Homer1a, and c-Fos transcription in rats' cortical regions 90 minutes after injection [305]. In a mouse model of depression, a subanaesthetic dose of ketamine reversed the reduction of Arc gene expression in the PFC 72 hours after injection [306]. Anaesthetic doses of ketamine were found to only produce an increase in the Arc gene and the protein expression levels 10 minutes after injection, but not at later time points. Also, chronic administration of various doses of ketamine produced a decrease in the Arc gene and the protein expression levels after 24 hours [304]. In the study by Nardou et al. (2023), RNA sequencing of the NAc 48 hours and 2 weeks after a single medium dose of ketamine showed that the IEGs cFos, Junb, Arc, and Dusp and several genes coding for components of the extracellular matrix were enriched 48 hours after ketamine, when the critical period of social reward learning is re-opened, as compared to 2 weeks, when the period is closed again [281].

3.3.3.2. Human research

In humans, it was observed that MDD patients carrying the Val66Met BDNF allele show reduced response to treatment with subanaesthetic doses of ketamine [307]. One study showed an increase in peripheral BDNF at 230 minutes post-administration of ketamine in MDD patients [308]. Another study in healthy individuals found that BDNF levels were higher following ketamine compared to placebo at 2 hours, but this was the function of a reduction of BDNF in the placebo group and BDNF did not significantly increase in the ketamine group [309]. Persisting increases at 2 weeks have also been found following six infusions of subanaesthetic doses of ketamine [310]. There is some evidence that increased BDNF may be isolated to treatment responders only, with evidence of specificity at 4 hours, 1 week and 1 month post infusion [311–313]. One study found reductions of BDNF 1 week following a single ketamine infusion in BP patients [314]. Many studies reported no change in serum or plasma BDNF levels following administration of

subanaesthetic doses of ketamine in the hours or days following administration [312,315–319,319–324]. Other studies have probed peripheral BDNF levels following the use of ketamine as an anaesthetic, either during surgery or electroconvulsive therapy. Three large studies demonstrated increased BDNF in the days following the administration of a high dose of ketamine compared to placebo [325–327]. Levels equalized with the placebo at day 5 in one study and persisted for a month in another [326,328]. One study found BDNF to significantly increase following a course of electroconvulsive therapy plus ketamine compared to electroconvulsive therapy plus placebo, but this was not replicated [327,329].

3.3.4. Discussion

The results presented on the subacute effects of ketamine on structural, functional, and molecular markers of neuroplasticity demonstrate that these compounds exert a complex and multifaceted impact on neuroplasticity, producing significant long-lasting biological changes in the organism with behavioural correlates and hence implications for clinical use of ketamine (Figure 5).

On the pre-clinical side, a framework is emerging to account for the subacute effect of a single therapeutically meaningful dose of ketamine on neuroplasticity. The acute pharmacological action of ketamine on neurotransmission would open a state of increased functional meta-plasticity, for a limited amount of hours, where higher sensitivity to environmental stimuli causes structural modifications lasting for weeks or months after a single administration. Such modifications are sustained by up-regulation of molecular and genetic factors and seem to mediate the reduction of behavioural symptoms of mood disorders as reproduced in animal models. In addition, ketamine was found to promote adult hippocampal neurogenesis, another mechanism associated with improvements in the symptomatology of mood disorders. The evidence for structural modifications following ketamine exposure is well-characterized both in vivo and in vitro, with increases in dendritic complexity, number of spines, and synapses emerging within a day and lasting for weeks. In vivo, the structural modifications are mostly defined for neurons within the PFC in the form of increased spinogenesis, where an association has been characterized between neuronal hypertrophy and the reduction of depression-like and anxiety-like animal behaviours. The sustained up-regulation of a molecular pathway involving NMDA and AMPA receptors and BDNF cycling seems to underline the hyperplastic modifications of ketamine.

In humans, translating the evidence on neuroplasticity induced by ketamine has proven to be challenging. Some research has successfully probed the heightened meta-plastic state and altered functional neural properties in the hours following exposure to ketamine with paradigms of non-invasive sensory stimulation. Mixed results were also obtained with regard to genetic and molecular modifications induced by the administration of ketamine, likely due to technical limitations in the measurements. On the structural side, limited evidence is available, which will be reviewed in Chapter 5. Therefore, a gap exists in the translation to humans of the working model emerging from the animal literature.

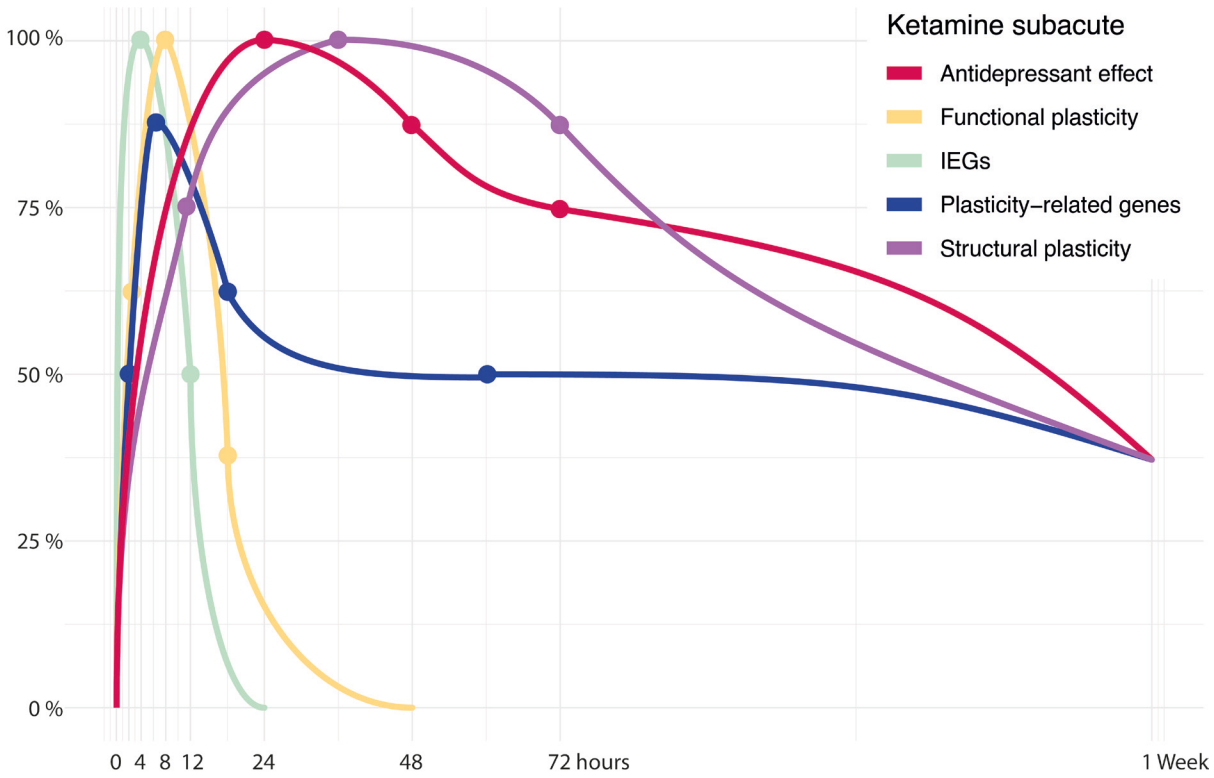

Figure 5: The subacute effects of ketamine. The timeline of the antidepressant, functional, structural, and genetic subacute effects of ketamine.

3.4. Conclusion

Overall, research has shown that ketamine produces profound and consistent neurophysiological modifications, with functional implications for its mood-related effects. Antagonism at the NDMA receptor by ketamine triggers a cascade of cellular pathways, leading to circuit-level disinhibition and glutamate release in frontocortical regions. The release of glutamate instantiates a positive feedback loop, leading to an acute increase in cortical excitability, functional network reorganization, and subacute cellular and genetic alterations. The acute emergence of dissociative and psychedelic-like effects following ketamine administration is paralleled by increased global functional connectivity and reduced integrity of canonical brain networks. In the hours following ketamine exposure, the subjective effects subside but a heightened state of sensitivity to environmental stimuli persists (i.e., functional meta-plasticity). During this state, functional and structural hyper-plastic modifications can instantiate, supported by the over-expression of plasticity-related factors. This period can lead to the re-opening of critical developmental windows, allowing for long-term reconfiguration of functional brain networks and behavioural patterns. Such neuroplastic modifications might vehicle the enduring improvements in depressive-like symptomatology observed after a single administration of ketamine.

While intriguing and valuable, this framework is mainly based on evidence coming from animal research and thus has limited power in addressing fundamental questions regarding the subjective and therapeutic effects of ketamine. Especially in the case of mood disorders, the validity of animal models is confined to the recapitulation of isolated symptoms, which are tested with too unspecific behavioural assays, and cannot investigate the subjective aspect of those illnesses and of the effects of mind-altering compounds. It is therefore necessary to capture the biological effects of ketamine in clinical research to appropriately test the validity of modern theories, such as the "excitatory/inhibitory imbalance" and the "neural atrophy" hypothesis of depression. Recently developed neuroimaging methods and techniques provide an opportunity to probe the acute and subacute effects of ketamine in humans.

4. Chapter 4: Neurophysiological correlates of ketamine-induced dissociative state in bipolar disorder

This chapter will present the results of an experiment measuring the acute neurophenomenology of a subanaesthetic dose of ketamine in patients with TR-BP undergoing an MDE.

4.1. Introduction

As neuropsychopharmacology delves into a new era marked by the exploration of mind-altering substances, important questions arise regarding the significance of the subjective experiences these substances produce. Their illicit status, coupled with societal attitudes toward altered states of consciousness induced by hallucinogens, profoundly influences the nature of innovation in this field. In the case of ketamine, the predominant approach in clinical trials has been to interpret the results obtained with the drug within a biomedical framework, often neglecting an emphasis on subjective experience and contextual factors [330]. In fact, the dissociative experience induced by ketamine is commonly regarded as a side effect in most of the scientific literature (e.g., [331]). Conversely, newer models inspired by research in psychedelic-assisted psychotherapy consider extra-pharmacological factors, such as the physical setting whereby the drug is administered, the subjective experience induced by the substance itself, and the psychophysical state of the patient, as potential mediators of antidepressant effects [332]. Further, the neural dynamics underpinning the alteration of consciousness of ketamine, particularly in clinical populations, also remain inadequately understood. The escalating use of ketamine as a prescription medication for TR-MDD and off-label for other mood disorders, such as TR-BP, raises the crucial issue of identifying trans-diagnostic biomarkers of ketamine's mechanism of action [333]. Importantly, therapeutically efficacious doses of ketamine are also in the range reported to produce drug-induced subjective experiences [330]. In this context, a comprehensive characterization of the acute neurophenomenology of ketamine across diverse patient populations and settings becomes essential to pinpoint robust markers indicative of its therapeutic efficacy.

The diverse pharmacological profile of ketamine is reflected in the intricate and varied phenomenology of the subjective experiences it induces. The duration and quality of the subjective effects of ketamine exhibit a tight dependence on dose, route of administration, and context. In recreational settings, subjective effects of low doses of ketamine (up to 0.5 mg/kg) are commonly reported by users as alcohol-like disinhibition and relaxation [35]. At subanaesthetic doses (0.5 to 1 mg/kg), typical to both recreational and psychiatric use, ketamine induces a psychedelic-like state, termed dissociation in medical terms (anecdotally known as a "k-hole") [331]. This state is often described as an "out-of-body" or "near-death" experience, accompanied by effects such as loss of motor control, pain relief, perceptual distortions, internal hallucinations, memory suppression, conceptual thinking, immersion enhancement, euphoria, and depersonalization [334,335]. A survey involving 30 frequent recreational users, 30 infrequent recreational users, and 30 ex-users revealed common experiences such as "melting into the surrounding", "visual hallucinations", "out-of-body experiences", and "giggliness". Undesirable effects for half of the users included "memory loss" and "decreased sociability" [336]. In clinical practice, the main themes reported by patients with depression after ketamine treatment include unusual bodily sensations, a sense of peace, disinhibition, and a sense of altered perception [337]. At anaesthetic doses (more than 1 mg/kg), ketamine induce a state of unresponsiveness to environmental stimuli but with reports of complex, long, vivid dreams upon recovery from the anaesthetic state [338]. Thus, ketamine appears to dose-dependently produce a state where the brain becomes progressively disconnected from the environment while maintaining vivid, internally generated experiences.

To measure the intensity and nature of the ketamine-induced experience at subanaesthetic antidepressive doses in clinical populations, the Clinician-Administered Dissociative States Scale (CADSS) stands out as the most commonly employed questionnaire [339]. This scale is subdivided into 3 primary factors: depersonalization, derealization, and amnesia. Originally developed for the examination of dissociative disorders, the CADSS index symptoms of gaps in memory (i.e., amnesia), out-of-body experiences and other distortions of the sense of one's own body and self (i.e., depersonalization), and distortions in perception, such as seeing things as if they are in a tunnel or seeing things in black and white (i.e., derealization) [340]. A validation analysis of the CADSS score in individuals with mood disorders undergoing ketamine treatment showed that the scale performs well in capturing some aspects of the ketamine experience, such as perceptual and bodily effects while failing to describe other aspects of the experience such as disinhibition and blissful experiences [337]. Another frequently employed scale in research settings

is the Brief Psychotic Rating Scale (BPRS) [228]. The utilization of the BPRS follows observations made in studies investigating subanaesthetic doses of ketamine to induce transient psychotomimetic symptoms in healthy volunteers. In these contexts, ketamine was reported to elicit behaviours akin to the positive and negative symptoms, as well as cognitive alterations, observed in schizophrenia. These manifestations encompass paranoia, tangentiality, loose associations, concreteness, ideas of reference, and unusual thought content [341]. The psychedelic-like attributes of subanaesthetic ketamine are commonly assessed through research utilizing the Altered States of Consciousness Questionnaire (ASCQ), a tool more commonly applied in studies with healthy volunteers [342]. Employing the 5-dimensional version of the scale, studies have demonstrated that ketamine generates a spectrum of psychedelic-like, dose-dependent psychological experiences. These experiences encompass a pleasurable loss of ego boundaries and feelings of oneness, as well as a more psychotic-like ego dissolution involving fear and paranoid ideation [211]. Another salient aspect of the ketamine experience, captured by the 11-dimensional version of the ASCQ, includes feelings of disembodiment and impaired control and cognition. Additionally, ketamine has been reported to induce unitive and spiritual experiences to an extent comparable to that of classic psychedelics [343].

A systematic review examining the association between ketamine-induced subjective effects and antidepressant response yielded mixed results. Among the analysed studies, only 3 observed a relationship between scores on the CADSS or BPRS and antidepressant response. Among those studies reporting a significant relationship, the explained variance of dissociative experiences for antidepressant response ranged from 12% to 21% [344]. Using the 5-dimensional ASCQ after repeated ketamine infusions, it was shown that the dread of ego dissolution experiences induced by ketamine was higher in non-responders [345]. Furthermore, another study revealed that a greater antidepressant response with ketamine was correlated with 3 subfactors of the 11-dimensional ASCQ: experience of unity, spirituality, and insight [346]. To summarise, the subjective effects of ketamine appear to be highly variable, influenced significantly by factors such as dosage, individual differences, and contextual elements like the administration setting and the instrument employed to assess the experience. Yet, certain aspects of the ketamine-induced experience seem to predict therapeutic response. This variability underscores the crucial need to identify reliable biomarkers of acute neurophenomenology of ketamine in diverse patient populations and contexts.

As discussed in Chapter 1, EEG stands out as the most appropriate non-invasive neuroimaging method to investigate the acute psychoactive effects of the drug in a flexible manner due to its high temporal resolution and portability. The neural signals recorded by EEG/MEG display a diverse combination of rhythmic and arrhythmic patterns [58]. Rhythmic patterns emerge from oscillatory network activity with a characteristic time scale [347], while arrhythmic patterns lack confinement to any specific scale, reflecting what is known as fractal (or scale-free) dynamics [348]. Upon subjecting the EEG/MEG signal to power spectra analysis via the discrete Fast Fourier Transform (FFT), the oscillatory component manifests as discrete peaks at specific frequencies, reflecting their relative spectral power in the signal. The oscillations within the human brain encompass rhythmic neuronal activity spanning a frequency range from 0.05 to 500 Hz [347]. The generation of slow, low frequency, oscillations requires the recruitment of large networks and leads to the functional coupling of neurons over large distances, while faster, high frequency, oscillations occur within smaller neuronal populations on smaller spatial scales [349]. Oscillatory activity measured at rest (i.e., resting-state EEG/MEG) is believed to be reflective of the intrinsic functioning of networks and can provide an index of pharmacological or pathological modulation of functioning. Although the generation of neural oscillations in each frequency band requires the synchronized activity of large populations of neurons, the mechanisms generating oscillations in each frequency band are distinct, providing information about diverse neural processes. Typically, the oscillatory component of the EEG/MEG signal is subdivided into five main functional frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-80 Hz) [350]. Delta is the slowest neural oscillation typically studied with EEG and is associated with sleep and anaesthesia states, including ketamine-induced anaesthesia [351]. However, it has also been linked to cognitive functions, including motivation and cognition [347]. Cortical delta oscillations are generated in layers II to VI of the cortex as a result of extended periods of depolarization and hyperpolarization. The extended depolarization of populations of pyramidal neurons occurs due to reciprocal interactions with GABAergic interneurons in local circuits. This depolarization progressively

depletes extracellular calcium, creating a reduction in synaptic excitation and hyperpolarization. All major cortical cell classes display this oscillation, with synchronization occurring across the cortex during a state of minimal consciousness, such as slow-wave sleep and anaesthesia. Delta oscillations recruit large networks of neurons and play an important role in facilitating functional connectivity between disparate brain regions [350]. Theta oscillations are particularly prominent in the limbic system, especially the hippocampus, where they play an important role in memory processes [347]. While hippocampal theta oscillations are the most commonly studied, the generation of theta oscillations by other cortical and subcortical areas has also been demonstrated. Substantial evidence suggests that the medial septum acts as a pacemaker in the generation of theta oscillations in behaving animals [352]. The cells in the medial septum that act as pacemakers are GABAergic inhibitory interneurons, which synapse on interneurons in the hippocampus, causing them to discharge within the theta frequency range and producing rhythmic IPSPs on hippocampal pyramidal cells [350]. Alpha oscillations dominate human neural activity during the brain's resting state [347]. Generated in the occipital cortex during periods of reduced visual attention, in the somatosensory cortex during relaxation (termed mu rhythms), and in the temporal cortex (referred to as tau rhythms), alpha oscillations are generally indicative of an idling state of the brain where cortical areas irrelevant to task performance are inhibited. A decrease in alpha spectral power occurs with environmental stimulation or task performance as the activity of alpha-generating neurons becomes desynchronized [353]. Alpha oscillations are most pronounced when the eyes are closed and decrease with visual input. Primate studies have revealed that infragranular cells located in layer V, particularly in the visual cortex, are the primary local generators of alpha oscillations, although generators are found in all layers of the visual cortex [354]. Beta oscillations are typically observed during wakeful states with a frontocentral distribution [347]. Despite their prevalence, beta oscillations remain one of the least understood in terms of their functional role, traditionally being associated with sensorimotor processes. In the predictive coding framework, beta oscillations are proposed to mediate feedback to the lower levels of the cortical hierarchy [350]. Gamma oscillations, the fastest of the typically defined neural oscillations, are functionally well-characterized but poorly standardized [347]. They have been described across all areas of the cortex, playing a crucial role in high-demanding cognitive tasks and information processing, especially within the hippocampal network. These oscillations are primarily generated in layers II and III of the cortex, particularly in the superficial layers that serve as the source of feedforward projections. Synchronous gamma activity can be generated locally through the activation of inhibitory networks, called the interneuron network gamma (ING) mechanisms, or through the activation of reciprocally connected inhibitory interneurons and excitatory neurons, known as the pyramidal interneuron network gamma (PING) mechanisms [355]. The PING mechanism involves a feedback loop encompassing excitatory pyramidal neurons and a fast-spiking type of parvalbumin-expressing (PV) GABAergic inhibitory interneurons, and it is mediated by NMDA receptor activity [355,356]. Thus, modulation of gamma activity is often interpreted as an index of cortical excitatory/inhibitory balance [357].

Spectral analysis of resting-state EEG/MEG has been extensively used to investigate the acute neural changes induced by ketamine in healthy subjects. It has been found that subanaesthetic doses of ketamine decrease spectral power in the delta frequency band [221,226,358–362], while anaesthetic doses increase delta spectral power [363]. The administration of ketamine as an intravenous bolus was found to induce a transient burst of increased spectral power in the theta frequency band [221,364,365]. However, ketamine administration with continuous infusion shows decreases in theta power [360,362,366,367]. One of the most consistent findings in the ketamine literature is a reduction in spectral power in the alpha band [221,226,358–362]. The reduction in alpha power was also found to correlate with depersonalization scores of the CADSS in one study [360], and elementary imagery scores of the ASCQ in another [362]. Ketamine typically induces a reduction [221,364–366,368,369] or no change in beta spectral power [360,366]. One investigation reported a significant correlation between elementary imagery scores and beta power reduction [366]. Lastly, there is a high level of consistency in the literature showing that ketamine increases spectral power in the gamma band [221,226,360,362,364–366,369]. A recent study measured intracranial EEG in epileptic patients who were administered a subanaesthetic dose of ketamine prior to induction of general anaesthesia with propofol for electrode removal surgery. They confirmed that ketamine induces a decrease in delta power in most of the analysed structures. The power of the theta frequencies increased in the insula cortex and decreased in frontal, parietal, temporal,

and occipital cortices [370]. Alpha power showed a decrease in most of the analysed cortical structures, with the largest reduction in postcentral and occipital cortices. Beta frequencies increased in hippocampus and amygdala, and decreased in frontal, parietal, temporal structures, and occipital cortices. Gamma frequencies showed an increase in frontal and temporal areas and a decrease in occipital areas [370]. In patients with TR-MDD, it was also observed that continuous infusion of ketamine reduces delta, theta, and alpha, and increases gamma power. Regional- and frequency-specific ketamine-induced EEG changes were related to, and predictive of, decreases in depressive symptoms (theta, alpha, gamma) and suicidal ideation (alpha) [371,372]. Using a bolus, ketamine increased theta, high beta, low and high gamma power, and decreased delta, alpha, and low beta power, but no significant associations between neural effects and antidepressant response were reported in MDD patients [373]. Overall, subanaesthetic doses of ketamine produce consistent changes in rhythmic EEG activity, characterized by a reduction of spectral power in the low-frequency bands, particularly alpha, and increases in the high frequencies, particularly gamma. Nonetheless, the relationship of the neural changes with the drug-induced subjective experience and antidepressant response is still unclear.

Resting-state EEG/MEG signals can provide additional information associated with brain activity that is not directly related to brain oscillations. In recent years, arrhythmic and non-linear brain dynamics have progressively been studied and characterized [374]. The arrhythmic component of the spectral power is evident as a descending straight line on the log-log plot [375]. This scale-free (also termed fractal) component adheres to a $1/f^{\beta}$ power-law relationship, where f is frequency and β is the power-law exponent (PLE). This relationship expresses the property of EEG/MEG signal to show an inverse relationship between power density and frequency [347]. The physiological mechanisms by which power-law scaling is generated in the brain are poorly understood and their significance remains controversial [58]. While typically regarded as "1/f noise" in most of the neurophysiological literature, others have proposed a functional role of the scale-free component of neural activity, possibly reflecting a state of self-organized criticality or as an excitatory/inhibitory index [376]. The potential functional importance of power-law scaling in the brain is underscored by its alteration in neuropsychiatric conditions [377–379], ageing [380,381], and its dynamic modification in task states and learning [348,382–385]. Interestingly, it was shown that the PLE correlates with the subjective sense of selfhood, as measured via the self-consciousness scale [386]. Muthukumaraswamy and Liley demonstrated that the PLE of the resting-state EEG/EMG signal is sensitive to various pharmacological manipulations, including ketamine. In particular, subanaesthetic doses of ketamine were shown to decrease the PLE exponent at frequencies between 5 and 100 Hz and decrease the PLE at lower frequencies in EEG/MEG recordings of non-human primates and healthy subjects [387]. To date, the relationship of the effect of ketamine on the $1/f^{\beta}$ power-law relationship with the subjective effects of the drug and therapeutic response in patients has not been investigated.

In addition to scale-free properties, the high temporal resolution provided by EEG/MEG makes it ideal for determining measures of non-linear dynamics, such as complexity and entropy of brain activity. One such measure is the Lempel-Ziv complexity (LZc), which assesses the level of compressibility of a deterministic signal. LZc is closely associated with entropy and indicates the diversity of a signal. To compute LZc the instantaneous amplitude of the signal needs to be determined, binarizing it based on its mean, to then apply the Lempel-Ziv compression algorithm to obtain unique non-overlapping substrings. A higher diversity of substrings corresponds to a higher LZc value [388]. The use of LZc has been increasingly incorporated into various pharmaco-EEG studies, including those involving ketamine and classic psychedelic drugs [388–392]. According to the "entropic brain" hypothesis, the entropy of spontaneous brain activity reflects the informational richness of conscious states. This hypothesis is grounded in evidence showing reduced spontaneous brain complexity in states of reduced consciousness and increased complexity in altered states of consciousness induced by psychedelic drugs, correlating with the intensity of the subjective experience [393].

A few studies have shown increased EEG/MEG spontaneous signal complexity measured via LZc upon subanaesthetic ketamine administration in healthy subjects [389,394,394,395]. Notably, the intensity of the subjective experience induced by ketamine was found to correlate with LZc, particularly in relation to

features such as ego dissolution, complex imagery, elementary imagery, experience of unity, and anxiety. It's interesting to note that ketamine-induced increases in complexity appear to be limited to spontaneous EEG, with complexity following perturbation using transcranial magnetic brain stimulation (TMS) being unchanged [394]. Additionally, TMS-EEG evoked signal complexity was not reduced under ketamine anaesthesia either, exhibiting a pattern close to wakefulness. Participants in this state reported long, vivid dreams unrelated to the external environment [338]. Conversely, during ketamine-induced anaesthesia, alternating low and high spontaneous complexity levels were observed, stabilizing upon recovery [395]. Since evoked complexity metrics measure the general capacity of the brain to sustain consciousness, these results suggest that ketamine mainly alters the content, rather than the structuring of conscious experience [396]. Regarding the effects of subanaesthetic ketamine on LZc in depression and its relationship with treatment outcome, a recent study in a cohort of late-life TR-MDD found that ketamine increased LZc. However, this increase did not correlate with antidepressant response [397]. While some evidence exists on the effects of subanaesthetic doses of ketamine on brain signal complexity in healthy subjects, the characterisation of these dynamics in the patient population is very limited. Also, the relationship of non-linear dynamics with subjective effects and therapeutic response to ketamine has not been investigated.

In summary, current literature supports the potential of EEG measures to provide robust biomarkers of ketamine-induced altered states of consciousness and therapeutic response. Both rhythmic and arrhythmic features of resting-state EEG activity have been associated with putative markers of depressive symptomatology and drug responses, allowing to probe of prominent theories of depression, such as the "excitatory/inhibitory balance" hypothesis. However, a complex relationship exists between the neurophenomenological and therapeutic effects of ketamine, which warrants further investigation. Further, very limited data are available on the effect of ketamine on arrhythmic and non-linear brain dynamics, their relationship with rhythmic neural activity, and their phenomenological and clinical significance. Critically, the neural basis of ketamine response in different patient populations, such as TRD-BP, remains largely overlooked. As the use of ketamine diffuses into clinical practice, real-world evidence is necessary to investigate the complex neuropsychopharmacological response to the drug outside standardized research contexts. In fact, limited evidence is available on ketamine response in complex patient populations with heterogenous demographics and often undergoing poly-pharmacological treatments with different therapeutic trajectories. The use of a portable EEG headset is particularly suitable to non-invasively study the neurophysiological effects of ketamine in patients receiving ketamine treatments in real-world, hospitalized settings.

4.2. Study Aim

In this study, a 32-channel EEG headset was employed to study the acute neurophysiological and dissociative effects of a subanaesthetic infusion of ketamine in patients with TR-BP undergoing ketamine therapy for depression. The aims of the current study were 1) to characterize the neurophysiological underpinnings of the dissociative state induced by a subanaesthetic dose of ketamine in TR-BP in a clinical, ecologically valid, setting. 2) Explore the relationship of such markers with the acute subjective effects and treatment response induced by ketamine.

4.3. Materials and methods

4.3.1. Patient population

The study included a total of 30 patients (Mean age = 51 ± 13, Females = 12; Table 1) with TR-BP type 2 with a history of failure of at least 2 conventional antidepressant treatments and currently undergoing a depressive episode requiring hospitalization. The severity of depression at inclusion was assessed with the Montgomery-Åsberg Depression Rating Scale (MADRS) and a semi-structured interview by the study psychiatrist. The criteria for inclusion were: having between 18 and 70 years of age, a diagnosis of BP type 2 in accordance with the DSM V criteria [10], an ongoing TR depressive episode defined as a history of failure of at least 2 conventional antidepressants, and a MADRS score at inclusion above 30 (i.e., moderate to severe depression). Acute suicidal ideation, presence of psychotic symptoms, current dependence on alcohol and other substances of abuse, and presence of other severe medical conditions were grounds for

exclusion. Comorbidity with other minor psychiatric conditions was allowed. All participants in the study were undergoing concomitant poly-psychotropic treatment during the study (Table 3). To be included, all patients were required to be in treatment with a psychiatrist at the moment of study enrolment. All patients provided informed consent to participate in the study, collected by the researcher and study psychiatrist. The study was approved by the research ethics committee of the University Hospital of Siena. All inclusion and experimental procedures were performed in the psychiatry unit within the Department of Mental Health of the University Hospital of Siena, Italy.

	TRD-BP (N = 30)	**ER (N = 18)**	**LR (N = 12)**
Sex	M = 18, F = 12	M = 11, F = 7	M = 7, F = 5
Age	51 ± 13	52 ± 11	48 ± 15
SSRI (T0)	77 %	78 %	75 %
SNRI (T0)	17 %	11 %	25 %
Lithium (T0)	80 %	72 %	92 %
Valproate (T0)	43 %	50 %	33 %
Antipsychotic (T0)	73 %	72 %	75 %
Antiepileptic (T0)	47 %	50 %	42 %
Benzodiazepines (T0)	47 %	44 %	50 %
Bupropion (T0)	13 %	17 %	8 %
Other medications (T0)	43 %	39 %	50 %
MADRS (T0)	38 ± 5	38 ± 5	38 ± 4
MADRS (T1)	21 ± 6	17 ± 3	27 ± 5
MADRS (T2)	13 ± 3	14 ± 3	12 ± 4
Ketamine dose mg/kg (T2)	0.7 ± 0.2	0.6 ± 0.1	0.9 ± 0.09
CADSS Tot (T2)	26 ± 15	29 ± 17	22 ± 13
CADSS Depersonalization (T2)	8 ± 3	9 ± 5	7 ± 5
CADSS Derealization (T2)	14 ± 8	15 ± 8	12 ± 6
CADSS Amnesia (T2)	2 ± 2	3 ± 2	1 ± 1
N° of previous ketamine administrations (T0-T2)	9 ± 8	7 ± 8	11 ± 7

Table 3: Demographics, Drug, and Psychometric measurements.

4.3.2. Study design

The patients included in this experiment were recruited from a cohort of in-patients with TR-BP undergoing off-label ketamine therapy for a current MDE. The treatment involved 30 minutes long i.v. continuous infusions of subanaesthetic doses of racemic ketamine (1:1 mixture of ketamine enantiomers) twice a week for a month, following which, the treatment was adjusted based on the patient's needs. The treatment started at dosages lower than 0.5 mg/kg and was titrated based on individual tolerability and clinical response to a maximum of 1 mg/kg. Depressive symptomatology was monitored by the study psychiatrist at baseline and bi-weekly through the administration of the MADRS until the end of treatment. For each patient, the EEG signal was measured once, during one of the ketamine administrations with a dosage between 0.5 and 1 mg/kg, known to reliably produce acute subjective effects [398]. All EEG

recordings were performed at least 1 week after the beginning of treatment. Hence, each participant had a variable dose and number of prior exposures to ketamine at the time of the EEG recording. To quantify the intensity and phenomenology of the subjective experience induced by ketamine on the day of the EEG, the CADSS was administered immediately at the end of the infusion. This scale was chosen as being the most commonly used in previous literature [337].

The EEG recording consisted of 6 minutes of baseline resting state before the start of ketamine infusion. The 6 minutes of baseline EEG recording were subdivided into 3 minutes of eyes open (Pre-OP) and 3 minutes of eyes closed (Pre-EC) conditions. Then, EEG was recorded throughout the 30 minutes of continuous i.v. infusion of a subanaesthetic dose of ketamine (0.5-1 mg/kg). Immediately at the end of the infusion, EEG recording continued for an additional 6 minutes, again subdivided into 3 minutes of eyes open (Post-EO) and 3 minutes of eyes closed (Post-EC) conditions. A summary of the study design is shown in Figure 6 A. Continuous i.v. infusion of a subanaesthetic dose of ketamine (0.5-1 mg/kg) of 30 minutes has been shown to have an elimination half-life of 2 to 3 hours, with dissociative/psychedelic-like effects lasting around 1 to 2 hours. Thus, the post-ketamine EEG condition captured the acute phase of the ketamine state.

4.3.3. CADSS

To quantify the intensity and character of the subjective experience induced by ketamine, the 25-item version of the CADSS was administered. The items were adapted to the Italian language. The scale was scored according to Bremner et al. (1998), resulting in the 3 factors of dissociation, namely depersonalization, derealization, and amnesia [340].

4.3.4. MADRS

To assess the clinical trajectory of the patients, the MADRS was administered by the study psychiatrist on a weekly basis from the start until the end of treatment. The time points of MADRS administration relevant for the current investigation were: time of recruitment (T0), 1 week after the beginning of the treatment (T1), and before the ketamine infusion with concurrent EEG recording (T2) (Figure 1 A). At the session where EEG recordings were made (T2), all patients had responded to the prior treatments, with all participants exhibiting MADRS scores below 19. Patients who showed a 50% reduction of MADRS after the first week of treatment were classified as early responders (ER), while the other portion of patients was classified as late responders (LR).

4.3.5. EEG setup

The EEG recording was performed with a Wireless, 32-channel Starstim device (Neuroelectrics®, Barcelona Spain). The montage included 32 Ag^{+}/ $Ag^{+}Cl^{-}$ passive electrodes (10-20 international EEG system). This portable and quick-to-setup EEG headset was chosen as it offers enough channel density to reliably measure good quality electrophysiological signal across the scalp with minimal invasiveness and distress for the patient, making it suitable for ecologically valid recording in a hospitalized population. The acquisition sampling rate was 500 Hz. Two reference electrodes were placed on the left mastoid. Data were acquired with the Neuroelectrics® Instrument Controller software.

4.3.6. EEG pre-processing

The continuous EEG signal was pre-processed off-line, retaining the sampling resolution of 500 Hz. The pre-processing steps followed a standard procedure. First, the data were baseline corrected and a 1 to 80 Hz band-pass filter was applied, with a notch filter at 50 Hz. Then, a semi-automatic artefact removal approach was applied. The process involved the automatic detection of portions of signal showing a standardized score of deviation from the mean above a pre-specified threshold. Then, the data were visualized for the manual removal of artefacts associated with movements and jaw clenches, as well as noisy channels. Then, independent component analysis (ICA), using the "runica" algorithm, was applied to remove from the signal EEG components of muscle activity, blinks, ocular movement, and cardiac activity. Removed channels were then interpolated using the weighted average of neighbouring electrodes. Lastly, the data were re-referenced to the average of all electrodes. A comparable amount of channels (Pre-

ketamine: M = 2, SD =2; Post-ketamine: M = 3, SD =2) and ICA components (Pre-ketamine: M = 6, SD =2; Post-ketamine: M = 8, SD =3) was removed before and after ketamine. Also, the different conditions had similar data lengths after pre-processing: Pre-EO (M = 182 s , SD = 7), Pre-EC (M = 177 s , SD = 7), Post-EO (M = 184 s , SD = 11), and Post-EC (M = 175 s , SD = 13). All pre-processing steps were implemented in Matlab software using the open-source toolbox FieldTrip [399].

4.3.7. EEG analysis

All analyses were performed on the clean EEG data recording of the 3-minute eye open and 3-minute eyes closed conditions before and immediately following ketamine infusion. Prior to all analysis, the continuous pre-processed EEG data were subdivided into non-overlapping epochs of 2 seconds.

For the spectral power density (SPD) analysis, data were Fast-Fourier transformed using a conventional single taper (e.g., Hanning) for frequencies between 1 and 30 Hz and using the multiple tapers based on discrete prolate spheroidal sequences for frequencies between 30 and 80 Hz. To determine the separate contribution of oscillatory and fractal components to the original spectral power, the signal was decomposed using the Irregularly Resampled Auto Spectral Analysis (IRASA) algorithm, as described by Wen and Liu [400]. The technique virtually compresses and expands the time-domain data with a set of non-integer resampling factors prior to FFT-based spectral decomposition. As a result, oscillatory components in the power-spectrum are redistributed while the fractal 1/f contribution is left intact. Taking the median of the resulting auto-spectral distributions extracts the power-spectral fractal component, and the subsequent removal of the fractal component from the original power-spectrum offers a power-spectral estimate of oscillatory content alone. Original, fractal, and oscillatory spectral power density were divided in the following canonical frequency bands for statistical analysis: delta (1-4Hz), theta (4-8 Hz), alpha (8-13 Hz), low beta (13-20 Hz), high beta (20-30 Hz), low gamma (30-45 Hz), high gamma (55-80 Hz), and broadband (1-80 Hz).

In order to estimate the PLE of the power spectrum, the fractal component $1/f^{\beta}$ was transformed to log-log coordinates. Taking the log brings the β down from the exponent, turning the relation to a linear one: $\log(1/f^{\beta}) = \beta \log(1/f)$. Then, a linear regression was performed to extract the β coefficient. Finally, taking the negative of the β coefficient turns f^{β} to $1/f^{\beta}$ (i.e., PLE). To avoid biasing regression estimates towards the higher frequencies, where more sampling points exist in logarithmic space, frequency estimates are resampled to be evenly spaced in logarithmic coordinates prior to computation of the regression. Also, visual inspection of the log-log distribution of the fractal power showed a "knee" frequency at 20 Hz, were the slope (i.e., β coefficient or PLE) of the spectrum showed a significant change. Thus, data were separated into frequency bands "by eye" into two spectral regions, a high-frequency region (20-80 Hz) for which the parameter PLE_{hf} was defined and a lower frequency range (1-20 Hz) for which PLE_{lf} was defined, following a similar approach to the one of Muthukumaraswamy and Liley [387].

For the quantification of LZc, the pre-processed data were first binarized by comparing each data point for epoch and channel to the mean value of that channel and epoch, with values above the mean transformed into 1s and values below into 0s. Then, the LZc 76 algorithm was applied to compute the number of distinct "patterns" (or substrings) in each binarized epoch and channel, and then normalized the resulting number for data length by a factor N/log2(N), where N is the data length in samples. Then, the normalized values were averaged over epochs to obtain a measure of brain entropy estimate (here referred as LZc) . Regular signals are characterized by a small number of patterns and hence have low LZc, while irregular signals contain many different patterns and hence have a high LZc. The analysis followed the original method described by Lempel A, Ziv J [401], applied to EEG data.

For the quantification of complexity contribution of each frequency band, the novel estimator called Complexity via State-space Entropy Rate (CSER) was computed. The CSER is a spectrally and temporally resolved estimation of neural signal diversity, recently introduced by Mediano et al [402]. Compared to LZc, CSER does not require the signal to be discretized, allowing it to fully exploit continuous signals and avoiding potential artefacts introduced by the discretization procedure. Also, CSER has the unique

advantage of allowing for complexity analysis within spectral frequency bands. First, the pre-processed data were down sampled to 160 Hz. Then data were normalized via z-scoring, by subtracting the mean and then dividing by the standard deviation in each channel. The CSER was applied to the z-scored data to obtain a CSER measure for each frequency band, which adds up to a total CSER. The frequency-specific CSER was computed on 2 Hz windows of the broadband and then averaged within the frequency bands of interest. For comparison, we replicated the LZc analysis with the same down-sampled and normalized data, showing comparable results to the LZc computed on the 500 Hz resolution data (data not shown). All analysis were performed in Matlab software using the open-source toolbox FieldTrip [399] for the EEG spectral analysis and the EntRate package for the LZc and CSER analysis [402].

4.3.8. Statistical analysis

The analysis of the original, fractal, and oscillatory spectral power density (divided in the main frequencies of interest), as well as the PLE and LZc measures of the EEG, involved group-level channel-specific comparisons between the eyes open closed conditions before and immediately after ketamine infusion ended. For each EEG metric and channel, a cluster-level permutation tests was computed between conditions, an approach shown to be the most efficient in addressing the multiple comparisons problem [403]. For every channel, the experimental conditions are compared by means of a t-value. All samples are selected whose t-value is larger than a cluster α value of 0.05. Then, selected samples are clustered in connected sets on the basis of temporal, spatial and spectral adjacency. Cluster-level statistics are calculated by taking the sum of the t-values within every cluster and the maximum of the cluster-level statistics is taken. The significance probability is calculated by means of the so-called Monte Carlo method. The method collect the trials of the different experimental conditions in a single set and randomly draw as many trials from this combined data set as there were trials in condition 1 and place them into subset 1. The remaining trials are placed in subset 2. On this randomized set, the test statistic is computed as described above (i.e., the maximum of the cluster-level summed t-values). These steps were repeated for 1000 random permutations to obtain a distribution of test statistics, from which the proportion of random partitions that resulted in a larger test statistic than the observed one is computed. If the probability value (i.e., p value) is smaller than the critical α-level of 0.025 (one tail for positive and one for negative clusters; total 0.05), then the data in the two experimental conditions were considered significantly different.

To analyse changes in CSER, an average CSER value across channels was obtained for each frequency and for the broadband data. A linear mixed-effect model was used to calculate the pre- vs post-ketamine difference in CSER, using a random slope for each subject to account for the within-subject design. This analysis was performed using the open-source programming language R.

For correlations between EEG metrics and between EEG metrics with dose, scores on the CADSS, and MADRS scores at T2, the electrodes belonging to statistically significant clusters computed with the permutation tests were extracted for each comparison and metric. Then, the difference (i.e., Δ value) between post- to pre-ketamine values was computed for those electrodes only. Both the raw and relative (corrected for the baseline value) differences were computed. For each contrast, non-parametric spearman correlation tests were performed between EEG metrics, total, depersonalization, derealization, and amnesia CADSS scores, MADRS scores at T2 (EEG day, before ketamine administration), and ketamine dosage. For the correlation with the CADSS scores only, the analysis was repeated with the exclusion of 1 patient who reported an abnormally high CADSS score compared to the others. The outlier was identified via analysis on the quantile distribution using the Tukey's rule [107]. Outliers were defined as data points laying 1.5 times the Interquartile range above the third quartile (75% of the distribution) or below the first quartile (25% of the distribution). To account for FDR inflation due to multiple comparisons, the p values resulting from spearman tests were adjusted independently using the Benjamini-Hochberg adjustment [110]. Results of the FDR correction are reported as "p adj. " in the main text.

For the analysis of early versus late responders, patients were stratified based on the difference in MADRS scores between the T0 and T1. Patients who showed a reduction of MADRS scores of more than 50 % between the 2 time points where considered ER, while the others were classified as LR. The significance

analysis for the comparison of baseline EEG metrics between groups was performed with cluster-based permutation statistics with a between-subject design. For the comparison of EEG changes induced by ketamine between groups, cluster-based permutation statistics with a mixed within-between subject design was employed. First, condition differences were computed for each EEG metric and contrasts (i.e., post-EO vs pre-EO; post-EC vs pre-EC). Then the difference between condition was compared between groups (i.e., ER vs LR). For CSER and for the inclusion of dosage as a covariate, the analysis was implemented with a linear mixed-effects model, using the channel-averaged EEG metrics as dependent variable, group and condition as independent variables, plus their interaction, dose as covariate, and a random effect for each subject.

4.4. Results

4.4.1. Demographics, Drugs, and Psychometric measures

All patients reported a clinically meaningful experience of dissociation during the EEG recorded ketamine administration, defined by a CADSS total score above 4 (based on a previously reported normative scores in heathy participants according to Bremner et al. [340]). No significant correlations between dose and CADSS total or CADSS subdimensions were observed. Also, there were no correlation between MADRS scores before ketamine administration and CADSS scores after ketamine infusion.

4.4.2. EEG metrics

The analysis of the acute neurophysiological changes induced by a single continuous infusion of ketamine (i.v. 0.5-1 mg/kg for 30 minutes) involved the comparison of rhythmic and arrhythmic components of the resting-state EEG signal. All EEG metrics were computed as averages within each of the 3 minute-long conditions, namely the pre ketamine eyes open condition (i.e., Pre-EO), pre-ketamine eyes closed condition (i.e., Pre-EC), post ketamine eyes open condition (i.e., Post-EO), and post-ketamine eyes closed condition (i.e., Post-EC). The main experimental contrasts were between the Pre-EO and Post-EO conditions and between the Pre-EC and Post-EC conditions. A plot of the power spectra and its oscillatory and fractal components is shown in Figure 6 B-D.

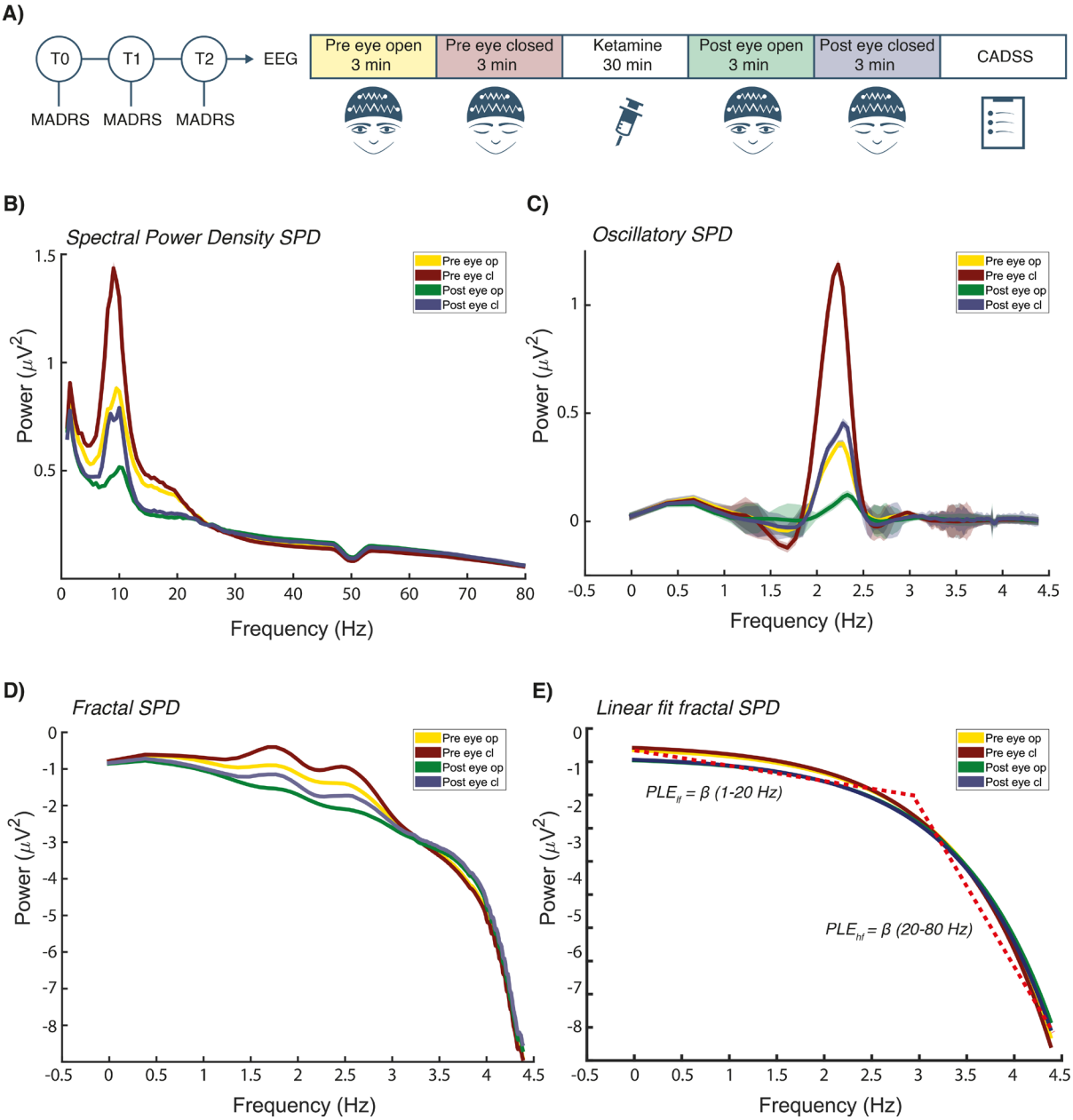

Figure 6: Study design and power spectra. A) The illustration shows the timeline and structure of the experiment (see materials and methods). B) The frequency/power plot of the SPD. C) The logarithm of the frequency is plotted over the oscillatory component of the power spectra. D) the log-log plot of the fractal (scale-free) component of the power spectra. E) The log-log plot of the linear fit of the fractal component of the power spectra. The dashed lines shows the interpolated regression lines used to estimate the PLE for the low (1-20 Hz) and high frequencies (20-80 Hz).

4.4.2.1. Spectral Power Density (SPD)

The analysis of the SPD for the Pre-EO vs Post-EO contrast revealed a significant reduction in broadband (i.e., 1 to 80 Hz) PSD during ketamine exposure (Cluster-based stats = -53.80.41; p = 0.002; CI = 0.004). Frequency-specific analysis showed a reduction in the delta (Cluster-based stats = -43.33; p < 0.001; CI = 0.002), theta (Cluster-based stats = -108.08; p < 0.001; CI = 0.002), alpha (Cluster-based stats = -191.30; p < 0.001; CI = 0.002), low beta (Cluster-based stats = -153.62; p < 0.001; CI = 0.002), and high beta (Cluster-based stats = -15.72; p= 0.016; CI = 0.008) bands. No statistically significant differences where observed for low and high gamma (All data are shown in Figure 7A). Similarly, for the Pre-EC vs Post EC contrast there was a broadband reduction of SPD during ketamine administration (Cluster-based stats = -74.76; p < 0.001; CI = 0.002), with reduction in delta (Cluster-based stats = -67.92; p = 0.002; CI = 0.003), theta

(Cluster-based stats = -151.57; p < 0.001; CI = 0.002), alpha (Cluster-based stats = - 221.26; p < 0.001; CI = 0.002), and low beta (Cluster-based stats = -152.81; p < 0.001; CI = 0.002). Consistent with the EO condition, no differences were found in high beta, low and high gamma power in the EC condition (All data are shown in Figure 7 B).

4.4.2.2. Irregular Resampling Auto-Spectral Analysis (IRASA)

The original SPD was decomposed using the IRASA method into its oscillatory and fractal components. As for the original SPD, the analysis of the oscillatory component of the spectral power for the Pre-EO vs Post-EO contrast showed a significant broadband reduction following ketamine administration (Cluster-based stats = -80.66; p < 0.001; CI = 0.002). However, frequency-specific oscillatory power showed significant reductions only for the alpha (Cluster-based stats = -137.01; p < 0.001; CI = 0.002) and low beta (Cluster-based stats = -73.77; p < 0.001; CI = 0.002) frequencies, while an increase was observed for the low gamma (Cluster-based stats = 64.73; p < 0.001; CI = 0.002) oscillatory power. No differences where observed for delta, theta, high beta, and high gamma (All data are shown in Figure 7 A). A similar reduction of broadband (Cluster-based stats = -119.30; p < 0.001; CI = 0.002), alpha (Cluster-based stats = -162.71; p < 0.001; CI = 0.002), and low beta (Cluster-based stats =-26.54; p = 0.014; CI = 0.007) and increase in low gamma (Cluster-based stats = 53.98; p < 0.001; CI = 0.002) oscillatory power was present for Pre-EC vs Post EC contrast. Interestingly, there was also a reduction of theta (Cluster-based stats = -47.72; p = 0.005; CI = 0.004) as well. No differences were observed for delta, high beta, and high gamma power (All data are shown in Figure 7 B).

The analysis of the fractal component of the spectral power for the Pre-EO vs Post-EO contrast showed a broadband (Cluster-based stats = -66.56; p < 0.001; CI = 0.002) reduction during ketamine, with reductions within the delta (Cluster-based stats = -11.90; p = 0.017; CI = 0.008), theta (Cluster-based stats = -105.12; p < 0.001; CI = 0.002), alpha (Cluster-based stats = -125.30; p < 0.001; CI = 0.002), low beta (Cluster-based stats = -99.45; p < 0.001; CI = 0.002), and high beta (Cluster-based stats = -17.38; p = 0.020; CI = 0.009) bands. No statistically significant differences were observed for low and high gamma fractal power (All data are shown in Figure 7A). In the Pre-EC vs Post-EC contrast, there was a significant broadband reduction of fractal power (Cluster-based stats = -85.42; p < 0.001; CI = 0.002), with similar reductions in the delta (Cluster-based stats = -37.82; p = 0.002; CI = 0.004), theta (Cluster-based stats = -135.60; p < 0.001; CI = 0.002), alpha (Cluster-based stats = -145.82; p < 0.001; CI = 0.002), low beta (Cluster-based stats = -108.32; p = 0.002; CI = 0.003) and high beta (Cluster-based stats = -9.93; p = 0.021; CI = 0.009) bands, as observed in the other contrast. There were no differences in low and high gamma (All data are shown in Figure 7 B).

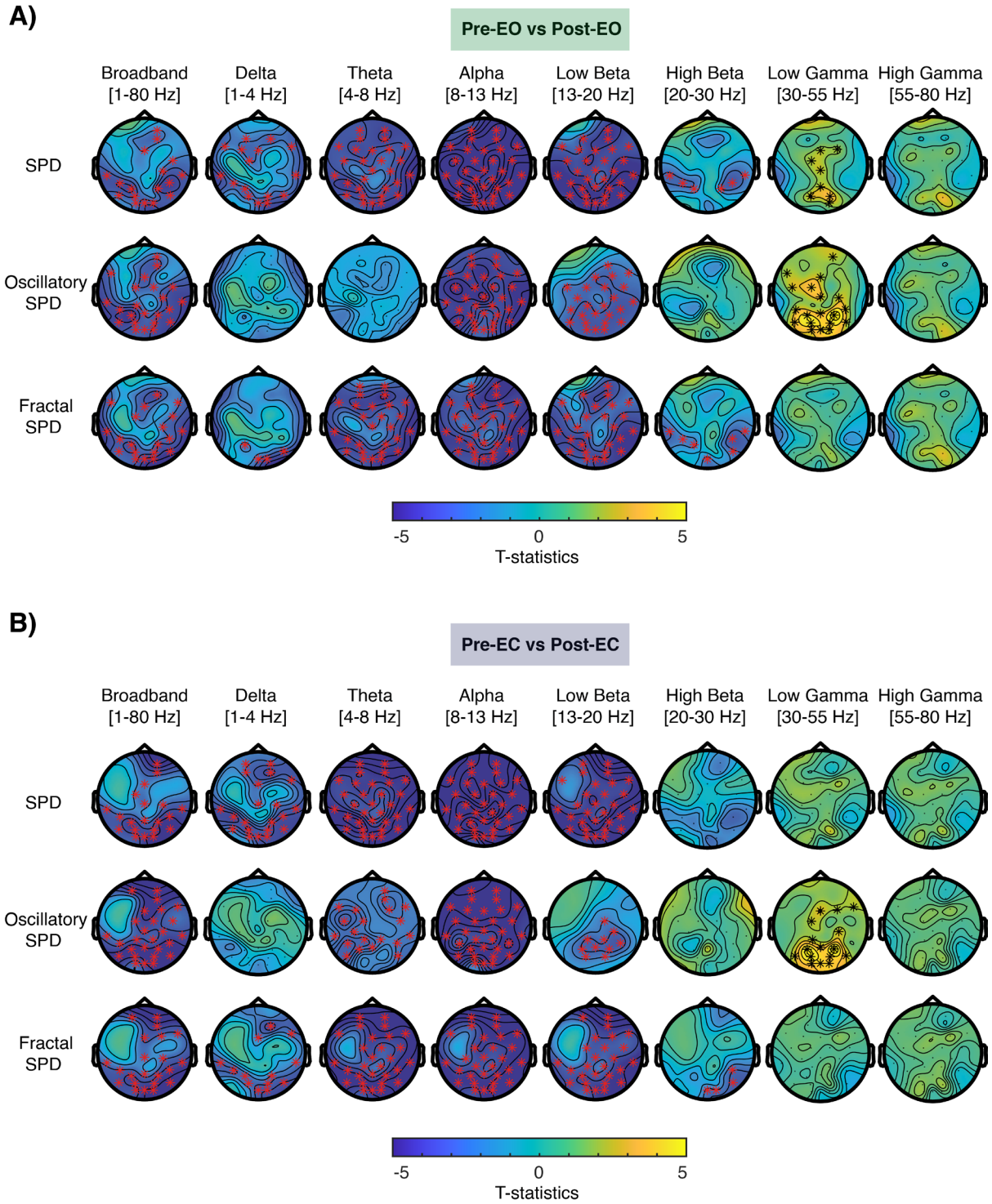

Figure 7: The rhythmic features of the EEG signal. A) The topoplots of the original, oscillatory, and fractal components of the spectral power density for the eyes open condition divided by frequency. B) The topoplots of the original, oscillatory, and fractal components of the spectral power density for the eyes closed condition divided by frequency. The values in the topoplots corresponds to the results of the cluster-based permutation T-statistics. Electrodes belonging to significant clusters are marked in red, if there was decrease from pre- to post-ketamine, and in black if there was an increase from pre- to post-ketamine. Clusters were considered statistically significant with an a value < 0.025.

4.4.2.3. Power-law Exponent (PLE)

The analysis of the slope of the fractal component of the EEG signal was performed by estimating the PLE for all frequencies (i.e., 1-80 Hz, PLE_{tot}), as well as for low frequencies (i.e., 1-20 Hz, PLE_{lf}) and high frequencies (20-80 Hz, PLE_{hf}) separately (Shown in Figure 6 E). A statistically significant reduction of the PLE_{tot} was observed following ketamine exposure for both the Pre-EO vs Post-EO (Cluster-based stats = -170.15; $p < 0.001$; CI = 0.002, Figure 3 A) and Pre-EC vs Post-EC contrasts (Cluster-based stats = -166.76; $p < 0.001$; CI = 0.002, Figure 3 B). Signifying a reduction in the steepness of the 1/f fractal distribution. In particular, there was no difference in the PLE_{lf} and a significant decrease in the PLE_{hf} in Pre-EO vs Post-EO (Cluster-based stats = - 139.88; $p < 0.001$; CI = 0.002, , Figure 3 A) and Pre-EC vs Post-EC (Cluster-based stats = -127.16; $p < 0.001$; CI = 0.002, Figure 3 B) contrasts. Further analysis of the PLE_{hf} showed that the change in slope was confined within the high beta (20-30 Hz) frequency band in both the Pre-EO vs Post-EO (Cluster-based stats = -179.71; $p < 0.001$; CI = 0.002) and Pre-EC vs Post-EC contrasts (Cluster-based stats = -201.75; $p < 0.001$; CI = 0.002). Therefore, the change in PLE within the high beta (PLE_{beta}) was later used for the correlation with the other EEG and behavioural metrics.

4.4.2.4. Lempel–Ziv Complexity (LZc)

Analysis of the broadband entropy of the EEG signal was performed via LZc computation. Both the Pre-EO vs Post-EO (Cluster-based stats = 165.68; $p < 0.001$; CI = 0.002, Figure 8 A) and Pre-EC vs Post-EC contrasts (Cluster-based stats = 203.35; $p < 0.001$; CI = 0.002, Figure 8 B) showed a marked increase in LZc following ketamine administration.

4.4.2.5. Complexity via State-space Entropy Rate (CSER)

Analysis of the broadband entropy of the EEG signal averaged across channels was performed via CSER, showing an with increases in both the Pre-EO vs Post-EO ($\beta = 0.21$, $p < 0.001$; $d = 1.29$, Figure 8 A) and Pre-EC vs Post-EC ($\beta = 0.30$; $p < 0.001$; $d = 1.55$, Figure 8 B) contrasts. The results of CSER were consistent with what observed for LZc averaged across channels (both pre-processed at 160 Hz and 500 Hz sampling rates, data not shown). Frequency-decomposed CSER showed an increase within the delta ($\beta = 0.003$; $p = 0.003$; $d = 0.55$), high beta ($\beta = 0.02$; $p < 0.001$; $d = 1.13$), low gamma ($\beta = 0.08$; $p < 0.001$; $d = 1.65$), and high gamma ($\beta = 0.11$; $p < 0.001$; $d = 1.24$) bands but a decrease within alpha ($\beta = - 0.02$; $p < 0.001$;$d = - 1.14$) and low beta ($\beta = - 0.01$; $p < 0.001$; $d = -0.66$) bands for the Pre-EO vs Post-EO contrast (Figure 8 A). Similar results were obtained for the Pre-EC vs Post-EC contrast (Figure 8 B), with the exception of no significant differences for low beta; delta ($\beta = 0.01$; $p < 0.001$; $d = 0.72$), theta (ns), alpha ($\beta = - 0.01$; $p < 0.001$; $d = -0.93$), high beta ($\beta = 0.03$; $p < 0.001$; $d = 1.62$), low gamma ($\beta = 0.10$; $p < 0.001$; d 1.79), high gamma ($\beta = 0.15$; $p < 0.001$; $d = 1.37$).

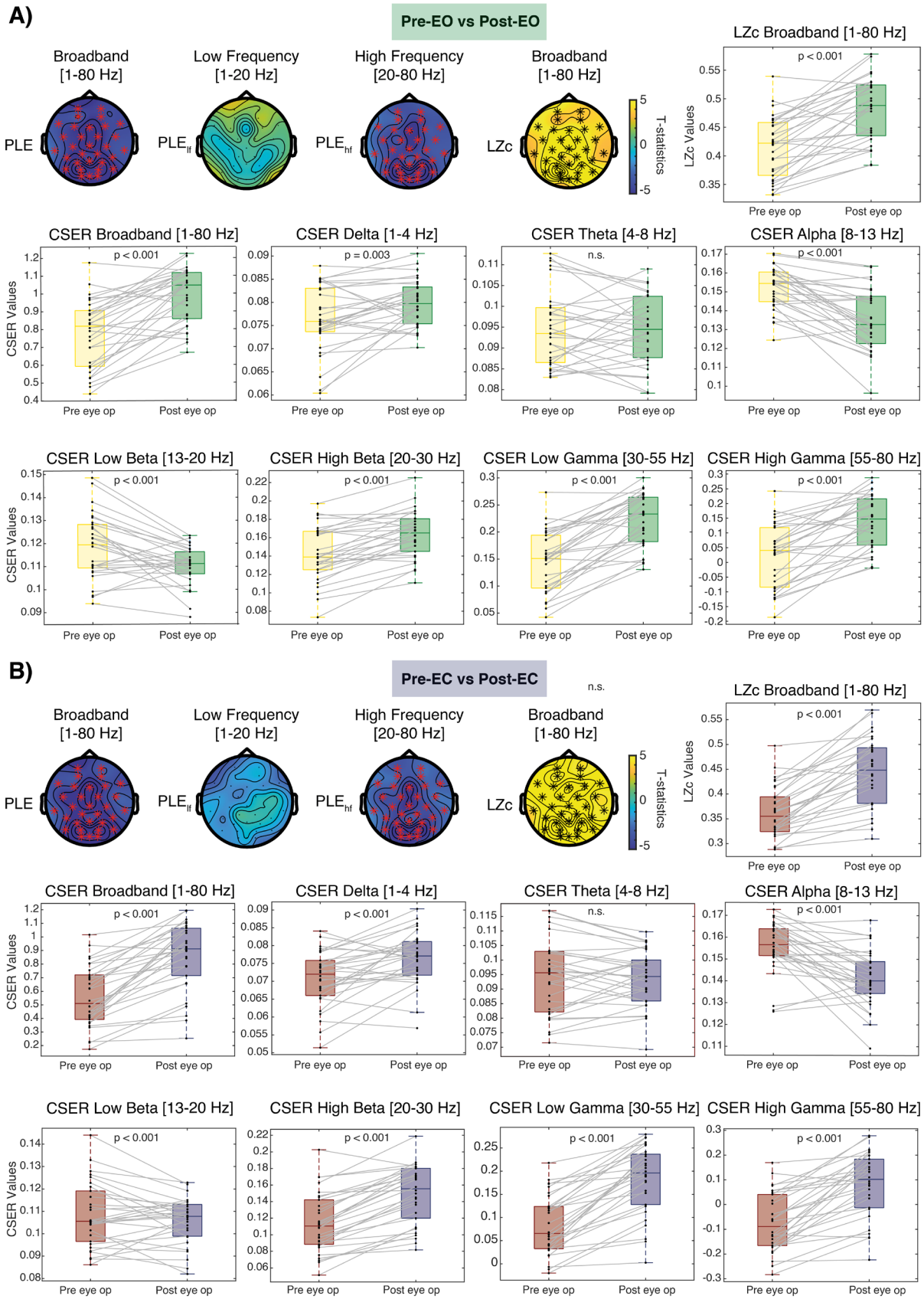

Figure 8: The arrhythmic features of the EEG signal. A) The difference in arrhythmic features of the EEG signal in the eyes open condition. B) The difference in arrhythmic features of the EEG signal in the eyes closed condition. Those include the topoplots of the PLE$_{lf}$ and PLE$_{hf}$, the topoplot of the normalized LZc, and the channel average of the LZc as well as broadband and frequency-decomposed CSER. The values in the topoplots corresponds to the results of the cluster-based

permutation T-statistics. Electrodes belonging to significant clusters are marked in red, if there was decrease from pre- to post-ketamine, and in black if there was an increase from pre- to post-ketamine. Clusters were considered statistically significant with an a value < 0.025.

4.4.3. Correlation between EEG metrics

To investigate the relationship between the changes induced by ketamine in EEG signal oscillatory (i.e., rhythmic), fractal, and complexity (i.e., arrhythmic) components, pair-wise correlations were performed between statistically significant broadband and frequency-specific Δ oscillatory power and CSER and Δ PLE_{beta}.

There was a statistically significant negative correlation between Δs in total CSER and PLE_{beta} in both the Pre-EO vs Post-EO (R = -0.50 ; p = 0.006; p.adj. = 0.016) and Pre-EC vs Post-EC (R = -0.84 ; p < 0.001; p adj. < 0.001) contrasts. The same was true when using the relative Δ of total CSER and PLE_{beta} only for the Pre-EC vs Post-EC (R = -0.76 ; p < 0.001; p adj. < 0.001) contrast. In the Pre-EO vs Post-EO contrast, there was a significant negative correlation between Δ broadband oscillatory power and total CSER (R = -0.50 ; p = 0.006; p adj. = 0.016). However, it was not significant when using the relative difference. In the delta and theta bands, no association was found between the EEG metrics which showed a significant change following ketamine administration. In the alpha band, the Δ alpha CSER and Δ PLE_{beta} correlated positively in the Pre-EO vs Post-EO (R = 0.65; p < 0.001: p adj. = 0.001) and Pre-EC vs Post-EC (R = 0.63; p < 0.001; p adj. < 0.001) contrasts. Using the relative difference, the correlation was significant in the Pre-EO vs Post-EO (R = 0.68; p < 0.001; p adj. < 0.001) and Pre-EC vs Post-EC (R = 0.60; p < 0.001; p adj. = 0.002) contrasts. Also, there was a positive correlation between Δ oscillatory alpha and Δ PLE_{beta} when using the relative difference for both the Pre-EO vs Post-EO (R = 0.69; p < 0.001; p adj. < 0.001) and the Pre-EC vs Post-EC (R = 0.52; p = 0.004; p adj. = 0.011) contrasts. There was a positive correlation between Δ low beta oscillatory power and low beta CSER in the Pre-EO vs Post-EO (R = 0.67; p < 0.001; p adj. = 0.001) and Pre-EC vs Post-EC (R = 0.57; p = 0.001; p adj. = 0.003) contrasts. The relationship was not maintained when using the relative change. A negative correlation was found between Δ high beta CSER and Δ PLE_{beta} in the Pre-EO vs Post-EO (R = - 0.47; p = 0.009; p adj. = 0.022) and Pre-EC vs Post-EC (R =- 0.60; p < 0.001; p.adj. = 0.002) contrasts. Using the relative difference, the effect was only present in the Pre-EC vs Post-EC (R = -0,47 ; p = 0.010; p adj. = 0.027) contrast. In the low gamma band, there was a negative correlation between Δ low gamma CSER and Δ PLE_{beta} in the EO vs Post-EO (R = - 0.60; p < 0.001; p adj. = 0.003) and Pre-EC vs Post-EC (R = - 0.84; p < 0.001; p adj.< 0.001) contrasts. The correlation was maintained only when using the relative difference in the Pre-EC vs Post-EC (R = - 0.75 ; p < 0.001; p adj. < 0.001) contrast. Also, Δ low gamma CSER correlated positively with Δ low gamma oscillatory power in the Pre-EC vs Post-EC (R = 0.41; p = 0.026; p adj. = 0.055) contrast, but it did not survive correction for multiple comparisons. This was not observed when using the relative change. There was a negative correlation between Δ low gamma oscillatory power and Δ PLE_{beta} in the Pre-EC vs Post-EC (R = - 0.39; p = 0.032; p adj. = 0.061) contrast, but it did not survived correction for multiple comparisons. The correlation was not observed when using the relative difference. There was a negative correlation between Δ high gamma CSER and Δ PLE_{beta} in the EO vs Post-EO (R = - 0.59; p < 0.001; p adj. = 0.003) and Pre-EC vs Post-EC (R = - 0.86; p < 0.001; p adj. < 0.001) contrasts. The result was confirmed when using the relative difference only in the Pre-EC vs Post-EC (R = - 0.80 ; p < 0.001; p adj. < 0.001) contrast.

4.4.4. Correlation between EEG metrics and ketamine dose

4.4.4.1. Spectral Power Density (SPD)

Analysis of the relationship between SPD and ketamine dose delivered during the EEG measurement showed a negative correlation between dosage with Δ alpha (R = -0.40; p = 0.028) and Δ low beta (R = -0.40; p = 0.031) in the Pre-EC vs Post-EC contrast only (Figure 9 B). This result was confirmed when using the relative Δ alpha (R = -0.41; p = 0.024) and Δ low beta (R = -0.36; p = 0.049) change.

4.4.4.2. Irregular Resampling Auto-Spectral Analysis (IRASA)

No significant correlations were found between dose and raw changes in oscillatory power in any of the analysed contrasts (Figure 9). When considering the relative difference, there was a statistically significant negative correlation between oscillatory Δ broadband ($R = -0.38$; $p = 0.039$) and Δ theta ($R = -0.40$; $p = 0.027$) in the Pre-EC vs Post-EC contrast.

4.4.4.3. Power-law exponent (PLE)

There was a significant negative correlation between dosage and the raw Δ PLE_{beta} range ($R = - 0.37$; $p = 0.047$) in the Pre-EC vs Post-EC contrast only (Figure 9 B).

4.4.4.4. Lempel–Ziv Complexity (LZc)

There was a statistically significant positive correlation between dose and raw ($R = 0.40$; $p = 0.028$; Figure 9 B) and relative ($R = 0.39$; $p = 0.034$) Δ LZc in the Pre-EC vs Post-EC contrast only.

4.4.4.5. Complexity via State-space Entropy Rate (CSER)

There was a negative correlation between dose and raw Δ CSER in the alpha ($R = -0.51$, $p = 0.004$) and low beta band ($R = -0.40$, $p = 0.027$) in the Pre-EO vs Post-EO contrast (Figure 9 A). Using the relative change the same correlations were observed: dose and Δ CSER alpha ($R = -0.49$, $p = 0.006$), dose and CSER low beta ($R = -0.39$, $p = 0.031$). A negative correlation was also present between dose and raw ($R = -0.41$, $p = 0.023$; Figure 9 B) and relative ($R = -0.41$, $p = 0.024$) Δ CSER and in the alpha band and dose in the Pre-EC vs Post-EC contrast. None of the observed correlations between EEG metrics and ketamine dosage survived correction for multiple comparisons.

4.4.5. Correlation between EEG metrics and CADDS

4.4.5.1. Spectral Power Density (SPD)

Analysis of the correlation between SPD and CADSS scores showed a statistically significant positive correlation between Δ broadband SPD and total CADSS ($R = 0.39$; $p = 0.035$) and derealization ($R = 0.52$; $p = 0.003$) scores in the Pre-EO vs Post-EO contrast. There was a positive correlation between Δ delta and CADSS derealization ($R = 0.39$; $p = 0.033$) in the Pre-EC vs Post-EC contrast. When using the relative difference, the correlations between CADSS derealization and Δ broadband in the Pre-EO vs Post-EO contrast ($R = 0.48$; $p = 0.007$) and with Δ delta and in the Pre-EC vs Post-EC contrast ($R = 0.36$; $p = 0.047$) held.

With the exclusion of the outlier on the CADSS, there was a positive correlation between total CADSS score and Δ broadband in the Pre-EO vs Post-EO ($R = 0.44$; $p = 0.016$; Figure 9 A) and Pre-EC vs Post-EC ($R = 0.40$; $p = 0.032$; Figure 4 B) contrasts. In particular, Δ broadband correlated positively with CADSS derealization scores in the Pre-EO vs Post-EO ($R = 0.62$; $p < 0.001$; Figure 9 A) and Pre-EC vs Post-EC ($R = 0.48$; $p = 0.009$; Figure 4 B) contrasts. Scores on CADSS derealization correlated positively with Δ theta ($R = 0.41$; $p = 0.027$), alpha ($R = 0.40$; $p = 0.030$), and high beta ($R = 0.40$; $p = 0.031$) in the Pre-EO vs Post-EO contrast (Figure 9 A) and with Δ delta ($R = 0.49$; $p = 0.007$) and theta ($R = 0.41$; $p = 0.028$) in the Pre-EC vs Post-EC contrast (Figure 9 B). Using the relative change, the correlation between CADSS derealization Δ broadband ($R = 0.47$; $p = 0.009$) and high beta ($R = 0.42$; $p = 0.022$) was maintained in the Pre-EO vs Post-EO contrast and with Δ delta in the Pre-EC vs Post-EC contrast ($R = 0.45$; $p = 0.014$).

4.4.5.2. Irregular Resampling Auto-Spectral Analysis (IRASA)

No significant correlations were found between CADSS scores and raw changes in oscillatory power in any of the analysed contrasts. With the relative change, there was a negative correlation between the oscillatory Δ low beta and CADSS depersonalization scores ($R = -0.37$; $p = 0.044$) in the Pre-EO vs Post-EO contrast.

Exclusion of the outlier lead to significant positive correlation between raw Δ oscillatory broadband and scores in total ($R = 0.41$; $p = 0.030$) and derealization ($R = 0.39$; $p = 0.034$) CADSS scores in the Pre-EC vs Post-EC contrast (Figure 9 B).

4.4.5.3. Power-law exponent (PLE)

No significant correlations were found between CADSS scores and changes in PLE_{beta} in any of the analysed contrasts (Figure 9).

4.4.5.4. Lempel–Ziv Complexity (LZc)

No significant correlations were found between CADSS scores and changes in LZc in any of the analysed contrasts (Figure 9).

4.4.5.5. Complexity via State-space Entropy Rate (CSER)

No significant correlations were found between CADSS scores and changes in CSER in any of the analysed contrasts (Figure 9).

Altogether, none of the observed correlations between CADSS and EEG metrics survived comparison for multiple comparisons.

4.4.6. Correlation between EEG metrics and MADRS T2 (EEG day)

No significant correlations were found between MADRS scores before ketamine and changes in EEG metrics in any of the analysed contrasts (Figure 9).

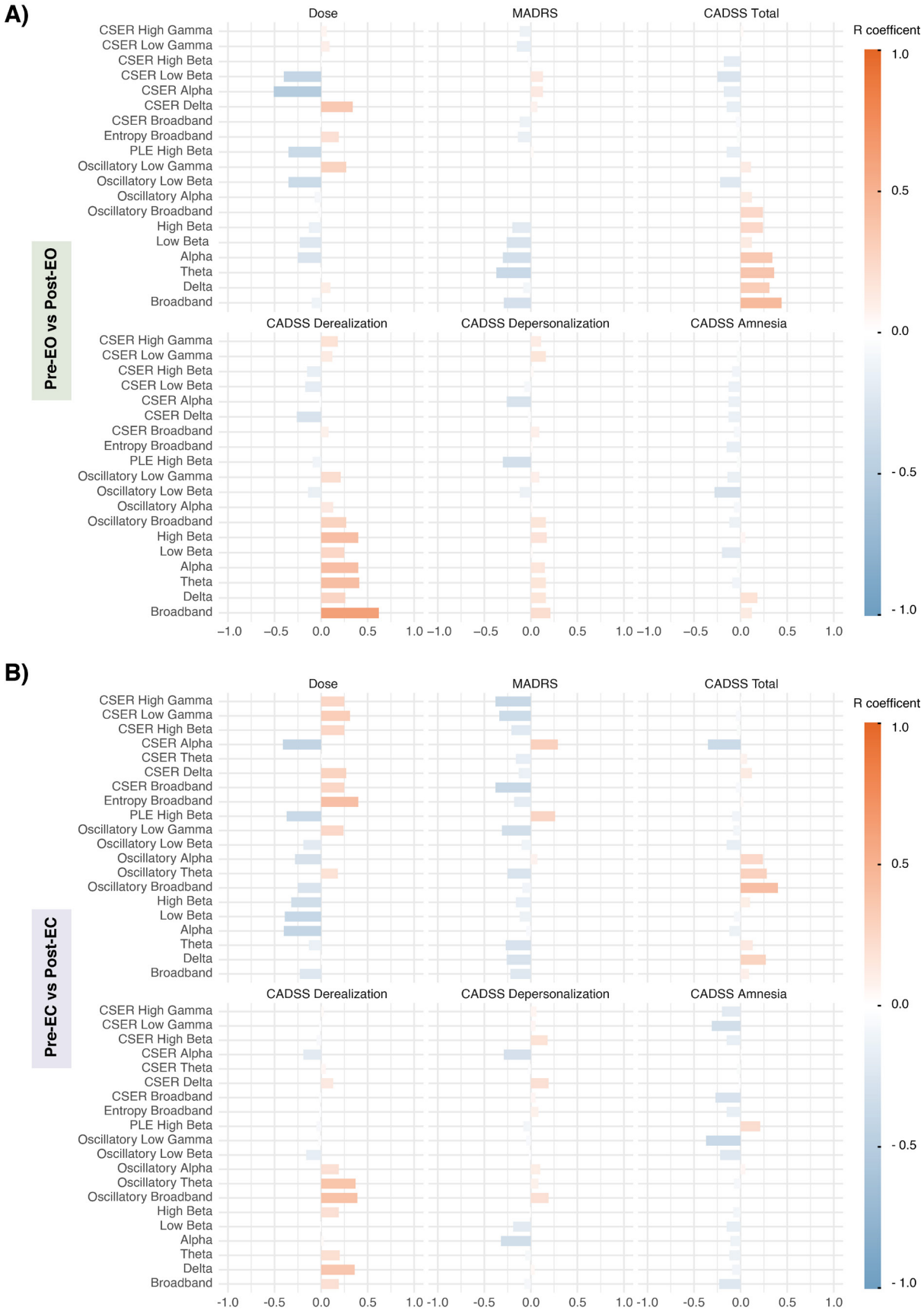

Figure 9: The correlations between EEG metrics and ketamine dose, CADSS scores, and MADRS scores at T2. A) The spearman correlation coefficient is plotted for each pairwise correlation between EEG metrics and ketamine dose, CADSS scores, and MADRS scores at T2 for the eyes open condition. B) The spearman correlation coefficient is plotted for each

pairwise correlation between EEG metrics and ketamine dose, CADSS scores, and MADRS scores at T2 for the eyes closed condition. All correlations are shown with the raw change in EEG metrics. All correlations with the CADSS are shown without the inclusion of the outlier.

4.4.7. Early vs Late responders

Modulation of EEG metrics induced by ketamine were compared between patients who responded to ketamine treatment at T1 (i.e., ER group) with patients who responded after repeated exposures (i.e., LR group). The groups showed no statistically significant differences in MADRS at T2 and baseline EEG metrics.

4.4.7.1. Spectral Power Density (SPD)

No statistically significant interactions were observed between raw changes in SPD and groups in any of the analysed contrasts.

4.4.7.2. Irregular Resampling Auto-Spectral Analysis (IRASA)

No statistically significant interactions were observed between raw or relative changes in oscillatory power and groups in any of the analysed contrasts.

4.4.7.3. Power-law exponent (PLE)

There was a significant difference between the ER and LR groups in PLE_{beta} decrease in the Pre-EC vs Post-EC contrast (Cluster-based stats = 43.40; p = 0.007; CI = 0.005). In particular, the LR group showed a higher decrease in PLE_{beta} as compared to the LR group following ketamine exposure. Given the previously reported correlation between PLE_{beta} decrease and dose in the Pre-EC vs Post-EC contrast and the fact that LR had higher dosages than ER due to the treatment schedule, we performed an analogue analysis correcting for dose. The interaction effect was still significant after using dose as a covariate (β= 0.02; p= 0.034; Figure 10 C). A similar trend was evident in the Pre-EO vs Post-EO contrast but did not reach statistical significance (β= 0.01; p = 0.066).

4.4.7.4. Lempel–Ziv Complexity (LZc)

There was a significant difference between the ER and LR groups in LZc increase in the Pre-EC vs Post-EC contrast (Cluster-based stats = -15.85; p = 0.031; CI = 0.011). In particular, the LR group showed a higher increase in LZc as compared to the ER group following ketamine exposure. After correcting for dose, the interaction effect was still significant (β = - 0.04; p = 0.018; Figure 10 D).

4.4.7.5. Complexity via State-space Entropy Rate (CSER)

The comparison of the change in broadband CSER averaged across channels did not show a statistically significant change between the ER and LR groups in either contrast, even though it followed the same trend of LZc but not as robustly (data not shown). Spectral decomposition of the CSER showed that the LR had a steeper decrease in CSER within the alpha band in both the Pre-EO vs Post-EO (β = 0.01; p= 0.001) and Pre-EC vs Post-EC (β = 0.02; p = 0.002) contrasts. Those effects were not altered when correcting for ketamine dose in both contrasts (Figure 10 E). The LR had a steeper decrease in CSER within the low beta (β = 0.01; p= 0.003; Figure 10 B) band in the Pre-EO vs Post-EO, which was not affected by dose. Also, LR had a higher increase in the low gamma CSER (β = - 0.04; p= 0.041; Figure 10 F) as compared to ER in the Pre-EC vs Post-EC contrast, independent of drug dosage.

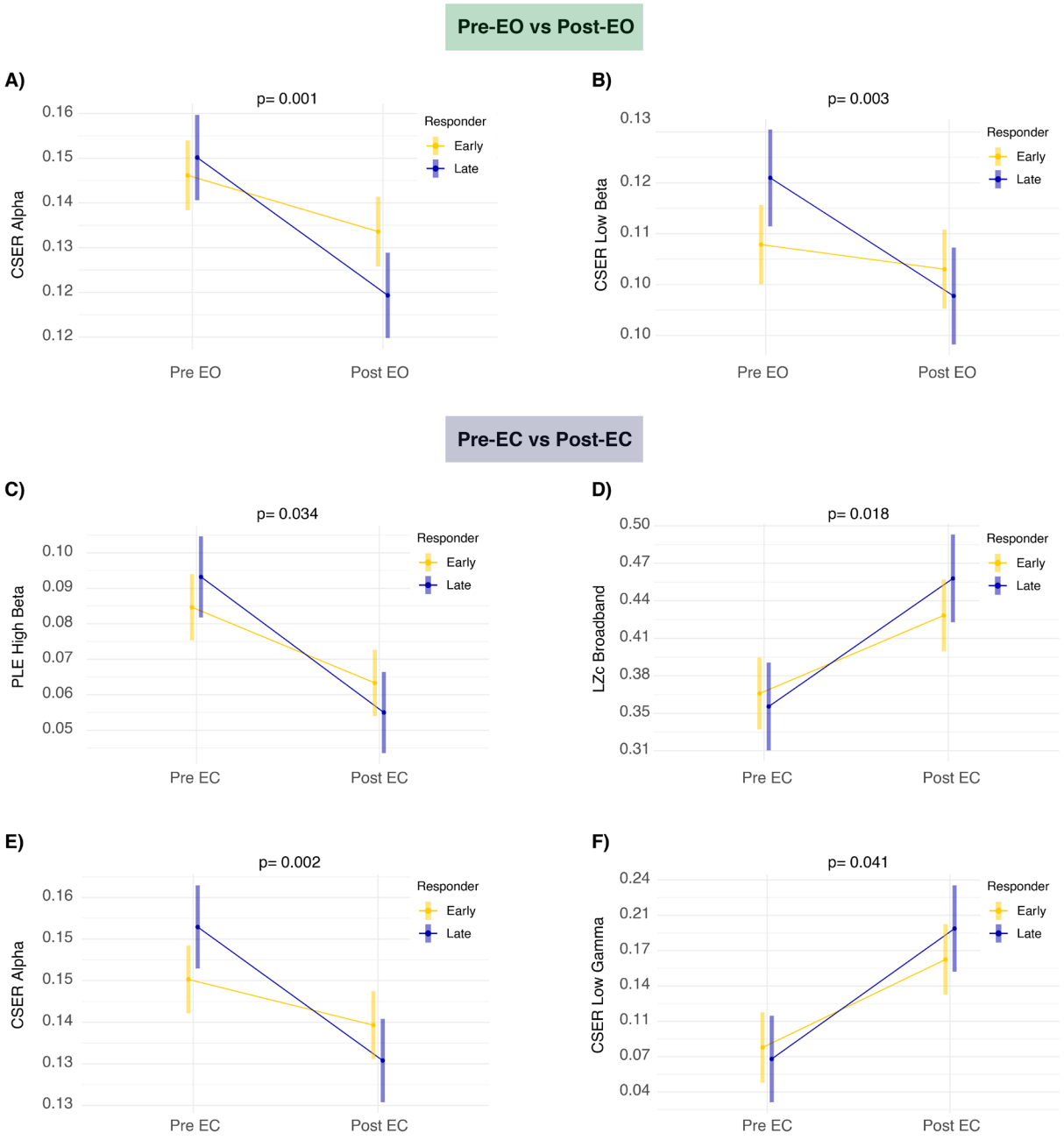

Figure 10: The difference in EEG metrics of ketamine action between early and late responders. A) The difference in CSER decrease within the alpha band in the eyes open condition. B) The difference in CSER decrease within the low beta band in the eyes open condition. C) The difference in PLE_{beta} reduction in the eyes closed condition. D) The difference in normalized Lempel–Ziv complexity (LZc) increase in the eyes closed condition. E) The difference in CSER decrease within the alpha band in the eyes closed condition. F) The difference in CSER increase within the low gamma band in the eyes closed condition. Significance levels for the interaction effects where considered with $a < 0.05$. All p values are corrected for ketamine dose.

4.5. Discussion

The EEG changes induced by a single administration of a subanaesthetic dose of ketamine were investigated in a cohort of hospitalized patients with TR-BP undergoing treatment for depression. At the time of the ketamine infusion with concurrent EEG measurement, all patients had manifested a clinical response to the repeated infusion treatment protocol. The chosen ketamine dose for acute EEG recording fell within the known range associated with clinically meaningful subjective effects, as corroborated by scores on the CADSS. The analysis focused on rhythmic and arrhythmic features of the resting-state EEG signal before and immediately after one of the infusions of ketamine envisaged by the treatment protocol

and related those metrics with the subjective experience induced by the drug. Additionally, the neurophysiological changes induced by ketamine were compared between patients who responded within one week of treatment and those who responded later.

The analysis revealed an intricate pattern of EEG changes induced by ketamine across various rhythmic and arrhythmic signal features. Most of the observed EEG changes in spectral power and its oscillatory and spectral components (shown in Figure 7) as well as measures of scale-free activity and entropy (shown in Figure 8) were distributed across the scalp. In fact, the use of cluster-based permutation as test statistics without the selection of a-priori regions of interest is not informative about spatially specific effects [404]. Therefore, the presented results and subsequent interpretation are to be understood as generalized across EEG channels.

Ketamine elicited a broad reduction in spectral power, particularly evident in the delta, theta, alpha, and beta bands, irrespective of whether patients had their eyes open or closed. This observation aligns with findings from prior studies employing similar dosing administration protocols in both healthy individuals and patients with depression [221,358–362,371–373]. Analysis of the oscillatory component of the EEG spectra revealed that ketamine predominantly diminished low-frequency oscillatory activity in the theta, alpha, and low beta bands, while concurrently increasing activity in the low gamma frequency band. This elevated oscillatory power in the high-frequency band is consistent with previously documented observations in both pre-clinical and human studies (for a review see [349]). These outcomes reinforce existing evidence regarding the ketamine-induced effects of low-frequency desynchronization and increased high-frequency activity.

The data presented herein align with the "disinhibition" model of ketamine's acute mechanism of action, as discussed in the previous chapter. At subanaesthetic doses, ketamine is postulated to diminish low-frequency synchronous activity by selectively inhibiting GluN2D-containing NMDA receptors expressed on GABA inhibitory interneurons. This inhibition results in the interruption of local cortical circuit firing due to the disinhibition of cortical pyramidal neurons. Consequently, there is a desynchronization of slow rhythmic activities, such as delta, theta, alpha, and low beta, coupled with an overall increase in local activity levels due to glutamate release, determining an elevation of gamma frequency [185]. Ketamine's impact on NMDA receptors expressed on fast-spiking interneurons is proposed to disrupt the regulatory feedback of the PING mechanism to generate the increase in tonic gamma activity. This is substantiated by evidence demonstrating that NMDA receptor antagonists enhance gamma activity across various brain regions [405]. Pharmacological restoration of GABAergic input to pyramidal neurons disrupts the ketamine-induced increase in cortical excitation and dysfunction of NMDA receptors expressed on inhibitory interneurons enhances baseline cortical gamma rhythms, as shown in-vivo and in-silico [356,406,407]. Consequently, alterations in gamma rhythms are posited to result from a shift in the cortical excitatory/inhibitory balance toward higher excitability, serving as a crucial index of cognitive and emotional functioning within the brain.

Several studies indicates that brain oscillations may serve as a potential biomarker for depression and the antidepressant effects of ketamine [408]. In a large-scale investigation conducted by Grin-Yatsenko et al. (2010), patients exhibited increased activity in theta, alpha, and beta bands compared to healthy subjects, suggesting a potential role for the modulation of slow-wave activity in the antidepressant action of ketamine [409]. This result was confirmed by later studies, even though some discrepancies exists in the literature [408]. Nonetheless, the result is intriguing in light of neuroimaging finding showing ketamine-induced decreases in low frequency activity and increases in high frequency activity in brain networks associated to depressive symptomatology, such as the DMN [226]. Recently, gamma rhythms have received considerable attention as potential biomarkers of depression and antidepressant action, as reviewed by Fitzgerald and Watson (2018) [410]. The authors also summarised evidence showing that gamma rhythms in individuals with unipolar depression are distinct (generally higher) from those found in bipolar depression. While mixed findings exist due to methodological heterogeneity and standardization challenges in the gamma frequency range, the notion of an optimal amount of gamma corresponding to stable mood states has been proposed. In this perspective, one possible mechanism of action of ketamine would involve the modulation of gamma

rhythms. However, caution is warranted in interpreting data from non-invasive EEG measures of gamma oscillations in humans due to the inherent difficulty in achieving reliable measurements [411]. To summarise, the obtained results regarding the effects of ketamine on rhythmic brain activity align largely with existing literature and extend these findings to a cohort TR-BP in a real-world hospital setting.

The modulation of oscillatory patterns of activity was accompanied by a reduction in fractal (scale-free) arrhythmic activity, particularly in the low the high frequency transition point. While the exact significance of the power of scale-free activity remains uncertain, the functional importance of the slope of the 1/f distribution has been proposed [348]. Ketamine produced a "flattening" of the slope of the power spectra, evident in both eyes open and closed conditions. This effect is consistent to what was reported in healthy subjects by the only available study having quantified the PLE with ketamine [387]. With the present experiment, the finding of Muthukumaraswamy and Liley have been extend to a patient population with TR-BP. In particular, the flattening of the slope was observed in the high frequency band (20 to 80 Hz) and was predominately localized in the high beta frequency band. The specificity of the effect above the "knee" frequency of 20 Hz in the data is in striking accordance with the modelling work of Gao et al. (2017) [412]. They demonstrated that the slope of the 1/f distribution of the fractal component of a spectrum, simulated by an excitatory and an inhibitory neural population, correlates with the excitatory/inhibitory ratio for frequencies above 20 Hz. Decreasing the excitatory/inhibitory ratio steepens the slope of the power spectra, as evidenced by invasive EEG recordings in macaques during propofol sedation [412]. Therefore, there is an intriguing possibility that the observed changes in the slope of the scale-free component of the power spectra are influenced by the stimulation of glutamatergic activity by ketamine, connecting rhythmic (i.e., gamma) and arrhythmic effects. While further research is necessary to substantiate this conclusion, the results of the present study support the "excitatory/inhibitory imbalance" hypothesis of depression and suggest a potential role for the scale-free properties of EEG as biomarkers of mood disorders and ketamine antidepressant effect.

The analysis of the temporal non-linear properties of the EEG signal revealed that ketamine induced a widespread increase in the entropy of brain activity. This effect was consistent for both eyes open and closed conditions and aligned with observations in healthy subjects and a cohort of late-life patients with depression [389,394,394,395,397]. Additionally, a novel measure of informational complexity was applied to achieve spectral decomposition of signal entropy [402]. The analysis indicated that the increase in broadband entropy induced by ketamine was driven by changes within the high frequencies, such as high beta and low and high gamma, while signal entropy tended to decrease in the low frequencies, particularly in alpha and low beta (despite an increase in delta). The observed increase in gamma and reduction in alpha is akin to the findings reported by Mediano et al. (2023) in the validation of the method on healthy individuals following acute administration of classic psychedelics [402]. The present study represents the first application of the novel estimator CSER to investigate the effects of ketamine on spectrally decomposed neural entropy. While little is known about the neurophysiological significance of complexity measures of circuit neural activity, alterations of such metrics has been reported in several neuropsychiatric conditions (for a review see Lau 2022 [413]). Modulations of brain signal entropy following exposure to ketamine and classic psychedelics have been proposed to underlie the "richness" of the content of conscious experience, indicating a state of higher meta-stability and cognitive flexibility, potentially providing a mechanism for the antidepressive effects of these compounds [393]. Previous studies have reported overall lower values of complexity in the EEG signals of individuals with depression compared to healthy controls, suggesting a link with depressive symptoms, such as rumination and a tendency to fixate on negative emotional states, both associated with lower EEG complexity [408]. However, a causal link between the acute state of complexity induced by ketamine and classic psychedelics and therapeutic response remains uncertain. Moreover, some studies report an opposite trend, with positive relationships between the severity of depressive symptoms and various EEG complexity indexes. For instance, a study found that depressed patients had higher baseline LZc compared to controls, and antidepressant treatment normalized brain complexity [414]. Discrepancies in the literature may arise from the heterogeneity of complexity measures used and the high state-dependency of these metrics. The present results support the proposition of

increased neural entropy as a mechanism of ketamine action, and future comparative research should address differences in this mechanism with conventional antidepressants.

The analysis of the interplay between rhythmic and arrhythmic components of the EEG signal revealed that the reduction in broadband oscillatory power (predominantly low-frequency) and the flattening of the slope of the fractal component of the spectra induced by ketamine were both associated with the increase in signal entropy. Those parameters were also modulated by ketamine dosage, providing evidence for a coherent neurophysiological profile of ketamine action. The frequency-specific investigation of the EEG signal revealed an intricate relationship between EEG metrics. The modulation of complexity and oscillatory power demonstrated concordant changes in the alpha, low beta, and gamma frequencies. Specifically, the reduction in oscillatory activity in alpha and low beta corresponded to a decrease in signal entropy within those frequencies, while increases in low gamma oscillatory activity were associated with heightened entropy. Given that entropy measures the unpredictability of the neural signal, an increase in its value with increases in high-frequency activity is perhaps to be expected. Conversely, the reduction of entropy alongside diminished slow-wave synchronous activity is noteworthy. However, in the obtained data, the reduction in complexity and oscillatory power was statistically significantly correlated only in the low beta, but not in alpha. Further research is necessary to elucidate the neurophysiological significance of these finding. Additionally, for frequencies such as delta, theta, high beta, and gamma, no linear relationship was discerned between oscillatory activity and signal entropy.

Regarding the relationship between oscillatory activity and the 1/f distribution of the spectra, interesting associations were identified specifically within alpha and low gamma activity. The correlation between alpha oscillatory power and the 1/f properties of the spectra supports the model proposed by Muthukumaraswamy and Liley, suggesting that the dampening of the alpha oscillator is a fundamental determinant of changes in the slope of the fractal distribution of power spectra [387]. The association between changes in PLE and low gamma oscillatory power also implies the involvement of high-frequency oscillators in modulating the slope of the fractal component of the spectra. However, the effect in gamma was smaller in magnitude compared to alpha and not consistent across conditions. Nevertheless, this result suggests that alterations in the excitatory/inhibitory balance, influenced by the glutamatergic action of ketamine, may represent a common mechanism underlying high-frequency rhythmic activity and the arrhythmic properties of the spectra. Notably, the reduction in brain entropy within alpha and the increase in gamma frequencies were the only ones associated with changes in the slope of the 1/f distribution, an association also observed at the broadband level. This relationship between signal entropy and PLE reductions introduces a novel possible effect of ketamine manifested in the non-linear dynamics of the signal. Specifically, since the power spectrum is equivalent to the FFT of the autocovariance function (as per the "Wiener-Khinchin theorem"), a reduced PLE indicates shorter/weaker autocorrelation in the time domain. According to He's proposition (2014), the reduction of temporal autocorrelation (i.e., redundancy) underlies higher online information processing. This finding might have important implications for the "entropic brain" hypothesis, providing a putative link with the "excitatory/inhibitory imbalance" hypothesis that warrants for further investigation [415].

Of note, a differential effect of ketamine was observed within the beta frequency, with low beta and high beta exhibiting markedly different EEG profiles. The intersection between low and high beta was the "knee" frequency of the fractal component of the spectra in the data, and the change in PLE was observed in the high beta frequency, suggesting an important role for the beta frequency in the overall neurophysiological effects of ketamine. Additionally, the differential behaviour within the beta frequency may help explain some of the inconsistencies in the literature regarding the effects of ketamine within this frequency band.

Taken together, the observed EEG effects of ketamine, including low-frequency desynchronization, increased gamma oscillatory activity, reduction of the slope of scale-free activity, and high-frequency increases in brain entropy, collectively indicate a global shift in brain activity toward a more autonomous and unpredictable state. There is suggestive evidence that these changes may be manifestations of an altered

excitatory/inhibitory balance induced by the glutamatergic action of ketamine. Such alterations could contribute to the disintegration and desegregation of higher-order functional networks, potentially underpinning the altered state of consciousness produced by subanaesthetic doses of ketamine. In this regard, the analysis of the relationship between modulation of EEG and reported experiences of dissociation induced by ketamine yielded unexpected results. The overall reduction in spectral power and its oscillatory component were predominantly positively correlated with scores on the CADSS, suggesting that a smaller magnitude of EEG effects of ketamine is associated with a higher intensity of subjective experience. However, these effects were not robust against correction for multiple comparisons. Additionally, no significant associations were found with arrhythmic components of the EEG. Limitations of the study might help to elucidate these findings. The study utilized the CADSS as a measure of the subjective effects of ketamine, chosen for its widespread use in similar studies, but the scale has several limitations. Inconsistent results have been reported when employing the CADSS to study the subjective effects of ketamine. In particular, comparisons of the CADSS with qualitative reports of the subjective experience induced by ketamine have indicated that the scale fails to capture important themes of the experience, and low scores on the CADSS were often associated with reports of clinically significant drug effects [337]. These limitations may stem from the fact that the CADSS was originally developed to capture symptoms of dissociation in conditions such as dissociative disorders and trauma [340], possibly making it unsuitable for capturing the transitory and psychedelic-like alteration of consciousness induced by ketamine. The unique nature of the ketamine subjective experience requires a more specific scale, which is currently lacking. Future research into ketamine action should also consider adopting the neurophenomenological approach to altered states of consciousness proposed by Timmerman et al. (2022), aimed at extracting fine-graded and specific properties of drug-induced experiences [416]. However, the application of thorough, yet time-consuming, methods for investigating subjective experience might be challenging in real-world, hospitalized settings. Finally, the present study employed a non-validated translation of the scale, introducing additional bias.

One of the most intriguing findings of the current study pertains to the divergence in neurophysiological responses to ketamine intervention between patients who exhibited a response after one week of treatment and those who responded later. Specifically, patients with a delayed response to repeated ketamine administration displayed a greater magnitude of change in EEG features following ketamine exposure compared to patients with an early response. Importantly, these effects were not attributable to ketamine dosage, nor were they determined by differences in neurophysiological markers or the severity of depression before drug administration. This distinctive response suggests that individual differences in the pathological endophenotype of patients may dictate a specific sensitivity to drug effects. Notably, the clinical effect observed in this investigation was specific to arrhythmic properties of the EEG signal, underscoring the functional relevance of these metrics. In particular, the major effects were detected for the modulation of the PLE and broadband, alpha, and low gamma complexity measures. Based on what discussed above, this suggest that the differences in response to ketamine between early and late responders might be due to a different response to shifts in excitatory/inhibitory balance. Although speculative, these conclusion may stimulate future research with important implications for personalized and precision psychiatry.

It is crucial to acknowledge the naturalistic nature of the study, which brings both strengths and weaknesses. On one hand, the study offers novel insights into the neurophysiological effects of ketamine in TR-BP in a real-world clinical setting. On the other hand, differences in the number of previous exposures to ketamine before the EEG recording, along with the concurrent and heterogeneous poly-pharmacological treatment of the patients, limit the generalizability of the results. Additionally, the relatively limited sample size and the absence of controls represent additional limitations of the study. Nevertheless, the replication of many previous findings in standardized and controlled settings underscores the high robustness of the adopted naturalistic approach.

In conclusion, the study provides comprehensive insights into the acute neurophysiological effects of ketamine in patients with TR-BP. The observed EEG changes across multiple rhythmic and arrhythmic components of the neurophysiological signal highlight the potential utility of EEG as a valuable tool for

assessing and monitor the neurobiological effects of ketamine in real-world, ecologically valid, clinical settings. Moreover, the differential responses between early and late responders promote the importance of considering individual differences in treatment outcomes and their neural correlates.

5. Chapter 5: Detecting synaptogenesis induced by ketamine in healthy subjects

In the following chapter, the results of a study measuring the subacute neuroplastic effects of a single subanaesthetic dose of ketamine in healthy volunteers will be presented. The study employed the PET tracer [^{11}C]-UCBJ, aimed at measuring the effects of ketamine on structural neuroplasticity 1 to 8 days following drug administration.

5.1. Introduction

Clinical trials on ketamine antidepressant effects have shown that the therapeutic action of the drug surface within a few hours and last for up to a week after drug exposure, with repeated exposures extending the duration significantly [179]. The long lasting effects of ketamine on mood are accompanied by persistent functional and structural changes in brain activity, as reviewed in Chapter 3. The presence of neuropsychological effects beyond the presence of the drug in the organism posit the question on what mechanism might sustain such enduring post-acute effects [237–239]. Reduced neuroplasticity within mood-regulating brain regions, like the PFC and the limbic system, has been linked with various neuropsychiatric disorders, including depression. Animal models and post-mortem clinical research showed an association between the severity of symptoms of depression and neural atrophy [243–245]. Thus, modern theories of depression propose reductions in neural plasticity as the root basis of depressive symptomatology, a "neural atrophy" hypothesis of depression [417].

Recent models suggest that ketamine's ability to enhance structural neuroplasticity and stimulate the development of new synapses its crucial for the instantiation of the therapeutic benefits. Those theories are grounded in pre-clinical research, which has provided compelling evidence regarding ketamine's role in promoting structural neuroplasticity in association with improvements in symptoms of mood disorders (see Chapter 3). Despite substantial animal work suggesting that ketamine's rapid and enduring antidepressant effects arise from increased synaptic density, the translation of such findings to the living human brain remains challenging. A common approach to measure structural modifications non-invasively in humans is through structural MRI and diffusion tensor imaging (DTI) (see Chapter 1). With structural MRI it was shown that ketamine reduces the volume of the left NAc but increases left hippocampal volume in depressed patients 24 hours post-dose. However, the results were only significant for patients achieving remission [418]. Repeated injections of subanaesthetic doses of ketamine resulted in an increase in MRI volumes of the right hippocampus and left amygdala in MDD patients 24 hours after the last administration. However, at baseline, the volume in those regions was smaller compared to healthy individuals [419]. Another study found that repeated administration of oral ketamine tablets resulted in a bilateral volume increase in the putamen, thalamus, caudate, NAc, and the periaqueductal grey in patients with chronic suicidality [420]. Using tensor-based morphometry, it was found that a single dose of ketamine increased volume in several brain regions in MDD patients, but not in healthy controls. Within those regions, the inferior frontal gyrus was found to be smaller in MDD compared with controls at baseline, and 24 hours after ketamine administration this abnormality was normalized, in association with antidepressant effects. However, another study found that the volume of the left lateral orbitofrontal cortex was significantly reduced in MDD after ketamine administration [421]. Using DTI to quantify increases in synaptic density via decreases in mean diffusivity within the grey matter, a study found that depressed patients treated with a subanaesthetic dose of ketamine showed no significant main effect on mean diffusivity 24 hours post-infusion. Relative reductions in mean diffusivity in the left Brodmann area 10 and left amygdala, representing putative increased plasticity in these regions, predicted greater improvements in depression scores in patients receiving ketamine. However, in the hippocampus, the results were in the opposite direction, with higher mean diffusivity predicting greater improvement in depression symptomatology [417].

Advancements in PET techniques have only recently allowed for its use for the in vivo quantification of synaptic density in the living human brain. This was achieved with the human validation of the novel radioligand [^{11}C]-UCBJ, a ligand binding to the synaptic vesicle protein 2A (SV2A), ubiquitously located in pre-synaptic vesicles [81]. The concentration of SV2A is believed to reflect the density of synapses in a given region of the brain. Compared to previous tracers, [^{11}C]-UCBJ offers superior selectivity and binding potential, enabling more accurate in vivo measurements of synaptic density [422]. Prior work utilizing this tracer has reported synaptic alterations in various neuropsychiatric conditions, including Alzheimer's

disease (AD) and schizophrenia [423,424]. In mood disorders, Holmes and colleagues (2019) demonstrated reduced synaptic density in the PFC and limbic cortices of patients with depression compared to healthy controls, and a negative correlation between severity of depressive symptoms and synaptic density [425]. In a subsequent study, they examined the change in synaptic density induced by a single subanaesthetic 0.5 mg/kg dose of ketamine in depressed patients, patients with trauma, and healthy subjects. 1 day after ketamine administration, no significant changes in the concentration of SV2A were detected in all groups. Interestingly, a post-hoc exploratory analysis showed that ketamine produced a significant increase in SV2A density only in those depressed patients showing the lowest SV2A density at baseline [426].

While suggestive of the presence of an effect, the results obtained so far on the neuroplastic modifications induced by ketamine are not robust. Further, most of the available literature assessed modulation of neuroplasticity at 1 or 2 days after drug administration. Since ketamine's antidepressant effect has been found to endure up to a week after a single administration, investigating the time-frame of the modulation of neuroplasticity at multiple time points is of outmost importance the unravel the neural basis of its mechanism of action.

5.2. Study aim

In the present study, the potential of the PET tracer [^{11}C]-UCBJ to quantify modulations in SV2A protein levels following the administration of a single subanaesthetic dose of ketamine was investigated in a cohort of healthy human volunteers at multiple time points. Building upon previous works from Holmes and colleagues [426], the aim was to better characterize the potential of [^{11}C]-UCBJ as a marker for the neuroplasticity-promoting effects of ketamine in healthy human subjects using a higher dosage (i.e., 1 mg/kg vs 0.5 mg/kg in the Holmes study) and measuring changes in [^{11}C]-UCBJ at 1 to 8 days following ketamine administration. Further, the association between structural brain changes with acute and subacute psychological effects of ketamine was assessed.

5.3. Materials and methods

5.3.1. Participant recruitment and screening

A total of 11 healthy male participants (Age 32 ± 10 years) were screened and included in the study. Participant's details are listed in Table 4. The inclusion criteria included: participants needed to be between 20 and 60 years of age, exhibit no physical or psychiatric medical conditions, and have no history or current incidence of substance abuse. Consumption of ketamine or classic psychedelics in the 6 months preceding the beginning of the study was a ground for exclusion. This was due to recent evidence suggesting a common mechanism of action of ketamine and classic psychedelics in modulating synaptic plasticity and avoiding carry-over effects from previous substance exposure [241]. Of the total sample, none of the participants were naïve to classic psychedelics, while N = 7 participants were ketamine naïve. Additionally, participants were required to abstain from alcohol and illicit substance intake for at least 1 week before and throughout the study period. Participants were screened for contraindications to MRI or research PET scanning. Ethical approval was granted by the Brent Research Ethics Committee, London, UK.

All participants (N = 11)		
Age	32 ± 10	
Psychedelic naive	N = 0	
Last psychedelic use (years ago)	7 ± 10	
Ketamine naive	N = 7	
Last ketamine use (years ago)	8 ± 5 (N = 4)	
Ketamine administered	78 ± 13 mg	
CADSS Total	16 ± 5	
BPRS	11 ± 7	
MOAA/S	19 ± 2	
	Scan 1 ([11C]-UCBJ)	**Scan 2 ([11C]-UCBJ)**
Injected dose (MBq)	231.47 ± 45.32	190.57 ± 70.14
Injected mass (ng/kg)	3.95 ± 1.55	3.57 ± 2.08

Table 4: *Demographic, drug, and radiotracer characteristics for all participants.*

5.3.2. Ketamine administration

Racemic ketamine (1:1 mixture of both enantiomers) was obtained from the St Charles Centre for Health & Wellbeing Pharmacy and administered intravenously by constant infusion over 40 minutes at a dose of 1 mg/kg. This dosage is subanaesthetic and produces a psychedelic-like experience often described as dissociative, and has confirmed anti-depressant effects [427]. The amount of ketamine administered was similar across participants, and all participant experienced the dissociative effects of the drug (Table 4). Vital signs (blood pressure and heart rate) were obtained before, during, and after ketamine infusion. The psychological safety of participants during the acute effects of ketamine was assessed through the Brief Psychiatric Rating Scale (BPRS) [428] and the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale [429], administered by the study physician following drug administration.

5.3.3. Study design

Each participant underwent 2 PET scans, where [11C]-UCBJ was administered intravenously for the quantification of brain SV2A. Participants had their first scan (i.e., Scan 1) approximately two weeks before the ketamine infusion. Of the 11 participants, a subset of N = 4 participants underwent their post-ketamine scan (i.e., Scan 2) 1 day after ketamine infusion. One participant had the scan 2 days after ketamine infusion due to tracer production failure 1-day post-ketamine administration. Participants who had their second scan either at 1 or 2 days after ketamine are referred to as the Day 1-2 group (N = 5). A subset of N = 4 participants had their Scan 2 at 7 days after ketamine infusion. Two participants had their Scan 2 at 8 days after ketamine infusion due to tracer production failure at 7 days post-ketamine administration. Those participants are referred to as the Day 7-8 group (N = 6).

5.3.4. Psychometric measures

At baseline, the following psychometric questionnaire were administered to assess the psychological profile of participants before ketamine exposure: the BDI [100], the State-Trait Anxiety Inventory for Adults Y-2 (STAI Y-2) [102], the Profile of Mood States Short Form (POMS) [101], the WEMWBS [104], the Modified Tellegen Absorption Questionnaire (MODTAS) [430], the Multidimensional Psychological Flexibility Inventory (MPFI) [431], the Five Facets of Mindfulness Questionnaire (FFMQ) [432], the Brief Experiential Avoidance Questionnaire (BEAQ) [433], and the WCS [97]. On day 1 following ketamine, the following

questionnaires were administered: the POMS, the STAY Y-2, the BEAQ, and the PIS. On day 7 following ketamine, the following questionnaires were administered: the POMS and the WEMWBS. At week 4, all questionnaires measured at baseline were administered again. Collectively, those metrics will be referred to as subacute measures. Immediately after exposure to ketamine, participants were asked to fill the following questionnaires to assess the intensity and nature of the acute subjective experience induced by the drug: the 5-Dimensional ASCQ (5D-ASCQ) [343], the Mystical Experience Questionnaire (MEQ) [434], the Challenging Experiences Questionnaire (CEQ) [435], the Psychotomimetic States Inventory (PSI) [436], the Emotional Breakthrough Inventory (EBI) [437], the Ego Dissolution Inventory (EDI) [438]. The 6-item version of the CADSS [439] was administered by the study physician. Collectively, those metrics will be referred to as acute measures.

5.3.5. PET data acquisition and analysis

The PET data were acquired using an integrated General Electric Signa 3 Tesla combined PET/MRI scanner with a 32-channel head coil at the Invicro Imaging Centre, London, UK. The [^{11}C]-UCBJ tracer was synthesized onsite and administered i.v. as a bolus over 20 seconds by the study physician. The PET scan acquisition time was 90 minutes in addition to 30 minutes of MRI scanning. The PET data were processed with the following steps. Continuous blood data were acquired through arterial cannulation and calibrated to align with overlapping discrete whole blood samples, forming a comprehensive whole blood activity curve that spanned the 90-minutes scan duration. Activity measurements from the discrete plasma samples were juxtaposed with the corresponding whole blood data, forming the plasma-over-blood (POB) data. This was then fit to a constant model, enabling interpolation of the relationship. The POB model fit curve was multiplied by the whole blood curve, culminating in an estimated total plasma curve. Plasma samples underwent HPLC analysis to determine the parent fraction, which was subsequently fit to a sigmoidal model. The parent plasma input function, derived by multiplying the ensuing parent fraction profile with the total plasma curve, was further refined by smoothing it post-peak using a multi-exponential fit. An incorporated time delay was adjusted for in the kinetic modelling. All image data were analysed using Invicro London in-house PET data quantification tool, MIAKATTM. MIAKATTM is implemented using Matlab and makes use of SPM12 functions for image segmentation and registration. Each participant's MRI image underwent grey matter segmentation and was then registered to an anatomical template image in MNI152. Dynamic PET images, registered to the MRI scans of participants, were corrected for any motion. An automated definition of ROIs was performed on the MNI152 space based on the CIC atlas [105]. ROIs, defined on the MRI images, were applied to the dynamic PET data to extract regional time-activity curves. The study employed the 1 tissue compartment model for reversible binding to correlate the parent plasma input function with tissue time-activity curves, producing estimates of the total VT for each predefined ROI. Pre-defined ROIs were the dorsolateral PFC, ventromedial PFC, ACC, hippocampus, and amygdala. Those ROIs were extracted from the CIC atlas and defined as follows: dlPFC included the anterior and posterior dlPFC, the vmPFC included anterior and posterior vmPFC, the ACC included the ventral cingulate subcallosal gyrus, the anterior cingulate gyrus, and the dorsal anterior cingulate grey matter. The amygdala and the hippocampus were taken directly from the CIC atlas. The selection of the ROIs was based on previously published work on [^{11}C]-UCBJ VT changes induced by ketamine [426]. The ROIs-specific VTs, corrected for subregional volume, were the primary outcome measures of the study. Additional PET metrics were the binding potential (BP) and the free-fraction corrected [^{11}C]UCB-J (FP). The FP is obtained by dividing the unspecific binding of [^{11}C]UCB-J in the plasma from the ROI-specific VTs, as in Equation 3. The BP of each ROI was obtained by dividing the ROI-specific VTs by the VT of the reference region, the centrum semiovale (CS), according to Equation 4.

Equation 3: [11C]UCB-J Free fraction correction

$$FP = \frac{VT\ (ROI)}{VT(Plasma)}$$

Equation 4: [11C]UCB-J Reference region correction

$$BP = \frac{VT\ (ROI)}{VT(CS)} - 1$$

5.3.6. Statistical analysis

The normal distribution of the ROIs [11C]-UCBJ PET data was assessed using the W test. A linear mixed-effect model was used for within-subject analysis measuring changes in plasma free-fraction, VT, BP, and FP measures of [11C]-UCBJ before and after ketamine administration. The VT, BP, or FP of [11C]-UCBJ within each ROIs was the dependent variable, the time-point was the independent variable (i.e., Scan 1 and Scan 2), inserted as a fixed effect, and a random intercept was added for each subject to account for the paired nature of the data. Statistical significance was considered at α values below 0.05, taken from a one-sided distribution. The choice of a one-sided statistics was justified by the a-priori definition of the ROIs. A similar model was used to test for difference in subacute psychometric measures, this time using two-sided test statistics. An interaction term was added to the model to test for the between-subject difference in [11C]-UCBJ metrics change before and after ketamine administration between participants who had their Scan 2 at 1 to 2 days following ketamine and those who had it at 7 to 8 days. For all models, the effect size was estimated using Cohen's d [108]. Also, the confidence of the result was estimated by computing the BF [109]. Pair-wise spearman correlation tests were used to analyse the correlation between [11C]-UCBJ VT, BP, and FP metrics, acute, and subacute psychometric measures. To account for FDR inflation due to multiple comparisons, the p-values resulting from spearman tests were adjusted independently using the Benjamini-Hochberg adjustment [110]. Results of the FDR correction are reported as "p adj. " in the main text.

5.4. Results

5.4.1. Difference in synaptic density before and after ketamine

The difference between the amount of [11C]-UCBJ injected before and after ketamine injection was normally distributed ($W = 0.955$; $p = 0.705$). There was no statistically significant difference in the injected mass of [11C]-UCBJ between the pre and post ketamine (Mean difference: -3.13 ± 53.92 %; ns).

5.4.2. All subjects

With all subjects included in the analysis, no difference in plasma free-fraction was detected following ketamine (Mean difference: 3.70 ± 24.91 %; ns ; Table 5). Also, no differences in [11C]-UCBJ VT (Mean difference: 7.51 ± 31.28 %; ns ; Table 5) and FP (Mean difference: 5.44 ± 39.55 %; ns ; Table 5) within the CS were found between pre- and post-ketamine scans. The distribution of the difference in [11C]-UCBJ VT concentration within the ROIs was checked for normality. Data were normally distributed in all ROIs: the dlPFC ($W = 0.980$; $p = 0.967$), the vmPFC ($W = 0.979$; $p = 0.963$), the ACC ($W = 0.955$; $p = 0.704$), the hippocampus ($W = 0.956$; $p = 0.719$), and the amygdala ($W = 0.909$; $p = 0.238$). The same was true for BP and FP metrics (data not shown). We observed no statistically significant increase in VT of [11C]-UCBJ following ketamine administration in any of the analysed ROIs (Table 5). Notably, there was a trend towards an increase in all ROIs, approaching statistical significance in the amygdala (Mean difference: 6.63 ± 10.94 %; $\beta = 1.10$; $p = 0.055$; $d = 0.528$; $BF = 0.949$) and the ACC (Mean difference: 7.98 ± 13.79 %; $\beta = 1.45$; $p = 0.059$; $d = 0.515$; $BF = 0.902$). Similar results were obtained when the [11C]-UCBJ VT was corrected for plasma free-fraction to obtain FP, and for VT within the reference region CS to obtain BP. However, for FP and BP no trends were observed. All results shown in Table 5.

All participants (N = 11)							
ROIs	[11]-UCBJ Metric	Scan 1 (SD)	Scan 2 (SD)	% Difference (SD)	p-value	Cohen's d	Bayes factor
dlPFC	VT	18.83 (3.50)	20.06 (3.64)	7.72 (15.54)	0.090	0.435	0.677
	FP	77.24 (14.05)	79.10 (12.66)	4.01 (15.74)	0.322	0.144	0.328
	BP	3.63 (1.08)	3.79 (1.15)	11.66 (46.20)	0.349	0.121	0.319
vmPFC	VT	18.63 (3.37)	19.74 (3.51)	7.04 (14.82)	0.100	0.413	0.628
	FP	76.54 (13.97)	78.04 (13.48)	3.42 (15.64)	0.352	0.118	0.318
	BP	3.58 (1.02)	3.72 (1.17)	10.48 (44.96)	0.356	0.114	0.317
ACC	VT	21.42 (4.17)	22.87 (4.04)	7.98 (13.79)	0.059	0.515	0.902
	FP	87.84 (16.44)	90.36 (15.10)	4.43 (15.90)	0.283	0.179	0.346
	BP	4.26 (1.22)	4.47 (1.32)	11.10 (43.14)	0.325	0.141	0.327
Amygdala	VT	18.93 (3.70)	20.03 (3.68)	6.63 (10.94)	0.055	0.528	0.949
	FP	87.84 (16.44)	79.12 (13.83)	3.56 (17.37)	0.357	0.114	0.316
	BP	3.65 (1.08)	3.78 (1.18)	9.94 (43.83)	0.363	0.108	0.315
Hippocampus	VT	16.09 (3.15)	16.97 (3.08)	6.63 (13.67)	0.096	0.422	0.647
	FP	65.96 (12.45)	67.00 (11.32)	3.20 (16.23)	0.379	0.096	0.311
	BP	2.95 (0.93)	3.05 (1.01)	11.05 (47.81)	0.380	0.095	0.310
CS	VT	4.14 (0.54)	4.32 (0.83)	7.51 (31.28)	0.269	0.152	0.332
	FP	17.11 (3.00)	17.39 (4.94)	5.44 (39.55)	0.435	0.049	0.301
Plasma	Free-fraction	0.24 (0.02)	0.26 (0.04)	4.82 (15.16)	0.174	0.298	0.445

Table 5: Changes in [11C]-UCBJ metrics before and after ketamine, all participants (N = 11). The table report the average VT, BP, and FP values within the pre-defined ROIs. The p-values are the result of one-sided linear mixed-effect model with a significance threshold set at 0.05 (see materials and methods). The effect size was computed via Cohen's d test. The statistical confidence of the result was computed via BF analysis.

5.4.3. Day 1-2 versus Day 7-8 groups

The difference in [^{11}C]-UCBJ VT when considering only participants scanned at scanned 1-2 days (Table 6) or 7-8 days (Table 7) after ketamine was not statistically significant in any of the analysed ROIs. The 2 groups also did not show a differences in the magnitude of change of [^{11}C]-UCBJ VT. Participants in the Day 1-2 group showed an increase in [^{11}C]-UCBJ VT within the reference region CS (Mean difference: 18.86 ± 16.72 %; β = 1.05; p < 0.001; d = 1.150; BF = 1.895), while the Day 7-8 group showed no statistically significant differences. The difference between the 2 groups was statistically significant (β = 1.59; p = 0.005; Figure 11 A), such that participants scanned at Day 1-2 after ketamine had bigger change (increase) in VT within the CS as compared to those scanned at Day 7-8. There was no significant change in plasma [^{11}C]-UCBJ free-fraction after ketamine in both Day 1-2 and Day 7-8 groups, and no significant differences between them. No difference in the [^{11}C]-UCBJ FP within the CS was observed in the Day 1-2 group (Table 6) but there was a trend towards a decrease of [^{11}C]-UCBJ FP within the CS in the Day 7-8 group (Mean difference: -15.67 ± 19.83 %; β = -3.02; p = 0.056; d = -0.789; BF = 1.181; Table 7). Further, there was a significant difference in FP change within the CS between groups (β = 7.28; p = 0.032; Figure 11 A). The difference in [^{11}C]-UCBJ FP after ketamine was not significant in the Day 1-2 group or Day 7-8 group in the analysed ROIs and no differences in [^{11}C]-UCBJ FP change were detected between Day 1-2 and Day 7-8 groups. In both groups, there were no statistically significant differences in [^{11}C]-UCBJ BP in any of the analysed ROIs, but a trend towards an increase was observed in the Day 7-8 group specifically (Table 7). Also, the two groups differed in the magnitude and direction of [^{11}C]-UCBJ BP change in the ACC (β =-1.77; p = 0.044; Figure 11 D), amygdala (β =-1.46; p = 0.039; Figure 11 E), and hippocampus (β =-1.27; p = 0.039; Figure 11 F), with a trend in the dlPFC (β =-1.46; p = 0.055; Figure 11 B) and vmPFC (β =-1.40; p = 0.061; Figure 11 C). In particular, [^{11}C]-UCBJ BP tended towards a decrease in the Day 1-2 group and towards an increase in the Day 7-8 group.

Day 1-2 group (N = 5)

ROIs	[^{11}]-UCBJ Metric	Scan 1 (SD)	Scan 2 (SD)	% Difference (SD)	p-value	Cohen's d	Bayes factor
dlPFC	VT	19.62 (4.09)	21.39 (5.04)	9.21 (14.14)	0.117	0.626	0.768
	FP	79.39 (14.86)	83.98 (15.19)	8.12 (17.73)	0.250	0.331	0.493
	BP	4.13 (1.00)	3.49 (1.36)	-16.87 (22.30)	0.087	-0.740	0.934
vmPFC	VT	19.22 (3.90)	20.92 (4.88)	9.05 (13.33)	0.109	0.655	0.806
	FP	77.93 (14.77)	82.58 (17.21)	8.00 (17.589)	0.245	0.340	0.498
	BP	4.02 (0.90)	3.40 (1.35)	-17.07 (22.82)	0.090	-0.726	0.912
ACC	VT	22.22 (5.06)	24.27 (5.50)	9.80 (12.79)	0.095	0.704	0.878
	FP	89.94 (18.76)	95.52 (17.75)	9.06 (19.62)	0.252	0.328	0.491
	BP	4.81 (1.21)	4.10 (1.54)	-15.90 (20.48)	0.083	-0.756	0.962
Amygdala	VT	19.68 (4.68)	21.34 (4.97)	9.05 (9.92)	0.081	0.764	0.975

	FP	79.59 (16.99)	84.02 (16.37)	8.95 (22.58)	0.290	0.269	0.460
	BP	4.15 (1.16)	3.48 (1.38)	-17.07 (20.45)	0.073	-0.807	1.053
Hippocampus	VT	16.78 (3.64)	18.06 (4.00)	8.15 (11.35)	0.111	0.645	0.793
	FP	67.93 (13.19)	71.06 (12.59)	7.51 (19.41)	0.304	0.249	0.450
	BP	3.38 (0.90)	2.79 (1.13)	-19.02 (21.17)	0.058	-0.897	1.233
CS	VT	3.82 (0.33)	4.87 (0.56)	29.71 (28.26)	0.005	1.150	1.895
	FP	15.55 (2.45)	19.81 (5.53)	30.77 (44.14)	0.085	0.747	0.947
Plasma	Free-fraction	0.25 (0.02)	0.26 (0.06)	2.97 (19.05)	0.360	0.172	0.422

Table 6: Changes in [^{11}C]-UCBJ metrics before and 1-2 days after ketamine, Day 1-2 group (N = 5). The table report the average VT, BP, and FP values within the pre-defined ROIs. The p-values are the result of one-sided linear mixed-effect model with a significance threshold set at 0.05 (see materials and methods). The effect size was computed via Cohen's d test. The statistical confidence of the result was computed via BF analysis.

Day 7-8 group (N = 6)							
ROIs	[^{11}]-UCBJ Metric	Scan 1 (SD)	Scan 2 (SD)	% Difference (SD)	p-value	Cohen's d	Bayes factor
dlPFC	VT	18.17 (3.01)	18.95 (1.69)	6.48 (17.86)	0.277	0.259	0.439
	FP	75.45 (10.35)	75.03 (9.60)	0.59 (14.60)	0.470	-0.032	0.374
	BP	3.22 (1.10)	4.04 (1.01)	35.43 (48.78)	0.085	0.655	0.884
vmPFC	VT	18.14 (2.89)	18.76 (1.75)	5.37 (17.02)	0.311	0.214	0.418
	FP	75.37 (10.01)	74.26 (9.43)	-0.40 (14.27)	0.419	-0.087	0.380
	BP	3.21 (1.07)	3.99 (1.03)	33.43 (47.27)	0.091	0.633	0.842
ACC	VT	20.75 (3.50)	21.71 (2.20)	6.46 (15.60)	0.229	0.328	0.482
	FP	86.09 (11.04)	86.07 (12.47)	0.57 (12.58)	0.498	-0.002	0.373
	BP	3.81 (1.21)	4.77 (1.17)	33.59 (45.29)	0.069	0.718	1.011

Amygdala	VT	18.30 (2.79)	18.93 (2.04)	4.62 (12.25)	0.245	0.304	0.466
	FP	76.04 (9.02)	75.04 (11.14)	-0.93 (11.91)	0.408	-0.100	0.383
	BP	3.24 (0.99)	4.04 (1.05)	32.44 (46.60)	0.071	0.713	1.000
Hippocampus	VT	15.51 (2.69)	16.06 (2.01)	5.36 (16.50)	0.292	0.239	0.429
	FP	64.32 (8.65)	63.62 (9.96)	-0.40 (13.82)	0.435	-0.070	0.378
	BP	2.60 (0.91)	3.27 (0.95)	36.10 (50.56)	0.080	0.674	0.919
CS	VT	4.40 (0.51)	3.87 (0.76)	-10.98 (20.36)	0.092	-0.547	0.707
	FP	18.40 (2.55)	15.38 (3.69)	-15.67 (19.83)	0.056	-0.789	1.181
Plasma	Free-fraction	0.24 (0.02)	0.25 (0.02)	6.36 (12.77)	0.139	0.450	0.588

Table 7: Changes in [^{11}C]-UCBJ metrics before and 7-8 days after ketamine, Day 7-8 group (N = 6). The table report the average VT, BP, and FP values within the pre-defined ROIs. The p-values are the result of one-sided linear mixed-effect model with a significance threshold set at 0.05 (see materials and methods). The effect size was computed via Cohen's d test. The statistical confidence of the result was computed via BF analysis.

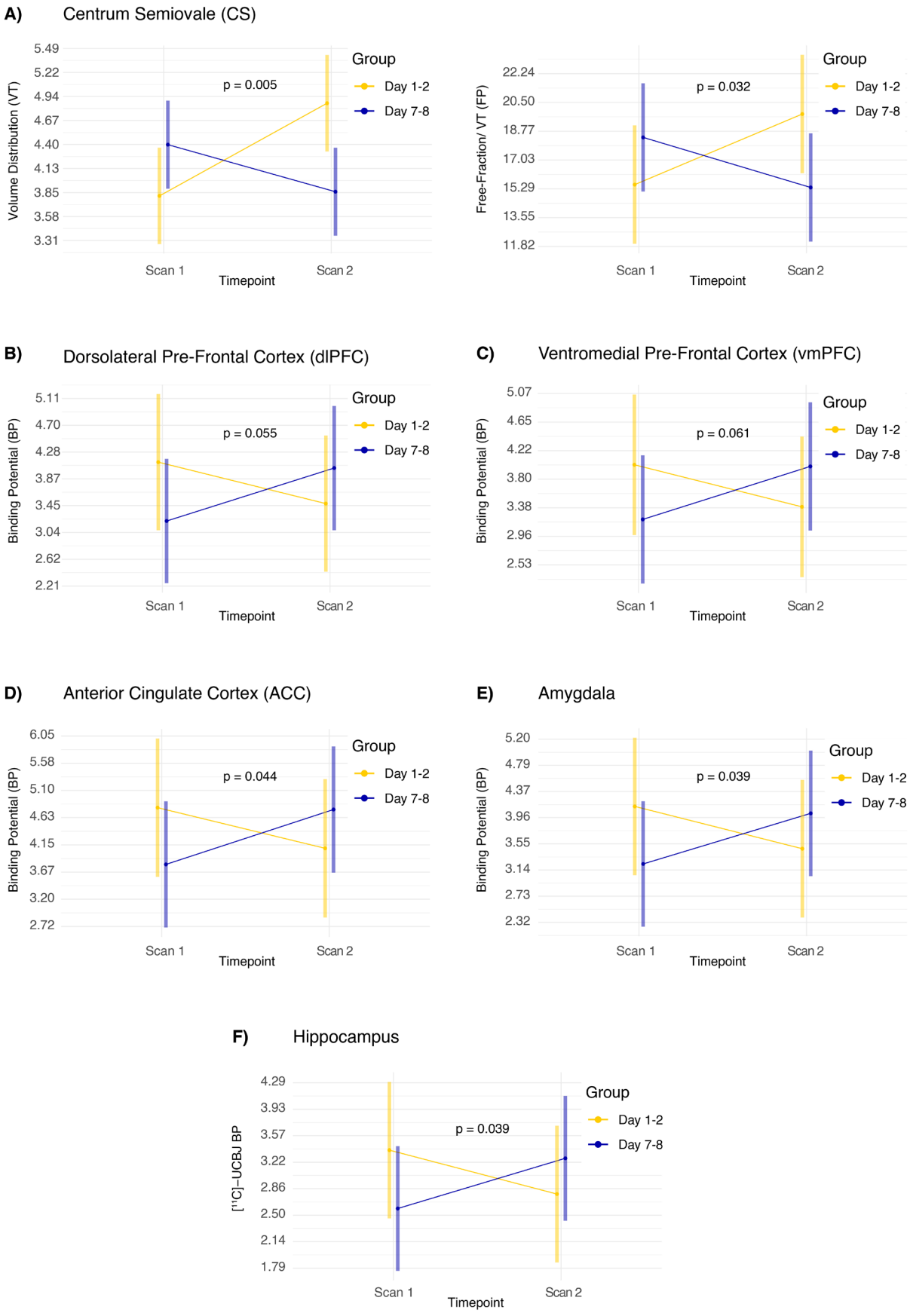

Figure 11: Difference in PET metrics between Day 1-2 and Day 7-8 groups. A) The difference in VT and FP within the reference region CS between groups. B) The difference in BP within the dlPFC between groups. B) The difference in BP within the dlPFC between groups. C) The difference in BP within the vmPFC between groups. D) The difference in BP within the

ACC between groups. E) The difference in BP within the Amygdala between groups. E) The difference in BP within the hippocampus between groups. Significance levels for the interaction effects where defined at a < 0.05.

5.4.4. Correlation between PET metrics

At baseline, the 3 PET metrics (i.e., VT, FP, and BP) were significantly correlated in all analysed ROIs. After ketamine the PET metrics were not significantly correlated, with the exception of a weak correlation between VT and BP in the dlPFC, vmPFC, and ACC, which did not survived correction for multiple comparisons. All results are shown in Table 8.

All participants (N = 11)						
ROIs	Timepoint	[11]-UCBJ Metric 1	[11]-UCBJ Metric 2	R coefficient	p value	p adj.
dlPFC	Scan 1	VT	BP	0.89	< 0.001	0.003
	Scan 1	VT	FP	0.95	< 0.001	<0.001
	Scan 2	VT	BP	0.64	0.040	0.065
	Scan 2	VT	FP	0.5	0.121	0.159
vmPFC	Scan 1	VT	BP	0.86	0.001	0.004
	Scan 1	VT	FP	0.85	0.002	0.004
	Scan 2	VT	BP	0.64	0.040	0.065
	Scan 2	VT	FP	0.45	0.163	0.191
ACC	Scan 1	VT	BP	0.88	0.001	0.003
	Scan 1	VT	FP	0.84	0.003	0.005
	Scan 2	VT	BP	0.64	0.040	0.065
	Scan 2	VT	FP	0.39	0.237	0.248
Amygdala	Scan 1	VT	BP	0.87	0.001	0.003
	Scan 1	VT	FP	0.88	0.001	0.003
	Scan 2	VT	BP	0.59	0.061	0.091
	Scan 2	VT	FP	0.38	0.248	0.248
Hippocampus	Scan 1	VT	BP	0.85	0.002	0.004
	Scan 1	VT	FP	0.92	< 0.001	< 0.001
	Scan 2	VT	BP	0.58	0.066	0.092
	Scan 2	VT	FP	0.48	0.137	0.170

Table 8: Correlations between [11C]-UCBJ metrics at Scan 1 and at Scan 2, all participants (N = 11). The correlation coefficients (R) and p-values results from pair-wise spearman correlations between the PET metrics. The p adj. are the results of FDR correction using the Benjamini-Hochberg adjustment (see materials and methods).

5.4.5. Correlation between PET metrics with acute psychometric measures

A statistically significant correlation was found between the scores on the 5D-ASCQ dimension of oceanic boundlessness and increase in [11C]-UCBJ VT within the hippocampus (R = 0.66; p = 0.031; p adj. = 0.589) and the amygdala (R = 0.65; p = 0.034; p adj. = 0.592), following a similar trend in the other ROIs.

However, the correlations did not survive correction for multiple comparisons and were not observed when using the FP and BP of [11C]-UCBJ. For BP, a positive correlation was found between scores of dissociation on the total CADSS and change in [11C]-UCBJ BP in all analysed ROIs: dlPFC (R = 0.72; p = 0.012; p adj. = 0.312), vmPFC (R = 0.72; p = 0.012; p adj. = 0.312), ACC (R = 0.70; p = 0.018; p adj. = 0.369), amygdala (R = 0.70; p = 0.018; p adj. = 0.369), hippocampus (R = 0.72; p = 0.012; p adj. = 0.312). The same was found for the derealization subscale: dlPFC (R = 0.80; p = 0.003; p adj. = 0.312), vmPFC (R = 0.78; p = 0.005; p.adj. = 0.312), ACC (R = 0.75; p = 0.008; p adj. = 0.312), amygdala (R = 0.75; p = 0.008; p adj. = 0.312), hippocampus (R = 0.78, p = 0.005; p adj. = 0.312). The correlations did not survive correction for multiple comparisons and were not present when using VT and FP of [11C]-UCBJ. All correlations are shown in Figure 12.

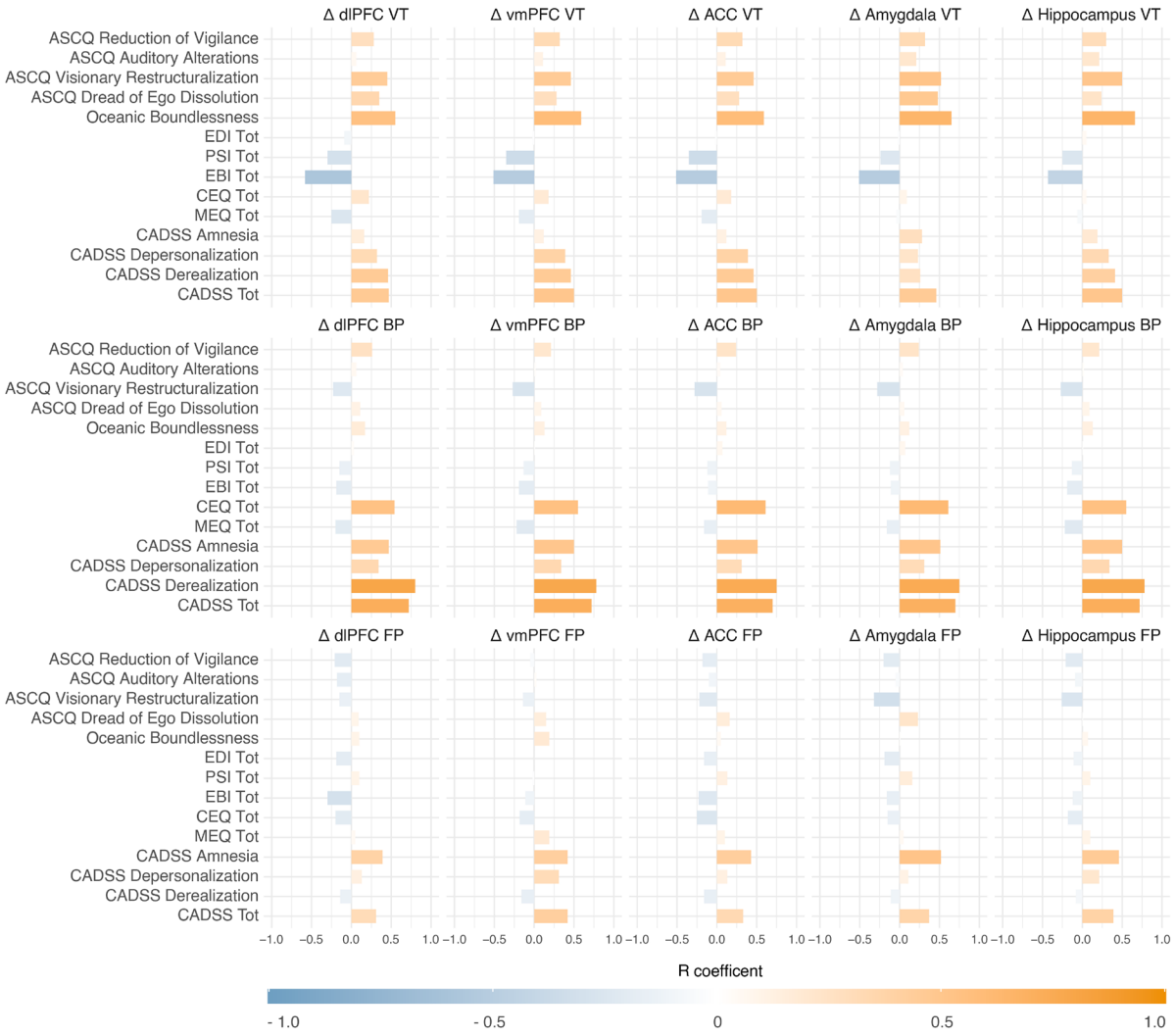

Figure 12: Correlation between PET metrics and Acute psychometric measures. The Spearman correlation coefficient is plotted for each pairwise correlation between [11C]-UCBJ VT, BP, and FP with the acute psychometric measures: the 5-Dimensional Altered States of Consciousness Questionnaire (5D-ASCQ), the Mystical Experience Questionnaire (MEQ), the Challenging Experiences Questionnaire (CEQ), the Psychotomimetic States Inventory (PSI), the Emotional Breakthrough Inventory (EBI), the Ego Dissolution Inventory (EDI). The 6-item version of the Administered Dissociative State Scale (CADSS).

5.4.6. Correlation between PET metrics and subacute psychometric measures

In the total sample, there was a significant reduction in total mood disturbance, as quantified by the POMS, at 1 day (Mean difference: -12.82 ± 24.96 ; β = -12.82; p = 0.033; d = 0.513; BF = 0.898), 7 days (Mean difference: -14.82 ± 19.61; β = -14.82; p = 0.015; d = 0.756 ; BF = 2.459), and 4 weeks (Mean difference:

-13.91 ± 14.82; β = -13.91; p = 0.021; d = 0.939; BF = 5.609) following ketamine treatment. Also, there was a significant increase in well-being, measured via the WEMWBS, at 7 days following ketamine (Mean difference: 5.64 ± 9.30 %; β = 5.636; p = 0.040; d = 0.606; BF = 1.296). There was a trend towards reductions in experiential avoidance facet of the MPFI at 4 weeks after ketamine (Mean difference: - 0.55 ± 0.84 %; β = -0.55; p = 0.052; d = -0.665; BF = 1.659). There was a significant correlation between total CADSS scores and reduction of POMS scores at 1 day (R = -0.63; p = 0.036; p adj. = 0.412) and 4 weeks (R = -0.71; p = 0.014; p adj. = 0.350) following ketamine administration. Also, the CADSS scores of depersonalization were associated with reduction of POMS scores at 7 days (R = -0.7; p = 0.017; p adj. = 0.350) and 4 weeks (R = -0.61; p = 0.047; p adj. = 0.412) following ketamine exposure. There was a statistically significant correlation between reduction in POMS at 4 weeks and increases in [^{11}C]-UCBJ BP in the vmPFC (R = -0.64; p = 0.034; p adj. = 0.295), ACC (R = -0.68; p = 0.022; p adj. = 0.295), Amygdala (R = -0.68; p = 0.022; p adj. = 0.295), and Hippocampus (R = -0.64; p = 0.034; p adj. = 0.295), with a similar trend in the dlPFC (R = -0.59; p = 0.056; p adj. = 0.317). However, none of the above correlations survived correction for multiple comparisons.

An exploratory analysis was performed to explore the moderating effect of acute experience in the relationship between changes in [^{11}C]-UCBJ and mood following ketamine administration. No significant interaction effect was found on CADSS total scores in moderating the relationship between reductions in POMS at 4 weeks and increases in [^{11}C]-UCBJ BP.

5.5. Discussion

In the current study, the PET tracer [^{11}C]-UCBJ was employed to examine the effects of a single subanaesthetic dose of ketamine on the modulation of SV2A protein levels in 11 healthy participants. No statistically significant alterations in synaptic density — indexed via the VT, FP, and BP of [^{11}C]-UCBJ — were observed after ketamine administration in any of the analysed brain regions. A trend towards an increase in VT, especially at day 1-2, and BP, specifically at day 7-8, was observed in all ROIs following ketamine. However, the effect size and the confidence of the result were relatively weak. A notable enhancement in mood was observed after ketamine administration, and this improvement correlated with the level of dissociation induced by the drug. Despite the limited sample size and the participants' healthy status, these findings align with previous evidence in clinical populations [185]. The trends towards an increase of [^{11}C]-UCBJ VT and BP correlated with measures of acute subjective effects (i.e., oceanic boundlessness and dissociation) and subacute changes in mood (i.e., reduction of mood disturbance) induced by ketamine. While potentially interesting in the context of ketamine effects on mood regulation, the observed findings were not robust and, due to the lack of statistical significance, limited sample size, and considerable individual variability, these observations are challenging to interpret conclusively.

Inspection of differences between participants scanned 1 to 2 days following ketamine compared to those scanned 7 to 8 days showed a differential trend in the binding of [^{11}C]-UCBJ in the reference region, which have been shown to have a certain degree of specific binding of the tracer [440]. In particular, participants scanned at 1-2 days after ketamine showed an increase in both total and free-fraction-corrected binding of [^{11}C]-UCBJ in in the reference region, as compared to those scanned at 7-8 days after ketamine. The increase in VT in the reference region at day 1-2 explains the trend towards a reduction in BP of [^{11}C]-UCBJ in all ROIs at that time point. On the contrary, at day 7-8, there was a trend towards a decrease in binding within the CS, resulting in an increase of BP in the analysed ROIs. These discrepancies were also confirmed by the loss of correlation across PET measures following ketamine administration. While no differences were detected in plasma free-fraction of [^{11}C]-UCBJ following ketamine, adjusting the VT for the free-fraction eliminated the trend towards an increase in all ROIs, suggesting that the increase of VT was possibly due to increases in unspecific binding. Together these observations might signify that the pharmacological activity of ketamine impacted the metabolism and binding of the PET tracer in the brain, a possible confounder that warrants for further investigation. In fact, such effect would be a crucial consideration in the design of experiments employing the [^{11}C]-UCBJ tracer to assess the effect of a pharmacological challenge on synaptic density, as also suggested by Holmes et al (2022) [426].

Overall, our findings do not offer support for a notable effect of a single subanaesthetic dose of ketamine on modulating synaptic density in healthy human brains, as indicated by changes in the PET radiotracer [^{11}C]-UCBJ in frontal and limbic regions at 1 to 8 days post-administration. This aligns with the sole published study by Holmes et al., which also provided inconclusive evidence regarding the effect of ketamine on synaptic density, measured with [^{11}C]-UCBJ, in both healthy and depressed individuals 1 day post-drug exposure [426]. The obtained results thus corroborate their findings using a similar methodology, but add important insight into a differential effect of ketamine on [^{11}C]-UCBJ based on the time of scanning following drug administration. Several factors could account for the absence of observable changes in synaptic density post-ketamine administration. As no significant main effects were detected in the study by Holmes et al. with a ketamine dose of 0.5 mg/kg, a doubled dosage was employed in the current study, corresponding to the end of titration dose used in the clinic [427]. However, comparable results with their study were obtained, suggesting that detectable changes in structural neuroplasticity in humans might require a full treatment course to manifest. In support to this observation, a recent study by Johansen et al. (2023), using [^{11}C]-UCBJ to measure neuroplasticity following SSRI treatment, observed that only longer treatment duration was associated with increases in [^{11}C]-UCBJ VT, as compared to shorter exposure to SSRIs [441]. Nonetheless, the small magnitude of the effect sizes reported across investigations suggest that the [^{11}C]-UCBJ tracer might have inherent limitations in detecting changes in SV2A concentration as induced by pharmacological compounds. Thus, the magnitude of the putative increase in synaptic density produced by a single subanaesthetic dose of ketamine might not be sufficient to be detected with the employed method. Also, by using [^{11}C]-UCBJ alone it is not possible to disambiguate if changes in tracer concentration correspond to changes in the number of synaptic vesicles, the expression of SV2A, or synaptic density per se, introducing a mechanistic bias. A significant body of animal research, primarily in rodents, demonstrates ketamine's substantial influence on enhancing structural neuroplasticity [210,260,261,442]. However, most of the available evidence points to a post-synaptic effect of ketamine, such as the increase in the number of dendritic spines, only a fraction of which would then develop into mature synapses. The binding site of [^{11}C]-UCBJ is thought to be the SV2A protein expressed on the vesicles carrying neurotransmitters [422]. Hence, its potential for capturing the post-synaptic effect of ketamine, as widely reported in animal studies, might be constrained since the majority of SV2A protein is expressed in the pre-synaptic compartment. In addition, there is some evidence showing an inhibitory effect of ketamine on neurotransmitter recycling and vesicle trafficking, possibly confounding the use of SV2A as a marker [196,443]. A pharmacological explanation might also account for the lack of results observed within the present investigation. Evidence coming from in-vivo animal work specifically localize the effect of ketamine on the glutamatergic system, showing that the increases in dendritic spines mainly involve excitatory pyramidal neurons within the PFC. Thus, the ubiquitous expression of SV2A across neural phenotypes might represent an additional limiting factor in capturing the effects of ketamine on neuroplasticity [185]. Lastly, there might be significant individual variability in response to ketamine. Previous studies in neuropsychiatric cohorts showed that patients with AD, MDD, and schizophrenia, exhibit diminished synaptic density, as indexed via [^{11}C]-UCBJ, compared to healthy controls [423–425]. However, other studies suggest that modulation of [^{11}C]-UCBJ are evident only for patients exhibiting severe symptomatology. In particular, Onwordi et al. (2023), failed to find a significant alteration of [^{11}C]-UCBJ in patients in the early phase of schizophrenia [444]. Accordingly, in the study by Holmes et al. (2022), the only significant effect of ketamine on synaptic density was observed in an exploratory analysis involving a subset of only the most severely depressed patients [426]. Therefore, the effect of ketamine captured via [^{11}C]-UCBJ might become evident only for those conditions of baseline impaired synaptic density.

In conclusion, elucidating the intricate and highly dynamic phenomenon of synapse formation and remodelling, which is incessantly modulated by various factors in the living brain, such as homeostatic constraints, biological rhythms, and individual variability, remains challenging when employing non-invasive imaging techniques in human subjects. The current investigation sought to both replicate and expand upon prior research examining the influence of the rapid-acting antidepressant, ketamine, on structural neuroplasticity in healthy human subjects. The results do not provide conclusive evidence supporting an augmentation in synaptic density following ketamine administration, assessed at 1 to 8 days

post-administration. Nonetheless, the presence of specific trends and the possible effects of ketamine on PET tracer binding might guide hypothesis and design formulation for future studies.

6. Chapter 6: General Discussion

" The aim of science is to seek the simplest explanations of complex facts. We are apt to fall into the error of thinking that the facts are simple because simplicity is the goal of our quest. The guiding motto in the life of every natural philosopher should be: seek simplicity and distrust it. "

Alfred North Whitehead, Process and Reality, 1929.

Taken together, the findings of the 3 original studies presented in this dissertation demonstrate the effective utilization of state-of-the-art neuroimaging techniques in conjunction with psychoactive pharmacological challenges to elucidate the aetiology of depression. Each study contributes valuable insights that are particularly pertinent to the scientific evaluation of prominent hypotheses regarding the neurobiological underpinnings of depressive disorder and the mechanism of action of the rapid-acting antidepressant ketamine in humans.

In the first study presented, a direct assessment of the "5-HT deficiency" theory of depression was achieved using an amphetamine challenge to probe the state of the 5-HT system imaged through the PET radioligand [^{11}C]Cimbi-36 in patients with depression and healthy subjects. The results yielded compelling evidence indicating a reduced 5-HT release capacity in individuals with depression compared to healthy controls. This pioneering finding provides support for the "5-HT deficiency" theory of depression, offering an evidence-based rationale for the underlying mechanism of action of serotonergic antidepressants, including SSRIs and classic psychedelics. Moreover, the study uncovered an intriguing difference in baseline 5-HT2A receptor availability between depressed patients and control subjects. Within the patient group, the amount of 5-HT2A receptor availability was found to correlate with the psychological trait of connectedness. These results indicate that high 5-HT2A receptor binding could serve as a vulnerability factor for mood disorders, with significant implications for the neurobiology of depressive symptomatology and for medications that target this receptor, such as the classic psychedelics.

In the second study, the acute neurophysiological effects of the novel rapid-acting antidepressant ketamine were investigated using a portable EEG apparatus in a population of hospitalized patients with bipolar depression. The administration of ketamine resulted in notable alterations in brain oscillatory activity, characterized by a pronounced desynchronization of low-frequency oscillations and hyper-synchronization of high-frequency oscillations. Furthermore, ketamine induced a substantial increase of brain signal entropy, particularly driven by high frequencies, along with a specific modulation of the fractal component of the power spectra. The observed changes in rhythmic and arrhythmic components of the EEG signal are suggestive of an underlying shift in cortical excitatory/inhibitory balance. Importantly, the magnitude of the modulation of EEG parameters induced by ketamine, specifically of the arrhythmic components, was found to differentiate between early and late responders to ketamine therapy. These findings provide supportive evidence for the "excitatory/inhibitory imbalance" hypothesis of depression, further strengthening our understanding of the neurobiological underpinnings of this condition. Moreover, the replication of similar results on the effect of ketamine on EEG signal, obtained from previous studies conducted in healthy subjects and medication-free depressed patients in standardized research settings, underscores the robustness of the ecologically-valid design of the study. These finding hold significant implications for the evidence-based monitoring and evaluation of novel psychoactive antidepressants like ketamine and classic psychedelics as they diffuse into medical practice and are implemented in real-world clinical settings.

Contrary to initial expectations, robust evidence for an increase in neuroplasticity was not observed in the third study. However, the presence of a trend, which exhibited correlation with acute and subacute psychological outcomes, warrants attention. Given the considerable body of pre-clinical evidence supporting the neuroplasticity-promoting effects of ketamine (reviewed in Chapter 3), the absence of significant results in the study may be attributed to methodological limitations inherent to the applied technique or dosing regimen. Still, no conclusions can be drawn with regard to the "neural atrophy" hypothesis of depression on the basis of the obtained results. Overall, the findings of the study align with evidence obtained by employing similar methodologies in patients with depression and other neuropsychiatric conditions, suggesting a high degree of heterogeneity within and across individuals to the neuroplasticity-enhancing effects of ketamine. Thus, while the study does not definitively affirm the proposed hypothesis, it underscores the complexity of neurobiological mechanisms underlying depression and highlights the need for further research to elucidate individual variability in response to ketamine and its potential implications for therapeutic interventions.

Altogether, the presented research contributes significantly to the understanding of depression's aetiology and the mechanism of action of the novel antidepressant ketamine. Through a comprehensive exploration of neurobiological theories with innovative neuroimaging methodologies, this body of work has not only validated established hypotheses regarding depression, but has also provided novel insights into the intricated nature of this debilitating condition.

6.1. Future directions

The multifaceted findings obtained across the various studies, which implicate different neurotransmitter systems at a brain-wide level, indicate that depression is characterized by alterations involving numerous brain regions and networks. This highlighting the need for a non-reductionist and multidisciplinary approach to studying brain function and dysfunction. The complexity of the brain's dynamics, arising from the interactions of billions of neurons and trillions of synapses, underscores the importance of adopting an integrative approach to studying depression and its underlying neurobiology. This approach recognizes the interconnectedness of various neurotransmitter systems and neural circuits in shaping cognitive and mental processes. Therefore, investigating "whole-brain" function, rather than focusing on isolated regions or pathways, is crucial for gaining insights into the complex mechanisms underlying depression. Further, while the research presented here has provided valuable correlative evidence on neural signatures of depression and antidepressant response, establishing causal relationships between mental states and brain function remains a challenge. To overcome those limitation, it is necessary to implement methods that allow for studying brain functioning across multiple spatial and temporal scales and for the precise manipulation of brain's spatiotemporal dynamics. Novel methods to study the brain as a dynamical system [445], along with advancements in NIBS techniques [446], hold great promise in this regard. By applying notions of complexity sciences, the brain can be studied as a unified entity, integrating across different levels of analysis and examining the behaviour of the system as a whole. This perspective views the brain as a hierarchical system with quantifiable spatiotemporal dynamics [445]. One of the key advantages of approaching the brain as an integrated dynamical system is the ability to apply powerful modelling techniques characterized by dimensionality reduction, such as whole-brain generative models, and integrative and multilevel computational neuroscience methods [447]. These modelling techniques allow to capture the complexity of brain activity and cognition as a unified entity, moving beyond simplistic neurotransmitter-phenotype associations. Simultaneously, recent advancements in NIBS methods, employing electromagnetic or sensory stimulations, offer precise manipulation of brain spatiotemporal dynamics [446]. Prominent techniques include TMS, transcranial direct current stimulation (tDCS), alternating current stimulation (tACS), random noise stimulation (tRNS), temporal interference (TI) [448], and magnetoelectric or sensory closed-loop stimulations [449]. These methods enable the investigation of brain and cognitive functioning with enhanced spatial and temporal specificity. By combining the robust yet unspecific action of pharmacological interventions with the precise effect of NIBS, novel targeted interventions can be developed. Altogether, dynamic brain modelling, NIBS, psychoactive pharmacological challenges, and non-invasive neuroimaging constitute a potent arsenal of experimental tools for studying brain diseases in humans. The integration of these methods holds the potential to identify novel treatment targets and interventions for depression and neuropsychiatric conditions, thus advancing precision psychiatry.

6.2. Individual variability in psychoactive drug response

Interestingly, a coherent framework emerges from the conclusions drawn across the diverse studies presented in the body of this thesis. Consistent findings point to the fundamental role of variability and individual predisposition in response to psychoactive drugs. This variability manifested in various ways: from differences in the state of the 5-HT system between healthy and patients and within depression endophenotypes to the distinct neurophysiological response to ketamine treatment observed between early and late responders, and the relative insensitivity to neuroplastic changes of ketamine in healthy subjects. These observations underscore the significance of considering individual variability when examining neurobiological responses to psychoactive drugs. Such findings align with prior evidence from animal [263] and clinical studies [426,444], where different endophenotypes manifested different neurophysiological trajectories in response to psychoactive drugs.

The concept of individual variability in drug response, rooted in individual-specific structural and functional neurophysiological characteristics, adds an additional layer to the well-established factors of "set" and "setting" introduced by research on classic psychedelics [450]. Converging evidence has shown that neurophysiological [451] and psychological [452] responses to psychoactive drugs are modulated by extra-pharmacological factors. The effect of individual's mindset is demonstrated by the role of expectancy and therapeutic intentions in mediating clinical responses to psychoactive drugs via the notorious placebo effect [453,454]. The role of physical setting is supported by research showing how environmental stimuli modulate the therapeutic response produced by psychoactive medications such as ketamine, classic psychedelics, and SSRIs [450,455]. Indeed, it has recently been shown that the physiological and therapeutic effect of SSRIs depended on the subjective perception of the environment in pre-clinical models of depression [456,457]. For ketamine [458] and classic psychedelic [459], exposure to music during the drug-induced experience is recognized as a fundamental mediator of therapeutic outcomes. Remarkedly, the recent experiment by Mediano et al. (2024), provided direct evidence on the role of context on the psychophysiological effects of classic psychedelics, showing that exposure to different intensities of acute sensory stimulations modulate the level of brain signal entropy and the subjective effect induced by the drug [451]. Consistent with these findings, the response to a psychoactive treatment hinges critically on the interpersonal context in which the psychoactive drug is administered, though mechanism such as the therapeutic alliance between the patient and the caregiver [460–462]. With the present work, an additional variable is introduced into the model, reflecting the constitutional differences that characterize individuals in terms of their brain structure and functioning, these findings shed light on the complexity of predicting responses to psychoactive compounds, as evidenced by the results of the 3 experiments presented herein. Beyond neurophysiological constituents, it has also been shown that the phenomenological and therapeutic response to psychoactive compounds, including ketamine and classic psychedelics, can be shaped by individual's past exposures to specific environmental stimuli, such as digital media, a phenomenon termed "imprinting" [463].

In essence, the picture that emerges resonates with a recent model proposed by Girn et al. (2023), suggesting that the spatiotemporal trajectory of neural dynamics in response to psychoactive drugs varies based on an individual's brain structural and functional architecture, a notion supported by the findings presented in this thesis [464]. In other words, the various brain traits and states associated with individual structural and functional characteristics exhibit specific dynamics in response to external perturbations, such as the administration of psychoactive compounds. Predicting the direction of the response to these perturbations, particularly the therapeutic response, relies heavily on identifying specific micro- and macroscopic determinants of neural and mental phenotypes. This concept could be extended with the constitutional variability of drug pharmacokinetics and pharmacodynamics, influenced by diverse genetic, metabolic, and receptor profiles. Although evidence in this area is still scarce, certain observations are beginning to surface. For instance, single point mutations of the 5-HT2A receptors have been found to modulate the efficacy and potency of classic psychedelics, potentially leading to differential psychophysiological responses [465]. This underscores the necessity for improved stratification and phenotyping of patient populations, not only based on constellations of symptoms but also at the biological level. Understanding patient heterogeneity requires a more holistic comprehension of organism functioning across various levels of analysis, transitioning from the brain to the entire organism. Achieving this necessitates the multimodal integration of diverse data sources, aiming to establish an organicist model of health and disease. To this end, it is crucial to combine different neuroimaging techniques, such as EEG, fMRI, and PET, alongside (epi)-genetic data to capture brain dynamics at multiple levels of organization simultaneously. Furthermore, beyond the brain, integrating physiological parameters related to inflammatory pathways, metabolomics, brain-heart and brain-gut interactions, and circadian rhythms is essential to attain a multidimensional fingerprint of the organism [445]. These steps are pivotal for enhancing patient diagnostics and care, moving psychiatry toward personalized medicine.

6.3. A bio-psycho-social model of depression

What are the implications of the obtained results for the understanding of the aetiology of depression and its treatment? If the neurophysiological and therapeutic response of psychoactive medications varies based

on an individual's brain architecture, mental state, as well as physical and interpersonal setting (current and past), then a purely somatogenic account of depression and antidepressant response is not sufficient. A comprehensive understanding of mental well-being necessitates a unified framework that accounts for the dynamic interplay between biological, psychological, and environmental factors. Such framework should integrate modern neurobiological evidence while acknowledging the top-down influence of psychological states and environmental factors on mental health outcomes. One such accounts is the Gene x Environment interaction model, which posits that the origin of mental disorders arises from a combination of genetic predisposition and environmental influences during development, including interpersonal relationship [466].

For example, socio-economic status [467,468], urbanization [469–471], and pollutants [472] have been shown to influence the prevalence of depression and mood disorders in the population. In the landmark Harvard Study of Adult Development it was demonstrated that the quality of interpersonal relationships during development was the most significant predictor of individual well-being over the lifespan, outperforming known morbid conditions such as cardiovascular diseases [473]. Indeed, interpersonal childhood traumas, such as sexual abuse, physical abuse, emotional abuse, and neglect, have been empirically linked to the emergence of mental disorders in adulthood [474,475]. Additionally, a reduced degree of social connectedness to others has been identified as a risk factor for developing depression [172]. A related construct of connectedness, which encompasses connections to oneself, others, and the world, is also implicated in the therapeutic process of psychedelic-like interventions [97,476] and may be linked to the 5-HT system (as observed in Chapter 2). In fact, loneliness and lack of connection are common traits of depression and related mental health conditions [477]. These findings underscore the need for an extended framework of mental health that considers the dynamic interplay between an organism and its "exposome", defined as the sum of all the environmental exposures an individual has experienced.

Combining this evidence with the demonstrated influence of individual variability and extra-pharmacological factors on drug therapeutic responses, a novel integrated model for the aetiology and treatment of depressive disorder begins to take shape. Initially, depressive symptomatology may stem from exposure to stressors and traumatic events, especially during critical developmental periods [478]. The impact of such external circumstances on an individual's physiology would be mediated by individual variabilities in structural and functional biological architecture and predisposition towards pathology, such as genetic vulnerability to mood disorders. In vulnerable individuals, external stressors could exert significant effects on the developing organism, resulting in pathophysiological changes, like shifts in neurotransmitter balance (e.g., 5-HT, glutamate, and GABA) and decreases in brain plasticity. Consequently, symptoms of the disorder would manifest, and the closure of critical developmental periods would cement these manifestations, rendering them chronic. In this context, the role of antidepressant medications, such as ketamine, classic psychedelics, and SSRIs, would be to re-open a therapeutic window via their pharmacological activity [30,241,281]. However, this process might be characterized by significant interindividual variabilities, potentially associated with the nature and severity of the illness. This variability is evidenced by the diverse neural responses to psychoactive drugs observed in the 3 experiments outlined throughout the dissertation. As a result, the neurophysiological effects on neurotransmitter balance and neuroplasticity resulting from psychoactive interventions might vary based on individual endophenotypes [464]. Furthermore, reopening the therapeutic window by itself would not inherently guarantee therapeutic benefit, as indicated by research highlighting the interdependency of neuroplasticity and contextual factors in mediating therapeutic responses [455]. Achieving an effective therapeutic response requires consideration of other factors beyond pharmacological action, such as the patient's psychological mindset and the interpersonal environment during treatment. The importance of the "set " is underscored by phenomena such as the placebo effect, widely reported in the psychopharmacological literature [453,454]. Additionally, the physical and interpersonal "setting" in which these medications are administered plays a pivotal role in the therapeutic outcome, evidenced by treatment response variations based on the physical and interpersonal context [461,462].

In summary, the proposed model advocates against the reductionist perspectives that view mental health conditions solely as biological impairments (i.e., "broken brains") or purely psychological phenomena devoid of physiological underpinnings. Instead, it suggests a more comprehensive understanding that integrates biological, psychological, and contextual factors in shaping mental well-being (Figure 13). The findings presented in this work contribute to the development of a unified framework for understanding the origins of depression and the therapeutic effects of antidepressants. However, to establish the scientific validity of such bio-psycho-social model of depression and mental well-being, it is essential to identify empirically-testable mechanisms that mediate the dynamic interplay between these factors.

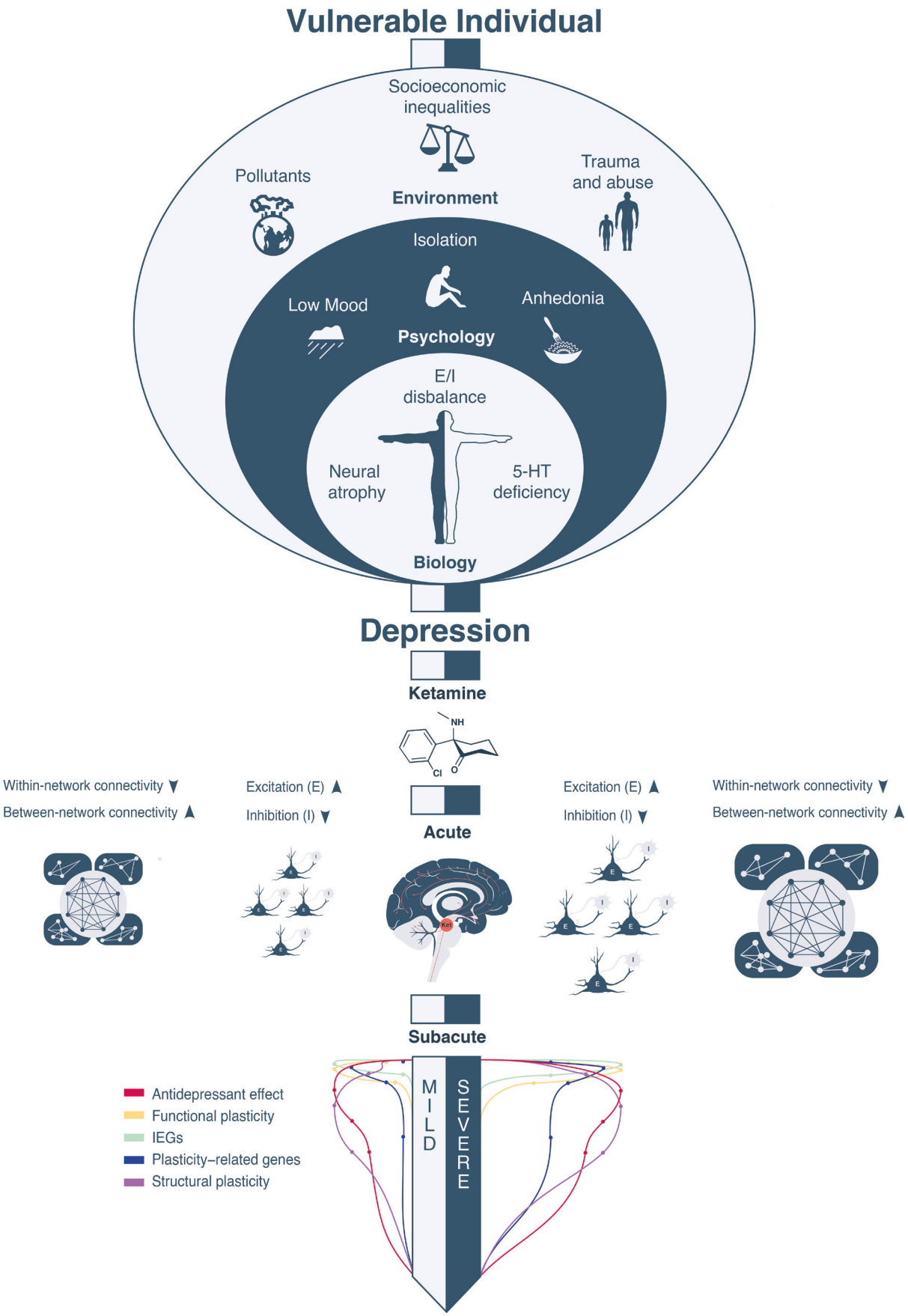

Figure 13: A bio-psycho-social model of depression and ketamine's antidepressant effect.

6.4. Information-theoretic prospectives of the mind

The proposed model of mental health necessarily implicate a dynamic interplay between mental and biological spheres. A mechanistic account for how this process might take place can be found in frameworks of brain functioning rooted in the complexity sciences and information-theory [445]. This method approaches the brain as an integrated dynamical system, including its collective properties such as mental operations and consciousness. At the core of this framework lies the co-identification of mentation with the quantity of information (from the latin informationem: "outline, concept, or idea") and of brain activity with information processing. The appeal and explanatory power of this approach lie in the quantifiable nature of information, which is a computable physical property. In this way, attention is given to the structure (i.e., the in-"form"-ation), rather than the content of mental properties. Such conceptualization of the mind in terms of information likely originated from the interdisciplinary movement known as Cybernetics during the 1950s [479]. The movement drew inspiration from the field of information-theory within telecommunication. Prominent figures in Cybernetics, such as Gregory Bateson, defined bits of information as the fundamental units of mental operations, famously describing them as "the difference that makes a difference" [480]. Cybernetics served as a fertile ground for significant technological and scientific advancements, laying the foundation for disciplines like artificial intelligence, now widely influential. The adoption of information-theoretic approaches to the neurosciences led to substantial progress and gave rise to successful fields of research, including computational neuroscience and computational psychiatry. Indeed, many influential theories of brain functioning made use of this framework, such as the free-energy principle [481] and the concept of the mind as emergent property of the brain [482], offering a possible account for the interaction between biological and psychological levels of explanation.

The free-energy principle, a highly influential theory of cognition operating within the predictive-coding framework, proposes that the nervous system guides an organism's behaviour by making inferences about the source of sensory information to minimize environmental uncertainty [481]. According to this model, the most effective strategy for an organism to achieve this minimization in a complex and ever-changing environment is by constructing an internally generated predictive model. This model simulates expected environmental circumstances and adjusts based on the mismatch (or "error signal") between the generated model (i.e., feed-back propagating signals) and newly incoming sensory information (i.e., feed-forward propagating signals). The framework also delineates a hierarchy of inferences ("priors"), with varying degrees of weight based on the expected variability of the information they encode. Neurobiologically, error signals and their weight are implemented through the activity of specific ascending and descending neural populations and their reciprocal connections. Some interpretations of the model suggest that these error signals constitute the mental property of the system, often equated with the qualia of subjective conscious experience, implying that consciousness is the awareness of the internally generated inferential model (though this view is debated by some) [483]. At the top of the hierarchy of the human brain sit the high-level abstract concepts with significant temporal depth, such as autobiographical memories and cultural beliefs. These concepts can exert top-down modulatory effects on lower priors, such as basic sensory and interoceptive information [484]. Experimental evidence supports the validity of the model, which has been applied as a powerful explanatory tool in health and disease across various brain functions [481]. For instance, in depression, the tendency towards ruminative thinking patterns and fixation on negative emotional states regardless of actual sensory information and environmental circumstances can be described in terms of the predictive coding framework [485]. In simple terms, the top-down modulatory power of abstract priors over raw sensory data can be regarded as the influence of mental states on brain activity, implemented as internally generated feed-back propagating error signals.

This perspective is closely linked to the widely held notion of the mind as an emergent property of brain activity, a concept also introduced by the complexity sciences [482]. Emergence refers to the phenomenon wherein complex patterns, properties, or behaviours arise from the interactions of simpler components within a system, often resulting in properties or behaviours that cannot be fully predicted or understood by examining the individual components alone. In the context of neuroscience, the emergent properties of the

mind are believed to stem from the dynamic and nonlinear interactions between neurons, wherein patterns of neural activity across various levels of organization give rise to higher-order cognitive functions and mental experiences [482]. This process aligns with the notion of a bottom-up generation of mental activity, adhering to the principles of upward causation. Conversely, downward causation suggests that behaviours at higher levels of complexity can influence outcomes at lower levels. In this context, high-order properties of the system, akin to the top-of-the-hierarchy priors in the predictive coding framework, can modulate lower-level constituents of the system, such as through top-down feedback propagating error signals. Thus, the informational properties of an individual's brain dynamics might serve as a bridge between physiological and psychological levels of description, facilitating the interplay between individual mental and biological states in determining mental health and responses to psychoactive medications.

A similarly consolidated account for how environmental factors, including social interactions, influence the body and mind relationship in mental health conditions and drug response is still lacking. In the recent work by Ibáñez et al. (2024), the need of neuroscience to account for an organism's mind and brain interaction with the environment is recognized as one of the most critical challenges for the advance of mental health research [445]. Current limitations in the field stem from oversimplified and reductive approaches to cognitive assessment, limited implementation of ecologically valid experimental designs, and a lack of strategies addressing integrated theoretical frameworks of naturalistic cognition. According to Ibáñez and colleagues, a pragmatic application of complexity science-based notions to the study of mental disorders as manifested in their naturalistic environment has the potential to address these limitations. The conclusion is supported by recent technological advancements that have expanded opportunities to observe human behaviour and neural activity in real-world settings and replicate real-world scenarios in controlled laboratory environments. An illustrative example of this was demonstrated in Chapter 4 of this thesis. Such technologies include portable neuroimaging devices, full-body biosensors, momentary sampling methods, and hyper-scanning setups. Additionally, employing virtual or ecologically valid environmental scenarios shows potential in the emerging fields of synergistic neuroscience and computational ethology [445]. These approaches, coupled with information-theoretic analytic tools, offer exciting prospects for deeper insights into the complex interplay between human behaviour, neural activity, and environmental factors in shaping mental health and responses to interventions.

In summary, the integration of complexity sciences within psychiatry offers a biologically plausible framework for understanding the aetiology of depression and the impact of mental and extra-pharmacological factors on responses to psychoactive medications. By taking into account the reciprocal influence between biological constituents, mental states, and contextual factors, this approach offers insight into phenomena such as the role of the interpersonal exposome in depression's aetiology and the influence of individual variability and extra-pharmacological factors in responses to psychoactive drugs. The identification of mental activity as a form of information processing in the brain provides a robust scientific basis for reconciling psychological and biological models of mental disorder aetiology within a naturalistic framework. It grounds mental phenomena in neurobiology while acknowledging the reciprocal influence of psychological and contextual factors on organism functioning.

6.5. Limitations

Importantly, a bio-psycho-social model that equates mentation with information processing is not without limitations. First, while influential, the empirical evidence on mapping the mind-brain relationship through informational processing is still limited. There remains an ongoing debate in the field, and while numerous new theories are rapidly emerging, a consensus has yet to be reached [483]. Furthermore, there are considerable limitations associated with current methodologies for evaluating the phenomenological aspects of psychiatric conditions like depression, as well as the therapeutic significance of the subjective experiences induced by psychoactive medications. This challenge is paramount to tackle given the pivotal role of subjective lived experiences in shaping mental health, as elucidated by Kyzar and Denfield [486]. In parallel, mounting evidence underscores the importance of subjective experiences induced by novel antidepressants like ketamine and classic psychedelics in mediating therapeutic outcomes [487]. Notably, there is ongoing debate within the field, mainly fuelled by preclinical evidence suggesting the possibility of

divorcing the subjective from the therapeutic effects induced by psychoactive compounds [280,488,489]. However, no evidence in humans of therapeutic effects of non-hallucinogenic psychedelics exists to date. At present, the available data from both healthy individuals and patients indicate that the psychoactive properties of these drugs profoundly contribute to improving mental well-being through psychological mechanisms like fostering insight [96], facilitating emotional release [437], and eliciting mystical-type experiences [487,490]. In principle, these findings are compatible with the information-theoretic framework of the mind-to-body relationship, but the quantitative methodologies of complexity science inherently lack the capability to fully grasp the phenomenological dimensions of conscious experience. The subjective experiences elicited by psychoactive drugs are often described as ineffable, presenting challenges to their systematic investigation though classical quantitative methods. Current methodologies for assessing the first-person experience in neuropsychopharmacology frequently rely on reductionistic questionnaires, which, while useful for establishing correlations between neural mechanisms and therapeutic response, possess notable limitations, as elaborated in Chapter 4. To mitigate these shortcomings, a particularly promising and pragmatic approach to studying the subjective experience of psychiatric conditions and psychoactive medications is offered by the science of neurophenomenology [416,491]. This approach advocates for a disciplined exploration of subjective experience combined with modern neuroimaging techniques. Taking inspiration from traditional contemplative practices, particularly meditation, neurophenomenology empirically analyses the nature of mental processes from a first-person perspective, affording them equal importance as manifested neural activity. Similar to information-based theories of the brain, the focus is placed on the structure of phenomenal experience, rather than its content, in an attempt to extrapolate principal components of mental processes. This approach offer a superior analytic compatibility with neurobiological measures as compared to less systematic qualitative and free-narrative methods. Notably, the integration of this methods with complexity science is beginning to delineate the novel and promising field of computational neurophenomenology [492].

An additional limitation pervading the discourse of this manuscript pertains to the characterization of depression as a static and isolated diagnostic category. According to the DSM, depression is conceptualized as a discrete disorder with uniform symptoms and aetiology across individuals [11]. However, this oversimplified view fails to capture the heterogeneity and dynamic nature of depressive experiences and their real-world manifestations. Viewing depression as a static entity overlooks its dynamic and gradational nature, wherein symptoms fluctuate over time and in response to various influences. Individuals may exhibit different symptom profiles at different stages of the illness or in different contexts, making it challenging to classify depression into rigid categories, as already discussed in the general introduction of this manuscript. Furthermore, the notion of depression as an isolated diagnostic category neglects its interconnectedness with other mental health conditions and broader psychosocial factors. Depressive disorder often co-occurs with other psychiatric and medical comorbidities, highlighting the need for a more integrated approach to its assessment and treatment [493]. The attainment of a nuanced understanding of the aetiology of depression in its various manifestations will crucially depend on the integration of multiple levels of analysis, encompassing the biological, psychological, phenomenological, and ecological dimensions of the individual.

6.6. The mind-body problem

A bio-psycho-social interpretation of mental health carries radical ontological implications for the definition of what constitutes the body and the mind. While delving into the various definitions of the so-called "mind-body problem" is beyond the scope of this work, it is nonetheless valuable to briefly contemplate the philosophical ramifications of the conclusions presented herein, as they lead to interesting considerations.

The unification and reciprocity of the biological, psychological, and sociological spheres require the adoption of a non-dualistic view of the mind-body problem. This necessitates identifying a monistic physical (or ideal) substrate that integrates both the body and the mind. Within this framework, a physicalist ontology, which excludes the existence of anything beyond the laws of physics, must provide a substrate for both the material and the mental worlds that adheres to these laws. While the description of biological

processes, including those of the brain, in terms of physical laws of cause and effect offers relatively precise explanations of an organism structure and function, achieving a similar account for the mind has proven significantly more challenging, partly due to difficulties in precisely defining the concept of the mind. In this regard, the conception of the of the brain as complex dynamical system and of mental activity as information processing is a powerful explanatory tool, but leads to some theoretical inconsistencies. As elucidated by Barutta et al. (2010), the interaction between the body and mind exhibits a level of circularity that transcends the conventional linear cause-and-effect relationship [482]. Indeed, framing the reciprocal influence between the brain and the mind within classical efficient causal terms, particularly for downward causation, proves challenging. Classical efficient causality depicts a linear relationship between two entities, cause and effect, along a temporal arrow. This model posits that entity A produces an effect on entity B, with a temporal delay between the cause and its effect. In the context of mind-body relations, the concept of downward causation suggests that emergent (mental) properties exert influence on a local (neurological or corporeal) level. Exemplified by phenomena such as the influence of interpersonal relationship during brain development on pathology, or the impact of "set" and "setting" in drug responses. However, sustaining the argument of downward causation in terms of efficient cause necessitates an understanding rooted in ontological dualism, wherein two distinct and separate entities (mind and brain) are linked by a bidirectional causal arrow. This conceptualization implies the amalgamation of two events, physical and non-physical, in a causal chain [482]. However, it's important to recognize that it's not the mental activity itself that directly triggers changes in brain activity, but rather the physiological adjustments initiated by that mental activity [445,482]. On the other hand, also the adoption of a pure reductionist physicalist position fails to provide a valid solution to this dilemma. Radical reductionism suggests that basal properties cause global properties, implying that the former should comprehensively explain the latter, thus relegating the mind to nonexistence or epiphenomenal status, without any causal power [482]. Both concepts of mind-brain causation (up and down), when construed in terms of efficient causality, yield inconsistent explanations. A viable alternative to dualistic or purely reductionistic physicalist positions necessitates the coexistence of different levels of analysis within the same system (i.e., neutral monism), where psychological and neurological properties manifest simultaneously as part of a singular phenomenon. Likewise, their causal properties should be understood within this unified framework, as there is no distinct mind A causing a change in another brain B. However, it becomes clear that the notion of efficient causality is contradictory when applied to causal relations between different levels of description of the same phenomenon, given that mental and neurological phenomena are viewed as a singular entity [445,482]. These theoretical challenges underscore the necessity for novel and more comprehensive explanatory frameworks to effectively reconcile biological and psychological levels of explanation. Pragmatic efforts have been undertaken by prominent scholars, exemplified by the works of Barutta et al. (2010) [482], Rosas et al. (2020) [494], and Ibáñez et al. (2024) [445], laying the groundwork for empirical testing of such concepts. These endeavours are crucial for the advancement of a nuanced understanding of the aetiology of mental health conditions within a coherent philosophical and scientific framework.

The association of the mind with information-theoretic metrics introduces another intriguing philosophical implication. Essentially, if mental operations arise from sufficiently complex information processing, then the mind could be conceptualized as a process potentially not unique to organic life. This idea aligns with the philosophy of mind known as Panpsychism, which implies that artificial forms of sentience might exist or could be created, carrying significant ethical implications [495]. Moreover, acknowledging information as the medium of mental processes is also congruent with the concept of the "extended mind", which suggests that mentality could potentially extend beyond an organism to encompass its environment. This notion further extends to the idea of a "collective mind" when applied to society and interpersonal relationship [480]. Many authors propose that everyday activities, such as interacting with other individuals or sophisticated electronic devices, can be viewed as forms of extended mentality, facilitated by the exchange of information [496]. While intriguing, empirical investigation into these concepts remains limited. Indeed, reconciling the notion of an extended mind with current neuroscientific theories of mental functioning poses significant challenges. Even in philosophical discourse, there are few examples of comprehensive metaphysical accounts able to integrate the physical and universal aspects of the mind without invoking a

dualistic prospective. A particularly interesting view is given by the Process philosophy of Alfred North Whitehead [497]. In his analytic approach, existing things exhibit both physical and mental attributes, but they are not conceived as separate entities. The interconnectedness and relational nature of reality is used to argue for the integration of the physical and mental dimensions on a universal scale. Moreover, the concept of an existing entity is considered specular to that of dynamic events. In a nutshell, the integrative metaphysical framework of Process philosophy underscores the irreducible, situated, and inherently temporal aspects of phenomena, viewing them as ongoing processes rather than static entities. While necessarily speculative and abstract, the emphasis on the relational aspect of reality is a powerful conceptual tool, with direct applications across various intellectual domains. Echoes of this philosophical framework can be found in cutting-edge approaches to many open scientific problems, spanning fields such as physics [498], chemistry [499], biology [500], psychology [501], anthropology [480], and neuroscience, as exemplified by the recent proposals by Ibáñez et al. (2024) [445]. At its core, this ecological perspective could lead to new ways in which individuals conceptualize their existential connection with themselves, their community, and their environment, carrying important implications for the development of a more holistic notion of well-being.

6.7. Conclusion

As a final consideration, it's essential to recognize the historical and cultural relativity inherent to any model or conception of mental disorders and deviant behaviours. As highlighted at the beginning of this manuscript, the understanding of the aetiology of mental illness, whether viewed through psychological, biological, or supernatural lenses, is deeply intertwined with the epoch and ethos of the society under examination [1]. Across centuries, these views have undergone cyclical shifts, reflecting the cultural, religious, or political context of the time. Similarly, contemporary neurobiological theories of mental disorders are constrained by the prevailing understanding and conceptualization of brain function. Throughout the history of neuroscience, various models and metaphors have been employed to elucidate the workings of the nervous system. Each era's scientific paradigms and technological advancements shape the prevailing theories, influencing how brain-related disorders are conceptualized and treated. Following scientific and technological developments, models of brain functioning have undergone significant evolution. Initially, analogical models portrayed the brain as resembling hydraulic systems, mechanical devices, or telephones. As neuroscientists delved deeper into the brain's informational processing capabilities, the analogy shifted towards digital systems, particularly computers. In the digital framework, the brain was envisioned as an algorithmic computational device, with neurons serving as binary switches akin to those found in early computers [502]. More recently, as our understanding of the brain's complexity has grown, the network analogy has gained prominence. This perspective reflects the brain's interconnectedness and emphasizes the importance of studying brain activity in terms of complex systems and networks. This shift aligns with modern technological advancements, such as artificial intelligence, which also rely on network-based architectures for processing information. It is evident that our models and conceptualizations of the brain and the mind are profoundly influenced by the cultural and technological outline of a particular society, rendering them inherently transient and incomplete. Indeed, reducing the vast array of human mental experiences to a computable operation, no matter how intricate and advanced, may ultimately prove to be an oversimplification. The mystery and complexity of life and the mind in all their manifestations remain largely ineffable. However, adhering to the pragmatic notion that "all models are wrong, but some are useful" [503], the exploration of unresolved questions, such as the nature of the mind, represents a crucial endeavour in advancing humanity's understanding of its own condition. In this prospective, a significant challenge for the advancement of mental health research lies in the interdisciplinary integration of diversified knowledge, fostering a dialogue with fields such as cultural anthropology, sociology, and philosophy. By engaging with these disciplines, we could gain deeper insights into the socio-cultural contexts that shape mental well-being and illness. This interdisciplinary approach offers a more unitary understanding of human experiences and opens avenues for more effective interventions and support systems tailored to diverse cultural and societal contexts.

In conclusion, the evidence presented in this manuscript represents a significant advancement in our understanding of depression's aetiology and the evolution of the field of neuropsychopharmacology. The findings not only shed light on key aspects of mental health but also prompt crucial questions that underscore the importance of interdisciplinary research in unravelling its complexities and enhancing treatment outcomes. Emerging research avenues offer novel and exciting prospects for addressing unresolved questions about depression and mental health. These endeavours are fuelled by novel scientific tools, revolutionary theoretical frameworks, and the progressive destigmatization of the exploration of the mind. By embracing these advancements, we can develop more effective interventions for individuals struggling with depression and related conditions, thereby facing the mental health crisis of our times.

7. References

1. Barlow, D. H. & Durand, V. M. Abnormal Psychology: An Integrative Approach. (Cengage Learning, Australia, 2015).
2. Restak, R. Mysteries of the Mind. (National Geographic Society, Washington, DC, 2000).
3. André, C. Evolving story: trepanation and self-trepanation to enhance brain function. Arq. Neuropsiquiatr. 75, 307–313 (2017).
4. Rucker, J. J. H., Iliff, J. & Nutt, D. J. Psychiatry & the psychedelic drugs. Past, present & future. Neuropharmacology 142, 200–218 (2018).
5. Tseng, W. S. The development of psychiatric concepts in traditional Chinese medicine. Arch. Gen. Psychiatry 29, 569–575 (1973).
6. Tasca, C., Rapetti, M., Carta, M. G. & Fadda, B. Women And Hysteria In The History Of Mental Health. Clin. Pract. Epidemiol. Ment. Health CP EMH 8, 110–119 (2012).
7. Opiela, K. FROM THE ASYLUM TO THE MENTAL HOSPITAL. Zesz. Glottodydaktyczne 103–126 (2018).
8. Hillhouse, T. M. & Porter, J. H. A brief history of the development of antidepressant drugs: From monoamines to glutamate. Exp. Clin. Psychopharmacol. 23, 1–21 (2015).
9. Macpherson, J. Psychiatrie: Ein Lehrbuch für Studirende und Aerzte. Edinb. Med. J. 6, 67–68 (1899).
10. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. (American Psychiatric Association, Washington, D.C, 2013).
11. Young, G. DSM-5: Basics and Critics. in Unifying Causality and Psychology: Being, Brain, and Behavior (ed. Young, G.) 565–590 (Springer International Publishing, Cham, 2016). doi:10.1007/978-3-319-24094-7_22.
12. Kraepelin, E. Manic Depressive Insanity and Paranoia. J. Nerv. Ment. Dis. 53, 350 (1921).
13. Hidaka, B. H. Depression as a disease of modernity: explanations for increasing prevalence. J. Affect. Disord. 140, 205–214 (2012).

14. Vos, T. et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet 396, 1204–1222 (2020).

15. Evans-Lacko, S. et al. Socio-economic variations in the mental health treatment gap for people with anxiety, mood, and substance use disorders: results from the WHO World Mental Health (WMH) surveys. Psychol. Med. 48, 1560–1571 (2018).

16. Karrouri, R., Hammani, Z., Benjelloun, R. & Otheman, Y. Major depressive disorder: Validated treatments and future challenges. World J. Clin. Cases 9, 9350–9367 (2021).

17. Presti, D. E. Psychoactive Drugs. in Encyclopedia of the Human Brain (ed. Ramachandran, V. S.) 75–82 (Academic Press, New York, 2002). doi:10.1016/B0-12-227210-2/00290-9.

18. Ban, T. A. In memory of three pioneers. Int. J. Neuropsychopharmacol. 9, 475–477 (2006).

19. Salzer, H. M. & Lurie, M. L. Anxiety and depressive states treated with isonicotinyl hydrazide (isoniazid). AMA Arch. Neurol. Psychiatry 70, 317–324 (1953).

20. López-Muñoz, F. & Alamo, C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr. Pharm. Des. 15, 1563–1586 (2009).

21. Berger, M., Gray, J. A. & Roth, B. L. The expanded biology of serotonin. Annu. Rev. Med. 60, 355–366 (2009).

22. Dahlström, A. & Fuxe, K. Localization of monoamines in the lower brain stem. Experientia 20, 398–399 (1964).

23. Törk, I. Anatomy of the serotonergic system. Ann. N. Y. Acad. Sci. 600, 9–34; discussion 34-35 (1990).

24. Hoyer, D. et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203 (1994).

25. Szegedy-Maszak, M. The career of a celebrity pill. As Prozac's long reign comes to an end, experts are questioning its legacy. US News World Rep. 131, 38–39 (2001).

26. Al-Harbi, K. S. Treatment-resistant depression: therapeutic trends, challenges, and future directions. Patient Prefer. Adherence 6, 369–388 (2012).

27. Keks, N. A. et al. Beyond the evidence: is there a place for antidepressant combinations in the pharmacotherapy of depression? Med. J. Aust. 186, 142–144 (2007).

28. Ferguson, J. M. SSRI Antidepressant Medications: Adverse Effects and Tolerability. Prim. Care Companion J. Clin. Psychiatry 3, 22–27 (2001).

29. Depression: How effective are antidepressants? in InformedHealth.org [Internet] (Institute for Quality and Efficiency in Health Care (IQWiG), 2020).

30. Nutt, D., Erritzoe, D. & Carhart-Harris, R. Psychedelic Psychiatry's Brave New World. Cell 181, 24–28 (2020).

31. Nutt, D. Illegal Drugs Laws: Clearing a 50-Year-Old Obstacle to Research. PLOS Biol. 13, e1002047 (2015).

32. Aghajanian, G. K. & Marek, G. J. Serotonin and Hallucinogens. Neuropsychopharmacology 21, 16–23 (1999).

33. Domino, E. F. & Warner, D. S. Taming the Ketamine Tiger. Anesthesiology 113, 678–684 (2010).

34. Corssen, G. & Domino, E. F. Dissociative anesthesia: further pharmacologic studies and first clinical experience with the phencyclidine derivative CI-581. Anesth. Analg. 45, 29–40 (1966).

35. Jansen, K. L. R. A Review of the Nonmedical Use of Ketamine: Use, Users and Consequences. J. Psychoactive Drugs 32, 419–433 (2000).

36. Sassano-Higgins, S., Baron, D., Juarez, G., Esmaili, N. & Gold, M. A Review of Ketamine Abuse and Diversion. Depress. Anxiety 33, 718–727 (2016).

37. Jelen, L. A. & Stone, J. M. Ketamine for depression. Int. Rev. Psychiatry Abingdon Engl. 33, 207–228 (2021).

38. Khorramzadeh, E. & Lotfy, A. O. The use of ketamine in psychiatry. Psychosomatics 14, 344–346 (1973).

39. Kolp, E. et al. Ketamine-Enhanced Psychotherapy: Preliminary Clinical Observations on its Effects in Treating Death Anxiety. Int. J. Transpers. Stud. 26, (2007).

40. Krupitsky, E. M. & Grinenko, A. Y. Ketamine psychedelic therapy (KPT): a review of the results of ten years of research. J. Psychoactive Drugs 29, 165–183 (1997).

41. Wei, Y., Chang, L. & Hashimoto, K. A historical review of antidepressant effects of ketamine and its enantiomers. Pharmacol. Biochem. Behav. 190, 172870 (2020).

42. Fonnum, F. Glutamate: a neurotransmitter in mammalian brain. J. Neurochem. 42, 1–11 (1984).

43. Anis, N. A., Berry, S. C., Burton, N. R. & Lodge, D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br. J. Pharmacol. 79, 565–575 (1983).

44. Page, C. E. & Coutellier, L. Prefrontal excitatory/inhibitory balance in stress and emotional disorders: Evidence for over-inhibition. Neurosci. Biobehav. Rev. 105, 39–51 (2019).

45. Petroff, O. A. C. GABA and glutamate in the human brain. Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry 8, 562–573 (2002).

46. Sohal, V. S. & Rubenstein, J. L. R. Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Mol. Psychiatry 24, 1248–1257 (2019).

47. Gonzalez-Burgos, G. & Lewis, D. A. GABA Neurons and the Mechanisms of Network Oscillations: Implications for Understanding Cortical Dysfunction in Schizophrenia. Schizophr. Bull. 34, 944–961 (2008).

48. Appelbaum, L. G., Shenasa, M. A., Stolz, L. & Daskalakis, Z. Synaptic plasticity and mental health: methods, challenges and opportunities. Neuropsychopharmacology 48, 113–120 (2023).

49. Cohen, E. J., Quarta, E., Bravi, R., Granato, A. & Minciacchi, D. Neural plasticity and network remodeling: From concepts to pathology. Neuroscience 344, 326–345 (2017).

50. Ismail, F. Y., Fatemi, A. & Johnston, M. V. Cerebral plasticity: Windows of opportunity in the developing brain. Eur. J. Paediatr. Neurol. 21, 23–48 (2017).

51. Rădulescu, I., Drăgoi, A. M., Trifu, S. C. & Cristea, M. B. Neuroplasticity and depression: Rewiring the brain's networks through pharmacological therapy (Review). Exp. Ther. Med. 22, 1131 (2021).

52. Claudio, A. & Andrea, F. Circadian neuromarkers of mood disorders. J. Affect. Disord. Rep. 10, 100384 (2022).

53. Maletic, V. et al. Neurobiology of depression: an integrated view of key findings. Int. J. Clin. Pract. 61, 2030–2040 (2007).

54. Iversen, L., Iversen, S., Bloom, F. E. & Roth, R. H. Introduction to Neuropsychopharmacology. (Oxford University Press, 2008).

55. Mucci, A., Volpe, U., Merlotti, E., Bucci, P. & Galderisi, S. Pharmaco-EEG in psychiatry. Clin. EEG Neurosci. 37, 81–98 (2006).

56. Nathan, P. J., Phan, K. L., Harmer, C. J., Mehta, M. A. & Bullmore, E. T. Increasing pharmacological knowledge about human neurological and psychiatric disorders through functional neuroimaging and its application in drug discovery. Curr. Opin. Pharmacol. 14, 54–61 (2014).

57. Talbot, P. S. & Laruelle, M. The role of in vivo molecular imaging with PET and SPECT in the elucidation of psychiatric drug action and new drug development. Eur. Neuropsychopharmacol. 12, 503–511 (2002).

58. Buzsáki, G., Anastassiou, C. A. & Koch, C. The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. 13, 407–420 (2012).

59. Proudfoot, M., Woolrich, M. W., Nobre, A. C. & Turner, M. R. Magnetoencephalography. Pract. Neurol. 14, 336–343 (2014).

60. Murakami, S. & Okada, Y. Contributions of principal neocortical neurons to magnetoencephalography and electroencephalography signals. J. Physiol. 575, 925–936 (2006).

61. Muthukumaraswamy, S. D. The use of magnetoencephalography in the study of psychopharmacology (pharmaco-MEG). J. Psychopharmacol. (Oxf.) 28, 815–829 (2014).

62. Koubeissi, M. Z. Niedermeyer's Electroencephalography, Basic Principles, Clinical Applications, and Related Fields, 6th ed. Arch. Neurol. 68, 1481 (2011).

63. Collura, T. F. Neocortical Dynamics and Human EEG Rhythms. J. Clin. Neurophysiol. 13, 177 (1996).

64. Boto, E. et al. Moving magnetoencephalography towards real-world applications with a wearable system. Nature 555, 657–661 (2018).

65. Grover, V. P. B. et al. Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. J. Clin. Exp. Hepatol. 5, 246–255 (2015).

66. Di Salle, F. et al. Exploring brain function with magnetic resonance imaging. Eur. J. Radiol. 30, 84–94 (1999).

67. Crosson, B. et al. Functional Imaging and Related Techniques: An Introduction for Rehabilitation Researchers. J. Rehabil. Res. Dev. 47, vii–xxxiv (2010).

68. Arakawa, R., Takano, A. & Halldin, C. PET technology for drug development in psychiatry. Neuropsychopharmacol. Rep. 40, 114–121 (2020).

69. Artigas, F., Nutt, D. J. & Shelton, R. Mechanism of action of antidepressants. Psychopharmacol Bull 36 Suppl 2, 123–32 (2002).

70. Shopsin, B., Gershon, S., Goldstein, M., Friedman, E. & Wilk, S. Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. Psychopharmacol Commun 1, 239–49 (1975).

71. Shopsin, B., Friedman, E. & Gershon, S. Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. Arch Gen Psychiatry 33, 811–9 (1976).

72. Smith, K. A., Fairburn, C. G. & Cowen, P. J. Relapse of depression after rapid depletion of tryptophan. Lancet 349, 915–9 (1997).

73. Delgado, P. L. et al. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. Biol Psychiatry 46, 212–20 (1999).

74. Ruhe, H. G., Mason, N. S. & Schene, A. H. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry 12, 331–59 (2007).

75. Zhang, X. et al. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron 45, 11–6 (2005).

76. Beaulieu, J. M. et al. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U A 105, 1333–8 (2008).

77. Fournet, V. et al. The deletion of STOP/MAP6 protein in mice triggers highly altered mood and impaired cognitive performances. J Neurochem 121, 99–114 (2012).

78. Chen, W. V. et al. Pcdhalphac2 is required for axonal tiling and assembly of serotonergic circuitries in mice. Science 356, 406–411 (2017).

79. Moncrieff, J. et al. The serotonin theory of depression: a systematic umbrella review of the evidence. Mol. Psychiatry 28, 3243–3256 (2023).

80. Jorgensen, L. M. et al. Cerebral 5-HT release correlates with [11C]Cimbi36 PET measures of 5-HT2A receptor occupancy in the pig brain. J Cereb Blood Flow Metab (2016) doi:10.1177/0271678X16629483.

81. Finnema, S. J. et al. Imaging synaptic density in the living human brain. Sci. Transl. Med. 8, 348ra96 (2016).

82. da Cunha-Bang, S. et al. Measuring endogenous changes in serotonergic neurotransmission with [(11)C]Cimbi-36 positron emission tomography in humans. Transl Psychiatry 9, 134 (2019).

83. Shotbolt, P. et al. Within-subject comparison of [(11)C]-(+)-PHNO and [(11)C]raclopride sensitivity to acute amphetamine challenge in healthy humans. J Cereb Blood Flow Metab 32, 127–36 (2012).

84. Erritzoe D., C. A. Serotonin release measured in the human brain: A PET study with [11C]Cimbi-36 and d-amphetamine challenge. (2017).

85. Kuczenski, R., Segal, D. S., Cho, A. K. & Melega, W. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. J Neurosci 15, 1308–17 (1995).

86. Heal, D. J., Cheetham, S. C., Prow, M. R., Martin, K. F. & Buckett, W. R. A comparison of the effects on central 5-HT function of sibutramine hydrochloride and other weight-modifying agents. Br J Pharmacol 125, 301–8 (1998).

87. Kehr, J. et al. Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. Br J Pharmacol 164, 1949–58 (2011).

88. Husain, M. I. et al. Serotonergic psychedelics for depression: What do we know about neurobiological mechanisms of action? Front. Psychiatry 13, 1076459 (2023).

89. Mans, K. et al. Sustained, Multifaceted Improvements in Mental Well-Being Following Psychedelic Experiences in a Prospective Opportunity Sample. Front. Psychiatry 12, (2021).

90. Roseman, L., Demetriou, L., Wall, M. B., Nutt, D. J. & Carhart-Harris, R. L. Increased amygdala responses to emotional faces after psilocybin for treatment-resistant depression. Neuropharmacology 142, 263–269 (2018).

91. Doss, M. K. et al. Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder. Transl. Psychiatry 11, 1–10 (2021).

92. Zeifman, R. J. et al. Post-Psychedelic Reductions in Experiential Avoidance Are Associated With Decreases in Depression Severity and Suicidal Ideation. Front. Psychiatry 11, 782 (2020).

93. Madsen, M. K. et al. A single psilocybin dose is associated with long-term increased mindfulness, preceded by a proportional change in neocortical 5-HT2A receptor binding. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 33, 71–80 (2020).

94. Soler, J. et al. Exploring the therapeutic potential of Ayahuasca: acute intake increases mindfulness-related capacities. Psychopharmacology (Berl.) 233, 823–829 (2016).

95. Erritzoe, D. et al. Recreational use of psychedelics is associated with elevated personality trait openness: Exploration of associations with brain serotonin markers. J. Psychopharmacol. (Oxf.) 33, 1068–1075 (2019).

96. Peill, J. M. et al. Validation of the Psychological Insight Scale: A new scale to assess psychological insight following a psychedelic experience. J. Psychopharmacol. (Oxf.) 36, 31–45 (2022).

97. Watts, R. et al. The Watts Connectedness Scale: a new scale for measuring a sense of connectedness to self, others, and world. Psychopharmacology (Berl.) 239, 3461–3483 (2022).

98. Erritzoe, D. et al. Brain Serotonin Release Is Reduced in Patients With Depression: A [11C]Cimbi-36 Positron Emission Tomography Study With a d-Amphetamine Challenge. Biol. Psychiatry 93, 1089–1098 (2023).

99. Hamilton, M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6, 278–96 (1967).

100. Dozois, D. J. A., Dobson, K. S. & Ahnberg, J. L. A psychometric evaluation of the Beck Depression Inventory–II. Psychol. Assess. 10, 83–89 (1998).

101. Curran, S. L., Andrykowski, M. A. & Studts, J. L. Short Form of the Profile of Mood States (POMS-SF): Psychometric information. Psychol. Assess. 7, 80–83 (1995).

102. Julian, L. J. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Arthritis Care Res. 63 Suppl 11, S467-472 (2011).

103. Parola, N. et al. Psychometric properties of the Ruminative Response Scale-short form in a clinical sample of patients with major depressive disorder. Patient Prefer. Adherence 11, 929–937 (2017).

104. Tennant, R. et al. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): development and UK validation. Health Qual. Life Outcomes 5, 63 (2007).

105. Tziortzi, A. C. et al. Imaging dopamine receptors in humans with [11C]-(+)-PHNO: Dissection of D3 signal and anatomy. NeuroImage 54, 264–277 (2011).

106. Ettrup, A. et al. Serotonin 2A receptor agonist binding in the human brain with [11C]Cimbi-36. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 34, 1188–1196 (2014).

107. Tukey, J. W. Some thoughts on clinical trials, especially problems of multiplicity. Science 198, 679–684 (1977).

108. Sullivan, G. M. & Feinn, R. Using Effect Size—or Why the P Value Is Not Enough. J. Grad. Med. Educ. 4, 279–282 (2012).

109. Keysers, C., Gazzola, V. & Wagenmakers, E.-J. Using Bayes factor hypothesis testing in neuroscience to establish evidence of absence. Nat. Neurosci. 23, 788–799 (2020).

110. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300 (1995).

111. Carhart-Harris, R. L. et al. Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin. Proc. Natl. Acad. Sci. U. S. A. 109, 2138–2143 (2012).

112. Mick, I. et al. Amphetamine induced endogenous opioid release in the human brain detected with [11C]carfentanil PET: replication in an independent cohort. Int J Neuropsychopharmacol 1–6 (2014) doi:10.1017/S1461145714000704.

113. Narendran, R. et al. Decreased prefrontal cortical dopamine transmission in alcoholism. Am J Psychiatry 171, 881–8 (2014).

114. Ettrup, A. et al. Serotonin 2A receptor agonist binding in the human brain with [(11)C]Cimbi-36: Test-retest reproducibility and head-to-head comparison with the antagonist [(18)F]altanserin. Neuroimage 130, 167–74 (2016).

115. Beliveau, V. et al. A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System. J Neurosci 37, 120–128 (2017).

116. Yang, Y., Ju, W., Zhang, H. & Sun, L. Effect of Ketamine on LTP and NMDAR EPSC in Hippocampus of the Chronic Social Defeat Stress Mice Model of Depression. Front. Behav. Neurosci. 12, (2018).

117. Toll, L. et al. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA Res Monogr 178, 440–66 (1998).

118. Tyacke, R. J. & Nutt, D. J. Optimising PET approaches to measuring 5-HT release in human brain. Synapse 69, 505–11 (2015).

119. Ettrup, A. et al. Radiosynthesis and in vivo evaluation of a series of substituted 11C-phenethylamines as 5-HT (2A) agonist PET tracers. Eur J Nucl Med Mol Imaging 38, 681–93 (2011).

120. Walther, D. J. & Bader, M. A unique central tryptophan hydroxylase isoform. Biochem Pharmacol 66, 1673–80 (2003).

121. Walther, D. J. et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 299, 76 (2003).

122. Harvey, M. et al. Support for the involvement of TPH2 gene in affective disorders. Mol Psychiatry 9, 980–1 (2004).

123. Zill, P. et al. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Mol Psychiatry 9, 1030–6 (2004).

124. Boldrini, M., Underwood, M. D., Mann, J. J. & Arango, V. More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. Brain Res 1041, 19–28 (2005).

125. Bach-Mizrachi, H. et al. Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. Mol Psychiatry 13, 507–13, 465 (2008).

126. Johnson, S. et al. The reduction of R1, a novel repressor protein for monoamine oxidase A, in major depressive disorder. Neuropsychopharmacology 36, 2139–48 (2011).

127. Meyer, J. H. et al. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Arch Gen Psychiatry 63, 1209–16 (2006).

128. Meyer, J. H. et al. Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence. Arch Gen Psychiatry 66, 1304–12 (2009).

129. Bacher, I. et al. Monoamine oxidase A binding in the prefrontal and anterior cingulate cortices during acute withdrawal from heavy cigarette smoking. Arch Gen Psychiatry 68, 817–26 (2011).

130. Underwood, M. D. et al. Neuron density and serotonin receptor binding in prefrontal cortex in suicide. Int J Neuropsychopharmacol 15, 435–47 (2012).

131. Rajkowska, G. et al. Length of axons expressing the serotonin transporter in orbitofrontal cortex is lower with age in depression. Neuroscience 359, 30–39 (2017).

132. Koenigs, M. & Grafman, J. The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. Behav Brain Res 201, 239–43 (2009).

133. Rolls, E. T., Cheng, W. & Feng, J. The orbitofrontal cortex: reward, emotion and depression. Brain Commun 2, fcaa196 (2020).

134. Hahn, A. et al. Attenuated serotonin transporter association between dorsal raphe and ventral striatum in major depression. Hum Brain Mapp 35, 3857–66 (2014).

135. Bhagwagar, Z. et al. 5-HTT binding in recovered depressed patients and healthy volunteers: a positron emission tomography study with [11C]DASB. Am J Psychiatry 164, 1858–65 (2007).

136. Savitz, J. B. & Drevets, W. C. Neuroreceptor imaging in depression. Neurobiol Dis 52, 49–65 (2013).

137. Politis, M. et al. Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study. Neurobiol Dis 40, 216–21 (2010).

138. Boileau, I. et al. Elevated serotonin transporter binding in depressed patients with Parkinson's disease: a preliminary PET study with [11C]DASB. Mov Disord 23, 1776–80 (2008).

139. Strecker, K. et al. Preserved serotonin transporter binding in de novo Parkinson's disease: negative correlation with the dopamine transporter. J Neurol 258, 19–26 (2011).

140. Fukui, M. et al. Vmat2 heterozygous mutant mice display a depressive-like phenotype. J Neurosci 27, 10520–9 (2007).

141. Taylor, T. N. et al. Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. J Neurosci 29, 8103–13 (2009).

142. Narboux-Neme, N. et al. Severe serotonin depletion after conditional deletion of the vesicular monoamine transporter 2 gene in serotonin neurons: neural and behavioral consequences. Neuropsychopharmacology 36, 2538–50 (2011).

143. Braak, H. et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24, 197–211 (2003).

144. Mayeux, R., Stern, Y., Cote, L. & Williams, J. B. Altered serotonin metabolism in depressed patients with parkinson's disease. Neurology 34, 642–6 (1984).

145. Kostic, V. S. et al. Depression and Parkinson's disease: possible role of serotonergic mechanisms. J Neurol 234, 94–6 (1987).

146. Tong, Q. et al. Reduced plasma serotonin and 5-hydroxyindoleacetic acid levels in Parkinson's disease are associated with nonmotor symptoms. Park. Relat Disord 21, 882–7 (2015).

147. Kish, S. J. et al. Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. Brain 131, 120–31 (2008).

148. Kerenyi, L. et al. Positron emission tomography of striatal serotonin transporters in Parkinson disease. Arch Neurol 60, 1223–9 (2003).

149. Guttman, M. et al. Brain serotonin transporter binding in non-depressed patients with Parkinson's disease. Eur J Neurol 14, 523–8 (2007).

150. Albin, R. L. et al. Spared caudal brainstem SERT binding in early Parkinson's disease. J Cereb Blood Flow Metab 28, 441–4 (2008).

151. Jorgensen, L. M. et al. Parkinson patients have a presynaptic serotonergic deficit: A dynamic deep brain stimulation PET study. J Cereb Blood Flow Metab 41, 1954–1963 (2021).

152. Eskow Jaunarajs, K. L. et al. Behavioral and neurochemical effects of chronic L-DOPA treatment on nonmotor sequelae in the hemiparkinsonian rat. Behav Pharmacol 21, 627–37 (2010).

153. Zhuo, C. et al. Efficacy of antidepressive medication for depression in Parkinson disease: a network meta-analysis. Med. Baltim. 96, e6698 (2017).

154. Drevets, W. C. et al. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nucl Med Biol 34, 865–77 (2007).

155. Bhagwagar, Z., Rabiner, E. A., Sargent, P. A., Grasby, P. M. & Cowen, P. J. Persistent reduction in brain serotonin1A receptor binding in recovered depressed men measured by positron emission tomography with [11C]WAY-100635. Mol Psychiatry 9, 386–92 (2004).

156. Sullivan, G. M. et al. Positron emission tomography quantification of serotonin-1A receptor binding in medication-free bipolar depression. Biol Psychiatry 66, 223–30 (2009).

157. Ballanger, B. et al. Role of serotonergic 1A receptor dysfunction in depression associated with Parkinson's disease. Mov Disord 27, 84–9 (2012).

158. Meyer, J. H. et al. Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. Am J Psychiatry 160, 90–9 (2003).

159. Bhagwagar, Z. et al. Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. Am J Psychiatry 163, 1580–7 (2006).

160. Biver, F. et al. Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. Br J Psychiatry 171, 444–8 (1997).

161. Attar-Levy, D. et al. The cortical serotonin2 receptors studied with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. Biol Psychiatry 45, 180–6 (1999).

162. Yatham, L. N. et al. Brain serotonin2 receptors in major depression: a positron emission tomography study. Arch Gen Psychiatry 57, 850–8 (2000).

163. Messa, C. et al. 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. Psychopharmacol. Berl 167, 72–8 (2003).

164. Mintun, M. A. et al. Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. Biol Psychiatry 55, 217–24 (2004).

165. Meyer, J. H. et al. Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. Am J Psychiatry 156, 1029–34 (1999).

166. Frokjaer, V. G. et al. Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder. Biol Psychiatry 63, 569–76 (2008).

167. Frokjaer, V. G. et al. Familial risk for mood disorder and the personality risk factor, neuroticism, interact in their association with frontolimbic serotonin 2A receptor binding. Neuropsychopharmacology 35, 1129–37 (2010).

168. Elmenhorst, D., Kroll, T., Matusch, A. & Bauer, A. Sleep deprivation increases cerebral serotonin 2A receptor binding in humans. Sleep 35, 1615–23 (2012).

169. Soloff, P. H., Price, J. C., Mason, N. S., Becker, C. & Meltzer, C. C. Gender, personality, and serotonin-2A receptor binding in healthy subjects. Psychiatry Res 181, 77–84 (2010).

170. Meyer, J. H. et al. The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study. Am J Psychiatry 158, 78–85 (2001).

171. Colom, M., Vidal, B. & Zimmer, L. Is There a Role for GPCR Agonist Radiotracers in PET Neuroimaging? Front. Mol. Neurosci. 12, 255 (2019).

172. Weziak-Bialowolska, D. et al. Prospective Associations Between Social Connectedness and Mental Health. Evidence From a Longitudinal Survey and Health Insurance Claims Data. Int. J. Public Health 67, 1604710 (2022).

173. Luppi, A. I. et al. A role for the serotonin 2A receptor in the expansion and functioning of human transmodal cortex. Brain J. Neurol. 147, 56–80 (2024).

174. Brouwer, A. & Carhart-Harris, R. L. Pivotal mental states. J. Psychopharmacol. Oxf. Engl. 35, 319–352 (2021).

175. Madsen, M. K. et al. Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. Neuropsychopharmacology 44, 1328–1334 (2019).

176. Becker, A. M. et al. Ketanserin Reverses the Acute Response to LSD in a Randomized, Double-Blind, Placebo-Controlled, Crossover Study in Healthy Participants. Int. J. Neuropsychopharmacol. 26, 97–106 (2023).

177. Stenbæk, D. S. et al. Brain serotonin 2A receptor binding predicts subjective temporal and mystical effects of psilocybin in healthy humans. J. Psychopharmacol. (Oxf.) 35, 459–468 (2021).

178. Berman, R. M. et al. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354 (2000).

179. Walsh, Z. et al. Ketamine for the treatment of mental health and substance use disorders: comprehensive systematic review. BJPsych Open 8, e19 (2022).

180. Terao, I., Tsuge, T., Endo, K. & Kodama, W. Comparative efficacy, tolerability and acceptability of intravenous racemic ketamine with intranasal esketamine, aripiprazole and lithium as augmentative treatments for treatment-resistant unipolar depression: A systematic review and network meta-analysis. J. Affect. Disord. 346, 49–56 (2024).

181. Zarate Jr., C. A. & Niciu, M. J. Ketamine for depression: evidence, challenges and promise. World Psychiatry 14, 348–350 (2015).

182. Hasselmann, H. W. W. Chapter 59 - Antidepressant and Abuse Potential of Ketamine. in Neuropathology of Drug Addictions and Substance Misuse (ed. Preedy, V. R.) 639–648 (Academic Press, San Diego, 2016). doi:10.1016/B978-0-12-800212-4.00059-5.

183. Zhdanava, M. et al. Esketamine nasal spray for major depressive disorder with acute suicidal ideation or behavior: description of treatment access, utilization, and claims-based outcomes in the United States. J. Med. Econ. 26, 691–700 (2023).

184. Mion, G. & Villevieille, T. Ketamine Pharmacology: An Update (Pharmacodynamics and Molecular Aspects, Recent Findings). CNS Neurosci. Ther. 19, 370–380 (2013).

185. Zanos, P. & Gould, T. D. Mechanisms of Ketamine Action as an Antidepressant. Mol. Psychiatry 23, 801–811 (2018).

186. Zarate, C. A. et al. Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. Biol. Psychiatry 72, 331–338 (2012).

187. Zanos, P. et al. Ketamine and Ketamine Metabolite Pharmacology: Insights into Therapeutic Mechanisms. Pharmacol. Rev. 70, 621–660 (2018).

188. Zhao, X. et al. Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. Br. J. Clin. Pharmacol. 74, 304–314 (2012).

189. Chen, X., Shu, S. & Bayliss, D. A. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. J. Neurosci. Off. J. Soc. Neurosci. 29, 600–609 (2009).

190. Zhang, Y. et al. Structural basis of ketamine action on human NMDA receptors. Nature 596, 301–305 (2021).

191. Moghaddam, B., Adams, B., Verma, A. & Daly, D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. Off. J. Soc. Neurosci. 17, 2921–2927 (1997).

192. Lorrain, D. S., Baccei, C. S., Bristow, L. J., Anderson, J. J. & Varney, M. A. Effects of ketamine and n-methyl-d-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 117, 697–706 (2003).

193. Pothula, S. et al. Cell-type specific modulation of NMDA receptors triggers antidepressant actions. Mol. Psychiatry 26, 5097–5111 (2021).

194. Rowland, L. M. et al. Effects of Ketamine on Anterior Cingulate Glutamate Metabolism in Healthy Humans: A 4-T Proton MRS Study. Am. J. Psychiatry 162, 394–396 (2005).

195. Abdallah, C. G. et al. The effects of ketamine on prefrontal glutamate neurotransmission in healthy and depressed subjects. Neuropsychopharmacology 43, 2154–2160 (2018).

196. Lazarevic, V., Yang, Y., Flais, I. & Svenningsson, P. Ketamine decreases neuronally released glutamate via retrograde stimulation of presynaptic adenosine A1 receptors. Mol. Psychiatry 26, 7425–7435 (2021).

197. Milak, M. S. et al. Assessment of Relationship of Ketamine Dose With Magnetic Resonance Spectroscopy of Glx and GABA Responses in Adults With Major Depression: A Randomized Clinical Trial. JAMA Netw. Open 3, e2013211 (2020).

198. Seamans, J. Losing inhibition with ketamine. Nat. Chem. Biol. 4, 91–93 (2008).

199. Khlestova, E., Johnson, J. W., Krystal, J. H. & Lisman, J. The Role of GluN2C-Containing NMDA Receptors in Ketamine's Psychotogenic Action and in Schizophrenia Models. J. Neurosci. Off. J. Soc. Neurosci. 36, 11151–11157 (2016).

200. Gerhard, D. M. et al. GABA interneurons are the cellular trigger for ketamine's rapid antidepressant actions. J. Clin. Invest. 130, 1336–1349 (2020).

201. Zhang, B. et al. Ketamine activated glutamatergic neurotransmission by GABAergic disinhibition in the medial prefrontal cortex. Neuropharmacology 194, 108382 (2021).

202. Cichon, J. et al. Ketamine triggers a switch in excitatory neuronal activity across neocortex. Nat. Neurosci. 26, 39–52 (2023).

203. Vesuna, S. et al. Deep posteromedial cortical rhythm in dissociation. Nature 586, 87–94 (2020).

204. Shen, G., Han, F. & Shi, W.-X. Effects of Low Doses of Ketamine on Pyramidal Neurons in Rat Prefrontal Cortex. Neuroscience 384, 178–187 (2018).

205. Zhang, X. X. & Shi, W. X. Dendritic glutamate-induced bursting in prefrontal pyramidal cells: role of NMDA and non-NMDA receptors. Zhongguo Yao Li Xue Bao 20, 1125–1131 (1999).

206. Nosyreva, E. et al. Acute Suppression of Spontaneous Neurotransmission Drives Synaptic Potentiation. J. Neurosci. 33, 6990–7002 (2013).

207. Miller, O. H. et al. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. eLife 3, e03581 (2014).

208. Cui, Y., Yang, Y., Dong, Y. & Hu, H. Decoding Depression: Insights from Glial and Ketamine Regulation of Neuronal Burst Firing in Lateral Habenula. Cold Spring Harb. Symp. Quant. Biol. 83, 141–150 (2018).

209. Hare, B. D. et al. Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. Nat. Commun. 10, 223 (2019).

210. Wu, M., Minkowicz, S., Dumrongprechachan, V., Hamilton, P. & Kozorovitskiy, Y. Ketamine Rapidly Enhances Glutamate-Evoked Dendritic Spinogenesis in Medial Prefrontal Cortex Through Dopaminergic Mechanisms. Biol. Psychiatry 89, 1096–1105 (2021).

211. Vollenweider, F. X., Leenders, K. L., Øye, I., Hell, D. & Angst, J. Differential psychopathology and patterns of cerebral glucose utilisation produced by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET). Eur. Neuropsychopharmacol. 7, 25–38 (1997).

212. Långsjö, J. W. et al. Effects of subanesthetic doses of ketamine on regional cerebral blood flow, oxygen consumption, and blood volume in humans. Anesthesiology 99, 614–623 (2003).

213. Lally, N. et al. Neural correlates of change in major depressive disorder anhedonia following open-label ketamine. J. Psychopharmacol. Oxf. Engl. 29, 596–607 (2015).

214. Ballard, E. D. et al. Neural correlates of suicidal ideation and its reduction in depression. Int. J. Neuropsychopharmacol. 18, pyu069 (2014).

215. Doyle, O. M. et al. Quantifying the Attenuation of the Ketamine Pharmacological Magnetic Resonance Imaging Response in Humans: A Validation Using Antipsychotic and Glutamatergic Agents. J. Pharmacol. Exp. Ther. 345, 151–160 (2013).

216. Deakin, J. F. W. et al. Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. Arch. Gen. Psychiatry 65, 154–164 (2008).

217. De Simoni, S. et al. Test-retest reliability of the BOLD pharmacological MRI response to ketamine in healthy volunteers. NeuroImage 64, 75–90 (2013).

218. Stone, J. et al. Perceptual distortions and delusional thinking following ketamine administration are related to increased pharmacological MRI signal changes in the parietal lobe. J. Psychopharmacol. Oxf. Engl. 29, 1025–1028 (2015).

219. Höflich, A. et al. Ketamine-dependent neuronal activation in healthy volunteers. Brain Struct. Funct. 222, 1533–1542 (2017).

220. Javitt, D. C. et al. Utility of Imaging-Based Biomarkers for Glutamate-Targeted Drug Development in Psychotic Disorders: A Randomized Clinical Trial. JAMA Psychiatry 75, 11–19 (2018).

221. McMillan, R. et al. Temporal dynamics of the pharmacological MRI response to subanaesthetic ketamine in healthy volunteers: A simultaneous EEG/fMRI study. J. Psychopharmacol. Oxf. Engl. 33, 219–229 (2019).

222. Alexander, L., Jelen, L. A., Mehta, M. A. & Young, A. H. The anterior cingulate cortex as a key locus of ketamine's antidepressant action. Neurosci. Biobehav. Rev. 127, 531–554 (2021).

223. A, A. et al. N-methyl-D-aspartate receptor antagonist effects on prefrontal cortical connectivity better model early than chronic schizophrenia. Biol. Psychiatry 77, (2015).

224. Driesen, N. R. et al. The Impact of NMDA Receptor Blockade on Human Working Memory-Related Prefrontal Function and Connectivity. Neuropsychopharmacology 38, 2613–2622 (2013).

225. Abdallah, C. G. et al. Ketamine Treatment and Global Brain Connectivity in Major Depression. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 42, 1210–1219 (2017).

226. Zacharias, N. et al. Ketamine effects on default mode network activity and vigilance: A randomized, placebo-controlled crossover simultaneous fMRI/EEG study. Hum. Brain Mapp. 41, 107–119 (2020).

227. Mueller, F. et al. Pharmacological fMRI: Effects of subanesthetic ketamine on resting-state functional connectivity in the default mode network, salience network, dorsal attention network and executive control network. NeuroImage Clin. 19, 745–757 (2018).

228. Marguilho, M., Figueiredo, I. & Castro-Rodrigues, P. A unified model of ketamine's dissociative and psychedelic properties. J. Psychopharmacol. Oxf. Engl. 37, 14–32 (2023).

229. Lehmann, M. et al. Differential effects of rumination and distraction on ketamine induced modulation of resting state functional connectivity and reactivity of regions within the default-mode network. Soc. Cogn. Affect. Neurosci. 11, 1227–1235 (2016).

230. Ionescu, D. F. et al. Ketamine-Associated Brain Changes: A Review of the Neuroimaging Literature. Harv. Rev. Psychiatry 26, 320–339 (2018).

231. Scheidegger, M. et al. Ketamine administration reduces amygdalo-hippocampal reactivity to emotional stimulation. Hum. Brain Mapp. 37, 1941–1952 (2016).

232. Cheng, P. et al. Evidence against mood-congruent attentional bias in Major Depressive Disorder. Psychiatry Res. 230, 496–505 (2015).

233. Krings, A., Heeren, A., Fontaine, P. & Blairy, S. Attentional biases in depression: Relation to disorder severity, rumination, and anhedonia. Compr. Psychiatry 100, 152173 (2020).

234. Fritzsche, A. et al. Specificity of cognitive biases in patients with current depression and remitted depression and in patients with asthma. Psychol. Med. 40, 815–826 (2010).

235. Gotlib, I. H., Krasnoperova, E., Yue, D. N. & Joormann, J. Attentional biases for negative interpersonal stimuli in clinical depression. J. Abnorm. Psychol. 113, 121–135 (2004).

236. Trapp, W., Kalzendorf, C., Baum, C., Hajak, G. & Lautenbacher, S. Attentional biases in patients suffering from unipolar depression: results of a dot probe task investigation. Psychiatry Res. 261, 325–331 (2018).

237. Zarate, C. A. et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864 (2006).

238. Feder, A. et al. Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 71, 681–688 (2014).

239. Kryst, J. et al. Efficacy of single and repeated administration of ketamine in unipolar and bipolar depression: a meta-analysis of randomized clinical trials. Pharmacol. Rep. PR 72, 543–562 (2020).

240. Castrén, E. & Antila, H. Neuronal plasticity and neurotrophic factors in drug responses. Mol. Psychiatry 22, 1085–1095 (2017).

241. Aleksandrova, L. R. & Phillips, A. G. Neuroplasticity as a convergent mechanism of ketamine and classical psychedelics. Trends Pharmacol. Sci. 42, 929–942 (2021).

242. Kavalali, E. T. & Monteggia, L. M. Targeting Homeostatic Synaptic Plasticity for Treatment of Mood Disorders. Neuron 106, 715–726 (2020).

243. Kalivas, P. W. & O'Brien, C. Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 33, 166–180 (2008).

244. Manji, H. K., Moore, G. J., Rajkowska, G. & Chen, G. Neuroplasticity and cellular resilience in mood disorders. Mol. Psychiatry 5, 578–593 (2000).

245. Moda-Sava, R. N. et al. Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. Science 364, eaat8078 (2019).

246. Thomas, M. J., Kalivas, P. W. & Shaham, Y. Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. Br. J. Pharmacol. 154, 327–342 (2008).

247. Rossi, R. et al. A single dose of cocaine raises SV2A density in hippocampus of adolescent rats. Acta Neuropsychiatr. 1–9 (2023) doi:10.1017/neu.2023.14.

248. Tardito, D. et al. Signaling Pathways Regulating Gene Expression, Neuroplasticity, and Neurotrophic Mechanisms in the Action of Antidepressants: A Critical Overview. Pharmacol. Rev. 58, 115–134 (2006).

249. Bernardinelli, Y., Nikonenko, I. & Muller, D. Structural plasticity: mechanisms and contribution to developmental psychiatric disorders. Front. Neuroanat. 8, (2014).

250. Götz, M. & Huttner, W. B. The cell biology of neurogenesis. Nat. Rev. Mol. Cell Biol. 6, 777–788 (2005).

251. Dong, C., Rovnaghi, C. R. & Anand, K. J. S. Ketamine alters the neurogenesis of rat cortical neural stem progenitor cells. Crit. Care Med. 40, 2407–2416 (2012).

252. Cavalleri, L. et al. Ketamine enhances structural plasticity in mouse mesencephalic and human iPSC-derived dopaminergic neurons via AMPAR-driven BDNF and mTOR signaling. Mol. Psychiatry 23, 812–823 (2018).

253. Ly, C. et al. Psychedelics Promote Structural and Functional Neural Plasticity. Cell Rep. 23, 3170–3182 (2018).

254. Ly, C. et al. Transient Stimulation with Psychoplastogens Is Sufficient to Initiate Neuronal Growth. ACS Pharmacol. Transl. Sci. 4, 452–460 (2021).

255. Zhang, Z. et al. Ketamine Regulates Phosphorylation of CRMP2 To Mediate Dendritic Spine Plasticity. J. Mol. Neurosci. 70, 353–364 (2020).

256. Ma, Z. et al. TrkB dependent adult hippocampal progenitor differentiation mediates sustained ketamine antidepressant response. Nat. Commun. 8, 1668 (2017).

257. Yamada, J. & Jinno, S. Potential link between antidepressant-like effects of ketamine and promotion of adult neurogenesis in the ventral hippocampus of mice. Neuropharmacology 158, 107710 (2019).

258. Rawat, R., Tunc-Ozcan, E., McGuire, T. L., Peng, C.-Y. & Kessler, J. A. Ketamine activates adult-born immature granule neurons to rapidly alleviate depression-like behaviors in mice. Nat. Commun. 13, 2650 (2022).

259. Li, N. et al. mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. Science 329, 959–964 (2010).

260. Ruddy, R. M., Chen, Y., Milenkovic, M. & Ramsey, A. J. Differential effects of NMDA receptor antagonism on spine density. Synap. N. Y. N 69, 52–56 (2015).

261. Phoumthipphavong, V., Barthas, F., Hassett, S. & Kwan, A. C. Longitudinal Effects of Ketamine on Dendritic Architecture In Vivo in the Mouse Medial Frontal Cortex. eNeuro 3, ENEURO.0133-15.2016 (2016).

262. Sarkar, A. & Kabbaj, M. Sex Differences in Effects of Ketamine on Behavior, Spine Density, and Synaptic Proteins in Socially Isolated Rats. Biol. Psychiatry 80, 448–456 (2016).

263. Treccani, G. et al. S-Ketamine Reverses Hippocampal Dendritic Spine Deficits in Flinders Sensitive Line Rats Within 1 h of Administration. Mol. Neurobiol. 56, 7368–7379 (2019).

264. Widman, A. J., Stewart, A. E., Erb, E. M., Gardner, E. & McMahon, L. L. Intravascular Ketamine Increases Theta-Burst but Not High Frequency Tetanus Induced LTP at CA3-CA1 Synapses Within Three Hours and Devoid of an Increase in Spine Density. Front. Synaptic Neurosci. 10, (2018).

265. Zou, X. et al. Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. Int. J. Dev. Neurosci. 27, 727–731 (2009).

266. Brown, B. P. et al. In vivo and in vitro ketamine exposure exhibits a dose-dependent induction of activity-dependent neuroprotective protein in rat neurons. Neuroscience 290, 31–40 (2015).

267. Huang, H. et al. Ketamine Affects the Neurogenesis of the Hippocampal Dentate Gyrus in 7-Day-Old Rats. Neurotox. Res. 30, 185–198 (2016).

268. Citri, A. & Malenka, R. C. Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. Neuropsychopharmacology 33, 18–41 (2008).

269. Zucker, R. S. & Regehr, W. G. Short-term synaptic plasticity. Annu. Rev. Physiol. 64, 355–405 (2002).

270. Hulme, S. R., Jones, O. D., Raymond, C. R., Sah, P. & Abraham, W. C. Mechanisms of heterosynaptic metaplasticity. Philos. Trans. R. Soc. B Biol. Sci. 369, 20130148 (2014).

271. Salami, M., Fathollahi, Y., Esteky, H., Motamedi, F. & Atapour, N. Effects of ketamine on synaptic transmission and long-term potentiation in layer II/III of rat visual cortex in vitro. Eur. J. Pharmacol. 390, 287–293 (2000).

272. Narimatsu, E., Kawamata, Y., Kawamata, M., Fujimura, N. & Namiki, A. NMDA receptor-mediated mechanism of ketamine-induced facilitation of glutamatergic excitatory synaptic transmission. Brain Res. 953, 272–275 (2002).

273. Izumi, Y. & Zorumski, C. F. Metaplastic effects of subanesthetic ketamine on CA1 hippocampal function. Neuropharmacology 86, 273–281 (2014).

274. Graef, J. D. et al. Effect of acute NR2B antagonist treatment on long-term potentiation in the rat hippocampus. Brain Res. 1609, 31–39 (2015).

275. Burgdorf, J. et al. GLYX-13, a NMDA Receptor Glycine-Site Functional Partial Agonist, Induces Antidepressant-Like Effects Without Ketamine-Like Side Effects. Neuropsychopharmacology 38, 729–742 (2013).

276. Aleksandrova, L. R., Wang, Y. T. & Phillips, A. G. Ketamine and its metabolite, (2R,6R)-HNK, restore hippocampal LTP and long-term spatial memory in the Wistar-Kyoto rat model of depression. Mol. Brain 13, 92 (2020).

277. Grieco, S. F. et al. Subanesthetic Ketamine Reactivates Adult Cortical Plasticity to Restore Vision from Amblyopia. Curr. Biol. 30, 3591-3603.e8 (2020).

278. Cannarozzo, C., Rubiolo, A., Casarotto, P. & Castrén, E. Ketamine and its metabolite 2R,6R-hydroxynorketamine promote ocular dominance plasticity and release tropomyosin-related kinase B from inhibitory control without reducing perineuronal nets enwrapping parvalbumin interneurons. Eur. J. Neurosci. 57, 940–950 (2023).

279. Casarotto, P. C. et al. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. Cell 184, 1299-1313.e19 (2021).

280. Moliner, R. et al. Psychedelics promote plasticity by directly binding to BDNF receptor TrkB. Nat. Neurosci. 26, 1032–1041 (2023).

281. Nardou, R. et al. Psychedelics reopen the social reward learning critical period. Nature 618, 790–798 (2023).

282. Ribeiro, P. O., Silva, H. B., Tomé, Â. R., Cunha, R. A. & Antunes, L. M. Hippocampal long-term potentiation in adult mice after recovery from ketamine anesthesia. Lab Anim. 43, 353–357 (2014).

283. Llamosas, N. et al. Ketamine promotes rapid and transient activation of AMPA receptor-mediated synaptic transmission in the dorsal raphe nucleus. Prog. Neuropsychopharmacol. Biol. Psychiatry 88, 243–252 (2019).

284. Guo, D. et al. Neonatal exposure of ketamine inhibited the induction of hippocampal long-term potentiation without impairing the spatial memory of adult rats. Cogn. Neurodyn. 12, 377–383 (2018).

285. Sumner, R. L. et al. Ketamine Enhances Visual Sensory Evoked Potential Long-term Potentiation in Patients With Major Depressive Disorder. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 5, 45–55 (2020).

286. Sumner, R. L. et al. Ketamine improves short-term plasticity in depression by enhancing sensitivity to prediction errors. Eur. Neuropsychopharmacol. 38, 73–85 (2020).

287. Cornwell, B. R. et al. Synaptic potentiation is critical for rapid antidepressant response to ketamine in treatment-resistant major depression. Biol. Psychiatry 72, 555–561 (2012).

288. Nugent, A. C., Wills, K. E., Gilbert, J. R. & Zarate, C. A. Synaptic potentiation and rapid antidepressant response to ketamine in treatment-resistant major depression: A replication study. Psychiatry Res. Neuroimaging 283, 64–66 (2019).

289. Gilbert, J. R., Yarrington, J. S., Wills, K. E., Nugent, A. C. & Zarate, C. A. Glutamatergic Signaling Drives Ketamine-Mediated Response in Depression: Evidence from Dynamic Causal Modeling. Int. J. Neuropsychopharmacol. 21, 740–747 (2018).

290. Scheidegger, M. et al. Ketamine decreases resting state functional network connectivity in healthy subjects: implications for antidepressant drug action. PloS One 7, e44799 (2012).

291. Bonhomme, V. et al. Resting-state Network-specific Breakdown of Functional Connectivity during Ketamine Alteration of Consciousness in Volunteers. Anesthesiology 125, 873–888 (2016).

292. Abdallah, C. G. et al. Ketamine, but Not the NMDAR Antagonist Lanicemine, Increases Prefrontal Global Connectivity in Depressed Patients. Chronic Stress Thousand Oaks Calif 2, 2470547018796102 (2018).

293. Kraus, C. et al. Evaluating global brain connectivity as an imaging marker for depression: influence of preprocessing strategies and placebo-controlled ketamine treatment. Neuropsychopharmacology 45, 982–989 (2020).

294. Lanahan, A. & Worley, P. Immediate-Early Genes and Synaptic Function. Neurobiol. Learn. Mem. 70, 37–43 (1998).

295. Ehrlich, D. E. & Josselyn, S. A. Plasticity-related genes in brain development and amygdala-dependent learning. Genes Brain Behav. 15, 125–143 (2016).

296. Broide, R. S. et al. Distribution of histone deacetylases 1-11 in the rat brain. J. Mol. Neurosci. MN 31, 47–58 (2007).

297. Lepack, A. E., Bang, E., Lee, B., Dwyer, J. M. & Duman, R. S. Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures. Neuropharmacology 111, 242–252 (2016).

298. Lepack, A. E., Fuchikami, M., Dwyer, J. M., Banasr, M. & Duman, R. S. BDNF Release Is Required for the Behavioral Actions of Ketamine. Int. J. Neuropsychopharmacol. 18, pyu033 (2014).

299. Choi, M. et al. Ketamine produces antidepressant-like effects through phosphorylation-dependent nuclear export of histone deacetylase 5 (HDAC5) in rats. Proc. Natl. Acad. Sci. 112, 15755–15760 (2015).

300. Kim, J.-W. & Monteggia, L. M. Increasing doses of ketamine curtail antidepressant responses and suppress associated synaptic signaling pathways. Behav. Brain Res. 380, 112378 (2020).

301. Viana, G. S. B. et al. Rapid and long-lasting antidepressant-like effects of ketamine and their relationship with the expression of brain enzymes, BDNF, and astrocytes. Braz. J. Med. Biol. Res. 54, e10107 (2020).

302. Autry, A. E. et al. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91–95 (2011).

303. Liu, R.-J. et al. Brain-Derived Neurotrophic Factor Val66Met Allele Impairs Basal and Ketamine-Stimulated Synaptogenesis in Prefrontal Cortex. Biol. Psychiatry 71, 996–1005 (2012).

304. Shi, M. et al. Effects of Ketamine on Learning and Memory in the Hippocampus of Rats through ERK, CREB, and Arc. Brain Sci. 11, 27 (2021).

305. de Bartolomeis, A. et al. Different effects of the NMDA receptor antagonists ketamine, MK-801, and memantine on postsynaptic density transcripts and their topography: Role of Homer signaling, and implications for novel antipsychotic and pro-cognitive targets in psychosis. Prog. Neuropsychopharmacol. Biol. Psychiatry 46, 1–12 (2013).

306. Bagot, R. C. et al. Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. Biol. Psychiatry 81, 285–295 (2017).

307. Laje, G. et al. Brain-Derived Neurotrophic Factor Val66Met Polymorphism and Antidepressant Efficacy of Ketamine in Depressed Patients. Biol. Psychiatry 72, e27–e28 (2012).

308. Duncan, W. C. et al. Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP 16, 301–311 (2013).

309. Woelfer, M. et al. Ketamine-induced changes in plasma brain-derived neurotrophic factor (BDNF) levels are associated with the resting-state functional connectivity of the prefrontal cortex. World J. Biol. Psychiatry Off. J. World Fed. Soc. Biol. Psychiatry 21, 696–710 (2020).

310. Zheng, W. et al. Plasma BDNF concentrations and the antidepressant effects of six ketamine infusions in unipolar and bipolar depression. PeerJ 9, e10989 (2021).

311. Haile, C. N. et al. Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. Int. J. Neuropsychopharmacol. 17, 331–336 (2014).

312. Allen, A. P. et al. Serum BDNF as a peripheral biomarker of treatment-resistant depression and the rapid antidepressant response: A comparison of ketamine and ECT. J. Affect. Disord. 186, 306–311 (2015).

313. Wang, M. et al. Sleep improvement is associated with the antidepressant efficacy of repeated-dose ketamine and serum BDNF levels: a post-hoc analysis. Pharmacol. Rep. 73, 594–603 (2021).

314. Rybakowski, J. K., Permoda-Osip, A., Skibinska, M., Adamski, R. & Bartkowska-Sniatkowska, A. Single ketamine infusion in bipolar depression resistant to antidepressants: are neurotrophins involved? Hum. Psychopharmacol. 28, 87–90 (2013).

315. Glue, P. et al. Safety and efficacy of extended release ketamine tablets in patients with treatment-resistant depression and anxiety: open label pilot study. Ther. Adv. Psychopharmacol. 10, 2045125320922474 (2020).

316. Glue, P. et al. Ascending-Dose Study of Controlled-Release Ketamine Tablets in Healthy Volunteers: Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability. J. Clin. Pharmacol. 60, 751–757 (2020).

317. Glue, P. et al. Effects of ketamine in patients with treatment-refractory generalized anxiety and social anxiety disorders: Exploratory double-blind psychoactive-controlled replication study. J. Psychopharmacol. Oxf. Engl. 34, 267–272 (2020).

318. Machado-Vieira, R. et al. Brain-derived neurotrophic factor and initial antidepressant response to an N-methyl-D-aspartate antagonist. J. Clin. Psychiatry 70, 1662–1666 (2009).

319. Medeiros, G. C. et al. Treatment of depression with ketamine does not change plasma levels of brain-derived neurotrophic factor or vascular endothelial growth factor. J. Affect. Disord. 280, 136–139 (2021).

320. Caliman-Fontes, A. T. et al. Brain-derived neurotrophic factor serum levels following ketamine and esketamine intervention for treatment-resistant depression: secondary analysis from a randomized trial. Trends Psychiatry Psychother. 45, e20210298 (2023).

321. Grunebaum, M. F. et al. Ketamine versus midazolam in bipolar depression with suicidal thoughts: A pilot midazolam-controlled randomized clinical trial. Bipolar Disord. 19, 176–183 (2017).

322. Jiang, H. et al. Plasma Levels of Brain-Derived Neurotrophic Factor and S100B in Relation to Antidepressant Response to Ketamine. Front. Neurosci. 15, 698633 (2021).

323. Zheng, W. et al. Baseline Plasma BDNF Levels are Associated with Antianhedonic Effects of Repeated-dose Intravenous Ketamine in Major Depressive Disorder. Curr. Neuropharmacol. (2022) doi:10.2174/1570159X20666220927085706.

324. Zheng, W. et al. Association between plasma levels of BDNF and the antisuicidal effects of repeated ketamine infusions in depression with suicidal ideation. Ther. Adv. Psychopharmacol. 10, 2045125320973794 (2020).

325. Jiang, M. et al. Effect of intraoperative application of ketamine on postoperative depressed mood in patients undergoing elective orthopedic surgery. J. Anesth. 30, 232–237 (2016).

326. Liu, P. et al. Effect of Pretreatment of S-Ketamine On Postoperative Depression for Breast Cancer Patients. J. Invest. Surg. 34, 883–888 (2021).

327. Zheng, W. et al. Serum BDNF levels and the antidepressant effects of electroconvulsive therapy with ketamine anaesthesia: a preliminary study. PeerJ 9, e10699 (2021).

328. Wang, J. et al. Use of Various Doses of S-Ketamine in Treatment of Depression and Pain in Cervical Carcinoma Patients with Mild/Moderate Depression After Laparoscopic Total Hysterectomy. Med. Sci. Monit. Int. Med. J. Exp. Clin. Res. 26, e922028-1-e922028-6 (2020).

329. Carspecken, C. W. et al. Ketamine Anesthesia Does Not Improve Depression Scores in Electroconvulsive Therapy: A Randomized Clinical Trial. J. Neurosurg. Anesthesiol. 30, 305–313 (2018).

330. Ballard, E. D. & Zarate, C. A. The role of dissociation in ketamine's antidepressant effects. Nat. Commun. 11, 6431 (2020).

331. Acevedo-Diaz, E. E. et al. Comprehensive assessment of side effects associated with a single dose of ketamine in treatment-resistant depression. J. Affect. Disord. 263, 568–575 (2020).

332. Garel, N. et al. The Montreal model: an integrative biomedical-psychedelic approach to ketamine for severe treatment-resistant depression. Front. Psychiatry 14, 1268832 (2023).

333. Wilkinson, S. T. et al. A Survey of the Clinical, Off-Label Use of Ketamine as a Treatment for Psychiatric Disorders. Am. J. Psychiatry 174, 695–696 (2017).

334. Harborne, G. C., Watson, F. L., Healy, D. T. & Groves, L. The effects of sub-anaesthetic doses of ketamine on memory, cognitive performance and subjective experience in healthy volunteers. J. Psychopharmacol. (Oxf.) 10, 134–140 (1996).

335. Griffiths, C., Walker, K., Reid, I., da Silva, K. M. & O'Neill-Kerr, A. A qualitative study of patients' experience of ketamine treatment for depression: The 'Ketamine and me' project. J. Affect. Disord. Rep. 4, 100079 (2021).

336. Muetzelfeldt, L. et al. Journey through the K-hole: Phenomenological aspects of ketamine use. Drug Alcohol Depend. 95, 219–229 (2008).

337. van Schalkwyk, G. I., Wilkinson, S. T., Davidson, L., Silverman, W. K. & Sanacora, G. Acute psychoactive effects of intravenous ketamine during treatment of mood disorders: Analysis of the Clinician Administered Dissociative State Scale. J. Affect. Disord. 227, 11–16 (2018).

338. Sarasso, S. et al. Consciousness and Complexity during Unresponsiveness Induced by Propofol, Xenon, and Ketamine. Curr. Biol. CB 25, 3099–3105 (2015).

339. Grabski, M., Borissova, A., Marsh, B., Morgan, C. J. A. & Curran, H. V. Ketamine as a mental health treatment: Are acute psychoactive effects associated with outcomes? A systematic review. Behav. Brain Res. 392, 112629 (2020).

340. Bremner, J. D. et al. Measurement of dissociative states with the Clinician-Administered Dissociative States Scale (CADSS). J. Trauma. Stress 11, 125–136 (1998).

341. Krystal, J. H. et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214 (1994).

342. Dittrich, A. The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. Pharmacopsychiatry 31 Suppl 2, 80–84 (1998).

343. Studerus, E., Gamma, A. & Vollenweider, F. X. Psychometric evaluation of the altered states of consciousness rating scale (OAV). PloS One 5, e12412 (2010).

344. Mathai, D. S., Meyer, M. J., Storch, E. A. & Kosten, T. R. The relationship between subjective effects induced by a single dose of ketamine and treatment response in patients with major depressive disorder: A systematic review. J. Affect. Disord. 264, 123–129 (2020).

345. Aust, S. et al. Anxiety during ketamine infusions is associated with negative treatment responses in major depressive disorder. Eur. Neuropsychopharmacol. 29, 529–538 (2019).

346. Sumner, R. L. et al. A qualitative and quantitative account of patient's experiences of ketamine and its antidepressant properties. J. Psychopharmacol. Oxf. Engl. 35, 946–961 (2021).

347. Buzsáki, G. & Draguhn, A. Neuronal oscillations in cortical networks. Science 304, 1926–1929 (2004).

348. He, B. J., Zempel, J. M., Snyder, A. Z. & Raichle, M. E. The Temporal Structures and Functional Significance of Scale-free Brain Activity. Neuron 66, 353–369 (2010).

349. McMillan, R. & Muthukumaraswamy, S. D. The neurophysiology of ketamine: an integrative review. Rev. Neurosci. 31, 457–503 (2020).

350. Hyafil, A., Giraud, A.-L., Fontolan, L. & Gutkin, B. Neural Cross-Frequency Coupling: Connecting Architectures, Mechanisms, and Functions. Trends Neurosci. 38, 725–740 (2015).

351. Kane, N. et al. A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. Revision 2017. Clin. Neurophysiol. Pract. 2, 170–185 (2017).

352. Colgin, L. L. Mechanisms and Functions of Theta Rhythms. Annu. Rev. Neurosci. 36, 295–312 (2013).

353. Herrmann, C. S. & Knight, R. T. Mechanisms of human attention: event-related potentials and oscillations. Neurosci. Biobehav. Rev. 25, 465–476 (2001).

354. Bollimunta, A., Chen, Y., Schroeder, C. E. & Ding, M. Neuronal Mechanisms of Cortical Alpha Oscillations in Awake-Behaving Macaques. J. Neurosci. 28, 9976–9988 (2008).

355. Tiesinga, P. & Sejnowski, T. J. Cortical Enlightenment: Are Attentional Gamma Oscillations Driven by ING or PING? Neuron 63, 727–732 (2009).

356. Carlén, M. et al. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. Mol. Psychiatry 17, 537–548 (2012).

357. Atallah, B. V. & Scanziani, M. Instantaneous modulation of gamma oscillation frequency by balancing excitation with inhibition. Neuron 62, 566–577 (2009).

358. Knott, V. J. et al. Separate and combined effects of low dose ketamine and nicotine on behavioural and neural correlates of sustained attention. Biol. Psychol. 88, 83–93 (2011).

359. Shaw, A. D. et al. Ketamine amplifies induced gamma frequency oscillations in the human cerebral cortex. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 25, 1136–1146 (2015).

360. de la Salle, S. et al. Effects of Ketamine on Resting-State EEG Activity and Their Relationship to Perceptual/Dissociative Symptoms in Healthy Humans. Front. Pharmacol. 7, 348 (2016).

361. Forsyth, A. E. M., McMillan, R., Dukart, J., Hipp, J. F. & Muthukumaraswamy, S. D. Effects of Ketamine and Midazolam on Simultaneous EEG/fMRI Data During Working Memory Processes. Brain Topogr. 34, 863–880 (2021).

362. Vlisides, P. E. et al. Subanaesthetic ketamine and altered states of consciousness in humans. Br. J. Anaesth. 121, 249–259 (2018).

363. Blain-Moraes, S., Lee, U., Ku, S., Noh, G. & Mashour, G. A. Electroencephalographic effects of ketamine on power, cross-frequency coupling, and connectivity in the alpha bandwidth. Front. Syst. Neurosci. 8, (2014).

364. Muthukumaraswamy, S. D. et al. Evidence that Subanesthetic Doses of Ketamine Cause Sustained Disruptions of NMDA and AMPA-Mediated Frontoparietal Connectivity in Humans. J. Neurosci. Off. J. Soc. Neurosci. 35, 11694–11706 (2015).

365. Forsyth, A. et al. Comparison of local spectral modulation, and temporal correlation, of simultaneously recorded EEG/fMRI signals during ketamine and midazolam sedation. Psychopharmacology (Berl.) 235, 3479–3493 (2018).

366. Vlisides, P. E. et al. Neurophysiologic Correlates of Ketamine Sedation and Anesthesia: A High-density Electroencephalography Study in Healthy Volunteers. Anesthesiology 127, 58–69 (2017).

367. Zacharias, N. et al. Ketamine effects on default mode network activity and vigilance: A randomized, placebo-controlled crossover simultaneous fMRI/EEG study. Hum. Brain Mapp. 41, 107–119 (2020).

368. Knott, V. et al. Nicotine and smoker status moderate brain electric and mood activation induced by ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist. Pharmacol. Biochem. Behav. 85, 228–242 (2006).

369. Rivolta, D. et al. Ketamine Dysregulates the Amplitude and Connectivity of High-Frequency Oscillations in Cortical-Subcortical Networks in Humans: Evidence From Resting-State Magnetoencephalography-Recordings. Schizophr. Bull. 41, 1105–1114 (2015).

370. Tian, F. et al. Characterizing brain dynamics during ketamine-induced dissociation and subsequent interactions with propofol using human intracranial neurophysiology. Nat. Commun. 14, 1748 (2023).

371. Lijffijt, M. et al. Identification of an optimal dose of intravenous ketamine for late-life treatment-resistant depression: a Bayesian adaptive randomization trial. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 47, 1088–1095 (2022).

372. de la Salle, S., Phillips, J. L., Blier, P. & Knott, V. Electrophysiological correlates and predictors of the antidepressant response to repeated ketamine infusions in treatment-resistant depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 115, 110507 (2022).

373. McMillan, R. et al. Simultaneous EEG/fMRI recorded during ketamine infusion in patients with major depressive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 99, 109838 (2020).

374. Stam, C. J. Nonlinear dynamical analysis of EEG and MEG: review of an emerging field. Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol. 116, 2266–2301 (2005).

375. Miller, K. J., Sorensen, L. B., Ojemann, J. G. & Nijs, M. den. Power-Law Scaling in the Brain Surface Electric Potential. PLOS Comput. Biol. 5, e1000609 (2009).

376. Beggs, J. M. The criticality hypothesis: how local cortical networks might optimize information processing. Philos. Trans. R. Soc. Math. Phys. Eng. Sci. 366, 329–343 (2007).

377. Lai, M.-C. et al. A shift to randomness of brain oscillations in people with autism. Biol. Psychiatry 68, 1092–1099 (2010).

378. Maxim, V. et al. Fractional Gaussian noise, functional MRI and Alzheimer's disease. NeuroImage 25, 141–158 (2005).

379. Wei, M. et al. Identifying major depressive disorder using Hurst exponent of resting-state brain networks. Psychiatry Res. 214, 306–312 (2013).

380. Voytek, B. et al. Age-Related Changes in 1/f Neural Electrophysiological Noise. J. Neurosci. Off. J. Soc. Neurosci. 35, 13257–13265 (2015).

381. Wink, A. M., Bernard, F., Salvador, R., Bullmore, E. & Suckling, J. Age and cholinergic effects on hemodynamics and functional coherence of human hippocampus. Neurobiol. Aging 27, 1395–1404 (2006).

382. Barnes, A., Bullmore, E. T. & Suckling, J. Endogenous Human Brain Dynamics Recover Slowly Following Cognitive Effort. PLOS ONE 4, e6626 (2009).

383. Churchill, N. W. et al. The suppression of scale-free fMRI brain dynamics across three different sources of effort: aging, task novelty and task difficulty. Sci. Rep. 6, 30895 (2016).

384. Bongers, A., Flynn, A. B. & Northoff, G. Is learning scale-free? Chemistry learning increases EEG fractal power and changes the power law exponent. Neurosci. Res. 156, 165–177 (2020).

385. Sheehan, T. C., Sreekumar, V., Inati, S. K. & Zaghloul, K. A. Signal Complexity of Human Intracranial EEG Tracks Successful Associative-Memory Formation across Individuals. J. Neurosci. Off. J. Soc. Neurosci. 38, 1744–1755 (2018).

386. Wolff, A. et al. The temporal signature of self: Temporal measures of resting-state EEG predict self-consciousness. Hum. Brain Mapp. 40, 789–803 (2019).

387. Muthukumaraswamy, S. D. & Liley, D. TJ. 1/f electrophysiological spectra in resting and drug-induced states can be explained by the dynamics of multiple oscillatory relaxation processes. NeuroImage 179, 582–595 (2018).

388. Schartner, M. et al. Complexity of Multi-Dimensional Spontaneous EEG Decreases during Propofol Induced General Anaesthesia. PloS One 10, e0133532 (2015).

389. Schartner, M. M., Carhart-Harris, R. L., Barrett, A. B., Seth, A. K. & Muthukumaraswamy, S. D. Increased spontaneous MEG signal diversity for psychoactive doses of ketamine, LSD and psilocybin. Sci. Rep. 7, 46421 (2017).

390. Cortes-Briones, J. A. et al. The Psychosis-like Effects of Δ9-Tetrahydrocannabinol Are Associated With Increased Cortical Noise in Healthy Humans. Biol. Psychiatry 78, 805–813 (2015).

391. Timmermann, C. et al. Human brain effects of DMT assessed via EEG-fMRI. Proc. Natl. Acad. Sci. U. S. A. 120, e2218949120 (2023).

392. Timmermann, C. et al. Neural correlates of the DMT experience assessed with multivariate EEG. Sci. Rep. 9, 16324 (2019).

393. Carhart-Harris, R. L. The entropic brain - revisited. Neuropharmacology 142, 167–178 (2018).

394. Farnes, N., Juel, B. E., Nilsen, A. S., Romundstad, L. G. & Storm, J. F. Increased signal diversity/complexity of spontaneous EEG, but not evoked EEG responses, in ketamine-induced psychedelic state in humans. PloS One 15, e0242056 (2020).

395. Li, D. & Mashour, G. A. Cortical dynamics during psychedelic and anesthetized states induced by ketamine. NeuroImage 196, 32–40 (2019).

396. Casali, A. G. et al. A Theoretically Based Index of Consciousness Independent of Sensory Processing and Behavior. Sci. Transl. Med. 5, 198ra105-198ra105 (2013).

397. Murphy, N. et al. Neural complexity EEG biomarkers of rapid and post-rapid ketamine effects in late-life treatment-resistant depression: a randomized control trial. Neuropsychopharmacology 48, 1586–1593 (2023).

398. Lavender, E., Hirasawa-Fujita, M. & Domino, E. F. Ketamine's dose related multiple mechanisms of actions: Dissociative anesthetic to rapid antidepressant. Behav. Brain Res. 390, 112631 (2020).

399. Oostenveld, R., Fries, P., Maris, E. & Schoffelen, J.-M. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput. Intell. Neurosci. 2011, 156869 (2011).

400. Wen, H. & Liu, Z. Separating Fractal and Oscillatory Components in the Power Spectrum of Neurophysiological Signal. Brain Topogr. 29, 13–26 (2016).

401. Kaspar, F. & Schuster, H. G. Easily calculable measure for the complexity of spatiotemporal patterns. Phys. Rev. A 36, 842–848 (1987).

402. Mediano, P. A. M. et al. Spectrally and temporally resolved estimation of neural signal diversity. eLife 12, (2023).

403. Maris, E. & Oostenveld, R. Nonparametric statistical testing of EEG- and MEG-data. J. Neurosci. Methods 164, 177–190 (2007).

404. Sassenhagen, J. & Draschkow, D. Cluster-based permutation tests of MEG/EEG data do not establish significance of effect latency or location. Psychophysiology 56, e13335 (2019).

405. Pinault, D. N-methyl d-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. Biol. Psychiatry 63, 730–735 (2008).

406. Susin, E. & Destexhe, A. A Network Model of the Modulation of γ Oscillations by NMDA Receptors in Cerebral Cortex. eNeuro 10, ENEURO.0157-23.2023 (2023).

407. Ma, J. & Leung, L. S. The supramammillo-septal-hippocampal pathway mediates sensorimotor gating impairment and hyperlocomotion induced by MK-801 and ketamine in rats. Psychopharmacology (Berl.) 191, 961–974 (2007).

408. de Aguiar Neto, F. S. & Rosa, J. L. G. Depression biomarkers using non-invasive EEG: A review. Neurosci. Biobehav. Rev. 105, 83–93 (2019).

409. Grin-Yatsenko, V. A., Baas, I., Ponomarev, V. A. & Kropotov, J. D. Independent component approach to the analysis of EEG recordings at early stages of depressive disorders. Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol. 121, 281–289 (2010).

410. Fitzgerald, P. J. & Watson, B. O. Gamma oscillations as a biomarker for major depression: an emerging topic. Transl. Psychiatry 8, 177 (2018).

411. Hipp, J. F. & Siegel, M. Dissociating neuronal gamma-band activity from cranial and ocular muscle activity in EEG. Front. Hum. Neurosci. 7, 338 (2013).

412. Gao, R., Peterson, E. J. & Voytek, B. Inferring synaptic excitation/inhibition balance from field potentials. NeuroImage 158, 70–78 (2017).

413. Lau, Z. J., Pham, T., Chen, S. H. A. & Makowski, D. Brain entropy, fractal dimensions and predictability: A review of complexity measures for EEG in healthy and neuropsychiatric populations. Eur. J. Neurosci. 56, 5047–5069 (2022).

414. Méndez, M. A. et al. Complexity analysis of spontaneous brain activity: effects of depression and antidepressant treatment. J. Psychopharmacol. Oxf. Engl. 26, 636–643 (2012).

415. He, B. J. Scale-free brain activity: past, present, and future. Trends Cogn. Sci. 18, 480–487 (2014).

416. Timmermann, C. et al. A neurophenomenological approach to non-ordinary states of consciousness: hypnosis, meditation, and psychedelics. Trends Cogn. Sci. 27, 139–159 (2023).

417. Kopelman, J. et al. Rapid neuroplasticity changes and response to intravenous ketamine: a randomized controlled trial in treatment-resistant depression. Transl. Psychiatry 13, 1–9 (2023).

418. Abdallah, C. G. et al. The Nucleus Accumbens and Ketamine Treatment in Major Depressive Disorder. Neuropsychopharmacology 42, 1739–1746 (2017).

419. Zhou, Y.-L. et al. Volumetric changes in subcortical structures following repeated ketamine treatment in patients with major depressive disorder: a longitudinal analysis. Transl. Psychiatry 10, 1–9 (2020).

420. Gallay, C. C. et al. Six-week oral ketamine treatment for chronic suicidality is associated with increased grey matter volume. Psychiatry Res. Neuroimaging 317, 111369 (2021).

421. Dai, D. et al. Ketamine Normalizes the Structural Alterations of Inferior Frontal Gyrus in Depression. Chronic Stress Thousand Oaks Calif 4, 2470547020980681 (2020).

422. Nabulsi, N. B. et al. Synthesis and Preclinical Evaluation of 11C-UCB-J as a PET Tracer for Imaging the Synaptic Vesicle Glycoprotein 2A in the Brain. J. Nucl. Med. 57, 777–784 (2016).

423. Chen, M.-K. et al. Comparison of [11C]UCB-J and [18F]FDG PET in Alzheimer's disease: A tracer kinetic modeling study. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 41, 2395–2409 (2021).

424. Radhakrishnan, R. et al. In vivo evidence of lower synaptic vesicle density in schizophrenia. Mol. Psychiatry 26, 7690–7698 (2021).

425. Holmes, S. E. et al. Lower synaptic density is associated with depression severity and network alterations. Nat. Commun. 10, 1529 (2019).

426. Holmes, S. E. et al. Imaging the effect of ketamine on synaptic density (SV2A) in the living brain. Mol. Psychiatry 27, 2273–2281 (2022).

427. Fava, M. et al. Double-blind, placebo-controlled, dose-ranging trial of intravenous ketamine as adjunctive therapy in treatment-resistant depression (TRD). Mol. Psychiatry 25, 1592–1603 (2020).

428. Overall, J. E. & Gorham, D. R. The Brief Psychiatric Rating Scale. Psychol. Rep. 10, 799–812 (1962).

429. Kowalski, R., Mahon, P., Boylan, G., McNamara, B. & Shorten, G. Validity of the modified observer's assessment of alertness/sedation scale (MOAA/S) during low dose propofol sedation: 3AP6-3. Eur. J. Anaesthesiol. EJA 24, 26 (2007).

430. Wickramasekera II, I. E. Empathic Features of Absorption and Incongruence. Am. J. Clin. Hypn. 50, 59–69 (2007).

431. Rolffs, J. L., Rogge, R. D. & Wilson, K. G. Disentangling Components of Flexibility via the Hexaflex Model: Development and Validation of the Multidimensional Psychological Flexibility Inventory (MPFI). Assessment 25, 458–482 (2018).

432. Christopher, M. S., Neuser, N. J., Michael, P. G. & Baitmangalkar, A. Exploring the psychometric properties of the Five Facet Mindfulness Questionnaire. Mindfulness 3, 124–131 (2012).

433. Gámez, W. et al. The brief experiential avoidance questionnaire: development and initial validation. Psychol. Assess. 26, 35–45 (2014).

434. Barrett, F. S., Johnson, M. W. & Griffiths, R. R. Validation of the revised Mystical Experience Questionnaire in experimental sessions with psilocybin. J. Psychopharmacol. Oxf. Engl. 29, 1182–1190 (2015).

435. Barrett, F. S., Bradstreet, M. P., Leoutsakos, J.-M. S., Johnson, M. W. & Griffiths, R. R. The Challenging Experience Questionnaire: Characterization of challenging experiences with psilocybin mushrooms. J. Psychopharmacol. Oxf. Engl. 30, 1279–1295 (2016).

436. Mason, O. J., Morgan, C. J. M., Stefanovic, A. & Curran, H. V. The psychotomimetic states inventory (PSI): measuring psychotic-type experiences from ketamine and cannabis. Schizophr. Res. 103, 138–142 (2008).

437. Roseman, L. et al. Emotional breakthrough and psychedelics: Validation of the Emotional Breakthrough Inventory. J. Psychopharmacol. Oxf. Engl. 33, 1076–1087 (2019).

438. Nour, M. M., Evans, L., Nutt, D. & Carhart-Harris, R. L. Ego-Dissolution and Psychedelics: Validation of the Ego-Dissolution Inventory (EDI). Front. Hum. Neurosci. 10, 190474 (2016).

439. Rodrigues, N. B. et al. A simplified 6-Item clinician administered dissociative symptom scale (CADSS-6) for monitoring dissociative effects of sub-anesthetic ketamine infusions. J. Affect. Disord. 282, 160–164 (2021).

440. Rossano, S. et al. Assessment of a white matter reference region for 11C-UCB-J PET quantification. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 40, 1890–1901 (2020).

441. Johansen, A. et al. Effects of escitalopram on synaptic density in the healthy human brain: a randomized controlled trial. Mol. Psychiatry 1–8 (2023) doi:10.1038/s41380-023-02285-8.

442. Li, N. et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol. Psychiatry 69, 754–761 (2011).

443. Müller, H. K. et al. Ketamine regulates the presynaptic release machinery in the hippocampus. J. Psychiatr. Res. 47, 892–899 (2013).

444. Onwordi, E. C. et al. Synaptic Terminal Density Early in the Course of Schizophrenia: An In Vivo UCB-J Positron Emission Tomographic Imaging Study of Synaptic Vesicle Glycoprotein 2A. Biol. Psychiatry (2023) doi:10.1016/j.biopsych.2023.05.022.

445. Ibanez, A., Kringelbach, M. L. & Deco, G. A synergetic turn in cognitive neuroscience of brain diseases. Trends Cogn. Sci. S1364-6613(23)00306–6 (2024) doi:10.1016/j.tics.2023.12.006.

446. Bhattacharya, A. et al. An Overview of Noninvasive Brain Stimulation: Basic Principles and Clinical Applications. Can. J. Neurol. Sci. J. Can. Sci. Neurol. 49, 479–492 (2022).

447. Deco, G. & Kringelbach, M. L. Great expectations: using whole-brain computational connectomics for understanding neuropsychiatric disorders. Neuron 84, 892–905 (2014).

448. Violante, I. R. et al. Non-invasive temporal interference electrical stimulation of the human hippocampus. 2022.09.14.507625 Preprint at https://doi.org/10.1101/2022.09.14.507625 (2022).

449. Nasr, K. et al. Breaking the boundaries of interacting with the human brain using adaptive closed-loop stimulation. Prog. Neurobiol. 216, 102311 (2022).

450. Carhart-Harris, R. L. et al. Psychedelics and the essential importance of context. J. Psychopharmacol. Oxf. Engl. 32, 725–731 (2018).

451. Mediano, P. A. M. et al. Effects of External Stimulation on Psychedelic State Neurodynamics. ACS Chem. Neurosci. (2024) doi:10.1021/acschemneuro.3c00289.

452. Weiss, B., Sleep, C. E., Beller, N. M., Erritzoe, D. & Campbell, W. K. Perceptions of psychedelic personality change, determinants of use, setting and drug moderation: Toward a holistic model. J. Psychedelic Stud. 7, 200–226 (2023).

453. Hartogsohn, I. Set and setting, psychedelics and the placebo response: An extra-pharmacological perspective on psychopharmacology. J. Psychopharmacol. (Oxf.) 30, 1259–1267 (2016).

454. Bernstein, M. H. & Brown, W. A. The placebo effect in psychiatric practice. Curr. Psychiatry 16, 29–34 (2017).

455. Branchi, I. Interplay between plasticity, environment and depression. Eur. Psychiatry 66, S15–S15 (2023).

456. Poggini, S. et al. Subjective experience of the environment determines serotoninergic antidepressant treatment outcome in male mice. J. Affect. Disord. 350, 900–908 (2024).

457. Alboni, S. et al. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol. Psychiatry 22, 552–561 (2017).

458. Greenway, K. T., Garel, N., Goyette, N., Turecki, G. & Richard-Devantoy, S. Adjunctive music improves the tolerability of intravenous ketamine for bipolar depression. Int. Clin. Psychopharmacol. 36, 218–220 (2021).

459. Kaelen, M. et al. The hidden therapist: evidence for a central role of music in psychedelic therapy. Psychopharmacology (Berl.) 235, 505–519 (2018).

460. Ardito, R. B. & Rabellino, D. Therapeutic Alliance and Outcome of Psychotherapy: Historical Excursus, Measurements, and Prospects for Research. Front. Psychol. 2, 270 (2011).

461. Zeifman, R. J. et al. Preliminary evidence for the importance of therapeutic alliance in MDMA-assisted psychotherapy for posttraumatic stress disorder. Eur. J. Psychotraumatology 15, 2297536 (2024).

462. Murphy, R. et al. Therapeutic Alliance and Rapport Modulate Responses to Psilocybin Assisted Therapy for Depression. Front. Pharmacol. 12, (2022).

463. Garel, N. et al. Imprinting: expanding the extra-pharmacological model of psychedelic drug action to incorporate delayed influences of sets and settings. Front. Hum. Neurosci. 17, 1200393 (2023).

464. Girn, M. et al. A complex systems perspective on psychedelic brain action. Trends Cogn. Sci. 27, 433–445 (2023).

465. Schmitz, G. P., Jain, M. K., Slocum, S. T. & Roth, B. L. 5-HT2A SNPs Alter the Pharmacological Signaling of Potentially Therapeutic Psychedelics. ACS Chem. Neurosci. 13, 2386–2398 (2022).

466. Tsuang, M. T., Bar, J. L., Stone, W. S. & Faraone, S. V. Gene-environment interactions in mental disorders. World Psychiatry 3, 73–83 (2004).

467. Uddin, M., Jansen, S. & Telzer, E. H. Adolescent depression linked to socioeconomic status? Molecular approaches for revealing premorbid risk factors. BioEssays 39, 1600194 (2017).

468. Generaal, E., Timmermans, E. J., Dekkers, J. E. C., Smit, J. H. & Penninx, B. W. J. H. Not urbanization level but socioeconomic, physical and social neighbourhood characteristics are associated with presence and severity of depressive and anxiety disorders. Psychol. Med. 49, 149–161 (2019).

469. Bui, Q. T. T., Vu, L. T. H. & Tran, D. M. Trajectories of depression in adolescents and young adults in Vietnam during rapid urbanisation : evidence from a longitudinal study. J. Child Adolesc. Ment. Health 30, 51–59 (2018).

470. Pun, V. C., Manjourides, J. & Suh, H. H. Close proximity to roadway and urbanicity associated with mental ill-health in older adults. Sci. Total Environ. 658, 854–860 (2019).

471. Arvind, B. A. et al. Prevalence and socioeconomic impact of depressive disorders in India: multisite population-based cross-sectional study. BMJ Open 9, e027250 (2019).

472. Borroni, E., Pesatori, A. C., Bollati, V., Buoli, M. & Carugno, M. Air pollution exposure and depression: A comprehensive updated systematic review and meta-analysis. Environ. Pollut. 292, 118245 (2022).

473. The Good Life. (2023).

474. Springer, K. W., Sheridan, J., Kuo, D. & Carnes, M. The Long-term Health Outcomes of Childhood Abuse. J. Gen. Intern. Med. 18, 864–870 (2003).

475. Penza, K. M., Heim, C. & Nemeroff, C. B. Neurobiological effects of childhood abuse: implications for the pathophysiology of depression and anxiety. Arch. Womens Ment. Health 6, 15–22 (2003).

476. Watts, R., Day, C., Krzanowski, J., Nutt, D. & Carhart-Harris, R. Patients' Accounts of Increased "Connectedness" and "Acceptance" After Psilocybin for Treatment-Resistant Depression. J. Humanist. Psychol. 57, 520–564 (2017).

477. Ge, L., Yap, C. W., Ong, R. & Heng, B. H. Social isolation, loneliness and their relationships with depressive symptoms: A population-based study. PLOS ONE 12, e0182145 (2017).

478. Saveanu, R. V. & Nemeroff, C. B. Etiology of Depression: Genetic and Environmental Factors. Psychiatr. Clin. 35, 51–71 (2012).

479. Steer, M. D. Cybernetics: Circular Causal and Feedback Mechanisms in Biological and Social Systems. Transactions of the Seventh Conference, March 23-24, 1950, New York. Heinz von

Foerster, Ed. New York: Josiah Macy, Jr. Foundation, 1951. 251 pp. $3.50. Science 115, 100–100 (1952).

480. Bateson, G. Steps to an Ecology of Mind: Collected Essays in Anthropology, Psychiatry, Evolution, and Epistemology. (University of Chicago Press, Chicago, IL, 2000).

481. Friston, K. The free-energy principle: a unified brain theory? Nat. Rev. Neurosci. 11, 127–138 (2010).

482. Barutta, J., Gleichgerrcht, E., Cornejo, C. & Ibáñez, A. Neurodynamics of Mind: The Arrow Illusion of Conscious Intentionality as Downward Causation. Integr. Psychol. Behav. Sci. 44, 127–143 (2010).

483. Seth, A. K. & Bayne, T. Theories of consciousness. Nat. Rev. Neurosci. 23, 439–452 (2022).

484. Laukkonen, R. E. & Slagter, H. A. From many to (n)one: Meditation and the plasticity of the predictive mind. Neurosci. Biobehav. Rev. 128, 199–217 (2021).

485. Carhart-Harris, R. L. et al. Canalization and plasticity in psychopathology. Neuropharmacology 226, 109398 (2023).

486. Kyzar, E. J. & Denfield, G. H. Taking subjectivity seriously: towards a unification of phenomenology, psychiatry, and neuroscience. Mol. Psychiatry 28, 10–16 (2023).

487. Yaden, D. B. & Griffiths, R. R. The Subjective Effects of Psychedelics Are Necessary for Their Enduring Therapeutic Effects. ACS Pharmacol. Transl. Sci. 4, 568–572 (2020).

488. Cameron, L. P. et al. A Non-Hallucinogenic Psychedelic Analog with Therapeutic Potential. Nature 589, 474–479 (2021).

489. Kaplan, A. L. et al. Bespoke library docking for 5-HT2A receptor agonists with antidepressant activity. Nature 610, 582–591 (2022).

490. McCulloch, D. E.-W. et al. Psilocybin-Induced Mystical-Type Experiences are Related to Persisting Positive Effects: A Quantitative and Qualitative Report. Front. Pharmacol. 13, (2022).

491. Varela, F. J. Neurophenomenology: A Methodological Remedy for the Hard Problem. J. Conscious. Stud. 3, 330–49 (1996).

492. Ramstead, M. J. D. et al. From Generative Models to Generative Passages: A Computational Approach to (Neuro) Phenomenology. Rev. Philos. Psychol. 13, 829–857 (2022).

493. Thaipisuttikul, P., Ittasakul, P., Waleeprakhon, P., Wisajun, P. & Jullagate, S. Psychiatric comorbidities in patients with major depressive disorder. Neuropsychiatr. Dis. Treat. 10, 2097–2103 (2014).

494. Rosas, F. E. et al. Reconciling emergences: An information-theoretic approach to identify causal emergence in multivariate data. PLOS Comput. Biol. 16, e1008289 (2020).

495. Seager, W. Consciousness, information and panpsychism. J. Conscious. Stud. 2, 272–288 (1995).

496. Clark, A. & Chalmers, D. J. The Extended Mind. Analysis 58, 7–19 (1998).

497. Whitehead, A. N. Process and Reality. xii, 545 (Macmillan, Oxford, England, 1929).

498. Rovelli, C. Helgoland: Making Sense of the Quantum Revolution. (Riverhead Books, New York, 2021).

499. Sharma, A. et al. Assembly theory explains and quantifies selection and evolution. Nature 622, 321–328 (2023).

500. Louie, A. H. Relational Biology. in Handbook of Anticipation: Theoretical and Applied Aspects of the Use of Future in Decision Making (ed. Poli, R.) 191–218 (Springer International Publishing, Cham, 2019). doi:10.1007/978-3-319-91554-8_17.

501. Hammen, C. L. & Shih, J. Depression and interpersonal processes. in Handbook of depression, 3rd ed 277–295 (The Guilford Press, New York, NY, US, 2014). doi:10.1097/00005053-200301000-00022.

502. Gomez-Marin, A. A history of the metaphorical brain. Science 368, 375–375 (2020).

503. Box, G. E. P. Robustness in the Strategy of Scientific Model Building. in Robustness in Statistics (eds. Launer, R. L. & Wilkinson, G. N.) 201–236 (Academic Press, 1979). doi:10.1016/B978-0-12-438150-6.50018-2.