Investigation of the rapid-acting antidepressant-like effects of reelin in parallel to ketamine

by

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Bachelor of Arts, York University, 2018

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Abstract

Ketamine, an *N*-methyl-D-aspartate antagonist, presented one of the first major breakthroughs in decades of antidepressant research. Ketamine's clinical effects take place within hours in patients with treatment-resistant depression. It is also able to ameliorate some of the most stubborn symptoms such as anhedonia and suicidal ideation. However, the exact underlying mechanisms are still yet unknown. In addition, extrapyramidal side effects and potential health risks limit the use of ketamine to certain populations.

Reelin, an extracellular matrix glycoprotein, has been demonstrated to be downregulated in the hippocampus of patients with depression. Preliminary research from our lab has demonstrated that exogenous reelin administration may have fast-acting antidepressant effects, though its signaling pathways and effects on synaptic plasticity are still unknown. However, a significant amount of research on fast-acting antidepressants has directed attention to the upregulation of excitatory signaling in areas such as the hippocampus, through various cellular signaling pathways such as mTORC1 activation. This increase in excitatory signaling, particularly through increases in AMPAR transmission, has been heavily implicated in ketamine's antidepressant, but not dissociative, effects.

With a lens to increasing the translatability of findings from bench to bedside, this dissertation first discusses the inclusion of people with lived experience in the earliest stages of research. Following this, the underlying mechanisms of reelin are researched in a variety of methods in order to provide a broad picture of reelin as a putative rapid-acting antidepressant.

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Glossary of abbreviations

(2R,6R)-HNK	(2R,6R)-hydroxynorketamine
5-HT	5-hydroxytryptamine (serotonin)
5-HT_{2A}R	Serotonin 2A receptor
ABC	Avidin-biotin complex
ACTH	Adrenocorticotrophic hormone
AMPA/R	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid / receptor
ANOVA	Analysis of variance
BDI	Beck depression inventory
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
CA	Cornu ammonis
cAMP	Cyclic adenosine monophosphate
CCL5	C-C motif chemokine 5
CSF	Cerebrospinal fluid
CORT	Corticosterone
CREB	cAMP response element-binding protein
CRH	Corticotropin-releasing hormone
DAB	3'-diaminobenzidine
Dab1	Disabled-1
DCX	Doublecortin
DG	Dentate gyrus
DSM-5	Diagnostic and statistical manual of mental disorders (5th Edition)
DST	Dexamethasone suppression test
EC	Entorhinal cortex
ECT	Electroconvulsive therapy
EPM	Elevated plus maze
ERK	Extracellular signal-related kinase

FDA	Food and drug administration
fEPSP	Field excitatory post-synaptic potential
FGF-basic	Fibroblast growth factor-basic
FST	Forced swim test
GABA	Gamma-aminobutyric acid
$GABA_{A/B}Rs$	$GABA_A$ or $GABA_B$ receptors
GAD65/67	Glutamate acid decarboxylase-65/67
GCL	Granule cell layer
G-CSF	Granulocyte colony-stimulating factor
GluA1/2	Glutamate A receptor ½
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GR	Glucocorticoid receptor
HDAC	Histone deacetylase
HDRS	Hamilton depression rating scale
HPA	Hypothalamic-pituitary-adrenal
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
ICC	Immunocytochemistry
IHC	Immunohistochemistry
IL	Interleukin
I/O	Input/output
i.p.	Intraperitoneal
IP-10	Interferon gamma-induced protein-10
iPSC	Induced pluripotent stem cell
i.v.	Intravenous
IR	Immunoreactive
JLA	James Lind Alliance
kDa	Kilodalton

LTD	Long-term depression
LTP	Long-term potentiation
MADRS	Montgomery-Åsberg depression rating scale
MAOI	Monoamine oxidase inhibitor
MCP	Monocyte chemoattractant protein
MDD	Major depressive disorder
MIP	Macrophage inflammatory protein
mPFC	Medial prefrontal cortex
MPC	Membrane protein clustering
MR	Mineralocorticoid receptors
mTORC1	Mechanistic target of rapamycin complex 1
NGS	Normal goat serum
NHS	Normal horse serum
NOS	Nitric oxide synthase
NMDA/R	*N*-methyl-D-aspartate/receptor
p-	phosphorylated-
PBS	Phosphate-buffered saline
PDGF	Platelet-derived growth factor
PFC	Prefrontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
PL	Polymorphic layer
POR	Patient-oriented research
PSD-95	Postsynaptic density-95
PTP	Post-tetanic potentiation
PVN	Paraventricular nucleus
PWLE	People with lived experience
RANTES	Regulated upon activation normal T cell expressed and secreted

RR	Reelin repeats
s.c.	Subcutaneous
SEM	Standard error of the mean
SERT	Serotonin transporter
SFKs	Src family of non-receptor tyrosine kinases
SGZ	Subgranular zone
SLC6A4	Solute carrier family 6 member 4
SNP	Synaptoneurosome
SNRI	Serotonin–noradrenaline reuptake inhibitor
SPSS	Statistical package for the social sciences
SPT	Sucrose preference test
SSRI	Selective serotonin reuptake inhibitor
ST	Sucrose Splash Test
SynI	Synapsin I
TBS	Tris-buffered saline
TCA	Tricyclic antidepressant
TNF-α	Tumor necrosis factor-α
TRD	Treatment-resistant depression
TrkB	Tropomyosin receptor kinase B
UCMS	Unpredictable chronic mild stress
VTA	Ventral tegmental area
VEGF	Vascular endothelial growth factor
WB	Western blot

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Chapter 1

Major Depressive Disorder and the search for novel therapeutics

1.1 Dissertation outline

Major Depressive Disorder (MDD) affects an estimated 350 million people, making it the leading cause of disease burden and disability worldwide (Richards, 2011; Zhang, 2010). Characterized by an ongoing depressed mood, anhedonia, cognitive deficits, fatigue and disturbances in sleep and appetite, MDD is a chronic and debilitating disorder. Current numbers estimate a lifetime prevalence rate of 16%, higher than any other neuropsychiatric disorder (Kessler et al., 1997; Lim et al., 2018; Richards, 2011). Despite this prevalence, knowledge on the pathophysiology of depression and effective therapeutic targets remains elusive. One of the largest issues in neuropsychiatric research has been overlapping symptom domains of various disorders (Goldberg, 2011). For example, depression is often diagnosed alongside anxiety, hypertensive, inflammatory, and metabolic disorders, which can worsen outcomes through increased suicidal ideation and higher relapse rates (Hirschfeld, 2001; Kessler et al., 1993, 1997; Krishnan et al., 2002). Other major obstacles include depression's heterogeneity in illness course, biomarkers, treatment-responsiveness, and genetic polymorphisms (Goldberg, 2011). Evidently, depression is a major societal burden that presents unique challenges to the development of appropriate treatments. For this reason, it is imperative that we elucidate the specific biological alterations underlying depression to inform proper biomarkers, therapeutic targets, and even non-pharmacological interventions.

It is essential to include the voices of those studied in all facets of research, particularly in the field of mental health where it has been historically neglected. Underlying stigma of certain diagnoses, such as depression, can affect perceptions of competency and create biases in research (Rüsch et al., 2005). While patient-oriented research (POR) has gained popularity in clinical research (James Lind Alliance, 2020; Lophatananon et al., 2011), there is still an evident gap in patient engagement in foundational lab-based scientific work. Frameworks such as the James Lind Alliance (JLA) priority-setting partnerships have been created to incorporate POR into clinical research, however there is a paucity of guidelines related to the engagement of people with lived experience in laboratory-based scientific research. With these inclusions in mind, this thesis will describe the involvement of patient partners and development of guidelines to better include patient voices at the beginning stages of laboratory-based mental health research.

The monoaminergic hypothesis was one of the first major breakthroughs in the study of depression, stemming from a serendipitous clinical observation that drugs which inhibit the breakdown of monoamines ameliorate depressive symptoms (Domino, 1999). This insight led to the development of the classes of antidepressants which are used as first line treatments today, including selective 5-HT reuptake inhibitors (SSRIs) and 5-HT–noradrenaline reuptake inhibitors (SNRIs). However, there are several limitations to the use of monoaminergic-based antidepressants. After administration, there is a significant therapeutic time-lag for weeks to months of continuous treatment, despite the upregulation of monoamines happening immediately (Warden et al., 2007). Over 30% of patients fail to respond adequately to first-line treatment, necessitating subsequent treatments and further delays (Rush et al., 2006). This is a particular risk for those experiencing suicidal ideation, where a delay in treatment effects could precipitate a suicide attempt (Huynh & McIntyre, 2008). The subset of patients with treatment-resistant depression often have a higher recurrence of depressive symptoms, a more severe course of illness, and increased suicidality, indicating the need for improved and novel therapeutics (Fava, 2003; Fava & Davidson, 1996; Papakostas et al., 2003).

Decades later, research to find novel antidepressant therapeutics and underlying pathophysiology of depression has remained somewhat stagnant. Recently, the discovery that subanesthetic doses of ketamine, a non-competitive allosteric N-methyl-D-aspartate receptor (NMDAR) antagonist has rapid and long-lasting antidepressant effects has renewed interest in discovering novel therapeutic targets (Hirota & Lambert, 2022). Within hours, ketamine can significantly improve depression scores in participants with treatment-resistant depression, a sharp contrast to the therapeutic delay of traditional antidepressants (Berman et al., 2000; Diazgranados et al., 2010; Ibrahim et al., 2011; Kishimoto et al., 2016; Zarate et al., 2006). A surge of preclinical research has followed this discovery, attempting to identify the cellular and molecular mechanisms that underlie ketamine's therapeutic efficacy. One of the key takeaways from this research has been the importance of glutamatergic signaling mediation, particularly an increase in AMPAR transmission (Browne & Lucki, 2013; Monteggia & Zarate, 2015; Zanos et al., 2018b). Certain downstream signaling pathways have also been highlighted, such as mechanistic target of rapamycin complex 1 (mTORC1) signaling, which leads to further enhanced excitatory signaling in the hippocampus and cortex (Li et al., 2010; Zanos et al., 2016; Zanos et al., 2018a; Zanos & Gould, 2018). However, undesirable side effects such as psychotomimetic symptoms, dissociation, and abuse

liability have led research to investigate novel therapeutics that work on similar mechanisms, without the undesirable side effects that are hypothesized to be mediated through ketamine's NMDAR antagonism (Bonaventura et al., 2021a; Kokane et al., 2020).

Throughout the last decade, our lab has shown compelling preclinical evidence that reelin, a large extracellular glycoprotein, is implicated in depression (Caruncho et al., 2016). Reelin plays an essential role in development through guiding proper cortical lamination. In adulthood, reelin appears to regulate many forms of neuroplasticity, such as the generation of new-born cells, the formation of dendritic spines, dendritic outgrowth, and increasing synaptic connections (Beffert et al., 2002; Bosch et al., 2016; Hethorn et al., 2015; Niu et al., 2008; Pujadas et al., 2010; Ventruti et al., 2011; Weeber et al., 2002). In post-mortem tissue from persons with depression, schizophrenia, and bipolar disorder, reelin was significantly downregulated in the hippocampus (Fatemi et al., 2000). Repeated corticosterone (CORT) injections create a progressive emergence of depressive-like behaviour, which is paralleled by decreases in subgranular zone (SGZ) reelin and neurogenesis (Lussier et al., 2013). Heterozygous reeler mice (haplo-insufficient for RELN whom express 40-60% of normal reelin levels) are also more vulnerable to the depressogenic effects of CORT (Lussier et al., 2011). Both conventional and unconventional antidepressants rescue reelin levels in the SGZ decreased through chronic CORT administration while decreasing depressive-like behaviour (Brymer et al., 2018; Fenton et al., 2015; Johnston et al., 2020). More recent findings demonstrate that both chronic and acute peripheral administration of reelin can rescue depressive-like behaviour within 24 hours, making it a promising candidate for a potential fast-acting antidepressant (Allen et al., 2022).

The purpose of this dissertation is to ascertain the molecular mechanisms of reelin's fast-acting antidepressant-like effects in contrast to those of ketamine and its metabolites. To understand these mechanisms, I report the effects that ketamine has on hippocampal reelin and related neurogenesis, how reelin and ketamine impact isolated synaptic compartments from CORT-treated rats, and the behavioural, molecular, and electrophysiological effects of reelin and ketamine in an *in vivo* model to study antidepressant-like effects. To increase the translatability of my results, I delineate guidelines for the further inclusion of patient voices in laboratory-based research. I also report on the effects of reelin and (2R,6R)-HNK, a major ketamine metabolite, on induced pluripotent stem cell-derived neurons from participants with TRD. These experiments lay the groundwork for the

further development of reelin as a potential fast-acting therapeutic, where subsequent studies can determine which proteins are essential to reelin's response and inform subsequent pharmacokinetic and pharmacodynamic studies.

1.2 Overview of Major Depressive Disorder

Depression is the leading cause of disability worldwide, and the most prevalent neuropsychiatric disorder. First described in writing in the Ebers papyrus (1550 BCE), which references symptoms and prognosis of patients with fractured skulls (Kandel, 2013), depression has evidently plagued humanity for millennia. Later, when demonic possession was blamed for psychiatric disturbances, those who were suffering from depressive symptoms had holes drilled into their skulls to allow the demons to vacate (Foerschner, 2010). Even before this, Hippocrates spoke of the four humors, and postulated that an excess of black bile could cause "Malankholia" – or as we know now, melancholia (Hippocrates, 1849) – one of the first to link a physiological state with a psychological phenomenon. The development of psychiatry in the 19th century led to an influx of hypotheses that shifted theories based on demonic possession and emotional weakness to framing depression as a disease of the brain. Even in the past two decades, research on depression and antidepressants has grown exponentially with the invention of new techniques and novel findings, leading us into a new era of depression research (Domino, 1999; Foerschner, 2010).

Worldwide lifetime prevalence rates for depression are approximately 7% - 12% for men and 20% - 25% for women, though this can be difficult to estimate properly due to difficulties in defining and diagnosing depression, as well as variance in access to healthcare globally (Richards, 2011). Despite this variability, rates are consistently 3- to 5-fold higher in females than males, a sexual dimorphism attributed to various biopsychosocial factors (Eid et al., 2019; Labaka et al., 2018). The average age of onset ranges from 18 to 29, with a first episode usually occurring during adolescence, causing major disruptions in critical developmental periods (Kessler & Bromet, 2013). The disease course of MDD is pleomorphic, with significant variability in rates of remission and chronicity. Those with an earlier age of onset also have greater chances of recurrence and higher severity of symptoms (Hollon et al., 2006; Zisook et al., 2004). However, rates of depression are 2-fold higher in older adults (65+) than in younger cohorts (18-65), potentially due to psychosocial factors and high-stress life experiences such as retirement, loss of loved ones, and declining cognitive function. Estimates place the number of people suffering from depression close

to 200-250 million people, however challenges such as people not seeking help or diagnosis, despite meeting diagnostic criteria, can confound these numbers. Depression also has a great economic impact, with the Conference Board of Canada reporting around $32.3 billion in lost gross domestic product (GBD, 2018).

While lowered mood is a common experience for everyone, for a percentage of the population these feelings can become overwhelming and persistent, often accompanied by a constellation of other symptoms which worsen day-to-day mood and functioning (Richards, 2011; Tolentino & Schmidt, 2018). In severe cases, depression can lead to self-harm – and is also the leading cause of suicide at any age (Cuijpers et al., 2014). Deaths by suicide have been increasing over the past few decades, an issue that urgently needs to be addressed (World Health Organization, 2019). As of 2015, depression was responsible for over 50% of psychiatric evaluations and 12% of hospitalizations in the US (Kuo et al., 2015). In addition, depression is often accompanied by serious comorbidities which can influence symptom patterns and disease severity. The most common comorbities include anxiety disorders, hypertension, inflammation-related diseases, and metabolic disorders (Kessler & Bromet, 2013; Krishnan et al., 2002; Rohde et al., 1991). Around ¾ of those diagnosed with depression will experience multiple episodes, often making depression a chronic disorder. Median episode length is 12 weeks, though this varies greatly (Eaton et al., 2008). Early diagnosis of MDD is essential, as early treatment can make a large difference in later chronicity and severity (Tolentino & Schmidt, 2018).

Diagnosis of MDD in North America follows the DSM-5 criteria, whereby 5 (or more) of the listed symptoms should be present during a two-week (or longer) period for nearly all days and show changes previous functioning. The criteria also identifies two critical components of depression. The first is a pathological depressed mood, which must be separate from a normal response to upsetting events. The second core symptom is anhedonia, or a loss of interest in activities which used to pleasurable such as hobbies, social connections, or sexual desire. These two core symptoms must be accompanied by at least 3 others, which can include fatigue, issues with sleeping, drastic changes in weight, feelings of worthlessness, and general somatic symptoms of feeling unwell. Significant variability can also exist in the symptoms outside of the two core criteria, as evidenced by the language used in the DSM-5. Symptoms can include weight loss or weight gain, insomnia or hypersomnia, and agitation or retardation – all opposing profiles which can complicate

diagnosis. Due to this variability, there are specifiers such as “with anxious distress”, “with melancholic features” and more, that allow for more targeted approaches to treatment. A full list of the DSM-5 criteria and specifiers can be found in table 1.1.

Table 1.1: DSM-5 MDD Diagnostic Criteria

Five (or more) of these symptoms, present for nearly everyday for at least 2 weeks and represent a change from previous level of functioning: *Must be evident for diagnosis	Other Criteria:	Specifiers of MDD:
1. Depressed Mood* 2. Markedly diminished interest or pleasure in all, or almost all, activities* 3. Considerable weight loss when not dieting, weight gain, or decrease or increase in appetite 4. Insomnia or hypersomnia 5. Psychomotor agitation or retardation. 6. Fatigue or loss of energy nearly every day. 7. Feelings of worthlessness or excessive or inappropriate guilt nearly every day. 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day. 9. Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.	• The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning • The episode is not attributable to the physiological effects of a substance or to another medical condition • The occurrence of the episode is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder or other psychotic disorders • The individual has never had a manic episode or a hypomanic episode	• Severity • With anxious distress • With mixed features • With melancholic features • With psychotic features • With peripartum onset • With seasonal pattern

While the DSM-5 defines MDD as a separate diagnosis, it is important to note the overlap of symptoms which are also associated with diagnoses of anxiety disorders, schizoaffective disorders, and many more. Clinician expertise is needed to tease apart both appropriate diagnostic labels and proper treatment course. In a clinical setting, patient wellness is often assessed through

standardized questions and scales to determine diagnosis and/or severity. Scales such as the Hamilton Depression Rating Scale (HDRS), Montgomery-Asberg Depression Rating Scale (MADRS), and the Beck Depression Inventory (BDI) are commonly used to assess severity of depression and treatment-response. The HDRS is made up of 21 items which are graded for severity from 0-2/3/4. An overall score of 0-7 is considered a normal or clinical remission score – a score of 20+ indicates moderate to severe depression. Certain limitations include the exclusion of atypical depressive symptoms such as hypersomnia or hyperphagia (Hamilton, 1960). The BDI is a commonly used 21 item scale, with each item scored between 0-3 (total scores: 1-10, normal mood; 17 – 20, borderline clinical depression; 31 – 40, severe depression; 40+, extreme depression). However, total BDI scores can be influenced by maladaptive personality traits (Beck et al., 1961). Lastly, the MADRS is the shortest scale which was developed to be more sensitive to changes induced by pharmacotherapy. Focusing on core symptoms, it has 10 items which are scored between 0-6 (Svanborg & Åsberg, 2001). Clinicians also focus on symptoms such as pessimistic thinking, memory complaints, house cleaning, and grooming behaviours – often indicators that functioning has been significantly impacted.

In an attempt to move away from strict diagnostic labels and include the impact that various comorbidities may have on disease course, the National Institute of Mental Health (NIMH) developed the Research Domain Criteria (RDoC) initiative, whereby classifications would be based on various behavioural dimensions and other biological indicators (NIMH, 2008). The RDoC matrix is a four-dimensional model of the original two-dimensional framework, which highlights how traditional domains of neuropsychiatric illness, and the units which we use to analyze them, exist within a specific environmental context that can develop throughout the lifetime. This viewpoint then encompasses the influence that genetics, molecules, physiology, and behaviour can have on depressive pathophysiology within different environmental contexts (Cuthbert & Insel, 2013). While initially developed in 2008, the lack of consistent biomarkers for depression and other neuropsychiatric disorders has stalled progress on the implementation of the RDoC into common clinical practice.

The development of biomarkers for depression could help to inform individual therapeutic response, necessity of pharmacotherapeutic treatments, and clarify diagnostic categories. However, the discovery of neuropsychiatric biomarkers has faced a significant number of

challenges, particularly given the heterogeneity of depression and inability to distinguish between different neuropsychiatric disorders (Gururajan et al., 2016; Schmidt et al., 2011; Strawbridge et al., 2017). A significant amount of research has been put towards the analysis of saliva, tissue, blood, cerebrospinal fluid (CSF), and other samples with little fruition towards a strict set of biomarkers that can be used for diagnosis or as a predictor of therapeutic response. In response to these failures, research has started to expand on the development of biomarker panels in lieu of a single-biomarker approach. Promising areas have included metabolic and endocrine systems, both impacted by exposure to stress. However, in order to further biomarker research to a place where it can be clinically useful, we must understand the potential underlying mechanisms of depression.

1.2.1 Hypotheses on the etiology of depression

As previously discussed, MDD is the most prevalent neuropsychiatric disorder globally. Despite this prevalence rate, the underlying pathophysiological mechanisms of depression are still unknown. Challenges in uncovering these mechanisms include the heterogeneity of MDD, as well as the differing genetic and environmental contributors that mediate the development of depression. Due to these variances, we cannot assume that depression has one etiological basis and should consider a variety of causes that may precede or contribute to the development of depressive pathophysiology. Biological factors which can influence depression include neurological, endocrinological, immunological, and genetic factors that are all influenced by sex, age, and exposure to stress (Kendall et al., 2021; Schmidt et al., 2011; Shadrina et al., 2018). Environmental factors can include exposure to stressors such as childhood trauma and long-term adversities, as well as lack of social support and different socioeconomic statuses. In addition, personal factors which can mediate depression onset include personality traits, cognitive ability, and sociability; for example, depression is more often observed in those who would be characterized as neurotic and conscientious (Klein et al., 2011). Therapeutic outcomes can also be influenced by personality traits, as those who score high in trait-based neuroticism respond better to pharmacotherapy when compared to psychotherapy (Bagby et al., 2008). Put together, the etiology of depression is complex and made up of a combination of biological, environmental, and personal factors which can influence onset of depression, symptom patterns, and course of disease.

MDD is considered to be moderately heritable, with twin studies suggesting a heritability rate between 40% - 50% (Sullivan et al., 2000). Relative risk (RR) ratios of MDD are around 2 to 3

when comparing those with diagnosed first-degree relatives in comparison to the general population (Gershon et al., 1982; Maier et al., 1992; Weissman et al., 1982). However, these RR ratios increase significantly in cases where MDD has an early onset and/or recurrent episodes (Marazita et al., 1997; Weissman et al., 1982), suggesting that moderate to severe cases of MDD have higher heritability rates. There are also sex differences across heritability rates, with twin studies determining MDD was significantly more heritable in women than men, though most of the genetic risk factors for depression were shared between sexes (Kendler et al., 1999, 2000, 2001). Genome-wide association studies (GWAS) have identified certain risk factors, however most of these studies have yet to be replicated (Shadrina et al., 2018). Meta-analyses have also failed to identify any one locus responsible for depressive disorders, indicating that genetic predisposition to MDD is controlled by coordination of a multitude of varying genes and their interactions with environmental stressors (Shadrina et al., 2018).

Given the early focus on the monoaminergic hypothesis (described in detail later in this section), a significant amount of research looked to genes which encode for monoaminergic-related signalers. *SLC6A4* encodes for the serotonin transporter (SERT), which drives the reuptake of serotonin from the synaptic cleft. A polymorphism in the promoter region of *SLC6A4* has been associated with availability or absence of a 44 bp fragment (Heils et al., 1995). The available (L) and absent (S) alleles mediate the expression of *SLC6A4* mRNA, whereby the L allele has an increased capacity to reuptake serotonin. Further research indicated a certain single nucleotide polymorphism rs25531 (A → G) increased the reuptake capacity of the S allele to parallel the abilities of the L allele (Kraft et al., 2005), further complicating and distorting earlier human studies. More recent meta-analyses found that S allele carriers have a 1.14-fold increased risk for depression, though significance was not met due to many conflicting results (Oo et al., 2016). This example demonstrates the heterogeneity and complexity needed to analyze genetic contributions to depression. Another focus for genetic studies has been HPA axis-associated genes, given the role that chronic stress plays in the development of MDD. Age of depression onset has been associated with polymorphisms in genes which encode for glucocorticoid and mineralocorticoid receptors (*NR3C1* and *NR3C2)* (Klok et al., 2011; Schatzberg et al., 2014). In addition, genes which encode CRH receptors (*CRHR1* and *CRHR2)*, a hormone associated with the stress response, have been implicated repeatedly in the development of depression and its reoccurrence (Liu et al., 2006; Szczepankiewicz et al., 2013; Xiao et al., 2011).

Lastly, one of the most commonly described polymorphisms is a functional missense polymorphism that substitutes methionine with valine in codon 66 of *BDNF* (Val66Met). BDNF has been consistently implicated in the pathophysiology of MDD, as well as treatment response to pharmacotherapeutic and non-pharmacotherapeutic interventions. Disturbances in the maturation of BDNF have been observed, and a certain single nucleotide polymorphism is generally associated with lowered BDNF activity (Baj et al., 2013; Chen et al., 2004). However, the appearance of this polymorphism is significantly higher than the prevalence of depression (25% - 32% of European populations and 40% - 50% of Asian populations) – indicating it does not solely explain the occurrence of MDD (Verhagen et al., 2010). Inconsistency between studies also prompted a meta-analysis which did not confirm previous associations between *BDNF* polymorphic variants and MDD (Liu et al., 2009; Verhagen et al., 2010). Evidently, variability in depression-associated genetics research complicates conclusions that can be drawn. Nevertheless, research has outlined the important role that genetics plays in vulnerability for depression – as there are often no obvious associations between relapses and various environmental factors such as demographic, familial, and psychosocial contributors (Burcusa & Iacono, 2007).

At the crux between environmental and genetic changes in depression is epigenetics, a field which has surged over the past two decades. DNA methylation, whereby a methyl group is added in the 5' position of cytosines in CpG dinucleotides (Newell-Price et al., 2000) via DNA methyltransferases (DNMT) was the first discovered mechanism of epigenetic modification. This methylation process decreases the access that transcription factors have to DNA regulatory elements and can provide a quantitative measure of transcriptional repression (Bibikova et al., 2011). In addition, posttranslational histone modifications including but not limited to methylation, acetylation, and phosphorylation can also affect the transcriptional state of the chromatin (Allis & Jenuwein, 2016). A multitude of research has demonstrated epigenetic changes after exposure to environmental stressors, particularly early adverse events (Klengel et al., 2014; Mill & Petronis, 2007; Tsankova et al., 2007). Demethylation of *FKBP5* and methylation of *NR3C1*, genes which regulate glucocorticoid receptor expression, have been associated with individuals who experienced child abuse and other early life trauma (Klengel et al., 2013; Labonté et al., 2012; Suderman et al., 2012). Decreased methylation of the *SLC6A4* promoter has also been associated with exposure to environmental stress (Alasaari et al., 2012) and correlated with depressive symptoms (Zhao et al., 2013). Associated to the genetic markers addressed above,

hypermethylation of the *BDNF* promoter was able to differentiate between suicide and non-suicide post-mortem samples (Keller et al., 2010), though peripherally there may be decreased methylation in depressed participants (Fuchikami et al., 2011). Various epigenetic modifications have also been associated with antidepressant efficacy (Menke & Binder, 2022), such as in the *P11* and *Homer1* promoter region (Melas et al., 2012; Sun et al., 2021). The epigenetic changes mentioned are just a few across the breadth of research of the role of epigenetics in the etiology of depression, particularly as the field is still relatively new (Mourtzi et al., 2021). Further complicating the role of epigenetics in response to chronic stress, the human brain has unique epigenetic changes not observed in the periphery, including high levels of noncanonical cytosine methylation (non-CG) which is inversely correlated with gene expression (Luo & Ecker, 2015). All of these complexities should be considered in the discussion of epigenetic modifications in neuropsychiatric disorders.

Twin studies have revealed that while shared environmental influences seem to have little effect on the development of depression, individual environmental factors appear to contribute to nearly 63% of MDD (Sullivan et al., 2000). One of the most significant environmental contributors to depression is chronic stress. While acute stress is adaptive and often beneficial, increasing short-term plasticity and memory, chronic stress or a severe stressor can cause normal responses to become dysregulated, harming the individual. Experiencing an extremely adverse life event, which is associated with later chronic stress, can increase the risk of developing depression in the following months by 5- to 16-fold (Kendler et al., 2001; Sullivan et al., 2000). Early adverse events are often a major predictor of the development and severity of depression, often related to childhood emotional, physical, or sexual abuse (Cheasty et al., 1998; Lindert et al., 2014; Shapero et al., 2014). Other lifetime stressors associated with the development of depression include financial issues, divorce, harassment, and the death of a loved one among many others. Related to financial difficulties and other social factors, groups with lower socioeconomic status often have higher rates of depression (Miech & Shanahan, 2000; Schiavone et al., 2015). Chronic social disconnectedness and perceived social isolation have recently been identified as strong mediators of symptom severity of depression and anxiety (Santini et al., 2020), an effect that is mimicked in certain animal models exposed to stress (Weintraub et al., 2010).

Chronic stress has also been hypothesized to mediate recurrent episodes of depression, with each episode decreasing the stress threshold to cause the subsequent one (Kendler et al., 1999; Morris

et al., 2010). A large twin study revealed that the role of stress in catalyzing another episode decreases with increasing episodes, as the relationship between adverse life event and depressive symptom onset decreased after the 5^{th} – 6^{th} episode (Kendler et al., 2000, 2001). This has been paralleled in chronic corticosterone administration paradigms, whereby rats exposed to repeated cycles of corticosterone are sensitized to the development of depressive-like behaviour, including exacerbation of neurochemical changes which struggle to recover after multiple cycles (Lebedeva et al., 2017, 2020).

Biological information also supports the role that stress response dysregulation plays in depression. High levels of cortisol have also been consistently associated with depression, as those diagnosed with Cushing's disease (elevated ACTH secretion and excessive cortisol levels) have significantly higher levels of depression than the general population (Kelly et al., 1983; Starkman et al., 1986). Hippocampal atrophy and other neurobiological changes have also been associated with the development of depression and increasing symptom severity over time (Bremner et al., 2000; Sheline et al., 2003; Videbech & Ravnkilde, 2004). The larger question becomes the discovery of what mediates the relationship between gene-environment dysregulation that can lead to depression. The following discussion describes two of the major theories: the monoaminergic hypothesis, which has largely fallen out of favour due to the challenges with monoaminergic-based antidepressants, and the more novel neuroplasticity hypothesis.

1.2.1.1 The monoaminergic hypothesis

The monoaminergic hypothesis was developed over 70 years ago (Schildkraut, 1965), based on the observations that reserpine (an antihypertensive agent) precipitated depressive symptoms alongside its depletion of serotonin (Freis, 1954). In addition, evidence that reserpine impacted the vesicular storage of serotonin and other monoamines strengthened the hypothesis that depressive symptoms may be related to a decrease in efficacy of the monoaminergic system. In addition, the antimycobacterial agent iproniazid increased mood in tubercular patients, leading to the discovery that iproniazid acted as a monoamine oxidase inhibitor (MAOI) (West & Dally, 1959). MAOIs function by inhibiting the degradation of free monoamines in presynaptic nerve terminals, once again implicating the monoaminergic system in depression. As a group, monoamines include 5-HT, dopamine, norepinephrine, and epinephrine which mediate functions such as mood, sleep, appetite, reward, the fight-or-flight response, and concentration – all implicated in the

pathophysiology of depression. While this led the basis for most antidepressants used today, as discussed later in this thesis, several major issues have led the monoaminergic hypothesis to fall out of favour. One of the primary issues is the discrepancy between the biological actions of monoaminergic-based antidepressants and the observed clinical effects, with increases in monoamine availability happening immediately in contrast to the delayed therapeutic actions. While medications such as serotonin-selective reuptake inhibitors (SSRIs) inhibit the serotonin transporter (SERT) and increasing monoamine availability within hours, therapeutic actions often take weeks to occur (Rush et al., 2006). Research has attempted to improve this efficacy by creating compounds which target both 5-HT1A receptors and SERT, such as vilazodone. However, no changes were observed in therapeutic time-course (Sahli et al., 2016).

Despite these challenges, the monoaminergic system is still implicated in the pathophysiology of depression. Serotonin has been the monoamine most extensively researched, with previous research showing significant alterations in 5-HTergic molecules (Stockmeier et al., 2004). Regional and age specificity has also been shown, with younger participants demonstrating an increased SERT availability in the hypothalamus, but older adults having decreases in brainstem SERT availability (Dahlström et al., 2000; Malison et al., 1998). Evidently, the majority of these studies are older, and the consistency of results related to serotonergic changes has been heavily debated (Moncrieff et al., 2022). More recently, there has been the initial promising actions of serotonergic psychedelics, whose hallucinogenic activity and potential therapeutic value is hypothesized to be mediated through 5-HT_{2A} receptor agonism (López-Giménez & González-Maeso, 2018; Rolland et al., 2014). Abolishing the 5-HT_{2A} receptor activity is also able to prevent their antidepressant-like effects in rodent model, suggesting that serotonergic signaling is involved in changes of behaviour and neuroplasticity (Ly et al., 2018; Pędzich et al., 2022).

Serotonin and related signaling also appears to be implicated in suicide, with peripheral and central biomarkers of tryptophan, SERT, and binding efficacy of receptors being able to differentiate between those with suicidal ideation or attempt, those who died by suicide, non-suicide participants with MDD, and HCs (Johnston et al., 2022). In addition, previous work from our group has demonstrated abnormalities in lymphocytes, with altered patterns of SERT and 5-HT_{2A} receptor membrane protein clustering (MPC) in lipid rafts (Caruncho et al., 2019). Specifically, we have shown that both the number and size of 5-HT_{2A} receptor clusters are increased in naïve

participants with depression, and the size of SERT clusters was also increased (Rivera-Baltanas et al., 2012, 2015). In addition, the distribution of SERT and 5-HT_{2A} receptor MPCs was able to differentiate two subpopulations of naïve depression patients, despite both groups having identical HDRS scores before treatment. However, these two subpopulations responded differently to treatment; those with a higher percentage of larger clusters had significantly higher response and remission rates (Rivera-Baltanas et al., 2015). These results indicate that MPC of 5-HTergic proteins may be an effective putative biomarker for therapeutic response rates (Caruncho et al., 2019). These alterations have also been paralleled in a chronic corticosterone model for the study of depressive-like behaviour, as well as mice with genetic vulnerabilities to stress exposure (Romay-Tallon et al., 2018).

Despite some of these promising results, a recent meta-analysis found no consistent results on the role of serotonin in depression since the inception of the monoaminergic hypothesis until the end of 2020; perhaps serotonin may be involved in subsets of depression, or as an indicator of therapeutic responsiveness, but it appears as a general biomarker serotonin and its related molecules have fallen out of favour (Moncrieff et al., 2022). Similarly, the lack of consistency in results regarding the role of dopamine and norepinephrine have cast doubt on the monoaminergic hypothesis. It is evident that research on mechanisms outside of monoaminergic changes is necessary in order to find effective therapeutics that have reliable and fast-acting effects in those diagnosed with depression.

1.2.1.2 The neuroplasticity hypothesis

The failures of the monoaminergic hypothesis to address the complexity of depression, researchers shifted to focus to other potential mechanisms that underlie depressive pathophysiology. One of the most consistent major findings was the parallels between the molecular changes induced by antidepressants and those that underlie synaptic plasticity. Neuroplasticity is the process by which the brain is able to reorganize connections in response to external experience and synaptic plasticity, how synapses are able to change strength or efficacy of their transmission, controls a significant amount of behaviour (Citri & Malenka, 2008). Chronic stress has been known to impact these systems for decades and affect neuroplasticity and neuroplasticity-related behaviours such as learning and memory (McEwen, 1999; Shors et al., 1989), which could account for many of the changes observed in depression.

Behaviourally, patients diagnosed with MDD exhibit significant deficits in cognition, attention, concentration, and memory which correspond with functional impairments in the dorsolateral prefrontal cortex (DLPFC), medial temporal lobe, and hippocampus (Baxter et al., 1989; Harvey et al., 2005; Kritchevsky et al., 2004; Zakzanis et al., 1998). Chronic stress or treatment with high-dose glucocorticoids are also known to decrease hippocampal-dependent memory, a process heavily dependent on neuroplasticity (Sapolsky, 2003; Shors, 2006). Long term potentiation (LTP) whereby increased excitatory transmission leads to a long-lasting increase in communication between neurons, and its opposite long-term depression (LTD), have both been implicated in the stress response (Kim & Diamond, 2002).

On a molecular level, a significant amount of research has implicated neuroplasticity, or lack thereof, in depression. Decreases in calcium and cyclic AMP (cAMP) have been noted in animal models used to study depression and are known to be mediated by monoaminergic signaling – potentially explaining some portion of the therapeutic response to traditional antidepressants (Shelton et al., 1996; Thome et al., 2002). Local calcium influx is considered a good measure of neuronal activity, given that it most commonly derives from NMDAR activation. NMDARs are considered "coincidence detectors" for excitatory signaling, as they can only be opened when both the presynaptic and postsynaptic cells are simultaneously depolarized – a coincidence which appears to be required for most forms of homosynaptic plasticity (Hebb, 2005). Calcium-calmodulin-dependent kinases, whose release is induced by calcium increases, can also promote the insertion of AMPAR receptors in the post-synaptic membrane, increasing excitatory transmission at the synapse (Colbran & Brown, 2004). The insertion of AMPAR into the post-synaptic membrane can also "unsilenced" so-called "silent synapses", a synapse where the post-synaptic membrane contains only NMDARs and therefore cannot be activated (Malenka & Nicoll, 1997). Increases in the expression of AMPARs, and particularly the GluA1 subunit, have been associated with both traditional and non-traditional antidepressant efficacy (Dong et al., 2018; Duman et al., 2019; Qi et al., 2009).

Many other molecular mechanisms of neuroplasticity have been implicated in depression and antidepressant efficacy (Liu et al., 2017; Pittenger & Duman, 2008), however some of the strongest evidence towards the neuroplasticity hypothesis of depression has been the discovery of ketamine, an NMDAR antagonist with fast-acting antidepressant effects previously unseen. The ability of

ketamine to significantly reduce depressive symptoms within an hour sparked a surge of renewed interest in antidepressant therapeutics. The elucidation of the underlying mechanisms of ketamine has found substantial effects on glutamate-driven neuroplasticity, with clinical effects within hours and molecular changes that can be observed within minutes in animal models (Aleksandrova & Phillips, 2021; Kavalali & Monteggia, 2015; Yao et al., 2017). Described in further detail later in this dissertation, the discovery and untangling of ketamine's effects have been some of the greatest contributions to the field of depression and neuroplasticity.

1.2.2 Treatment-resistant depression

Today's commonly prescribed therapeutics, based on the monoaminergic hypothesis, are often not effective within the first antidepressant trial. In clinical practice, around 50 – 60% of patients do not reach an adequate response with first-line treatment (Rush et al., 2006), which has led to the diagnostic concept of treatment-resistant depression (TRD). While there is no set definition, TRD is typically defined as a failure to respond (usually determined as reaching remission) to at least two antidepressant trials of adequate duration and dose (Berlim & Turecki, 2007; Fava, 2003; Fava & Davidson, 1996; Thase et al., 2001). Degrees of treatment-resistance are also often classified, taking into account criteria such as the amount of initial response, which therapies were used, and whether combination or augmentation strategies were employed (Fava, 2003). There have been multiple predictors described for treatment-resistance, though findings have been inconsistent. A recent meta-analysis found that there are different clinical and sociodemographic predictors for non-responders and non-remitters. Non-response was associated with longer depressive episodes, greater amounts of hospitalizations, suicidal ideation, and a comorbid anxiety disorder. The response of non-remitters was mediated by comorbid anxiety symptoms and personality disorder diagnosis, as well as severity of illness – though these were interestingly modulated by marital status in certain cases (de Carlo et al., 2016). Patients who are treatment-resistant also often have an increased severity of disease course and a greater number of reported somatic symptoms (Papakostas et al., 2003).

Interestingly, patients with TRD appear to have certain distinct underlying pathophysiology when compared to those who respond to first-line treatment (Smith, 2013). Inflammation appears to be uniquely associated with TRD, with elevated levels of molecules such as C-Reactive Protein, TNF-α, and various pro-inflammatory cytokines often being associated with treatment-resistance

(Strawbridge et al., 2015; Yang et al., 2019). *BDNF* polymorphisms in combination with specific 5-HT_{1A} genotypes have also been implicated in TRD (Anttila et al., 2007), though serum levels of BDNF were not predictive of later treatment-response to electro-convulsive therapy (Fernandes et al., 2009). Metabolites of monoamines can also differentiate between first-line responders and TRD participants, particularly an elevation in CSF levels of the dopaminergic-derived homovanillic acid (Aklillu et al., 2009). More recently, neuroimaging has revealed that gamma power could be a powerful predictor of antidepressant treatment response, particularly to novel antidepressants such as ketamine (Gilbert & Zarate, 2020). Evidently, the need to consider treatment-resistance in the search for novel therapeutics and underlying mechanisms of depression is imperative. Insight from a multitude of sources, including clinicians, researchers, administrators, and most importantly – patients themselves, is necessary to begin the next phase of research on diagnosis and treatment of depression.

1.3 Importance of patient-oriented research

Over the past 5 years, our laboratory has prioritized the inclusion of patient partners in our research. Despite not treating patients directly, we are foundational researchers who focus on the discovery and investigation of basic mechanisms and are in the unique position to work alongside patients with lived experience (PWLE) in mental health disorders. Before the beginning of this section, it is important to note that those who have been diagnosed with mental health disorders encompasses a great amount of people; often, there are no boundaries between the terms “patient”, “clinician”, and “researcher”, and it is important to consider that we are not discussing disparate groups and overlap may create unique stigma.

The benefits of including patients into healthcare research is innumerable: the relevance and quality of research increases due to the experiential knowledge of patients, shared decision-making leads to increased participation, satisfaction, and treatment adherence (Sacristán, 2013), and patient partners can often bring their own individual skills to the research group, such as statistical expertise or creating promotional materials by those with backgrounds in mathematics or graphic design.

Defining patient-oriented research (POR) is essential to properly conduct and incorporate it into laboratory-based, referred to as foundational here, research. Loosely, POR supports prioritizing active partnership between various stakeholders such as patients, clinicians, and researchers to

advance improvements in our current healthcare models. Current "evidence-based" models of foundational research leave little room for patient-input, particularly in Canada (Krahn & Naglie, 2008; Schünemann et al., 2006; Whall et al., 2021). The United Kingdom's National Institute of Health Research (NIHR) has focused more on POR over the past few decades and have set up six comprehensive standards for public involvement. These six standards provide an in-depth framework to support relationships between those who work in healthcare and the public, as well as function as a checkpoint to ensure that these relationships are on track. The six standards reflect communications, governance, impact, working together, inclusive opportunities, and support and learning. Internationally, the International Association for Public Participation (IAP2) has provided a strong set of core values and guidelines for the inclusion of the public in research, however for the purpose of this dissertation I focus on Canadian guidelines and goals.

Following the development of international standards, the Canadian Institute of Health Research (CIHR) developed the Strategy for Patient-Oriented Research (SPOR). One of SPOR's central tenets is that of patient engagement, which is defined as "occurring when patients meaningfully and actively collaborate in the governance, priority setting, and conduct of research, as well as in summarizing, distributing, sharing, and applying its resulting knowledge" (CIHR, 2014). In addition, their hopes to shift the role of the patient from passive receptor of treatment to proactive research partner greatly aligns with our goals to incorporate POR into foundational research. Despite growth and funding towards inclusion of patient partners, there are still significant challenges that can impede on the proper incorporation of POR into healthcare research.

Some of the main exclusion mechanisms encountered by PWLE are behaviours (e.g. affording less respect, speaking time, or attention) and communications (e.g. having concerns dismissed or translational barriers) that disregard their input (Elberse et al., 2011; Williamson, 2008). The translational gaps between laboratory-based research, clinical sciences, public health administration, and the public can create inadvertent barriers that exclude PWLE. Jargon and specialized techniques of each separate group creates difficulties when attempting to collaborate and merge various fields of study to gain input from more diverse groups. Other deficits in clinical practice guidelines can often be attributed to lack of consideration of patient preferences, as key factors such as socioeconomic or work status are rarely incorporated into treatment plans. Recent research demonstrated that even with reported positive attitudes towards patient engagement,

biomedical scientists and service workers still ranked perceived expertise over experience, disregarding input from PWLE (Boaz et al., 2016).

Contrasts in research priorities between clinicians and PWLE have recently been described by the "Alberta Depression Research Priority Setting Project", which aimed to gain insight into the most important unanswered questions for clinicians and patients in depression research. For instance, where clinicians prioritized understanding mechanisms underlying antidepressants and contributing genetic factors, PWLE were more invested in determining long-term physical implications of pharmacotherapy and cost-benefit analyses of specific therapeutics (Breault et al., 2018). It is evident that realigning our priorities as healthcare practitioners and researchers to include patient priorities is essential, particularly those of diverse backgrounds.

This lack of consideration has led to the proposal of a patient emancipation movement, which favours greater personal autonomy, more control over medical decisions, increased knowledge sharing and respect from medical professionals and healthcare systems (Williamson, 2008). In particular, patients who have lived experience with neuropsychiatric disorders often face unique challenges in comparison to other patients, as stigma against those diagnosed with a mental health disorder is still highly prevalent. In general, people with lived experience (PWLE) are viewed as less competent to make autonomous decisions, a bias which does not account for differences in disorder severity or changes in cyclical well-being. This systematic discrimination has created barriers in forming equal partnerships between PWLE and researchers. While there are risks related to capacity of each individual to participate in research, thorough and consistent discussions on roles and responsibilities can help prevent any potential negative effects or unfair burdens on PWLE. In circumstances such as this, the inclusion of family members and caregivers can be extremely valuable, such as with shared decision-making with in-patients diagnosed with schizophrenia (Hamann et al., 2006, 2007).

The participation of diverse PWLE can also be impeded by facets of systemic discrimination. Disparities in access to health research are well-documented, however the specific exclusions or discrimination in POR has yet to be studied. Systemic discrimination has been repeatedly recorded in healthcare settings including lower quality of treatment, clinical trial participation bias, socioeconomic barriers, and barriers to proper healthcare access (Ayhan et al., 2020; Casey et al., 2019; Hall et al., 2022; Shavers et al., 2012; Thornicroft et al., 2007), which could all impact

patient engagement in research. For instance, research has shown that patients are more comfortable when a clinician is of the same race and gender (Owens et al., 2020), which could transfer to a PWLE-researcher relationship. However, lack of representation amongst Canadian University faculty members could contribute to greater discomfort for patient partners. For instance, data from 88 Canadian Universities show that out of full-time faculty, 40.2% were women, 20.9% were non-white, 1.3% were indigenous, 21.8% had diagnosed disabilities (Universities Canada, 2019; UVic, 2018), and no data was collected for LGBTQIA2S+ members. Particularly in STEM-based research, an umbrella which includes biomedical sciences, the "leaky pipeline" phenomenon leads to loss of diverse faculty members due to greater marginalization. Increasing diverse representation in university research should be prioritized in order to promote diverse patient participation and input.

Other challenges include attempts to quantify patient involvement, which is often of a qualitative nature. While many fields including psychology and sociology welcome qualitative methods, biomedical fields (particularly laboratory-based) are still biased towards quantitative measures, which could ignore essential information (Caron-Flinterman et al., 2005). Foundational scientists would also need education on qualitative measures and meaningful extraction of data. Related to this, experience and training on how to adapt experiential data to experimental questions that can be addressed in a laboratory setting is still missing from the field. One of the biggest challenges in this translation is the erasure of individual variables from foundational research, which often aims for consistency in comparison to the heterogeneity present in the majority of neuropsychiatric disorders. While steps are being made to increase patient engagement and involvement in clinical and public health research, the paucity of patient participation in laboratory-based foundational research is clear. Specific barriers that are present for PWLE with neuropsychiatric disorders and different marginalized groups also need to be addressed through proper representation and breakdown of gaps in translation to encourage full engagement of patient partners.

1.4 HPA Axis and MDD

Stress, whether chronic or acute, is an emotional or physiological response to perceived threats. In acute stress conditions, this response can help respond to dangerous situations or anticipate proper responses to threats. Exposure to a stressor stimulates an endocrinological cascade that is called the hypothalamic-pituitary-adrenal (HPA)-axis, which attempts to maintain homeostasis in the

brain and body (Herman & Cullinan, 1997). Generally, activation of the prefrontal cortex and hippocampus is needed to later suppress HPA-axis activity in a negative feedback loop that prevents overactivation. However, under chronic stress, this homeostatic mechanism is disrupted, leading to interruptions in functions such as the inflammatory response, emotionality, neuroplasticity, digestion, and energy storage. As pictured in Fig. 1.1, after exposure to an acute stressor, axonal terminals in the hypothalamic paraventricular nucleus (PVN) release corticotropin-releasing factor (CRH), which stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH is then able to stimulate glucocorticoid release from the zona fasciculata of the adrenal cortex. In humans, the primary stress hormone is cortisol, analogous to corticosterone (CORT) in rodents. Normal cortisol release is cyclical, with increased levels in the mornings and decreasing throughout the evening, a cycle controlled by the suprachiasmatic nucleus (Adam et al., 2010). Release of cortisol can happen in the span of minutes after synthesis, as it is not stored in vesicles (Gjerstad et al., 2018), which allows for the quick mobilization of energy stores to escape direct threats. This fast shift in energy stores from non-essential functions to those needed to escape can become deleterious under conditions of chronic exposure to stress, which has been linked to the development of depression (Holsboer, 2001; Pariante, 2003).

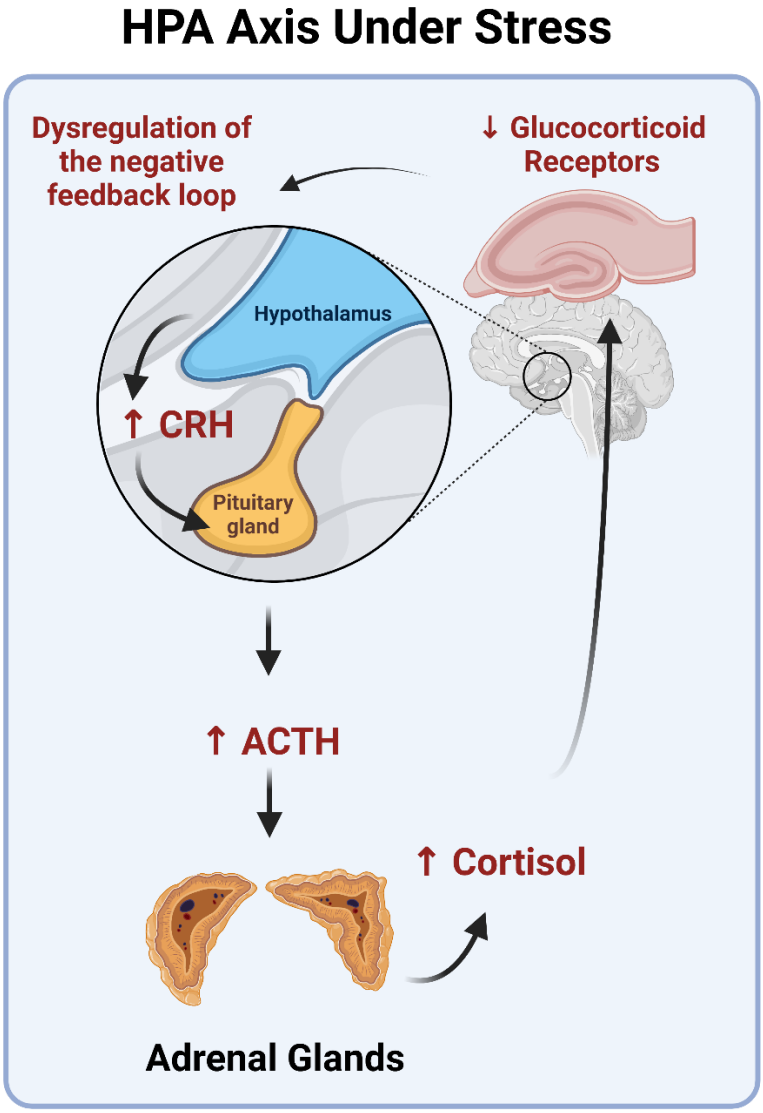


Figure 1.1 The HPA axis under stress conditions. After an acute stressor, the paraventricular nucleus in the hypothalamus secretes CRH, targeting the anterior pituitary gland. The pituitary then releases ACTH to stimulate the release of cortisol from the adrenal glands. Cortisol is able to diffuse through the blood brain barrier and bind to glucocorticoid receptors (GRs) in the cytosol of target cells, activating the negative feedback loop to shut off the stress response. However, in times of chronic stress, increases in CRH, ACTH, and cortisol can be maladaptive, leading to a downregulation of GRs and dysregulation of the negative feedback loop. Hypercortisolemia is observed in nearly 60% of patients diagnosed with depression, heavily implicating the stress response in depression. Figure created in BioRender by author.

As stress hormones are lipid soluble, they can pass through cellular membranes with ease where they bind to cytosolic mineralocorticoid (MR) and glucocorticoid receptors (GR) (de Kloet et al., 1990; Koning et al., 2019). As glucocorticoids have ~10-fold greater binding affinity to MRs than

GRs, MRs are generally activated in basal conditions and GRs under stress conditions (Reul & de Kloet, 1985). While MRs are expressed primarily in peripheral tissues and limbic brain regions, GRs are expressed nearly ubiquitously, with particularly high levels in the prefrontal cotex, amygdala, and hippocampus (de Kloet et al., 2005; Reul & de Kloet, 1985). Chronically high-levels of glucocorticoids desensitize GRs, which decreases the functionality of the negative feedback system of the HPA-axis (de Kloet et al., 1998). Following the overactivation of the HPA axis, research has shown decreases in dendritic arborization and increased neuronal apoptosis in the hippocampus and prefrontal cortex (McEwen, 2017; McEwen & Morrison, 2013; Mitra & Sapolsky, 2008; Sapolsky, 2003). In addition, dendritic hypertrophy has been observed in the amygdala, an area associated with fear response (Hoffman et al., 2017; Mitra & Sapolsky, 2008).

Unusually stressful events, such as grief or divorce, are well-known risk factors for the development of depression (Kessing, 2004). High levels of plasma cortisol have also been consistently reported in patients diagnosed with MDD (Gibbons & McHugh, 1962; Heim et al., 2008; Pariante & Lightman, 2008), findings which corroborate with research demonstrating increases in CRH and ACTH in both central and peripheral tissue (Banki et al., 1987; Carroll et al., 2007; Nestler et al., 2002). Those who died by suicide or who are experiencing suicidal ideation also have increases in levels of CRH and cortisol in blood, hair, and saliva (Johnston et al., 2022). Responsiveness to antidepressant treatment has not been consistently associated with cortisol levels (Nandam et al., 2020), but ECT and antidepressants have been shown to normalize HPA-axis function (Du & Pang, 2015; Holsboer, 2001; Mason & Pariante, 2006; Pariante, 2003; Pariante & Lightman, 2008). Discussed in further detail later in this thesis, chronic injections of CORT or exposure to chronic stressors in rodents can also lead to reliable models for the study of depression. In pre-clinical models, chronic antidepressant treatment can normalize GR density and stimulate GR gene transcription (Heiske et al., 2003). Lastly, hypercortisolemia is seen in ~60% of depressed patients, though it is unlikely that this directly causes depression (Carroll et al., 2007; Holsboer, 2001). It is evident that the HPA-axis plays a major role in depression and needs to be explored further.

1.5 Glutamatergic signaling and MDD

Over the past two decades, the direct modulation of the glutamatergic system has received considerable attention as a novel approach for treating major depressive disorder (MDD). This

attention was catalyzed by emerging clinical evidence showing that subanesthetic doses of the non-competitive allosteric N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine have rapid and long-lasting antidepressant effects in patients with treatment-resistant depression. As the primary excitatory neurotransmitter, glutamatergic neurons make up over half of excitatory neurons. Synthesized from glutamine by glutaminase, a mitochondrial enzyme, glutamate is packaged in vesicles to be released after cell depolarization. Glutamate is unable to be degraded in the extracellular space, so it is taken up by excitatory amino acid transporters (EAATs) that are present on presynaptic neurons and astrocytes. Astrocytes are able to reconvert glutamate to glutamine, which is then trafficked back to the neuron to restart the cycle.

Glutamate binds to three main subtypes of ionotropic receptors: AMPARs, NMDARs, and kainic acid receptors which open to allow sodium, potassium, and calcium ions to pass through, gating a depolarizing current. In addition, glutamate is able to activate slower-acting metabotropic receptors (8 subtypes of 3 main groups, mGluR I/II/III). These mGluRs are linked to varying G-proteins and second messenger signaling pathways, with mGluR1 and mGluR5 being associated with G_q and G_i with mGluR2, mGluR3, mGluR4, mGluR6, mGluR7, and mGluR8 (Niswender & Conn, 2010). Of note, certain ionotropic receptors also hold metabotropic functions. Kainate receptors are unique in that they can either increase or decrease excitatory signaling dependent on receptor location (pre- vs. post-synaptic neurons) (Contractor et al., 2011; Valbuena & Lerma, 2021). In the context of depression, most research has focused on the dysregulation of AMPARs and NMDARs.

In general, plasma levels of glutamate, glutamine, and glycine are increased in patients with depression – an increase that is associated with Hamilton Depression Rating Scale scores (Mitani et al., 2006; Hunag et al., 2021). Other studies have found elevated glutamate expression in the frontal cortex, where it may contribute to excitotoxicity (Hashimoto et al., 2007; Kantrowitz et al, 2021). However, most research has focused on determining the roles of glutamate receptors in depression and antidepressant therapeutics.

Rapid excitatory transmission is primarily mediated through AMPAR activation (Thompson et al., 2015), which are ubiquitously expressed throughout the brain and spinal cord and are made up of four receptor subunits (GluA1 – 4) that impart distinct ion selectivity, function, kinetic properties, and trafficking. GluA2 subunit-containing receptors restrict Ca^{2+} permeability, which are able to

decrease channel conductance and play an important role in consolidation of long-term potentiation. Increasing throughput of both Ca^{2+} permeable and impermeable AMPARs have been prioritized in antidepressant research, as significant changes in synaptic connectivity have been associated with both symptom presentation and therapeutic response (Liu & Zukin, 2007). The affinity of glutamate to each subunit is increased with increased binding site occupancy, and the channel requires 2 or more subunits to have bound glutamate to open (Prieto & Wollmuth, 2010). Importantly, AMPAR activation is also an important component of NMDAR signaling, which requires cellular depolarization to remove a voltage-sensitive magnesium block and become calcium permeable (Banke et al., 2005; Hayashi et al., 2014).

Excitatory transmission can lead to increased trafficking of AMPARs to the synapse, where it is inserted in the post-synaptic membrane and situated in the post-synaptic density (PSD). Increased surface insertion of AMPARs is able to further increase excitatory transmission and promote processes such as long-term potentiation (Lu et al., 2001). In addition, the trafficking and insertion of AMPARs into "silent synapses" (synapses which contain only NMDARs that cannot be activated alone due to their voltage-gated magnesium block) has been hypothesized to be a key process underlying learning and memory (Malenka & Nicoll, 1997). GluA1 knockout mice demonstrate significant reductions in hippocampal LTP and performance in memory tasks (Krugers et al., 2010; Sanderson et al., 2009, 2010; Sanderson & Bannerman, 2012). Phosphorylation of GluA1 subunits by CaMKII, PKA, and/or PKC also enhances AMPAR throughput through changes in subunit composition, localization, and channel kinetics (Banke et al., 2000; Derkach et al., 2007; Shi et al., 2001).

Deficits in AMPAR-mediated signaling have been consistently reported in depression and animal models for the study of depression. GluA1 and GluA3 mRNA expression was decreased in the hippocampus and perirhinal cortex of post-mortem tissue from patients with depression (Duric et al., 2013), a finding that has been complemented in chronic stress rodent models. In other chronic stress models, GluA1 expression and phosphorylation are decreased in the dorsal hippocampus and medial prefrontal cortex but increased in the ventral hippocampus and amygdala (Allen et al., 2022; Brymer et al., 2020; Kallarackal et al., 2013; Toth et al., 2008), demonstrating the importance of studies with regional specificity. Antagonizing AMPARs is also able to eliminate the actions of most traditional (fluoxetine, imipramine, etc.) and novel (ketamine, etanercept, etc.)

antidepressants (Aleksandrova et al., 2017; Brymer et al., 2018; Koike et al., 2011; Koike & Chaki, 2014).

The evident involvement of AMPARs in depression led to the development of AMPAR positive allosteric modulators (PAMs) as therapeutics for depression. While initial preclinical studies demonstrated that AMPAR PAMs rescued memory deficits, dendritic arborization, and neurogenesis – clinical trials demonstrated few effects (Kadriu et al., 2021). These clinical trial failures have led to a general decrease in interest surrounding direct AMPAR modulation, though certain newer therapeutics are still being developed with preliminary success in early-stage clinical trials.

NMDARs are considered a signaling "coincidence detector" whereby their activation requires the co-occurrence of glutamate and glycine co-agonism and cellular depolarization for removal of the inner pore magnesium block (Traynelis et al., 2010). The channel opening allows for an influx of Ca^{2+}, which plays an essential role in strengthening synaptic conduction. It appears that NMDAR function is location-dependent, associated with varying intracellular signaling cascades (Hardingham & Bading, 2002, 2010), and subunit composition in a similar manner to AMPARs (Liu & Zukin, 2007; Massey et al., 2004). At the synapse, NMDAR activity is associated with cell survival through inhibition of p38MAPK, which increases the phosphorylation of ERK1/2 and BDNF expression. Extrasynaptic NMDARs can promote p38MAPK activity, leading to increases in cell death (Dieterich et al., 2008; Hardingham & Bading, 2010; Liu et al., 2007; Myung et al., 2005; Parsons & Raymond, 2014; Xu et al., 2009).

Interestingly, these differences in cell survival roles arise from differences in subunit composition; GluN2B subunits are preferentially expressed extrasynaptically, whereas GluN2A subunits are most often expressed at the synapse (Rumbaugh & Vicini, 1999; Stocca & Vicini, 1998). GluN2A-containing NMDARs promote surface insertion of GluA1-AMPARs, which can be inhibited by GluN2B-NMDARs (Massey et al., 2004). Increases in tau toxicity (a hallmark of different neuropsychiatric and neurological disorders) have also been linked to GluN2B-NMDARs, which increase GSK-3β activity upstream of tau expression (Tackenberg et al., 2013).

Nevertheless, NMDARs are critical for learning and memory processes, particularly memory formation (Howland & Cazakoff, 2010), which can be impaired by NMDAR antagonism (Danysz et al., 1988; Hliák & Krejčí, 2002). Potentiation of NMDAR currents is able to enhance

hippocampal long-term potentiation, which is notably decreased in MDD (Collingridge & Bliss, 1987; Cui et al., 2013; Lüscher et al., 2012). GluN2A and GluN2B knockout mice also display strong deficits in behavioural measures of learning and memory (Cui et al., 2013). There is evident dysregulation of glutamatergic signaling in depression and homeostatic balance is essential to address deficits properly. While downregulation of AMPAR and NMDAR-mediated currents causes impairments in learning and memory, excessive glutamate and Ca^{2+} influx can lead to significant consequences, such as increases in reactive-oxygen species production and mitochondrial apoptosis which can damage cellular compartments and formation as well as DNA (Jaiswal et al., 2009). Further understanding of the role of the glutamatergic system in depression is necessary for the development of effective novel therapeutics and is discussed later in this dissertation.

1.6 Hippocampus: General structure, circuitry, major roles

The hippocampus, dubbed as such due to its resemblance to a seahorse (in Greek, "hippos" – horse, and "kampos" – sea monster) is a grey matter limbic structure heavily involved in mood regulation and consolidation of memory. Located in the medial temporal lobe of both hemispheres, the hippocampus is heavily involved in mood regulation and memory consolidation. It receives many connections from most cortical and subcortical regions to provide emotional and cognizant contextual information to experiences (Knierim, 2015; Lisman et al., 2017). Interest on the role of the hippocampus in memory consolidation began in 1957, where the patient H.M. underwent removal of his hippocampi to reduce epileptic seizures. After the procedure, H.M developed anterograde amnesia, unable to develop new semantic or episodic memories, despite retention of full cognitive function (Wixted & Squire, 2010). Difficulties with episodic and spatial memory impairments are paralleled in other patients with hippocampal lesions, even with generally normal levels of intelligence.

The hippocampal formation is composed of four major regions: the Cornu Ammonis (CA) and dentate gyrus (DG), the entorhinal cortex (EC), and the subiculum. All of these regions are essential for the proper relay of information between the hippocampus, other limbic regions, and the prefrontal cortex, and the hypothalamus (Amaral & Witter, 1989; Insausti & Amaral, 2003). Uniquely, the hippocampus has a unidirectional flow of information through each disparate region

through either the perforant pathway or via the tri-synaptic circuit which runs through the DG (Knierim, 2015). See Figure 1.2 for general structure and pathways of the hippocampal formation.

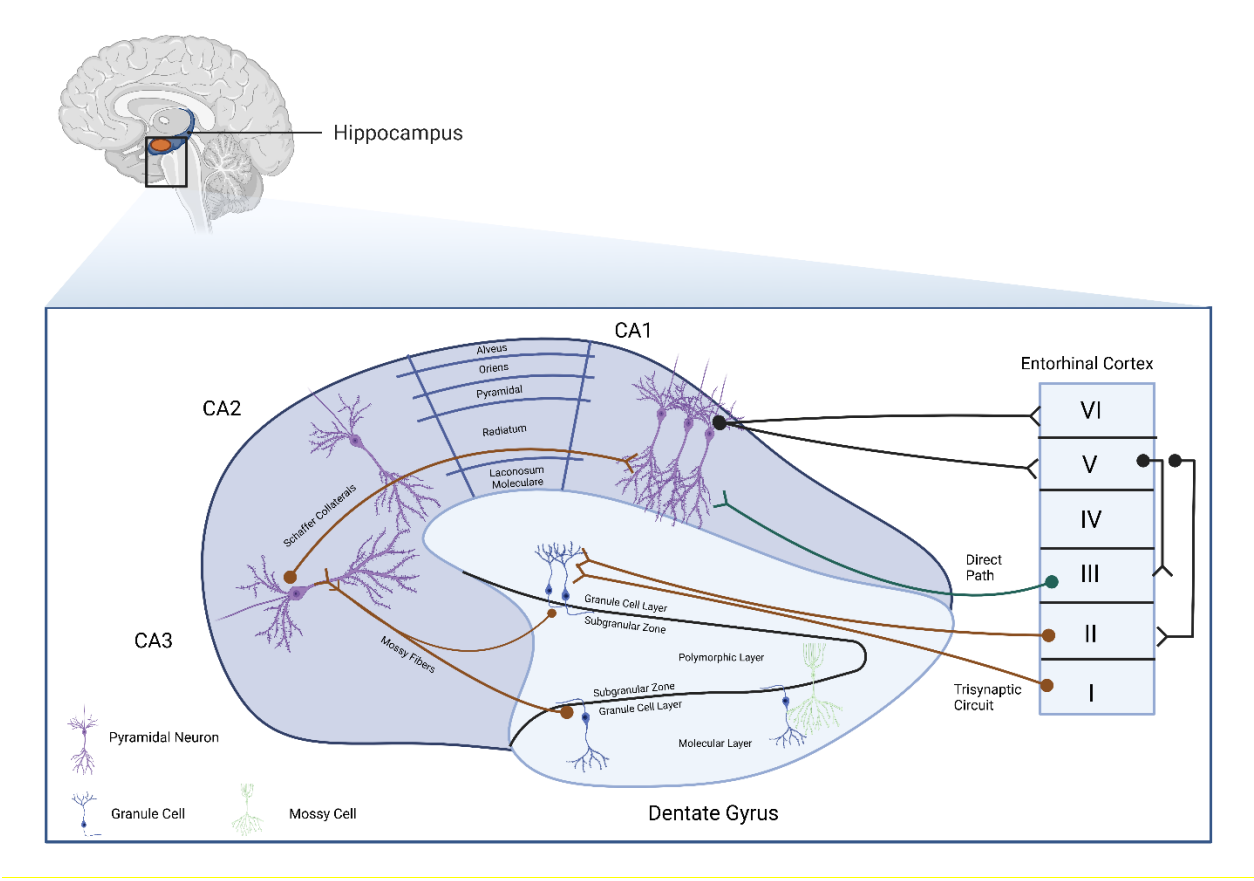


Figure 1.2. General structure and circuitry of the hippocampus. The hippocampus proper is made up of 3 subdivisions (CA1 – 3), which are further divided by layers (stratum oriens, stratum pyramidal, stratum radiatum, stratum lucidum (not pictured, localized to CA3), and stratum lacunosum-moleculare). The dentate gyrus (DG), or CA4, is made up of 3 distinct layers: the molecular layer, the granule cell layer, and the polymorphic layer. Imaged in brown here is the tri-synaptic circuit, a unidirectional circuit of the hippocampus which transfers information from layers I and II of the entorhinal cortex to the granule cells of the DG, then through the mossy fibers to pyramidal cells in CA3. From the CA3, the Schaffer collaterals connect to pyramidal cells in the CA1 which then output to layers V and VI of the entorhinal cortex. A more direct path (pictured in green) is the communication from layer III of the entorhinal cortex directly to the pyramidal cells of CA1. Figure was created in BioRender by author.

The CA, or hippocampus proper, is made up of 3 subdivisions (CA1 – 3) which are further separated by five strata: stratum oriens, stratum pyramidal, stratum radiatum, stratum lucidum, and stratum lacunosum-moleculare. The pyramidal contains the soma of excitatory pyramidal cells whose dendrites are located in the oriens. The activity of these pyramidal neurons is finely controlled by GABAergic interneurons that project to different CA subdivisions. The lucidum receives axonal projections (called mossy fibers) from the DG, whereas the radiatum houses

Schaffer collateral fibers (axonal projections of CA3 pyramidal cells to CA1/2). Schaffer collaterals also inhabit the lacunosum-moleculare wherein they activate GABAergic interneurons to provide feed-forward inhibition on pyramidal cells. CA1- and CA1/2-located dendrites receive direct innervation from the entorhinal cortex through either the temperoammonic (layer III) or perforant pathway (layer II) (Amaral & Witter, 1989; Basu & Siegelbaum, 2015).

The DG is a trilaminate structure, composed of the polymorphic layer (occasionally referred to as CA4 or the hilus), the granule cell layer, and the molecular layer. Layer II EC cells excite granule-cell dendrites in the molecular layer through the perforant pathway. Molecular layer perforant path-associated interneurons (MOPP) are able to mediate neurotransmission in this first synapse of the tri-synaptic pathway through feed-forward inhibition of granule cells (Li et al., 2009). The subgranular zone is located between the granule cell and polymorphic layers and is one of only two locations which is able to generate adult-born neurons. Mature dentate granule cells project to CA3 pyramidal cells, making up the second tri-synaptic synapse. The third synapse are the Schaffer collateral fibers from the CA3 which provide information to the surrounding CA2 and CA1 pyramidal cells. Lastly, the pyramidal cells in the CA1 project to layers V and VI of the EC, which can complete the circuit through projections to layer II and III of the EC (Amaral & Witter, 1989; Insausti & Amaral, 2003).

The structure and function of the hippocampus is decently conserved between species such as human, monkey, and rat, despite significant differences in size (Clark & Squire, 2013). However, the complexity of this structure leaves it both difficult to study and vulnerable to damage. It is estimated that a typical CA1 pyramidal cell receives over 30,000 glutamatergic and 1700 GABAergic synaptic inputs (Megías et al., 2001). Clinical and pre-clinical research has consistently demonstrated that the hippocampus is critical for memory consolidation and active recall (Squire et al., 2010). CA3 lesions and damage have been implicated in impaired spatial memory tasks and object-placed paired associative learning (Gilbert & Brushfield, 2009; Handelmann & Olton, 1981; Jarrard, 1983). In contrast, the CA2 seems more essential for social recognition memory (Hitti & Siegelbaum, 2014; Lehr et al., 2021). Working memory is often associated with CA1 function, as region-specific pyramidal neuron loss significantly impairs both working memory and spatial navigation (Auer et al., 1989; Chen et al., 2011; Olsen et al., 1994; Whishaw et al., 1994) CA1 lesions also impair autobiographical memory, autonoetic conscious

awareness, and mental time travel (Bartsch et al., 2011) – memory processes that are often interrupted in Alzheimer's disease, which often displays significant reductions in CA1 neuronal density (Padurariu et al., 2012). Finally, the dentate gyrus appears to process incoming signals by grouping selective activation of granule cells to use for input discrimination (Kesner, 2013). Examples of this selective grouping and storage includes novelty detection and linking spatial contexts to current environmental information (Kesner, 2013; Lee & Jung, 2017). The importance of the hippocampus for memory encoding and storage is one of the main reasons that it has been an area of major interest for depression.

1.6.1 Dysfunction in depression

A multitude of research has implicated the hippocampus in major depressive disorder (Sheline, 2011; Sheline et al., 2003, 2019). One of the first major reports was the finding that hippocampal volumes were reduced in patients with MDD compared to healthy controls (Sheline et al., 1996), a finding that has been replicated in the majority of related studies (Videbech & Ravnkilde, 2004). This reduction in hippocampal volume also appears to be associated with earlier onsets of depression, more recurrent episodes, and a longer total duration of depression (MacQueen & Frodl, 2011; Sheline et al., 2003), though it is uncertain whether it is a cause or symptom of depression. Observations of cellular changes have shown a decrease in neuropil and higher packing density of glia, granule cells, and pyramidal neurons which could contribute to volume reduction (Stockmeier et al., 2004). Recent fMRI studies have showed substructure-specific changes, with larger bilateral changes in the CA1 – CA4, dentate gyrus, and subiculum for those with recurrent depression. In first-presentation depression, the structures most affected were CA2 – CA4. Across all these subregions, changes were most pronounced on the left side – and changes to the CA1 were the largest overall and a significant predictor of illness duration (Roddy et al., 2019).

Many theories for the role of this reduction in volume have been hypothesized throughout the past few decades, with one of the most prominent being the neurotoxicity hypothesis (Sapolsky, 2000). The neurotoxicity hypothesis posits that increased and prolonged exposure to glucocorticoids, such as in chronic stress, increases neuronal vulnerability which leads to an increase in cell death from challenges or attrition. A bidirectional relationship between the HPA axis and hippocampal formation also exists, with optimal function of the hippocampal formation necessary for proper HPA axis signaling (Sapolsky, 2003). In contrast, the vulnerability hypothesis posits that a smaller

hippocampal volume confers a predisposition to the development of depression and later recurrence (Charney, 2004).

It appears that a decrease in hippocampal neurogenesis may also contribute to the deleterious effects of chronic stress, as well as the behavioural effects of certain antidepressants (Sahay & Hen, 2007). In fact, the interest for hippocampal neurogenesis in depression was spurred by observations that both pharmaceuticals and other interventions with antidepressant-like effects stimulated adult hippocampal neurogenesis (Eisch & Petrik, 2012; Sahay & Hen, 2007), and the time course of neuron generation in the dentate gyrus follows the time course of the delayed onset of monoaminergic-based antidepressants (Duman et al., 2001). In models where hippocampal neurogenesis is ablated, hippocampal-dependent learning is impacted – particularly for certain forms of fear conditioning, working memory, and long-term spatial memory (Becker & Wojtowicz, 2007). Neurogenesis has also been shown to have a role in pattern separation, which can lead to impaired recollection, a commonly observed trait in patients with depression (Gould & Tanapat, 1999). Despite promise in animal models for the study of depression, the involvement of neurogenesis in MDD is still heavily debated. Reliance on post-mortem tissue and heterogeneity of populations have produced mixed results, with many finding no differences (Berger et al., 2020). In addition, given the differential stages of neurogenesis, it is difficult to interpret information from single-stage markers.

Behaviourally, it is well-documented that patients with MDD have deficits in memory that is encoded by the hippocampus (Burt et al., 1995). Typically, those diagnosed with depression have impaired memory of positive events and recollection, as well as potentiated memory for events with negative associations (Burt et al., 1995; Matt et al., 1992; Ramponi et al., 2010). These memory impairments can also predict a more severe disease course (Sumner et al., 2010), and enhancement of memory can also improve depressive symptoms (Dalgleish & Werner-Seidler, 2014). Cognitive impairments are also present in around 2/3 of depression patients and are predictive of poor remission rates (Austin et al., 2001). In addition, cognitive impairments often persist even after clinical remission criteria is met (Rock et al., 2014).

In humans, positron emission tomography (PET) imaging has also revealed that those diagnosed with MDD have changes in metabolic activity in regions including the hippocampus, subgenual cingulate, and prefrontal cortex (Kennedy et al., 1997; Mayberg et al., 2013). Post-mortem

research has revealed consistent changes in glial cell density and neuronal numbers, density, orientation, and size (Cotter et al., 2002; Öngür et al., 1998; Rajkowska & Miguel-Hidalgo, 2008). On a molecular level, there has been a significant amount of pre-clinical observations that chronic stress can cause significant cellular atrophy in the hippocampus, often in a region-specific manner (Allen et al., 2022; Fenton et al., 2015; Goodman & McIntyre, 2017; Kleen et al., 2006; Lee et al., 2009; Lussier et al. 2013a; Rahman et al., 2016). Elevated glucocorticoids are also able to eliminate activity-driven increases of BDNF, an effect that is reversed by antidepressant treatments (MacQueen et al., 2003). In addition, changes in cyclic adenosine monophosphate (cAMP) phosphorylation (Stewart et al., 2001) and mRNA levels of cAMP response element-binding protein (CREB) in the CA1, CA3, and DG appear after antidepressant treatment (Dowlatshahi et al., 1998). Future research needs to aim towards further delineation of the role of the hippocampus and changes observed in human patients diagnosed with MDD.

1.7 Animal models for MDD

Though MDD is a human disorder, we are unfortunately not yet advanced enough to study many molecular and circuital aspects of the disorder in humans. Post-mortem tissue is difficult to obtain and may not reflect the true circumstances of the disorder, particularly in the case of depression, where death by suicide or other causes can cause significant changes in the brain. Animal models can provide us a bridge to cross the gap in knowledge, creating experimental models that may help reflect what is happening in various neuropsychiatric disorders. The focus of this section will be the use of rats to model depression, as this is the modeling that our lab uses, but there are a multitude of other models that have been proven useful in the study of depression (Willner, 1990). Although rats and humans diverged evolutionarily around 80 million years ago, nearly all genes associated with human disease are highly conserved in the rat genome (Huang et al., 2004). Rodents (such as rats and mice) are also used primarily to model human conditions due to fast reproductive rates, low-cost, replicability, and size.

Difficulties with using rodents as a model for depression however include an inability of animals to fully self-reflect (or at least describe this reflection to us) – making it nearly impossible to mimic certain hallmark symptoms such as suicidal ideation, feelings of worthlessness, low self-esteem, and excessive guilt. Depression is also a highly heterogenous disorder and cannot be fully capitulated in models where variables are kept relatively constant. Humans are exposed to a variety

of genetic predisposition factors, environmental stressors, and socioeconomic statuses that can all influence a person's experience and the underlying pathophysiology of depression.

Due to these challenges, animal models generally focus on a specific facet of a neuropsychiatric disorder they are modeling. For this reason, there are many different models to study depressive-like behaviour, with no clear superior model as it depends on each lab's specific research question. These models can vary from chronic and acute exposure to stressors (during development and/or adulthood), biological manipulations (surgical procedures such as removal of the olfactory bulb and optogenetic controls), and targeted gene manipulations (deletion, overexpression).

However, to ensure reproducibility and consistency, certain standards have been developed for the use of animals to model human disorders. McKinney & Bunney (1969) first proposed a set of criteria for valid animal models: they should exhibit phenotypes that can be reasonably compared to human symptoms; those behavioral changes can be measured objectively; behavioral abnormalities can be reversed by treatments that are effective in humans; and the results of animal studies should be easily replicable between researchers.

These initial tenets transformed into what we now refer to as face, predictive, and construct validity, terms first proposed by Willner (1990). Face validity determines whether behaviour or biology observed in the animal model is similar to what is observed in humans. It is important to note that this does not necessarily mean exact comparability – for example, a rat licking itself is considered grooming – perhaps equivalent to a human taking a shower. Changes in both behaviours can accompany depression and a depressive-like behaviour, however they are not identical. An important distinction related to this is that while cortisol is the primary stress hormone for humans, the analogous molecule in rodents is corticosterone (CORT). Humans are able to express CORT, however it has a weaker affinity to GRs, and acts mainly as an intermediary in the steroidogenic path (Miller & Auchus, 2011). Finding appropriate behavioral equivalents to human symptoms has been one of the greater challenges in the use of animal models.

Predictive validity is the concept that there is similarity in the predisposition factors of the disorder and occurrence of the disease (such as the link between chronic stress and depression), and similarities between the disorder and therapeutic agents that are efficacious in humans. For depressive-like behaviour, this is often measured through the Forced Swim Test (FST), an inescapable forced-swimming task that assesses coping behaviour exhibited by rodents as a proxy

for despair-like behaviour. For good predictive validity, therapeutics that decrease depressive symptoms in humans should parallel decreases in FST-immobility in rodents. Chronic (but not acute) treatment with traditional antidepressants, such as imipramine, is able to decrease this immobility in a similar time course to human experience (Fenton et al., 2015; Holick et al., 2007). Ketamine is also able to rescue this behaviour rapidly (Browne & Lucki, 2013) and preliminary research with psychedelics suggests rapid and prolonged effects in both humans and rodents (Hibicke et al., 2020; Kadriu et al., 2021). While this demonstrates some form of predictive validity, it is also important to note that there are differences in therapeutic response between rodents and human participants. While the depressive-like behaviour of most rodents can be rescued with traditional and non-traditional antidepressants, human participants have much more varied responses, with 30 – 50% not responding to initial treatment. This is due to the heterogeneity of the human population in comparison to rodent colonies – effects such as genetics, environmental exposures, and social supports are much more varied in humans. Even in rats, there are differences in response between strains, sexes, and method of stress exposure (Ma et al., 2019; Wu & Wang, 2010). Future research should make strong efforts to create a model that may mimic forms of treatment-resistance (Planchez et al., 2019).

Lastly, construct validity is defined as similarities of etiology, behavioural and/or cognitive processes, and biological changes between the human disorder and animal model. The hope is that with more similarities between animal and human, the more reliable a model will be for any specific disorder. Examples of this in animal models for depression could include a reduction in hippocampal volume, decreased dendritic complexity, dysfunction in memory processes, and many more. Despite similarities between behavioural and biological processes, it is still important to note that the reliability of animal models have been questioned due to difficulties in distinguishing models of different disorders (depression, schizophrenia, autism, etc.) (Belzung & Lemoine, 2011) and inability to mimic certain presentations of the disorder that impact severity and treatment-response (treatment-resistance, cyclicity and chronicity). Differences between lab facilities, data collection and analysis, researcher experience, and researcher sex can also have an impact on results collected from both clinical and laboratory-based research.

To improve upon these methods of validity, others have proposed three other concepts: homological validity (validity of the species as strain), pathogenic validity (biomarker validity,

similar in concept to construct validity), and mechanistic validity (similar underlying mechanisms) (Belzung & Lemoine, 2011). While these would definitely add to the reliability of animal models, challenges in neuropsychiatric research in particular include the lack of knowledge held surrounding the underlying etiology and mechanisms of depression, which makes it extremely difficult to re-enact in an animal system.

1.7.1 Behavioural tests to evaluate depressive-like behaviours

There is an incredible array of behaviours that can be observed in animals to evaluate depressive-like behaviour, such as escape response, fear-conditioning, sucrose preference for anhedonia, exploratory behaviour, and memory-reliant tasks. Three major tests of depressive-like behaviour used in this thesis will be described in-depth here, but there are many other common measures of depressive-like behaviour in rodents. The FST, demonstrated first by Porsolt and colleagues (Porsolt et al., 1978), is the most ubiquitous behavioural test for antidepressant efficacy. On day one, rodents are placed in a cylinder filled with water deeply enough that they cannot escape or use their tails to balance on the bottom for 15 minutes. The day after, they are placed in the tank again for 5 minutes to determine "despair-like" behaviour that was induced by the previous day's session. Researchers score the amount of time spent swimming, climbing (struggling – trying to climb the walls of the tank), immobility, and latency to immobility (the amount of time before going immobile). Porsolt's argument was that the 15 minutes of pre-swim brought on behavioural despair through learned helplessness, and that time spent immobile could be rescued through antidepressant treatment. However, some have argued that immobility on the second day of testing could be an adaptive, rather than despair-like, response as they learn to conserve energy because they realized they would be removed from the tank. To remove potential confounding effects of adaptation, a 1-day protocol has been established where rats are placed in the tank for 10 minutes and again assessed for swimming, climbing, immobility, and latency to immobility. In this updated protocol, stressed animals are consistently more immobile than those not exposed to stressed, and the immobility can be reversed with traditional and experimental antidepressants (Allen et al., 2022; Brymer et al., 2018, 2020; Fenton et al., 2015; Hibicke et al., 2020; Lebedeva et al., 2020; Marks et al., 2009). Other criticisms of the FST have focused on which human symptom it may mimic, with certain researchers arguing it better reflects coping behaviour rather than despair (Commons et al., 2017), the subjectivity of immobility analysis, and, as mentioned above,

antidepressants are able to consistently rescue FST behaviour within rodents but do not reflect a treatment-resistant population.

The sucrose preference test (SPT) has been developed as a measure of anhedonic behaviour, with the loss of pleasure in things that used to be pleasurable. Anhedonia is particularly present in the treatment-resistant population, making it a good screening tool for novel therapeutics that may be effective at targeting traditionally hard to treat symptoms. In fact, sucrose preference is most often rescued in chronically stressed rats with therapeutics that are effective in treatment-resistant depression (Hesselgrave et al., 2021; Zhang et al., 2014). For the test itself, rats are habituated to having 2 bottles of water, then the sucrose solution (usually 1%). For the test phase, 1 bottle of sucrose solution and 1 bottle of water are placed on the cage and switched halfway through to control for any potential location preference.

The splash test has been used as a measure for motivational and self-care behaviour, whereby animals that display depressive-like behaviour as reduced grooming and taking a longer period of time to begin grooming (Hu et al., 2017). In brief, sucrose (usually 10%) is sprayed on the back of the rodent, and the stickiness of the solution usually spurs grooming behaviour. Time spent grooming, as well as latency to groom, is usually scored across 5 minutes. This test has strong face validity, as a decrease in grooming/self-care/outward appearance is observed across participants with depression. Often, motivation to do daily tasks such as showering and brushing teeth decreases as other depressive symptoms worsen. Certain limitations of this are variations in the amount of sucrose sprayed (which can vary the necessity in grooming behaviour) as well as subjectivity in scoring what consists of grooming vs. non-grooming. The taste of the sucrose may also motivate certain rodents, but not others, to continue grooming for longer – on the other hand, anything with an aversive taste may impact grooming behaviour as well.

1.7.2 Chronic stress models of depression

With the benefits and limitations of animal models in mind, there have been a multitude of animal models developed for the study of depression, particularly in relation to chronic stress. Unpredictable chronic mild stress (UCMS), developed by Katz and colleagues (1981), creates an animal model of depression through repeated exposure to various mild stressors over the period of several weeks. These can include tilting of the cage, food and water deprivation, long periods of bright light, flashing lights, mild shocks, and other mild stressors across the period of the protocol.

With unpredictable exposures, animals are unable to lose sensitivity to the stressors and tend to develop depressive-like behaviours such as anhedonia, memory impairment, increases in the threshold for intracranial self-stimulation, and loss of weight and appetite. Accompanying the behavioural changes, biological changes such as increased inflammatory markers appear over time (You et al., 2011). While UCMS demonstrates good predictive and construct validity, differences in protocols across labs and changes in stressors challenge its face validity. In addition, protocols are often long and complex, limiting its use. Chronic restraint stress has also been used similarly, however the results are often unpredictable due to the predictability of the stressor (Bravo et al., 2009; Gregus et al., 2005; Lussier et al., 2013; Regenthal et al., 2009), leading UCMS to be more advantageous.

In a similar vein as UCMS, a model of learned helplessness was first introduced by Seligman and colleagues (Nuvvula, 2016), whereby animals were placed in chambers with electric shocks applied to their paws with no method of escape. After exposure to the no-escape box, 2/3 of animals would not escape the shock when placed in another box with a visible method of escape – he coined this "learned helplessness". This model does mimic quite a few aspects of human depression, including decreased motor activity (Seligman et al., 1980), lowered grooming behaviour, and decreased reward sensitivity (Zacharko et al., 1983) as well as – perhaps most interestingly – provides a model that allows the differentiation between animals who were resilient to the learned helplessness models and those who were not. The underlying biology also confirms similarities between the learned helplessness model and patients with depression, including a reduction dopaminergic signaling and overactivation of the habenula (Ilango et al., 2013; Shumake et al., 2005). The learned helplessness phenotype is also able to be reversed using chronic (but not acute) treatment with monoaminergic-based antidepressants (Kitada et al., 1981). However, there are disadvantages such as a need for larger animal groups to compensate for the differences between resilient and non-resilient animals and the requirement of specialized equipment.

To address some of the developmental influences of depression (Lindert et al., 2014; Shapero et al., 2014; Smith & Pollak, 2020), early life stress models have been developed. These include separating the pups from the dams, handling at an extremely early age, exposure to prenatal stress, or providing limited nesting materials. These methods lead to higher incidences of learned helplessness and depressive-like behaviours such as a reduction in grooming (Ivy et al., 2008;

Schmidt et al., 2011). Other models, such as lesion-based (e.g., olfactory bulbectomized rats) and genetic-based (e.g. Wistar Kyoto strain rats or transgenic models) are also used regularly to screen for antidepressant efficacy, however an in-depth review of these models are beyond the scope of this thesis.

1.7.2.1 Exogenous CORT as a model for depression

Over the past two decades, our lab has been using the chronic corticosterone administration paradigm to model depressive-like behaviour. As previously discussed, stress (particularly chronic) is a major predisposition factor for depression, causing an overactivation of the HPA axis that leads to a dysfunctional stress response. Due to variability in animal response to physiological and psychological stressors, it is difficult to evaluate certain aspects of behaviour and neurochemistry. To circumvent differences in reactions to stressors, our laboratory uses a chronic corticosterone administration paradigm, whereby rats are given subcutaneous injections of 40mg/kg of CORT daily over the span of 3 weeks. This allows us to control the amount of CORT given dependent on bodyweight, and more directly examine the detrimental effects of stress on brain and behaviour (Sterner & Kalynchuk, 2010). While CORT can be administered in a multitude of ways, including through food, water, pellet implantation, or osmotic pump infusion, subcutaneous injections balance more control over administration with the less invasive methods.

Research regarding the face validity of the CORT model is steadily growing, demonstrating that chronic CORT administration can trigger many parallels between patients with depression and depressive-like rats. Chronic CORT increases despair-like behaviour as measured with the FST (Ali et al., 2015; Allen et al., 2022; Brummelte & Galea, 2010; David et al., 2009; Gourley & Taylor, 2009; Gregus et al., 2005) in a dose- and time-dependent manner, as 40mg/kg causes significantly more immobility than 5mg/kg, 10mg/kg, and 20mg/kg (Johnson et al., 2006; Marks et al., 2015) and administration of CORT over 21 days produces a more severe phenotype than shorter durations. In addition, cyclical CORT administration worsens depressive-like behaviour and biology with each subsequent treatment cycle (Lebedeva et al., 2017, 2020). Differences simply in bodyweight and muscle strength are unable to explain these differences in the FST, as immobility, but not bodyweight, improved during recovery cycles (Lebedeva et al., 2017) and rats treated with CORT are able to hold onto a wire suspended in air for as long as vehicle rats (Marks et al., 2009). Paradoxically, low-doses of CORT (20mg/kg, over 10 days) increases immobility in

males but decreases it in females (Brotto et al., 2001), emphasizing the importance of making sex-specific observations.

Anhedonic-like behaviour has also been consistently measured in animals treated with chronic CORT. Rats usually prefer sucrose-sweetened water over regular water. However, CORT-treated animals drink significantly less sucrose water than regular water when compared to vehicle-treated animals (Gourley et al., 2008; Gourley & Taylor, 2009; Kvarta et al., 2015; Ma et al., 2018). Social behaviours, food-seeking, and sexual behaviours, normally rewarding interactions for rats, are also disrupted with CORT administration (Berger et al., 2019; Chan et al., 2017; Gorzalka et al., 2001; Peng et al., 2021). It is worth noting that while sexual behaviour is reduced in male rats, CORT can actually increase sexual behaviour in females (Gorzalka & Hanson, 1998; Hanson & Gorzalka, 1999).

Depression is also highly associated with comorbid anxiety, with around 51% of patients with depression also having a diagnosis of an anxiety disorder (Kessler et al., 1996). Comorbid anxiety can have a significant impact on course of illness, including delaying recovery and greater risk of relapses (Hirschfeld et al., 2001), making it important to also model this large population of patients. Anxiety-like behaviour, as measured through tests such as the open-field test (OFT), light-dark test (LDT), and elevated plus maze (EPM), is increased after chronic CORT administration. If displaying anxious-like behaviour, rodents will tend to avoid open areas (OFT) (Li et al., 2017a) and lessen exploratory behaviour (LDT, EPM) (Luo et al., 2017; Murray et al., 2008; Peng et al., 2021). Another anxiety-like behaviour is hyponeophagia, or inability to feed in a novel environment, is also induced by the CORT model (Berger et al., 2019). While mice often present increased anxiety-like behaviour after CORT administration, it is less effective in producing similar behaviours in rats (Allen et al., 2022; Gregus et al., 2005; Hill et al., 2014; Kalynchuk et al., 2004), demonstrating the importance of carefully choosing animal species and strains when designing your experimental plans to address your research question. Other behavioural and biological changes induced by the CORT model and human parallels can be noted in Table 1.2.

There are also many physiological and biological changes in the chronic CORT paradigm which parallel observations from patients with depression. Bodyweight is significantly decreased with CORT-administration, again in a dose- and time-dependent manner (Brummelte & Galea, 2010;

Gregus et al., 2005; Johnson et al., 2006; Lebedeva et al., 2020), potentially due to increases in in levels of leptin, a hunger-inhibiting hormone (Perry et al., 2019) or CORT mobilizing energy stores through the inhibition of insulin. Bodyweight is not rescued during recovery periods in cyclical CORT administration (Lebedeva et al., 2020), suggesting that these changes may be more persistent than the behavioural effects of CORT.

Human symptom	**Animal Test**	**CORT alterations**	**Reference**
Anxiety	Open Field Test	Lower entries & time in centre	(Li et al., 2017; Rainer et al., 2012)
	Elevated Plus Maze	Less time spent in open arms	(Luo et al., 2017; Myers & Greenwood-Van Meerveld, 2007)
	Light-Dark Test	Less time in light section	(Luo et al., 2017)
	Predator Odour Test	Less contact with aversive stimuli	(Kalynchuk et al., 2004)
Anhedonia	Sucrose Preference Test	Decreased sucrose consumption	(Gorzalka et al., 2003; Gourley et al., 2008; Gourley & Taylor, 2009)
	Novelty-Suppressed Feeding Test	Increased latency to feed	(David et al., 2009; Rainer et al., 2012)
Despair	Splash test	Decreased grooming	
	Forced Swim Test	Increased immobility	(Brymer et al., 2018; Gregus et al., 2005; Kalynchuk et al., 2004; Lussier et al., 2013; Rainer et al., 2012)
	Tail Suspension Test		(David et al., 2009; Zhao et al., 2008)
Learning and memory deficits	Y-maze	No change in spatial memory	(Hill et al., 2014)
		Decrease in learning/memory	(Notaras et al., 2020)
	Novel Object Recognition Task		(Darcet et al., 2014)
	Barnes Maze		
	Morris Water Maze		(Sousa et al., 2000)
	Object Location Test		(Brymer et al., 2018)
	Object in Place Task		

Sociability deficits	Social Interaction Test	No change in social interaction	(Gregus et al., 2005)
Decreased libido	Sexual Behaviour Test	Decreased sexual behaviour	(Gorzalka et al., 2001)
Weight gain/loss	Weight	Decreased weight	(Gourley & Taylor, 2009; Gregus et al., 2005; Johnson et al., 2006; Kalynchuk et al., 2004; Lebedeva et al., 2017; Lussier et al., 2013b)
Decreases in hippocampal reelin expression	Reelin-IR cells	Decreases in SGZ reelin-IR cells	(Allen et al., 2022; Brymer et al., 2018, 2020; Fenton et al., 2015; Johnston et al., 2020; Lebedeva et al., 2020; Lussier et al., 2011; Lussier et al., 2013a)
Decreases in dendritic complexity	Dendritic branching and length	Dendritic branching and length are decreased	(Cook & Wellman, 2004; Fenton et al., 2015; Fraga et al., 2021; Yau et al., 2016)
Decreases in hippocampal neurogenesis	DCX-IR cells	Decreases in number and complexity of DCX-IR cells	(Allen et al., 2022; Fenton et al., 2015; Lebedeva et al., 2020; Lussier et al., 2013a)
Decreases in hippocampal volume	Hippocampal volume	Decreases in hippocampal volume	(Murray et al., 2008; Zhang et al., 2015)
Changes in SERT clustering on lymphocytes	SERT clustering	Increases in size of SERT clusters	(Romay-Tallon et al., 2018)

Table 1.2. Comparison of human symptoms of depression with CORT alterations. Abbreviations: DCX = Doublecortin, FST = Forced Swim Test, IR = immunoreactive, SERT = Serotonin Transporter

1.8 iPSC modeling for neuropsychiatric disorders

While animal models are an invaluable resource, they cannot address certain aspects of complex neuropsychiatric disorders. Individual genetic contributions to disease course are unable to be captured in animal models, complicating the discovery of both common and disparate disease mechanisms. The discovery of methodology to reprogram cells to into induced pluripotent stem cells (iPSCs) has spurred the establishment of lines derived from patients in a multitude of disorders, including those primarily neuropsychiatric (O'Shea & McInnis, 2016; Wang et al., 2020). The ability to reprogram these iPSCs into any somatic cell type allows for the examination of genetic contributions to disease mechanisms and cell type-specific responses to traditional and novel therapeutics. In addition, the advent of iPSC modeling allows for a step towards individualized medicine, allowing researchers to capture the full complement of inherited genetic variation present in each participant's cells. Gene editing techniques also allow the ability to establish causal variants, providing a powerful tool to determine underlying disease state mechanisms (Tian et al., 2020).

Since the first induction of human iPSCs from the lab of Yamanaka and colleagues from human fibroblasts, a variety of methods by which iPSCs can be collected, reprogrammed, and differentiated into the cell-type(s) of choice have been developed. Human iPSCs can be collected from multiple sources, including peripheral blood mononuclear cells (PBMCs), dermal fibroblasts, and keratinocytes (Aasen et al., 2008; Seki et al., 2010; Takahashi et al., 2007). While more common and highly proliferative, dermal fibroblasts require a skin biopsy from patients, making this technique significantly more invasive than a blood draw/prick (PBMCs) or hair sample (keratinocytes). Reprogramming efficiency of PBMCs is lower than fibroblasts, however work in finger-prick collection has found efficient reprogramming with extremely low invasiveness (Tan et al., 2014). Lastly, keratinocytes from hair follicles are a promising method of collection that could be established in wide-spread use (for an in-depth comparison of methods, see: (Raab et al., 2014).

Based off the seminal work of the Yamanaka lab, the first methods to reprogram cells into iPSCs were retroviral and lentiviral. However, due to the necessity of genomic integration, new methods have been optimized to avoid integration. These methods include plasmid vectors (Okita et al., 2010), adenovirus (Stadtfeld et al., 2008), piggyBac transposons (Woltjen et al., 2011), synthesized

RNAs (Warren et al., 2010), recombinant proteins (Kim et al., 2009), and the Sendai virus (Fusaki et al., 2009). The most common factors used to reprogram cells into iPSCs are the "Yamanaka factors" Oct3/4, Sox2, Klf4, and c-Myc (OSKM) and "Thomson's factors" of Oct3/4, Sox2, Nanog, and Lin28. These factors all play different roles – Oct3/4 regains and maintains stem cell pluripotency, Sox2 regulates Oct3/4 expression, Klf4 prevents apoptosis, and c-Myc helps histone acetylation to aid Oct3/4 and Sox2. In the Thomson factors, Nanog controls the transcriptional network alongside Oct3/4 and Lin28 regulates mRNA translation and self-renewal of iPSCs. A significant amount of discussion has focused on different methods and factors to reprogram iPSCs and is beyond the scope of this dissertation (Buganim et al., 2014; Huang et al., 2019; Malik & Rao, 2013).

The majority of iPSC-derived studies for neuropsychiatric disorders have focused on disorders with a clear genetic pathogenesis, such as schizophrenia and certain autism subtypes (Wang et al., 2020). iPSCs from participants diagnosed with schizophrenia have lower neurite numbers, atypical vesicle release, and abnormal synaptic formation (Brennand et al., 2014; Deans & Brennand, 2021; Stachowiak et al., 2017) that mimic animal models and human post-mortem tissue. Other research has focused on known genetic risk factors for schizophrenia, such as 22q11.2 deletion and translocation of DISC1 (Disrupted in Schizophrenia 1), or interactions of anti-psychotics with iPSC-derived patient cell lines (LaMarca et al., 2018) Studies using iPSC lines from participants with bipolar disorder have found alterations in Wnt, Hedgehog, and Nodal signaling – demonstrating the potential of these models to identify disease mechanisms that may not be evident otherwise (O'Shea & McInnis, 2016).

Addressing depression with iPSC-derived models has proven more complicated due to significant heterogeneity. Even though genetic factors may contribute up to 50% of depressive etiology, very few replicated variants have been described in genome-wide association studies (Ormel et al., 2019; Shi et al., 2011). Despite this, iPSC models, particularly of TRD participants have been quite useful to determine mechanisms behind treatment responsiveness. Non-responder neurons have been found to display 5-HT-induced hyperactivity in comparison to SSRI-responders, due to an upregulation in multiple serotonergic receptors (Vadodaria,et al., 2019a), as well as aberrant neurite growth and morphology (Vadodaria,et al., 2019b). Other labs have demonstrated that ketamine was able to increase the proliferation of neural progenitor cells derived from iPSCs

alongside an increase in cAMP-IGF2 signaling, however it is not clear if these cells were from participants with depression (Grossert et al., 2019). In iPSC-derived dopaminergic neurons, ketamine was able to increase dendritic arborization and soma size through an induction of p70S6k, an effect that was blocked by inhibitors of the mTORC1-signaling pathway (Cavalleri et al., 2018). While iPSCs can evidently be used as models to determine therapeutic mechanisms, characterization of iPSCs from participants with MDD and TRD requires further study.

There are some limitations to iPSC-derived models. A primary issue is that most neuropsychiatric disorders are not clear-cut, and there are variations to the amount of influence that genetics hold (Flint & Kendler, 2014). Unfortunately, the loss of epigenetic markers with cellular reprogramming can limit certain inferences about disorder mechanisms or actual participant responses to therapeutics. As mentioned above, neural circuitry is often essential in disease pathogenesis – generating single-cell types from iPSCs could disregard the important role of this circuitry. Lastly, the cost and labour associated with generating large usable data sets through protein quantification, single cell RNA sequencing, and electrophysiological techniques are prohibitive.

Recent technology has attempted to address these issues in order to improve upon iPSC-based investigations. Utilizing 2D cultures which contain multiple cell types present in specific brain regions could help understand underlying circuitry. Improving on these heterogenous models are the advent of 3D brain organoids which can encompass multiple brain regions that are important for the neuropsychiatric disorder being studied. A few labs have begun development and characterization of these organoids, with a focus on substance abuse disorders (Ho et al., 2022) and ASD (Schafer et al., 2019). A downside to the development of organoids is the time necessary to mature, and some have suggested they may be more of use for studying early-life development and related disorders. In terms of cost and labour, higher throughput methodologies such as DropSeq allow for rapid characterization of single cells for lower costs than traditional methods. Through the separation of cells into nanoliter drops, DropSeq is able to assign a form of "barcode" to each cell which allows for simultaneous analysis of mRNA transcripts from thousands of cells at a time (Macosko et al., 2015). Still, given the relatively low invasiveness of the methodology, iPSCs hold great promise for high-throughput methods to assess underlying pathology and treatment response.

1.9 Therapeutics for MDD

1.9.1 Discovery

Depressive disorders have plagued humanity for centuries, but until the late 1930s there were seemingly no effective pharmacological therapies to address any of the related symptomology. Initial non-pharmacology based therapies were more common, however more severe cases were unresponsive to psychological treatments. Precipitating the first antidepressants was the discovery of therapeutics that could target catatonia in patients with schizophrenia (Lehmann & Ban, 1997) and the synthesis of antihistamines in 1937 following the discovery of histamine structure (Cozanitis, 2016). The basis for the first antidepressants was the synthesis of the antipsychotic chlorpromazine, after chlorine was added to promethazine – a sedative with strong antihistaminic properties (Domino, 1999). The trials of various antihistamines for use in schizophrenia continued until Dr. Roland Kuhn discovered G22355, a weak antihistamine with similar structure to chlorpromazine. Unfortunately, G22344 (later dubbed imipramine) was ineffective as an antipsychotic, but had potential antidepressant properties (Kuhn, 1958) – leading to the development of the first tricyclic antidepressant.

In parallel to the discovery of imipramine, researchers in New York were studying the effects of anti-tubercular agents (particularly isoniazid) on depressive symptoms (Salzer & Lurie, 1953). After presenting the effects of isoniazid on depressed patients in Syracuse, New York in 1957, trials grew rapidly. One year after the first ideas came to light, over 400,000 patients with depression were treated with what became the first monoamine oxidase inhibitor (MAOI). Discoveries found that the effects of TCAs and MAOIs were both mediated through the noradrenergic and serotonergic systems (Brodie, 1966; Coppen et al., 1967; Schildkraut, 1974; Schildkraut & Kety, 1967), leading to the development of the catecholamine hypothesis. Thus, the first two classes of antidepressants were developed, leading to a surge in research to find monoaminergic-based therapeutics which could target depressive symptomatology.

Despite initial promise with MAOIs such as iproniazid and pheniprazine, the accompanying hepatotoxicity and hypertension made use of these for depression somewhat obsolete (Lòpez-Muñoz et al., 2007). On the other hand, the synthesis of new tricyclics such as desipramine, protriptyline, iprindole, doxepin, trimipramine, and dothiepin caught hold (Fangmann et al., 2008) and are still commonly prescribed to this day, despite substantial adverse effects of administration,

such as cardiovascular toxicity. Findings which described the effects of MAOIs and TCAs being blocked by administration of serotonergic synthesis inhibitors (Shopsin et al., 1975, 1976) spurred more research into novel therapeutics that may work to increase serotonin availability. Serotonin selective reuptake inhibitors (SSRIs) were some of the first psychiatric therapeutics developed which followed a rational drug design, leading to a drug with more direct therapeutic effects and less extrapyramidal side effects (Lòpez-Muñoz et al., 2007). The most prominent SSRI released was fluoxetine hydrochloride, otherwise known as Prozac – which by 1990 became the most prescribed drug in North America.

1.9.2 Mechanisms of monoamine-based antidepressants

While the discovery of monoaminergic antidepressants was somewhat serendipitously based, significantly more research has now been conducted on the underlying mechanisms of monoaminergic-based antidepressants. Monoamine oxidase inhibitors, as the name suggests, inhibit the oxidative deamination of various monoamines, such as 5-HT, dopamine, and noradrenaline (Shih & Thompson, 1999; Tipton et al., 2012), keeping them bioavailable for longer periods of time. However, side effects that included severe hypertension, liver toxicity, and even death discontinued their common use. Despite this, the underlying mechanisms of monoamine oxidases (MAO). Despite initial drawbacks, understanding more about MAO biology may help provide alternate solutions. MAO have two isoenzymes (MAOA and MAOB), which are inhibited by different acetylenic inhibitors. MAOA is generally inhibited by clorgyline, and MAOB is typically inhibited using *l*-deprenyl. While both are able to deaminate the majority of monoamines, there are substrate specific differences that can contribute to variances in therapeutic action by MAOIs (Youdim et al., 2006). Levels of MAOA in particular are greatly increased by corticosterone, whereas MAOB levels are generally unaffected (Edelstein & Breakefield, 1986; Youdim et al., 1989). In contrast, mitogen-activated protein kinase pathway activation mediates levels of MAOB (Wong et al., 2002; Zhu et al., 1994). These differences in isoenzyme actions allowed for the development of other MAOI-based therapeutics that did not have as detrimental side effects. For instance, selective MAOB inhibitors do not produce the “cheese reaction” – whereby fermented foods enter the circulation and potentiate noradrenaline release (da Prada et al., 1988), as they are not as heavily localized in the intestine (Youdim & Weinstock, 2004). In addition, the development of reversible MAOA inhibitors has shown promise in targeting the CNS

somewhat exclusively (Anderson et al., 1993), allowing for the targeting of depressive symptoms without the peripheral-associated side effects. Nevertheless, the development of therapeutics such as SSRIs have outpaced much of the use of MAOIs, though early research suggested they may have use in certain circumstances, such as in an aging population (Gareri et al., 2000) and in those with Parkinson's disease (Birkmayer et al., 1985).

As described above, tricyclic antidepressants (TCAs) were discovered around the same time as MAOIs. While side effects of administration are less severe, their more general pharmacodynamics and risk of overdose make them less favoured in comparison to SSRIs. However, TCAs have proven equally efficacious to SSRIs in treating major depressive disorders, though they are most often used currently to treat conditions such as migraine, OCD, and chronic pain (Christensen et al., 1987; Jackson et al., 2010; Ramasubbu et al., 2022). Mechanistically, TCAs primarily block the reuptake of serotonin and norepinephrine, as well as acting as an antagonist on cholinergic, muscarinic, and histaminergic receptors (Ramasubbu et al., 2022). Due to these varied actions, side effects including confusion, tachycardia, blurred vision, and cardiovascular complicatons are common (Marshall & Forker, 1982). In addition to these side effects, links to increases in suicidal ideation make TCAs less than desirable for use in specific patient populations (Grunebaum et al., 2004).

SSRIs spurred the movement of rational drug design – rather than simply by luck, SSRIs were developed to specifically target the reuptake of serotonin to address depressive symptomatology. There are six major SSRIs in use today: citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline. Pharmacologically, SSRIs target the reuptake of serotonin via the inhibition of the presynaptic serotonin transporter (SERT) in a similar manner to TCAs, however they do not have unintended targets that contribute to most TCA-related side effects. Despite this, SSRIs do have off-target effects which can impact a variety of processes, including sexual and vascular function (Culbertson, 2018; Dimoula et al., 2021; Giatti et al., 2022). Inhibiting the neuronal uptake of 5-HT affects many post-synaptic 5-HT receptors (5-HT1A, 5-HT1D, 5-HT2A, 5-HT2C and 5-HT3), which in turn can have diverse effects on downstream signaling (Hoyer et al., 1994). In addition, the past two decades have determined that the decrease of neuronal SERT is heterogenous throughout brain regions, which may also account for certain differences in antidepressant responses (Kambeitz & Howes, 2015).

Despite the fact that SSRI administration prompts an immediate uptick in serotonergic signaling, the clinical effects of SSRIs often take weeks to begin – suggesting that they may spur longer term changes through mechanisms further downstream. However, it is important to consider that most of the immediate actions of these therapeutics are adverse side effects, which can lead to cessation of use and further worsening of depressive symptoms (Edinoff et al., 2021). Adverse effects commonly reported include increased suicidality, sexual dysfunction, sleep disturbances, weight changes, and more (David & Gourion, 2016). More severely, certain SSRIs (such as citalopram and escitalopram) have been associated with QT prolongation, a precursor to serious heart arrhythmias and serotonin syndrome, which can cause life-threatening excitotoxicity (de La Gándara et al., 2005; Funk & Botswick, 2013). In contrast, some recent research has demonstrated a beneficial effect of SSRIs on vascular inflammation, arterial stiffening, and endothelial function due to off-target effects (Dimoula et al., 2021). Clinically, SSRIs are often used in conjunction with other medications such as mirtazapine (Kessler et al., 2018), or as an adjunct to psychotherapy. A meta-analysis found that the combination of psychotherapy and SSRI administration improved treatment outcomes over a span of 2 years (Cuijpers et al., 2014).

There are many signaling effects of SSRIs not associated singularly with depression – for example, the proposed immunoregulatory effects were recently hypothesized to help those diagnosed with COVID-19, to help tackle the cytokine storms characteristic of the disease (Hamed & Hagag, 2020; Pashaei, 2021). In addition, SSRIs are approved for clinical use in a multitude of neuropsychiatric disorders, including but not limited to generalized anxiety disorder, bipolar disorder, bulimia nervosa, and post-traumatic stress disorder (Ogata et al., 2022).

Following the rational drug design of SSRIs, serotonin and norepinephrine reuptake inhibitors (SNRIs) were developed. As the name suggests, these therapeutics inhibit the reuptake of both serotonin and norepinephrine, though most currently marketed, such as venlafaxine and duloxetine, predominantly act on serotonergic mechanisms. In addition, the inhibition of serotonin reuptake usually precedes the inhibition of norepinephrine reuptake, which can lead to a unique side effect profile (Sansone & Sansone, 2014). Similar to SSRIs, they are prescribed for many different conditions, but may be particularly effective in cases of comorbid depression and chronic pain (Marks et al., 2009). However, they encounter many of the same problems as SSRIs including

delays in therapeutic efficacy and adverse side effects, which has prioritized the development of novel antidepressants over the past decade.

1.9.3 Novel therapeutic approaches

1.9.3.1 Ketamine

Ketamine was first developed in 1962 as a shorter-acting and less psychoactive sedative agent to replace the use of phencyclidine (Mion, 2017). As a non-competitive NMDAR antagonist, ketamine exerts its analgesic actions through activating inhibitory descending monoaminergic pain pathways. In addition, anesthetic doses of ketamine antagonises both μ- and κ-opioid receptors (Hirota & Lambert, 2022). The use of ketamine in human patients began in 1964 and was soon being described as a "dissociative anesthetic" given the patient descriptions of floating sensations and environmental disconnection (Domino & Warner, 2010). While initially unknown, this dissociation is most likely due to a functional disconnection between limbic and thalamocortical systems (Mion, 2017). Due to its large safety margins, ketamine was approved for human anesthesia in 1970 by the Food and Drug Administration, spurred by its use as a battlefield medication in the Vietnam war. However, lower patient tolerability due to its psychotomimetic actions, increases in intracranial pressure, and seizures led to discontinuation of common use outside of specific circumstances (Mion, 2017). In addition, the inclusion of ketamine in 1999 in the US Controlled Substances Act as a class III substance mirrored social concerns of ketamine as a drug of abuse. As an anesthetic, ketamine balances a mix of beneficial and adverse effects. Beneficially, ketamine has neuroprotective effects, anti-inflammatory effects, anti-tumor actions, increases opioid analgesia, prevents shock, and decreases hyperalgesia and acute tolerance. On the opposing side, ketamine has certain sympathomimetic actions that can lead to hypertension and tachycardia, as well as the potential for development of ulcerative cystitis in chronic recreational ketamine users (Hirota & Lambert, 2022). As an anesthetic, ketamine is currently most often used in veterinarian medicine. In humans, ketamine is still used as an intravenous induction agent in shocked patients, for those with reactive airways disease, for children with congenital heart diseases that are characterized by a right to left shunt, for severe burns, in battlefield analgesia, and as a conjunctive medication to benzodiazepines for anesthesia (Kurdi et al., 2014).

1.9.3.1.1 Ketamine structure/pharmacology

Ketamine is highly water and lipid soluble phencyclidine derivative which is rapidly broken down for distribution after administration. Containing an asymmetric carbon atom, ketamine has two enantiomers: S(+) isomer and R(-) isomer. Primarily, ketamine acts as a non-competitive N-methyl D-aspartic acid (NMDA) receptor antagonist, though it has also been shown to interact with opioid receptors, monoaminergic systems, and many other major signaling systems throughout the central and peripheral nervous system (Persson, 2010). Similar to phencyclidine, ketamine binds to a site deep within the ion channel, producing an open channel block which can occlude ions from moving through the open channel (Huettner & Bean, 1988). However, this property necessitates binding to a channel that is already in an open conformation with the magnesium block already removed (MacDonald et al., 1987). (S)-ketamine has a 4x stronger affinity to NMDARs than (R)-ketamine and 2x more than the racemic mixture of isomers. Generally metabolized in the liver through hydroxylation and N-demethylation, the major metabolites of ketamine are norketamine and hydroxoynorketamine. Due to its solubility, it easily crosses the blood brain barrier making for a rapid onset of effect (Herd et al., 2008). The elimination clearance of ketamine is also quite high (about equal to liver blood flow), and its half-life is 2 – 3 hours (Domino et al., 1984; Schüttler et al., 1987). Interestingly, clearance rate of ketamine may be 20% faster in women (Sigtermans et al., 2009).

1.9.3.1.2 Discovery of ketamine as an antidepressant

In the early 1990s, studies were beginning to characterize the antidepressant-like actions of MK-801 in animal models, an NMDAR antagonist (Nowak et al., 1993). Accompanying this were findings that exposure to stress led to glutamate surges in specific brain regions (Moghaddam, 2002), leading to a surge of interest in glutamatergic modulation for antidepressant effects. Cementing the role of glutamate in antidepressant effects were the changes in NMDARs in regions important for mood after treatment with monoaminergic-based antidepressants (Nowak et al., 1993; Skolnick et al., 1996). While the discovery of ketamine as a fast-acting antidepressant is often viewed as somewhat serendipitous, a strong preclinical foundation was set before early clinical findings. However, this does not make the discovery of the antidepressant effects of ketamine any less profound. At the break of the 21st century, Berman and colleagues demonstrated that the use of ketamine at subanesthetic doses could elicit rapid (within hours) and long-lasting (up to 3 days) antidepressant effects in TRD participants – a response that was previously unheard

of with all monoaminergic antidepressants (Berman et al., 2000). Following closely after the discovery of Berman and colleagues, Dr. Carlos Zarate's group quickly verified the effects of ketamine in a treatment-resistant depression and bipolar depression participants (Zarate et al., 2006, 2012), with response and remission rates significantly higher than that of traditional antidepressants. These clinical breakthroughs led to a surge of clinical and pre-clinical research to establish ketamine as a promising novel therapeutic.

1.9.3.1.3 Clinical effects

In recent years, multiple randomized, placebo-controlled trials have validated its robust antidepressant effects in individuals with MDD (Alnefeesi et al., 2022; McIntyre et al., 2020), those with treatment-resistant depression (TRD) who had not previously responded to conventional therapeutics (Dai et al., 2022; Zarate et al., 2006), and those with treatment-resistant bipolar depression (Diazgranados et al., 2010; Zarate et al., 2012). These antidepressant effects were found to be sustained well beyond the half-life of the drug and its peak pharmacokinetic exposure in the body, suggesting that its effects are maintained via a timely activation of signaling cascades in the brain. One meta-analysis demonstrated that ketamine's antidepressant effects peaked at 24 hours post-infusion and faded after 10 to 12 days (Kishimoto et al., 2016). Ketamine has also been found to successfully treat traditionally treatment-refractive symptoms domains such as anhedonia, suicidality, and amotivation (Monteggia & Zarate, 2015)

These findings led the FDA to approve intranasal esketamine (Spravato)—the (S)-enantiomer of ketamine—for adults with TRD in 2019, and for adults with MDD and acute suicidal ideation or behavior in 2020 (U.S. Food & Drug Administration, 2019) under a Risk Evaluation and Management Schedule (REMS). This agent has also been approved by the European Union for the same indications. With recent research on serotonergic psychedelics showing promise, attention has also shifted to what role psychoactive effects of ketamine may play in its antidepressant efficacy. The association between ketamine's efficacy and its psychoactive effects appears to be weak, and most data indicate that its psychotomimetic effects are not related to its antidepressant outcomes (Acevedo-Diaz et al., 2020; Ballard & Zarate, 2020). Furthermore, one recent study found no interaction between reported adverse events post-ketamine and later MADRS scores (Greenwald et al., 2020).

Despite its encouraging antidepressant profile, concerns regarding the use of ketamine as an antidepressant persist, particularly given its potential for abuse (Kokane et al., 2020). Ketamine also generates a transient psychoactive state that peaks around 40 minutes post-infusion, including changes in perception, mood, thought, and self-awareness, which necessitate its administration under medical supervision (Acevedo-Diaz et al., 2020; Kraus et al., 2017; Sassano-Higgins et al., 2016). A potential solution for this has been the promise observed with administration of (R)-ketamine, an enantiomer that has lower NMDAR potency and shown initial promise in clinical trials (He et al., 2022). Nevertheless, the re-purposing of ketamine from an anesthetic to an antidepressant created a paradigm shift, sparking a surge in research to develop and/or repurpose other therapeutic compounds with rapid and robust actions and similar behavioral and biological effects (Kadriu et al., 2021).

1.9.3.1.4 Hypothesized biological effects

1.9.3.1.4.1 Large-scale effects

The discovery of ketamine as a fast-acting antidepressant launched a new era of research which hoped to determine the underlying mechanisms of fast-acting antidepressants. In humans, neurophysiological techniques which can measure macroscopic brain activity, such as electroencephalography (EEG), magnetoencephalography (MEG), and/or functional magnetic resonance imaging (fMRI) have provided some of the greatest insight into ketamine's antidepressant efficacy. Amplitude changes of sensory evoked to potentials to a variety of stimuli (e.g. visual, auditory, and/or somatosensory) can be measured as a proxy of synaptic efficacy and long-term potentiation. As discussed previously in great detail, dysregulated neuroplasticity is a biological hallmark of depression, and these neuroimaging techniques can provide evidence towards region-specific deficits and mechanisms of novel therapeutics (Liu et al., 2017; Price & Duman, 2020).

Ketamine has been found to increase visual sensory evoked potentials of MDD participants within 3 – 4 hours relative to an active placebo, though this was not associated with treatment response (Sumner et al., 2020a). Ketamine has also been shown to rescue proxies of shorter-term plasticity, as measured by the mismatch negativity task (MMN) which measures detection of deviance within repeated presentation of auditory or visual stimuli. The MMN event-related response has been shown to be significantly increased 3 – 4 hours after ketamine infusion in participants with MDD

(Sumner et al., 2020b), though this result is not always consistent when conducted in healthy controls (Rosburg et al., 2004; Rosburg & Kreitschmann-Andermahr, 2016; Schmidt et al., 2011).

A significant amount of research has looked at the impact ketamine has on gamma power, especially as recent studies have shown that gamma power can be used as a diagnostic biomarker for MDD, differentiating between healthy controls as well as other neuropsychiatric disorders (Fitzgerald & Watson, 2018). In addition, gamma rhythms appear to correlate with generation of action potentials and are associated with interactions of GABAergic inhibition and glutamatergic excitation (Buzśaki & Wang, 2012). Robust increases in gamma power have been consistently observed after ketamine administration in clinical trials with comparisons to both active and saline placebos (de la Salle et al., 2022; Farmer et al., 2020; Gilbert & Zarate, 2020; Nugent et al., 2018). Changes in gamma power after ketamine administration are also reliably associated with treatment response, making it a potential promising biomarker of therapeutic efficacy (de la Salle et al., 2022; Gilbert & Zarate, 2020; Nugent et al., 2018).

Lastly, fMRI measurements of resting-state functional connectivity allow for regional insights into the antidepressant effects of ketamine. fMRI research has highlighted the importance of the default mode network (DMN) in depression, which includes regions such as the medial prefrontal cortex, anterior cingulate cortex, and posterior cingulate cortex (Marchetti et al., 2012; Sheline et al., 2010). Reduced connectivity between these regions is consistently found in participants with MDD in comparison to healthy controls (Abdallah et al., 2016; Kraus et al., 2020), and ketamine has been shown to increase global pre-cortical connectivity within one day (Abdallah et al., 2017). In addition, ketamine has demonstrated a rapid increase of DMN and insular connectivity to the level of healthy controls two days after administration (Evans et al., 2018). Prefrontal cortex and striatal connectivity in TRD participants were normalized after ketamine infusion and associated with sustained improvements in symptoms such as anhedonia (Mkrtchian et al., 2021). Evidently, large-scale measures of macroscopic brain activity can provide valuable insight into the mechanisms of ketamine's fast-acting therapeutic effects. However, defining molecular changes is also of utmost importance to the development of novel fast-acting therapeutics.

1.9.3.1.4.2 Small-scale

While initially thought to primarily exert its antidepressant effects through NMDAR antagonism, research suggests that ketamine has several potentially relevant mechanisms of antidepressant

action (Zanos et al., 2018). For example, therapeutic concentrations of the ketamine metabolite (2R,6R)-HNK are unable to inhibit NMDARs (Lumsden et al., 2019) and ketamine itself may only inhibit a fraction of NMDARS even at peak concentration (Dravid et al., 2007; Zhao et al., 2012). Other NMDAR antagonists have not been completely able to mimic ketamine's antidepressant effects in clinical trials, with compounds such as MK-801, memantine, lanicemine, and AV-101 failing in phase II or III despite initial pre-clinical promise (Autry et al., 2011; Piva et al., 2021; Pochwat et al., 2019).

Currently, two main hypotheses exist for ketamine's effects: the disinhibition hypothesis and the direct inhibition hypothesis (Miller et al., 2016). The disinhibition hypothesis states that, at subanesthetic doses, ketamine preferentially antagonizes NMDARs on gamma-aminobutyric acid (GABA)-ergic interneurons, potentially through a stronger affinity to GluN1/GluN2C containing interneurons (Pothula et al., 2021). The blockade of these inhibitory interneurons increases firing of excitatory pyramidal neurons, which increases glutamate release AMPAR transmission; this, in turn, activates integral synaptic signaling pathways that contribute to ketamine's therapeutic effects (Zanos & Gould, 2018). The direct inhibition hypothesis posits that ketamine's antagonism of NMDARs on excitatory pyramidal neurons lessens tonic NMDAR activation by circulating glutamate, which subsequently increases protein synthesis by decreasing suppression of eukaryotic elongation factor 2 (eEF2) (Autry et al., 2011; Nosyreva et al., 2013). In addition to its glutamatergic mechanisms, ketamine inhibits monoamine reuptake and is an opioid receptor (mu, delta, kappa) agonist, a dopamine receptor agonist, and a muscarinic receptor antagonist (Matveychuk et al., 2020).

More recently, attention has turned to the AMPAR-mediated mechanisms that may underlie the actions of both ketamine and SPs. The role of AMPAR transmission in the effects of rapid-acting antidepressants has been extensively described (Abdallah et al., 2018; Zanos et al., 2018). Briefly, inhibition of GABA signaling disinhibits glutamate release from excitatory pyramidal neurons. The increased glutamate binds to post-synaptic AMPARs, which increase brain-derived neurotrophic factor (BDNF) release through a rise in Ca2+ influx. BDNF then binds to tropomyosin-related kinase B (TrkB) which, through downstream signaling molecules, activates the mTOR complex 1 (mTORC1). This transient activation upregulates proteins related to increased excitatory transmission, such as post-synaptic density-95 (PSD-95), Synapsin I, and

increased membrane insertion of GluA1. These molecular changes ultimately lead to increases in excitatory signaling in areas such as the hippocampus and prefrontal cortex, two areas which demonstrate decreased signaling in depression.

Many preclinical studies support this mechanism of action for ketamine (Li et al., 2010; Zanos et al., 2016; Zhou et al., 2014). There has also been a drive to classify the actions of ketamine's enantiomers and metabolites, particularly (R)-ketamine and (2R,6R)-HNK (Yao et al., 2017; Zhang et al., 2014). Notably, the actions of (R)-ketamine, which induced longer-lasting antidepressant effects in a chronic stress model, appeared to depend on AMPA and TrkB activation. (S)-ketamine, in turn, activated mTOR independently of TrkB activation (Rafało-Ulińska & Pałucha-Poniewiera, 2022). The inactivation of eukaryotic initiation factor 4E-binding proteins (4E-BPs) through mTOR is essential for the antidepressant-like effects of both ketamine and (2R,6R)-HNK (Aguilar-Valles et al., 2021). Recent research into the role of mTORC1 on ketamine's effects have produced somewhat unexpected results. Specifically, rapamycin (which inhibits the actions of mTOR) prolonged ketamine's antidepressant effects in participants with TRD (Abdallah et al., 2020). A recent clinical trial similarly found that pre-treatment with rapamycin prolonged ketamine's antidepressant, but not anti-suicidal, effects (Averill et al., 2022). This may be due, in part, to rapamycin's anti-inflammatory effects in the periphery, which could contribute to ketamine's antidepressant effects (Attur et al., 2000; Chen et al., 2013).

Finally, while ionotropic glutamatergic transmission is clearly essential for the antidepressant effects of both ketamine, metabotropic glutamate receptors (mGluRs) may also play an important role (Chaki, 2021; Musazzi, 2020). mGluR levels have consistently been linked to stress vulnerability (Peterlik et al., 2015). Interestingly, (2R,6R)-HNK was found to exert mGlu2R-dependent antidepressant effects (Zanos et al., 2019). Another recent study found that while ketamine did not alter mRNA or the protein expression of ionotropic glutamate receptors, both chronic stress and ketamine administration altered mGluR2 expression (Elhussiny et al., 2021). Co-administration of ketamine and an mGluR2/3 antagonist also sustained antidepressant response in animal models (Pałucha-Poniewiera et al., 2021; Rafało-Ulińska et al., 2022).

1.9.3.1.4.2.3 GABAergic signaling

GABAergic signaling deficiencies have been consistently implicated in depression (Duman et al., 2019) and ketamine's antidepressant effects via the disinhibition hypothesis (Zanos & Gould,

2018). Other fast-acting antidepressants also appear to deficits in GABAergic signaling to restore E/I balance in animal models for the study of depression (Ren et al., 2016). The exact method of how ketamine is affecting GABAergic signaling has been of great interest. Pre-clinical studies suggest that ketamine may upregulate $GABA_AR$ activity in the hippocampus and cortex, as well as increasing hippocampal GABA turnover (Silberbauer et al., 2020; Wang et al., 2017). Therapeutics known to regulate $GABA_ARs$, such as allopregnanolone (brexanolone), have also shown to be effective at targeting certain depressive symptoms. In fact, brexanolone was approved by the FDA in 2019 for postpartum depression (Lüscher & Möhler, 2019; Pinna, 2020). It is currently unclear whether its $GABA_AR$-mediated mechanisms are responsible for its potential as an antidepressant, though future preclinical and clinical studies hope to fully elucidate this relationship. Negative allosteric modulators of $GABA_AR$ are able also able to strengthen excitatory signaling in a similar method to ketamine (Fischell et al., 2015). Interestingly, significantly increasing $GABA_AR$ transmission through high dose benzodiazepines dampens ketamine's antidepressant effects (Andrashko et al., 2020; Frye et al., 2015). Regardless of the underlying mechanisms, ketamine is evidently able to alleviate changes in inhibitory post-synaptic signaling and deficits in various synaptic GABAergic markers.

1.9.3.1.4.2.4 Other mediators of ketamine signaling

Ketamine does not solely modulate glutamatergic signaling, with recent research demonstrating that ketamine has effects in the monoaminergic and opioid systems among others. Racemic ketamine, its enantiomers, and/or its metabolites were able to increase extracellular levels of dopamine, serotonin, and norepinephrine in the mouse prefrontal cortex (Ago et al., 2019), an effect which may be mediated by glutamatergic projections from the mPFC to the locus coeruleus and dorsal raphe (López-Gil et al., 2019). Depletion of serotonin by a tryptophan hydroxylase inhibitor was also able to block some of the antidepressant-like effects of ketamine (du Jardin et al., 2018). Both preclinical and clinical PET-imaging research has demonstrated an increase in SERT occupancy and 5-HT1B receptor binding after ketamine administration, though some findings are inconsistent (Spies et al., 2018; Tiger et al., 2020; Yamanaka et al., 2014). Behavioural evidence through ketamine's rescue of motivational dysfunction and anhedonia suggests that it may also effectively impact dopaminergic reward circuitry (Abdallah et al., 2017; Lally et al., 2014; Mkrtchian et al., 2021; Nogo et al., 2022). Following this, application of dopamine signaling

inhibitors was able to prevent the behavioural effects of ketamine administration (Hare et al., 2019; Wu et al., 2021a, b). Repeated administration of ketamine has also been shown to increase dopaminergic and norepinephrinergic neuron firing activity (Iro et al., 2021). While not the main mechanism, it appears that monoaminergic signaling could be another contributor to ketamine's antidepressant effects.

In recent years, there has been a shift in focus to the role of the opioid system in depression (Perez-Caballero et al., 2020), in part spurred by observation of ketamine's antidepressant effects and actions on opioid receptors (Williams et al., 2018). Treatment-resistant depression has been associated with reductions in mu-opioid receptor availability, and certain symptoms can be resolved using low-dose buprenorphine, an opioid partial agonist (Peciña et al., 2018). In addition, public health research has found strong associations between depression and opiate use – over half of all opiate prescriptions in the US are given to those diagnosed with mood disorders (Davis et al., 2017). Ketamine also directly binds with strong affinity to mu-opioid receptors and weakly to kappa-opioid receptors (Bonaventura et al., 2021), which are only 5- to 20-fold weaker than ketamine's affinity to NMDAR. Recent pre-clinical research has demonstrated links between ketamine's actions on opioid receptors and its antidepressant effects, as opioid antagonists are able to abolish the fast-acting behavioural effects of ketamine (Klein et al., 2020; Wulf et al., 2022; Zhang et al., 2021). Clinically, administration of naltrexone diminished ketamine's antidepressant effects in participants with TRD (Williams et al., 2018), which will soon be correlated with neuroimaging data from a recent clinical trial (NCT04977674). Clinical trials are currently underway to determine the effects of ketamine in conjunction with methadone or buprenorphine for comorbid depression and opioid use disorder (NCT04177706, NCT05051449).

Evidently, ketamine may work through a multitude of mechanisms that are not quite fully understood. In addition, determining the mechanisms by which ketamine has its psychotomimetic effects and/or antidepressant effects is essential for developing further novel therapeutics that may be more accessible to more diverse populations.

1.10 Reelin

In Edinburgh in 1951, a spontaneous autosomal recessive mutation arose in a mouse colony, where the mice exhibited severe neuronal abnormalities that accompanied a "reeling" gate (Falconer, 1951; Hamburgh, 1963). These "reeling" mice were better characterized in the 1990s, where they

found a complete loss of *RELN* gene transcription (D'Arcangelo et al., 1995), which produced the extracellular matrix glycoprotein reelin. The loss of *RELN* led to neuronal ectopia in laminated brain structures. Due to abnormalities in the hippocampus, cortex, and cerebellum, these mice presented with severe deficits in motor coordination and generally leads to death around the time of weaning (Cooper, 2008). A mutation with similar deficits was later found to have a structural malformation in the 8th "reelin repeat" (RR) and failed transcription of the C-terminus (Ranaivoson et al., 2016). *RELN* mutations in humans are rare, and often lead to lissencephaly due to the deficits in proper neuronal migration (Kato & Dobyns, 2003). However, decreases in reelin protein expression are not uncommon, leading scientists to examine the role of how reelin maintains and controls biological process in adulthood, particularly in neurodevelopmental and associated psychiatric disorders.

Composed of 3461 amino acids and with a molecular mass of 388 kDa, the reelin sequence is coded by the *RELN* gene which is localized to chromosome 7 in humans and 5 in rodents (de Bergeyck et al., 1998; Ranaivoson et al., 2016). The amino acid structure is decently conserved between rodents and humans, with a similarity of 94.2% (DeSilva et al., 1997). Reelin is composed structurally by 8 RRs, a short C-terminal, and an N-terminal (Ichihara et al., 2001). Reelin can be cleaved in two locations by the Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMT) family. The N-t site is cleaved between the 2nd and 3rd RR by ADAMTS2/3, whereas the C-t site is cleaved between the 6th and 7th RR by ADAMTS4/5 (Koie et al., 2014; Sato et al., 2016). The central fragment of reelin contains the 3rd – 6th RR, the site which contains the necessary binding elements for reelin to activate its receptors (Knuesel, 2010; Nakano et al., 2007). It appears that each fragment of reelin may regulate different processes and cleavage can occur in endosomes or extracellular space, though these are still far from being fully understood (Koie et al., 2014). A Furin recognition site is also present on the C-t, which can release the ending 6 amino acids on the chain, however the function remains elusive (Ranaivoson et al., 2016).

Reelin's canonical receptors are part of the lipoprotein superfamily: very-low-density-lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) (Beffert et al., 2005, 2006). Research has suggested that reelin has a stronger affinity for ApoER2 than VLDLR, however certain studies have found similar affinities (Benhayon et al., 2003; Hiesberger et al., 1999; Yasui et al., 2010). In addition, there is evidence that the N-t of reelin binds to integrin receptors such as

α3β1, which contributes to neuronal positioning and synaptic strengthening (Kramár et al., 2002; Qiu et al., 2006; Rodriguez et al., 2000). Due to the observations of improper laminar migration, the first investigations into reelin focused on the role of reelin expression in development.

1.10.2 Developmental role of reelin

In prenatal development, reelin is secreted by Cajal-Retzius cells in the marginal zone of the cortex and hippocampus, as well as by glutamatergic cerebellar cells (Lacor et al., 2000; Schiffmann et al., 1997; Tissir & Goffinet, 2003). Reelin is released into the extracellular space at a synthesis-dependent rate and binds to its receptors mentioned above. When reelin is bound, it drives the VLDLR and ApoER2 receptors together to activate disabled-1 (Dab1), an intracellular adaptor protein which is phosphorylated through the Src family kinases (SFKs) Fyn and Src (Bock & May, 2016). Dab1 is the common factor which begins signaling pathways important for both developmental and post-developmental phases, such as the PI3k/Akt/mTORC1 and Crk/CrkL/Rap1 pathways (Gotthardt et al., 2000; Rice et al., 1998; Jossin, 2020).

As observed from the deficits present in homozygous reeler mice, reelin is an important cyto-architect which arranges the inside-out lamination of the cortex and other layered areas through control of cell-cell interactions to position migrating neurons (Chameau et al., 2009; Kohno et al., 2020). The presence of reelin initiates a specific radial glia phenotype from progenitor cells in the ventricular zone. These radial glia have projection fibers that act as scaffolds for new-born neurons to ascend towards their destinations (Chai et al., 2009). In proper development, early-born cells occupy deeper layers of the cortex, whereas later-born cells migrate to more superficial layers, where reelin may act as an attractant for neurons to progress past their predecessors (Gilmore & Herrup, 2000; Vilchez-Acosta et al., 2022). The canonical lipoprotein reelin receptors appear to have disparate effects in cortical migration, as knockout of VLDLR causes neurons to invade the marginal zone and ApoER2 knockouts have later-stage migration deficits (Hack et al., 2007; Arimitsu et al., 2021). VLDLR is hypothesized to act as a "stop signal" which terminates migration by detaching the neuron from the radial glial cells (Dulabon et al., 2000; Qiu et al., 2006). Interestingly, the central fragment of reelin is sufficient for proper cortical organization (Jossin et al., 2004; Wasser & Herz, 2017; Hattori & Kohno, 2021). Taken together, it demonstrates why reelin-deficient mice display inverted cortical lamination and how reelin is essential for proper neural development.

1.10.3 Reelin in adolescence and adulthood

In the post-natal brain, production of reelin by Cajal-Retzius cells is shifted to hippocampal and cortical GABAergic interneurons and glutamatergic cells in the olfactory bulb, cerebellum, and layer II pyramidal cells in the piriform and entorhinal cortex (Knuesel, 2010; Wasser & Herz, 2017). In adulthood, it appears that reelin regulates dendritogenesis and dendritic spinogenesis, and synaptogenesis (Beffert et al., 2006; Niu et al., 2008; Rogers et al., 2011, 2012; Ventruti et al., 2011; Weeber et al., 2002), as evidenced by reelin overexpression models which demonstrate increased neurogenesis, hypertrophy of dendrites, and synaptic contacts (Pujadas et al., 2010). Changes in reelin expression can also affect the morphology of dendritic spines, changing configurations of presynaptic boutons that can affect function of synapses (Bosch et al., 2016). Reelin's activation of the Crk family proteins through tyrosine phosphorylation may also impact dendritic outgrowth through regulation of membrane protein trafficking and the actin cytoskeleton. Low levels of Crk have been associated with decreased dendritic complexity in the hippocampus (Matsuki et al., 2008). Interestingly, heterozygous reeler mice and double Crk/CrkL mutant mice exhibit extremely similar cortical phenotypes (Park & Curran, 2008). Most likely due to its dendritogenic effects, an intraventricular injection of reelin was able to enhance LTP and cognitive function in wild-type mice (Rogers et al., 2011), heterozygous reeler mice (Rogers et al., 2013), and a mouse model of Angelman syndrome (Hethorn et al., 2015). A knockout of reelin's canonical receptors (VLDLR & ApoER2) produces reductions in synaptic density and memory impairments (Mulder et al., 2004; Trommsdorff et al., 1999).

A potential mechanism by which reelin could enhance synaptic plasticity is through the regulation of glutamate receptor activity. Dab1 activation is able to stimulate SFKs, which are physically connected to NMDARs and phosphorylate both GluN2B and GluN2A subunits to enhance current (Chen et al., 2005). In *ex vivo* hippocampal slices, reelin is able to enhance CA1 AMPAR- and NMDAR-synaptic signaling – however, this effect was blocked if the canonical receptors were knocked out (Weeber et al., 2002), or if slices had a pre-treatment of PI3K or Rap1 inhibitors (Qiu et al., 2006). In addition, treatment of hippocampal slices with reelin regulated neuronal excitability through prevention of GluA2 dephosphorylation (Durakoglugil et al., 2009). Pre-synaptically, reelin may influence neurotransmitter release through mediating levels of SNAP25, a protein required for vesicles to bind to the plasma membrane (Hellwig et al., 2011). Another

mechanism by which reelin could affect neuroplasticity is through activation of the PI3K/Akt pathway, upstream effectors of mTORC1 and a mode by which it increases AMPAR membrane insertion and dendritic branching in the hippocampus (Jossin & Goffinet, 2007). Reelin is also able to prevent amyloid-β induced LTD by inhibition of GSK-3β to protect hyperphosphorylation of Tau (Beffert et al., 2002; Durakoglugil et al., 2009; Kramár et al., 2002).

The N-t of reelin binds with a high affinity to the α3β1 intergrin receptor, which co-localizes to dendritic spines and post-synaptic densities (Rodriguez et al., 2000). Integrins mediate synaptic plasticity by shaping the formation and stabilization of synapses (Qiu et al., 2006). Mice deficient in the α3 integrin display deficits in CA1 LTP and spatial memory (Chan et al., 2003). In contrast to reelin's canonical receptors, deletion of α3- or β1-class integrins does not produce a reeler-like phenotype, and shows normal cortical lamination (Jossin et al., 2004). Reelin is also able to promote a developmental "switch" through SFK activation or decrease the ratio of NMDARs which express higher GluN2B than GluN2A (Sinagra et al., 2005). SFK activation is able to increase GluN2B surface mobility, reducing their presence at the synapse (Qiu et al., 2006; Sinagra et al., 2005), an effect which is abolished by blocking both the canonical receptors or β3 integrins (Groc et al., 2007).

There are still significant questions regarding the role of each reelin fragment in its actions. Once full-length reelin has bound to the receptor, it is internalized into endocytic vesicles. In these vesicles, reelin undergoes further proteolysis which cleaves the N-t for re-release (Hibi & Hattori, 2009). Some research has suggested that cleavage of the N-t site may impede Dab1 phosphorylation (Kohno et al., 2009; Kubo et al., 2002), however others have shown no change (Jossin et al., 2004; Koie et al., 2014). The phosphorylation of Dab1 is equally effective by binding of the central fragment of reelin or the full-length protein. Potentially, the cleavage of reelin could aid the diffusion of the central fragment in order to reach target cells at a further distance (Jossin et al., 2020). The cleavage of reelin may also be necessary for proper feedback mechanisms, as reelin cleavage is necessary for reelin clearance and halting the activation of Dab1 (Koie et al., 2014). Another negative feedback mechanism for reelin signaling is the cleavage of ApoER2, whereby the intracellular domain can translocate to the nucleus to regulate the transcription of genes (including *RELN*) and the extracellular domain is able to inhibit reelin activity in primary neurons (Balmaceda et al., 2014; Koch et al., 2002; Telese et al., 2015; Wasser & Herz, 2017).

Evidently, all processes which reelin is thought to regulate have been implicated in neuropsychiatric disorders, making reelin of great interest in the context of depression.

1.10.4 Reelin in depression

After the discovery of the importance of reelin in development, interest turned to uncovering the role of reelin in various neurodevelopmental and neuropsychiatric disorders such as autism, schizophrenia, bipolar disorder, Alzheimer's disease, and depression. Major breakthroughs were made when decreased levels of *RELN* mRNA were found throughout the brain in schizophrenia (Impagnatiello et al., 1998), the cerebral cortex of those diagnosed with bipolar disorder (Guidotti et al., 2000), and in the hippocampus of schizophrenia, bipolar, and MDD patients (Fatemi et al., 2000). The decrease in reelin was particularly strong in CA4, or DG, of the hippocampal region. This central downregulation was also paralleled by lowered serum reelin levels (Fatemi et al., 2001), which may be mediated by a hypermethylation of the *RELN* promotor caused by psychosocial stress (Abdolmaleky et al., 2005; Veldic et al., 2004). It appears that reelin-related methylation changes in the blood are parallel to those in the brain in schizophrenia, representing a potential promising biomarker which may hold true in mood disorders such as depression (Auta et al., 2013; Nabil-Fikri et al., 2017).

Both homozygous ($RELN^{-/-}$) and heterozygous ($RELN^{+/-}$) reeler mice are valuable models to study the role of reelin in various neuropsychiatric disorders. While homozygous reeler mice have obvious physiological and neurochemical abnormalities, heterozygous reeler mice (HRM) (who express 40 – 60% less reelin) exhibit only subtle changes. Behavioural tests such as the OFT, black-white box, FST, and novelty-suppressed feeding tests are unable to differentiate between HRM and wild-type mice (Teixeira et al., 2011). However, certain memory tests are able to reveal differences, with reelin-deficient mice having worse memory spans (Iemolo et al., 2021) and VLDLR/ApoER2 knockouts showing deficits in fear conditioning (Weeber et al., 2002). Despite no large baseline behavioural differences, HRM are quite vulnerable to the development of a depressive-like phenotype. HRM who were exposed to a chronic corticosterone administration had increased immobility on the FST and decreases in neurogenesis in a dose-dependent manner than was not found in wild-type mice (Lussier et al., 2011). This vulnerability has been corroborated in other studies that showed changes in Y-maze performance and social recognition between HRM

and wild-type mice after chronic CORT administration in drinking water (Notaras et al., 2020; Schroeder et al., 2015).

The administration of chronic corticosterone seems to have distinct biochemical effects in HRM vs. wild-type mice – co-expression of neuronal nitrous oxide synthase (NOS) and reelin in the hippocampus is decreased after CORT exposure in wild-type mice but increased in HRM. This could be one mechanism by which stress could worsen oxidative events in vulnerable phenotypes (Caruncho et al., 2010; Romay-Tallon et al., 2015). In addition, reductions in PSD-95, parvalbumin, activity-regulated cytoskeletal protein (Arc), and the dendritic number and complexity have been observed in HRM (Dong et al., 2003; Liu et al., 2001; Tueting et al., 2006; Ventruti et al., 2011). Lowered reelin signaling has also been linked to deficits in monoaminergic signaling, particularly serotonergic and dopaminergic signaling (Michetti et al., 2014; Varela et al., 2015). Glutamatergic signaling is also intertwined with reelin's pathways, as heterozygous reeler mice express higher protein levels of GluN2A and GluN2b than wild-type mice (Isosaka et al., 2006). On the other side, induced overexpression of reelin provides a resilience to chronic stress and promotes dendritic outgrowth and circuit establishment of granule cells from the dentate gyrus (Teixeira et al., 2011). Taken together, the vulnerability of reelin-deficient mice and the potential neuroprotective benefits of reelin overexpression heavily implicate reelin to have a role in depressive phenotypes.

Outside of genetic studies, reductions of reelin have been consistently associated with chronic stress (Caruncho et al., 2016). Our laboratory has shown that chronic corticosterone administration was able to decrease reelin-immunoreactive cells by 21% in the CA1 stratum-lacunosum-moleculare and by 26% in the subgranular zone of the dentate gyrus (Lussier et al., 2013a). Chronic restraint stress was not able to reduce reelin, however it also failed to induce depressive-like behaviours and changes in both GABAergic and glutamatergic neurotransmission generally associated with depression (Gregus et al., 2005; Lussier et al., 2013b). In addition, time-dependent increases in FST-immobility, indicative of despair-like behaviour, were found to be paralleled with decreases in reelin expression and an indicator of hippocampal neurogenesis (Lebedeva et al., 2017, 2020). Cyclical corticosterone administration also causes progressive decreases in reelin levels, which parallels with the worsening of depressive-like behaviour (Lebedeva et al., 2020). There appear to be sex differences in the response of reelin to stress, as basal levels of reelin are

lower in females than males in the paraventricular nucleus, and region-specific female reelin expression remains unchanged after chronic corticosterone (Sánchez-Lafuente et al., 2022). Analyses have not determined specific reelin epitope function in depression, and future research should pursue epitope-specific analyses.

The observed decreases in hippocampal reelin are able to be rescued by both traditional and non-traditional antidepressant administration. Chronic administration of imipramine, a TCA, and etanercept, a TNF-α inhibitor, were both able to rescue deficits in reelin after chronic corticosterone administration (Brymer et al., 2018; Fenton et al., 2015). Treatment with citalopram, an SSRI, was also able to counteract the deleterious effects of kainic acid on reelin mRNA and protein levels (Jaako et al., 2011). Lastly, solid evidence has implicated the role of reelin in ketamine's antidepressant effects: genetic depletion of reelin, its receptor ApoER2, or certain downstream effectors is able to abolish the behavioural and biochemical antidepressant-like effects of ketamine (Kim et al., 2021). This is some of the strongest evidence that reelin signaling may play a role in generating therapeutic response.

All of the evidence towards reelin's role in depression led our lab to posit that the administration of exogenous reelin could help treat a depressive-like phenotype. To first investigate the potential therapeutic effects of reelin, 1 µg of central fragment reelin was infused directly into the hippocampus in a repeated (1/week over 3 weeks) or acute (24 hours before FST) manner. Both repeated and acute doses of central fragment intrahippocampal reelin was able to rescue despair-like behaviour (FST immobility) and cognitive deficits (object location task), as well as neurochemical deficits in NMDARs, AMPARs, and $GABA_A$Rs (Brymer et al., 2020). Repeated injections were able to rescue the complexity and number of newborn granule cells, however this was not found after a single injection of reelin, suggesting that increases in neurogenesis are not necessary for reelin's short-term antidepressant effects. Interestingly, the reversal of behaviours and neurochemical deficits was blocked if CNQX, an AMPAR antagonist, was administered (Brymer et al., 2020). The reliance on AMPARs have been repeatedly implicated in fast-acting antidepressant therapeutics, including ketamine (Kadriu et al., 2021). As previously described, AMPARs are usually co-expressed with NMDARs and are essential for synaptic plasticity (Malenka & Nicoll, 1997). Other intracranial infusions of reelin, including intrahippocampal, intra-amygdalar, and intraventricular, have also found therapeutic effects in mouse models for the

study of depression that have been associated with increases in glutamatergic and GABAergic tone (Hethorn et al., 2015; Ibi et al., 2020; Rogers et al., 2011, 2013). Preclinical studies on the effects of exogenous reelin administration can be found in Table 1.3.

Model	Route of Administration	Dose & Timing	Findings
Chronic corticosterone model	Peripheral intravenous adminstration	Repeated 3 µg, 3x, once every 7 days Acute 3 µg 1 - 2 days after acute injection	Repeated and acute reelin administration rescued behaviour and neurochemical abnormalities (hippocampal GABAA2/3β, GluA1, and GluN2B receptors), as well as SERT MPC (Allen et al., 2022)
Chronic corticosterone model	Intrahippocampal infusion	Repeated 1 µg, 3x, once every 7 days Acute 1 µg Behaviour 1 – 7 days after infusion after acute injection	Repeated and acute reelin rescued deficits in behaviour and hippocampal AMPA, NMDA, and GABAA receptor expression, which could be blocked with CNQX (Brymer et al., 2020)
Maternal immune activation model	Intrahippocampal infusion	0.2 pmol/ hemisphere 4 and 10 days after injection	Rescued memory and anxiety-like behaviour, and recruited hippocampal synaptoporin (Ibi et al., 2020)
Angelman syndrome mouse model	Bilateral intraventricular injection	5 nM total hemisphere concentration 5 days after injection	Enhanced LTP, dendritic spine density, spatial learning and memory (Hethorn et al., 2015)
Heterozygous reeler mice	Bilateral intraventricular injection	5 nM total hemisphere concentration 5 days after injection	Recover deficits in heterozygous reeler mice, including synaptic function, sensorimotor gating, and performance on learning and memory tasks (Rogers et al., 2013)
Wild-type mice	Bilateral intraventricular injection	5 nM total hemisphere concentration 15 min, 3 hours, and 5 days after injection	Enhanced LTP, dendritic spine density and improve associative learning and memory (Rogers et al., 2012)
Hippocampal slices from wild-type mice	Wash-on	5 nM	Increased LTP, dependent on presence of both VLDLR and ApoER2 (Weeber et al., 2002)

Table 1.3. Pre-clinical studies with exogenous reelin administration. AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, ApoER2 = Apolipoprotein E Receptor 2, CNQX = cyanquixaline (6-cyano-7-nitroquinoxaline-2,3-dione), GABA = gamma-aminobutyric acid, LTP = long-term potentiation, NMDA = *N*-methyl-D-aspartate, VLDLR = very low-density lipoprotein receptor.

Evidently, direct hippocampal infusions are not translatable to a human population. Due to this, our lab established a peripheral administration paradigm, whereby reelin is injected intraventricularly (i.v.) into the lateral tail vein in the chronic corticosterone model (Allen et al., 2022). Repeated i.v. injections of reelin were able to rescue FST immobility, hippocampal reelin, GABA $_{A}$β2/3, GluA1, and GluN2B receptors, as well as serotonin transporter (SERT) clustering in peripheral lymphocytes. Most promisingly, a single i.v. injection of reelin was also able to rescue the behavioural and biochemical deficits induced by CORT, further implicating reelin as a potential fast-acting antidepressant. Reelin's actions on PI3k signaling, an upstream activator of mTORC1, have been of extreme interest to better understand its fast-acting antidepressant-like effects. To activate mTORC1, reelin binds to its canonical receptors very-low-density-lipoprotein-receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) (D'Arcangelo et al., 1999). Upon binding, Src/Fyn kinases phosphorylate cytosolic docking protein disabled1 (Dab1), which in turn activates downstream pathways including the PI3K/Akt pathway, an upstream activator of mTORC1 (Beffert et al., 2002). During development and in neuronal cultures, reelin promotes protein translation through the PI3K/Akt pathway, inducing a robust activation of mTORC1 and p70S6K, likely contributing to dendrite growth (Jossin & Goffinet, 2007). Please refer to Figure 1.3 for reelin and ketamine's hypothesized parallel signaling pathways. However, less is known regarding the role of reelin on mTORC1 signaling in adulthood, disease states, or depression, and requires further investigation.

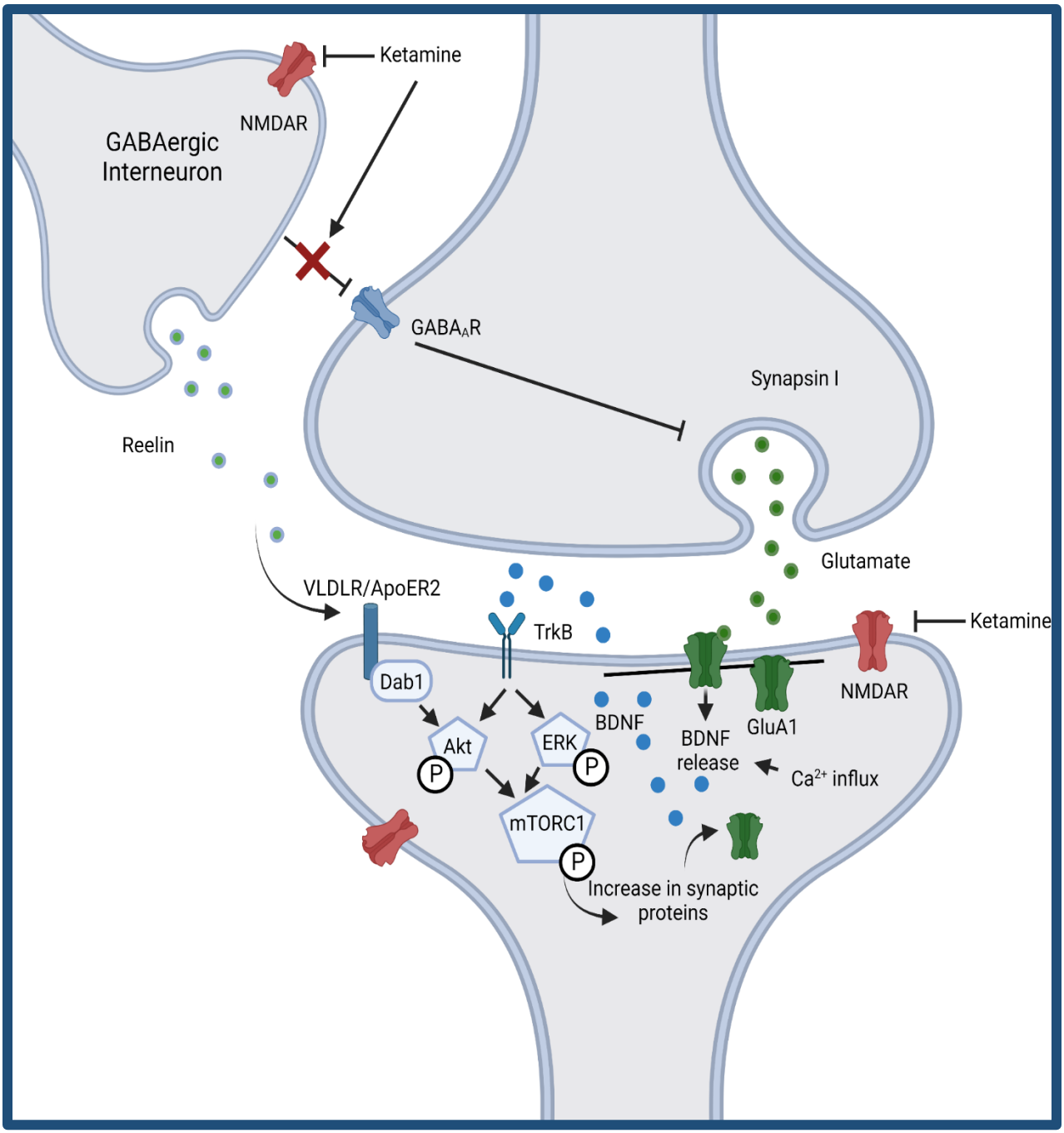


Figure 1.3 Hypothesized overlapping signaling pathways of reelin and ketamine. Reelin is constitutively released from GABAergic interneurons and goes on to bind to its canonical receptors VLDLR (very low-density lipoprotein receptor) and ApoER2 (Apolipoprotein E receptor 2). This binding activates Dab1, an intracellular signal adaptor protein, which in turn activates phosphoinositide-3' kinase (PI3k) to initiate downstream activation of mTORC1. Once phosphorylated, mTORC1 increases synaptic expression of proteins such as GluA1 and PSD-95, which contribute to further excitatory signaling. Ketamine may preferentially antagonize NMDAR receptors on GABAergic interneurons, which disinhibit excitatory cells and lead to an influx of glutamate in the synapse. This increase in glutamate release triggers a Ca^{2+} influx which increases BDNF release. BDNF binds to TrkB receptors, which activate parallel pathways to reelin in order to upregulate mTORC1 activity. Figure was created in BioRender by author.

1.11 Inflammation and depression

Though most research has focused on the impact that MDD has in the central nervous system, decades of research have found links between depressive symptoms and inflammatory states (Majd et al., 2020; Miller & Raison, 2015; Raison et al., 2006; Zunszain et al., 2013). As pleiotropic messengers, inflammatory molecules such as cytokines can promote further neuroinflammation through disruption of microglial and astrocytic function, integrity of the blood brain barrier, and apoptosis (Lugo-Huitrón et al., 2013; Schwarcz et al., 2012; Yirmiya & Goshen, 2011).

Initial interest of the link between depression and inflammation was spurred after administration of interferon-alpha (IFN-α) to treat Hepatitis C precipitated depressive symptoms that were able to respond to traditional antidepressant intervention (Hoyo-Becerra et al., 2014). The use of the endotoxin lipopolysaccharide (LPS) can also reliably induce "depressive-like" behaviours in preclinical research and consistently trigger large inflammatory responses (Zhao et al., 2019). As mentioned above, chronic stress plays a large role in the pathophysiology of depression and chronic, low-grade, inflammation could also contribute to the underlying etiology of depression. Pre-clinical models have found that the upregulation of stress hormones was able to significantly increase markers of inflammation such as tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β), and induce CNS inflammation (Bomholt et al., 2004; Kubera et al., 2011; Song & Wang, 2011). Our laboratory also demonstrated that peripheral administration of etanercept, a TNF-α inhibitor which cannot cross the blood brain barrier, reversed stress-induced behaviours and neurochemical alterations including levels of hippocampal reelin and glutamate receptors (Brymer et al., 2018).

Despite overwhelming preclinical evidence, heightened inflammation does not appear in all patients with depression. Instead, the presence of heightened inflammatory markers appears to represent a specific subset of patients, such as those with TRD (Strawbridge et al., 2019; Yang et al., 2019). Longitudinal studies have suggested that increased inflammation is associated with a higher recurrence of depressive symptoms, worsened outcomes, a more severe course of illness, increased suicidality, and a resistance to traditional therapies (Strawbridge et al., 2019). Depressive symptoms that are more typically associated with TRD, such as anhedonia, suicidality, and reduced motivation, have also been associated with increases in central IL-6 soluble receptors and TNF-α, as well as peripheral C-reactive protein levels (CRP) (Köhler-Forsberg et al., 2017; Ma et

al., 2016; Rengasamy et al., 2021). Higher baseline levels of CRP have also been found to predict treatment non-response (Chang et al., 2012; Zhang et al., 2019) and higher levels of six inflammasome related mRNAs (P2RX7, IL-1β, IL-6, TNF-α, CXCL12, and GR) were able to differentiate between treatment-responsive MDD and TRD (Cattaneo et al., 2020). A meta-analysis supported these studies, finding that higher levels of inflammatory markers, in particular elevated TNF-α, were associated with a poor treatment response (Strawbridge et al., 2015). While these are measurements of peripheral inflammation, recent imaging studies have begun to confirm similar increases in inflammation in the CNS. Positron emission tomography (PET) studies of TSPO (translocator protein 18 kDa) find increased TSPO binding in the PFC and ACC of those currently experiencing a major depressive episode (Richards et al., 2018), though not all findings regarding TSPO binding in MDD and TRD are consistent (Schubert et al., 2021; Turkheimer et al., 2021). Early research has also investigated inflammatory responses to psychological therapies such as cognitive behavioural therapy – where once again poor response was associated with higher levels of CRP, IL-6, and TNF-α (Lopresti, 2017).

Two potential mediators of the link between chronic stress, depression, and inflammation are the HPA axis and kynurenine pathway. As previously discussed, HPA axis overactivation is characteristic of chronic stress (Stetler & Miller, 2011). Higher biomarkers of both HPA axis activity and inflammation (cortisol and CRP) have been associated with the presence of somatic depressive symptoms (Iob et al., 2019), a symptom presentation most often associated with TRD. In women, increased hair cortisol concentrations were associated with poorer performance on memory and cognition tasks, an effect mediated by elevated CRP levels (Iob et al., 2019). The kynurenine pathway is a major modulator of glutamatergic signaling and is heavily impacted by inflammatory processes (Kowalczyk et al., 2019). TDO (tryptophan-2,3-dioxygenase) is a main enzyme of the kynurenine pathway which catabolizes of tryptophan, the precursor for serotonin synthesis. Pro-inflammatory states induce TDO production (Chen & Guillemin, 2009), which can lead to a tryptophan depletion. This depletion can cause depressive symptoms in vulnerable populations (Wichers et al., 2004). The biologically active products of the kynurenine pathway, kynurenic acid (KA) and quinolinic acid (QA) have different effects on inflammation, as KA functions as a neuroprotective agent (Foster et al., 1984) in contrast to QA, an endogenous neurotoxin (Lugo-Huitrón et al., 2013). In participants with depression, ratios of KA and QA have repeatedly found to be altered (Doolin et al., 2018; Moaddel et al., 2018; Zhou et al., 2018).

Ketamine, as discussed, is extremely effective at targeting TRD, making it an ideal therapeutic candidate for examining changes in depression-associated inflammation (Johnston et al., 2023, *in press*). In pre-clinical models, *(R)*-ketamine (though not *(S)*-ketamine) was able to decrease peripheral levels of IL-6 in a model of ulcerative colitis, an effect that was abolished by administration of a TrkB antagonist (Fujita et al., 2021). Similarly, *(R)*-ketamine significantly reduced central and peripheral pro-inflammatory cytokines after LPS administration (Zhang et al., 2021). Heightened levels of cytokines after maternal deprivation stress was also reversed by ketamine administration, or ketamine administered in conjunction with electroconvulsive stimulation in a sex-dependent manner, though other inflammatory measures such as catalase activity and carbonyl levels were not rescued (Abelaira et al., 2022).

Ketamine could also mediate inflammation through the HPA axis and kynurenine pathway. In a chronic stress model, ketamine helped restore the HPA axis' negative feedback loop through restoration of hippocampal glucocorticoid receptor expression (Wang et al., 2019) and was also able to decrease levels of ACTH and corticosterone after LPS administration (Besnier et al., 2017). In clinical research, ketamine has increased a rapid, but transient, burst of cortisol in healthy volunteers (Hergovich et al., 2001; Khalili-Mahani et al., 2015) – however research should investigate this in MDD and TRD. Ketamine directly impacts the actions of QA through its blockade of NMDARs, as QA needs to bind to NMDARs to have its pro-inflammatory actions. In addition, ketamine has been shown to decrease the kynurenine:tryptophan ratio in both clinical and pre-clinical research (Moaddel et al., 2018; Wang et al., 2015). Ketamine-associated increases in KA also correlate with short- and long-term MADRS reductions (Zhou et al., 2018).

Lastly, some studies suggest that ketamine can reduce the reactivity of inflammatory signaling in response to aversive stimuli, such as chronic stress. Administered prophylactically, ketamine can prevent stress-induced behavioural changes and increase resilience to LPS or TNF-α administration (Brachman et al., 2016; Camargo et al., 2021). Clinically, ketamine was also able to decrease levels of cortisol and alpha amylase in healthy volunteers if administered before the stressful stimuli was applied (Costi et al., 2023). While prophylactic ketamine seems promising, clinical applicability of this research may be limited due to the unpredictability of stressful and aversive lifetime experiences.

Less is known about the role of reelin in inflammatory processes, particularly in depression. Reelin is expressed nearly ubiquitously throughout the body, including immune cells in the periphery. Throughout development, reelin is essential for proper formation of the hippocampal radial glial scaffold (Förster et al., 2002) and mutations in reelin's signaling path lead to mutations in microglial immunoglobulin G Fc receptors (Rahimi-Balaei et al., 2020). In aging and schizophrenia, some have hypothesized that prenatal inflammation decreases reelin-expressing neurons, leading to protein aggregation and cognitive impairments in adulthood (Knuesel et al., 2009). Additionally, decreases in inflammation through inhibition of TNF-α, were able to increase reelin immunoreactivity in the hippocampus after chronic stress (Brymer et al., 2018). Offspring from pregnant mice treated with polyinosinic:polycytidylic acid (an immunostimulant) also showed reduced reelin reactivity at post-natal day 28 (Harvey & Boksa, 2012). A controlled cortical impact model of traumatic brain injury found decreased reelin expression in the hippocampus and found that reelin could protect hippocampal cells from glutamate-induced neurotoxicity *in vitro* (Dal Pozzo et al., 2020). Reelin administration was also able to rescue cognitive and behavioural measures in mouse offspring exposed to antenatal inflammation (Ibi et al., 2020), however it is yet unknown whether increasing levels of reelin would decrease inflammation directly in a depressive-like phenotype.

In contrast, some evidence suggests that a depletion of reelin could aid different inflammatory-based disorders such as atherosclerosis and autoimmune encephalomyelitis (Calvier et al., 2020, 2021). This is because high levels of reelin and Apoer2 are associated with endothelial cell dysfunction, promoting leukocyte-endothelial cell adhesion (Calvier et al., 2020). Accumulation of reelin during the aging process has also been associated with Alzheimer's disease, with reelin aggregates co-localizing with non-fibrillary amyloid plaques (Knuesel et al., 2009). It is evident that finding a homeostatic balance of reelin may be essential to regulate inflammation in the periphery and central nervous system. Taken together, the evidence suggests that inflammation appears to contribute to a subset of depressive pathology and could inform future biomarker and therapeutic research.

1.11.2 SERT clustering in the periphery

Due to the proposed involvement of inflammation in depression, significant amounts of research has been conducted to determine potential panels of inflammatory-related biomarkers, and whether

they are able to distinguish MDD from other neuropsychiatric disorders such as anxiety (Martin et al., 2014). Our laboratory has found that serotonin transporter (SERT) clustering on the lipid rafts of peripheral lymphocytes may provide a promising therapeutic biomarker. Serotonin, both synthesized and released by lymphocytes, appears to regulate natural killer cell function and promotes the function of chemotactic factors such as cytokines in early stages of the inflammatory processes (Marazziti et al., 2010). In relation to this, the expression of SERT on lymphocytes appears to regulate serotonin-induced lymphocyte proliferation (Slauson et al., 1984), though more an update in the research is necessary.

Lipid microdomains play important roles in neurotransmitter activity as well as reuptake (Magnani et al., 2004), and may be an important peripheral system affected by MDD. The redistribution of proteins in lipid rafts is actually believed to be critical for antidepressant response (Allen et al., 2006; Czysz et al., 2014; Erb et al., 2016), and could provide valuable clinically useful biomarkers. Alterations in SERT levels have also been repeatedly implicated in depression, particularly as a biomarker for suicidality (Purselle & Nemeroff, 2002) – which is often higher in those with TRD. In those who died by suicide, SERT binding was significantly lower independent of an MDD diagnosis (Underwood et al., 2018).

In the lipid raft microdomains on PBMCs, average SERT cluster size is increased in participants with treatment-naïve depression, a finding that is paralleled in pre-clinical models of chronic stress (Caruncho et al., 2019). In addition, the distribution pattern (percent clusters within a modal peak) was able to predict treatment response in patients with depression (Rivera-Baltanas et al., 2014). In both groups of patients (responders and non-responders), baseline HDRS and self-assessed anhedonia scores were equivalent – however all participants with larger clusters (a lower percentage of clusters within 0.05 to 0.1 μm) responded to treatment, with 75% achieving remission. Those with a higher proportion of smaller clusters had much worse response rates, with only 45% reaching the criteria for response and 22% reaching remission (Rivera-Baltanas et al., 2014). However, differences in membrane protein clustering in PBMCs is not completely unique to SERT clustering – participants with MDD show dysregulation in patterning of dopamine transporters, GluN2b-containing NMDARs, prion cellular protein, and Pannexin 1 (Romay-Tallon et al., 2017, 2018). Of importance, there is evidence that reelin is able to induce membrane protein clustering (Dong et al., 2003) and heterozygous reeler mice display significant increases in cluster

sizes (Caruncho et al., 2016) which parallel findings from chronic stress models and participants with depression. To my knowledge, no previous studies have looked at the effects of ketamine on SERT membrane protein clustering.

A large consideration is also the ease of implementation into SERT clustering analysis into a clinical setting. The ability to measure the clustering on PBMCs from a blood smear, which only requires a drop of blood from a finger, is quite non-invasive and does not take a significantly increased amount of time or materials (Romay-Tallon et al., 2017). In addition, our lab has created a macro which allows for automatic analysis of MPC, including size, number, and distribution of clusters (unpublished data). This macro runs through Fiji, a free program which is available worldwide to increase accessibility.

1.12 Specific research aims

Experiment 1: Due to the historical lack of involvement of patients in laboratory-based research, we aimed to create guidelines that would shape the introduction and involvement of people with lived experience into our research. To accomplish this, interviews and regular meetings were arranged with participants diagnosed with treatment-resistant depression and psychosis both inside and outside of the laboratory setting. The guidelines were developed in conjunction with these patient partners. In addition, the emergence of COVID-19 in 2020 impacted much of the patient-oriented research we were able to conduct but spurred structured discussions in a patient-oriented research advisory committee (consisting of people with lived experience, caregivers, researchers, clinicians, and healthcare administrators) that informed the development of guidelines to support patients diagnosed with neuropsychiatric disorders throughout the pandemic.

Experiment 2: As the evidence that ketamine and reelin may be working in a parallel through the mTOR pathway was preliminary, I started with the creation of synaptoneurosomes from vehicle and CORT-treated animals and *in vitro* incubations of reelin and ketamine to assess synaptic-specific changes related to the mTORC1 signaling pathway. Synaptoneurosomes from the hippocampus and cerebellum were created using sequential filtrations and treated with varying concentrations of reelin and ketamine (hippocampus: reelin – 5 nM, 10 nM, 50 nM, ketamine – 50 nM, 100 nM, 500 nM and cerebellum - 5 nM, 10 nM, 50 nM, ketamine - 5 nM, 10 nM, 50 nM). Western blotting was used to analyze proteins related to the mTORC1 signaling pathway, such as mTORC1, p-mTORC1, and PSD-95. In a similar manner, PBMCs were isolated from blood

collected from our vehicle animals and treated with 1 µm of CORT, then ketamine or reelin to observe changes in SERT clustering on lymphocytes after treatment.

Experiment 3: While initial *in vitro* research looked to determine parallel pathways, I also wanted to ascertain the impact that ketamine has on reelin expression, and whether this could mediate some of its fast-acting antidepressant effects. Animals underwent the chronic CORT-administration paradigm, and then were treated with ketamine. A fear conditioning and extinction protocol was used to verify ketamine's antidepressant effects. Immunohistochemical analyses were performed to analyze the expression of reelin and GluA1 in the subgranular zone of the hippocampus, as well as the count and complexity of DCX-immunoreactive cells to determine any impact ketamine may have on neurogenesis.

Experiment 4: To parallel the *in vitro* studies on synaptoneurosomes, I aimed to confirm the preliminary results we saw regarding the actions of ketamine and reelin on the mTORC1 pathway, as well as more large-scale excitatory transmission in the hippocampus. After chronic stress, animals received either reelin or ketamine and behavioural measurements were used to assess anhedonic-like and despair-like behaviour. *In vivo* electrophysiology was conducted on a subset of the animals to ascertain any deficits or rescues of long-term potentiation in the hippocampus. Reelin-immunoreactive cells were assess in the SGZ to observe the short-term effects exogenous reelin and ketamine administration would have on central reelin expression. Lastly, synaptoneurosomes were created to determine the effect reelin and ketamine had *in vivo* on the mTORC1 pathway, as assessed through Western blotting.

Experiment 5: While animal models can provide a valuable resource for the study of depression, novel approaches and methodologies to increase translation are invaluable. In this study, my aim was to begin characterizing an iPSC-model of TRD patients, as well as determining the responses of iPSC-derived neurons to ketamine and reelin treatment. Cells were reprogrammed and cultured into cortical neurons, then various proteins measured through Western blotting and immunocytochemistry to characterize the cell lines and treatment responses. In the interest of increasing translation, samples from TRD participants who had received ketamine treatment and assessed for various levels of cytokines and other inflammatory markers, to potentially provide future biomarkers for the study of depression.

A portion of the work outlined in this dissertation has been previously published:

Johnston, J. N., Ridgway, L., Cary-Barnard, S., Allen, J., Sanchez-Lafuente, C. L., Reive, B., ... & Caruncho, H. J. (2021). Patient oriented research in mental health: matching laboratory to life and beyond in Canada. *Research Involvement and Engagement*, 7(1), 1-11.

Johnston, J. N., Thacker, J. S., Desjardins, C., Kulyk, B. D., Romay-Tallon, R., Kalynchuk, L. E., & Caruncho, H. J. (2020). Ketamine rescues hippocampal reelin expression and synaptic markers in the repeated-corticosterone chronic stress paradigm. *Frontiers in Pharmacology*, 11, 1387.

Brymer, K. J., Johnston, J., Botterill, J. J., Romay-Tallon, R., Mitchell, M. A., Allen, J., ... & Kalynchuk, L. E. (2020). Fast-acting antidepressant-like effects of Reelin evaluated in the repeated-corticosterone chronic stress paradigm. *Neuropsychopharmacology*, 45(10), 1707-1716.

Chapter 2

The incorporation of patient-oriented research into laboratory-based studies

2.1 Abstract

As patient-oriented research gains popularity in clinical research, the lack of patient input in foundational science grows more evident. Research has shown great utility in active partnerships between patient partners and scientists, yet many researchers are still hesitant about listening to the voices of those with lived experience guide and shape their experiments. Mental health has been a leading area for patient movements such as survivor-led research, however the stigma experienced by these patients creates difficulties not present in other health disciplines. The emergence of COVID-19 has also created unique circumstances that need to be addressed. Through this lens, we have taken experiences from our patient partners, students, and primary investigator to create recommendations for the better facilitation of patient-oriented research in foundational science in Canada. With these guidelines, from initial recruitment and leading to sustaining meaningful partnerships, we hope to encourage other researchers that patient-oriented research is necessary for the future of mental health research and foundational science.

2.2 Introduction

Patient-oriented research (POR) aims to improve the healthcare system through prioritizing active partnerships between various stakeholders such as patients, clinicians, researchers, administrators. While the need for the incorporation of patient perspectives into the healthcare system seems clear, current evidence-based models and clinical practices in Canada often leave out the voices of people with lived experience (PWLE) (Krahn & Naglie, 2008; Schünemann et al., 2006).

Over the past two decades, patient lobbying has played a large part in shifting views towards the inclusion of PWLE, increasing patient empowerment and greater democratization of science (Elberse et al., 2011). In Canada, the Strategy for Patient-Oriented Research (SPOR) has been released by the Canadian Institute of Health Research (CIHR). Patient engagement is a central tenet of this framework, which is defined as "occurring when patients meaningfully and actively collaborate in the governance, priority setting, and conduct of research, as well as in summarizing, distributing, sharing, and applying its resulting knowledge" (CIHR, 2019). Throughout this section, the term patient partners (the term preferred by our PWLE collaborators) is used to describe those who are actively engaged in the development of novel research.

In addition to being directly impacted by the research, the value of patient input is multifaceted: experiential knowledge increases the relevance and quality of health research and participation of patient partners can lead to more widespread acceptance of research outcomes (Sacristán, 2013). The unique background and experiences of patient partners also provide new perspectives for experimental avenues and co-production of research ideas that are not limited to their own experiences with specific disorders. For example, patient partners with graphic design expertise could create materials for further recruitment or those with mathematical expertise could aid in statistical analyses. Despite these numerous benefits, there are still many barriers to the inclusion of patient partners in healthcare research, particularly laboratory-based science. Understanding these barriers and exclusion mechanisms that patient partners face needs to be a priority. Major barriers can be classified as behaviours (e.g. granting less attention, respect, or speaking time) and/or verbal communications (e.g. jargon-heavy speech, sidelining issues brought forth by patient partners) (Elberse et al., 2011; Williamson, 2008). In part, the exclusion of qualitative evidence can hinder advancements in patient inclusion through dismissal of experiential results. A recent study has demonstrated this clearly, showing that the majority of biomedical scientists and health care service workers still prioritized experience below perceived expertise, despite stating outward positive attitudes towards patient and public involvement (Boaz et al., 2016). The necessity to educate primarily lab-based scientists on the benefits of qualitative research and experiential expertise is evident.

To bridge these gaps in patient inclusion, emphasis needs to be placed on effective and inclusive knowledge translation. Successful knowledge translation is one of the greatest challenges in science, with the common “valleys of death” metaphor used to describe the significant amount of information lost between research stages (Butler, 2008). As community engagement and basic laboratory research are often a few steps away from each other, failures in translation are not uncommon (Eisenmann, 2017; Moore, 2008; Tkacs & Thompson, 2006). Clinical practice guidelines and research priorities can even go against patient preferences, with researchers rarely factoring in aspects such as socioeconomic or work status into treatment options (Krahn & Naglie, 2008). This lack of consideration of patient voices has led some to propose a patient emancipation movement, emphasizing the need for greater control over medical decisions, autonomy, informed consent, and respect from the healthcare system (Williamson, 2008). A potential solution for this is Priority Setting Partnerships, established by the James Lind Alliance, which brings together

PWLE, caregivers, researchers, and clinicians to establish top research priorities that have been jointly agreed upon (James Lind Alliance, 2012.; Hollis et al., 2018; Kelly et al., 2015; Knight et al., 2016; Madden & Morley, 2016). This consists of regular structured meetings, for equal sharing of ideas and decision-making between patient partners and clinicians or researchers. Priority Setting Partnerships also emphasize the need for education and knowledge transfer through education on language that is more specialized, or finding alternatives to jargon which could exclude certain participants, a major challenge in POR work.

Patient partners with lived experience of mental health disorders face additional unique challenges, such as being viewed as less competent to make decisions about their own care. This perceptual bias does not account for differences in disorder severity, or fluctuating cycles of wellbeing. This bias and potential discrimination emphasizes the need to ensure the relationship between patient partners and researchers is egalitarian. Depending on the capacity of each individual to engage in research, there may be risks related to involvement that need to be discussed before beginning a partnership. In certain instances, the inclusion of family members and/or caregivers in decision-making processes can be extremely valuable, as demonstrated by studies on shared decision-making with acutely ill in-patients diagnosed with schizophrenia (Hamann et al., 2006, 2007). In addition, this form of research is susceptible to participant bias, where those who are more likely to participate exemplify certain attributes that may not represent entire patient populations. It is also important to acknowledge that there are no boundaries to those diagnosed with a mental health disorder, and this can encompass a wide array of people that include clinicians and researchers. There are no boundaries between “patient”, “clinician”, and “researcher”. While these labels are used separately, please keep in mind that there is overlap and stigma faced by people who fit into multiple categories.

With these challenges in mind, my goal was to develop a set of guidelines alongside our patient partners that could be used to incorporate patient-oriented research into a laboratory setting. In addition, a major aim was to translate discussions from the Patient-Oriented Research Advisory Committee (POR-AC) on Vancouver Island into actionable requests and research ideas informed by patient partners, clinicians, administrators, and researchers.

2.3 Methodology

2.3.1 Recruitment of patient partners

Initial contact to patient partners was between Dr. Hector Caruncho and Lisa Ridgway. Following this, the recruitment of other patient partners was through word-of-mouth via initial patient partners and clinicians and presentations at conferences such as the 22nd Annual Vancouver Island Psychosis Conference. Before beginning patient-oriented research in the laboratory setting, patients were informally screened to determine if they would be an appropriate match for the laboratory. Questions such as time available, attitudes towards animal research, and decision-making capacity were considered.

2.3.2 Formation of the POR-AC

The composition of the POR-AC was built on the foundational principles of Canada's Strategy for Patient-Oriented Research – where patients, researchers, and health practitioners work together to contribute to improved and sustainable health systems and outcomes. The committee composed of 2 clinicians, 1 administrator, 6 people with lived experience, and 4 basic researchers. The committee was first formed at the beginning of 2020 and held monthly meetings until March 2022. While the initial plan was to meet in person, the majority of meetings were conducted online via Zoom due to the pandemic. Verbatim notes were taken at each meeting to be transposed and analyzed. Compensation for patient partners was provided through funding from the BC SUPPORT Unit.

2.4 Results

2.4.1 Guidelines for the incorporation of POR into laboratory-based sciences

Please keep in mind that these recommendations primarily reflect the incorporation of POR in the context of Canada, particularly in BC, as our patient partners and researchers share their experiences from within that administrative framework. They are based off the experiences of our research group and advisory committee, and we understand many labs will have varying experiences. The degree of involvement and engagement of each person with lived experience will need to be evaluated at an individual level, so please take caution in the generalization of these guidelines. These guidelines have been previously published in Johnston et al., 2020.

2.4.1.1 Initial recruitment

Advertisement and increased dissemination of POR opportunities are an essential first step to the inclusion of patient partners in laboratory research. Many patient partners have reported that they had no idea that they could be involved in this form of research. Local clinicians, conferences that are open to patients and caregivers, and various outreach groups that work with different communities are invaluable resources to advertising possible patient engagement. Conferences such as the 22nd Annual Vancouver Island Psychosis Conference has been effective in spurring initial participation interest from a variety of patient groups.

In the case where there is already an established patient partner in the laboratory, having this person "meet and greet" the new PWLE or caregiver after initial contact helps to build trust and provide a safe and secure forum to discuss research. To avoid potential barriers such as enforcing differential power dynamics, the first meeting(s) should take place outside of the lab setting, in a neutral location such as a coffee shop.

2.4.1.2 Building the partnership

Following the meeting with the established patient partner, the new potential partner is introduced in either a one-on-one (with the principal investigator) or in a group (inclusion of students in the lab, or research technicians for example) setting, depending on their levels of comfort. To also assist in comfort, a family member or caregiver may be present if preferred by the patient partner. The inclusion of a family member can help in building the support system and provide information that may be useful to both the patient partner and those who care for them. In the experience of our lab, once the patient partner has started to feel comfortable, they will start open conversations regarding ideas for research and begin to co-build the relationship between patient partner and researcher. One of the patient partners involved in our lab presented a graduate-level seminar on their journey with mental health, as well as co-leading a tutorial for graduate students to review their research from a lens of someone with lived experience with treatment-resistant depression.

Time and effort from both parties is required to co-build an equal relationship between patient partners and researchers. To help students improve their communication skills and interactions with patient partners, a translational neuroscience course has begun at the University of Victoria to allow interactions between researchers, graduate students, patients, and members of patient organizations in a more casual setting. In this setting, the patient partners are the leaders and students share their research to develop a shared and equal language to discuss their laboratory-

based scientific endeavours and hear opinions and ideas from the patient partner. Training such as this should be implemented in most, if not all, graduate programs that center mental health research. Earlier training in proper translation of research and center patient voices allows a greater amount of future researchers to incorporate POR into their later studies.

2.4.1.3 Conducting POR in laboratory-based science

Early in the process, patient partners should indicate their comfort and amount of involvement they would like to have in various projects. This comfort can depend on multiple factors such as social comfort, availability, previous knowledge in the field, and more. Privacy concerns are also an important issue, as some patient partners may not want to be identified due to surrounding stigma. In contrast, other patient partners may value sharing their experiences and journey with larger audiences, which can, as a side benefit, help spread information on POR and recruitment. Two of the patient partners that work closely in our laboratory setting have co-authored papers and make regular presentations to wide audiences. Regular and in-depth discussions regarding preferences of each patient partner should help circumvent any potential issues of discomfort or boundary-setting. Another barrier may be restrictions to certain lab areas, where direct observation of research may not be possible. If patient partners express interest, they could gain certifications that grant access to the restricted areas, or a neutral meeting place could be suggested so that the research can still be discussed and patient feedback would still have a direct impact on the studies being conducted inside of the lab.

Animal research can also pose a unique issue. As this is a sensitive topic, it is necessary to establish the comfort levels of patient partners with engaging in animal research. The patient partners I have worked closely with emphasize the need to discuss animal use ethics and the direct attempts of researchers to minimize the suffering and number of animals used. In addition, the understanding that the use of animal models is necessary for novel drug development and biomarkers also helped minimize hesitancy surrounding animal research. Another patient partner has suggested that researchers prioritize keeping up to date with non-animal substitutions, and design research questions accordingly. As discussed later in this thesis, switching from animal models to an iPSC-derived model was heavily informed by this viewpoint. If a patient partner is still not comfortable with any form of animal use, they should still be able to engage in the research laboratory if there are opportunities that are removed from animal research. For example, patient partners have been

more involved in biomarker studies that use human blood samples, which they reported significantly more comfort with. Again, it is essential to establish and re-establish boundaries regularly for each individual partner. After the initial meetings, contact may begin to fade naturally. To prevent this, researchers need to make clear that they are open to the engagement of patient partners and open to ideas and collaboration. Contact information for phone and email should be shared and readily available to all. In addition, setting up regular meeting times with no penalty for change or cancellation can encourage consistency in patient partner engagement.

Determining compensation is also an integral part of patient engagement. Patient partners dedicate their time and labour to the research conduct, policy, and dissemination and should therefore be appropriately compensated. This is supported by research demonstrating five important reasons for compensation: equity, different motivations than researchers, respect for vulnerability, commitment, and barrier removal (Breault et al., 2018; Hamann et al., 2006). Richards and colleagues have provided a detailed roadmap on patient compensation in Canada, which was contributed to by researchers and patient partners (Richards et al., 2018, 2020).

2.4.2 The effects of COVID-19 in mental health: as informed by people with lived experience

Following the development of guidelines to incorporate patient partners into laboratory science, discussions in the POR-AC shifted to the impact of the COVID-19 pandemic on people with lived experience as the pandemic begun. From these discussions, four priority themes emerged that have now been put together in a report for dissemination to various administrative bodies:

1. How has COVID-19 impacted the stigma surrounding mental health?
2. How important was support (familial, financial, etc.) for mental health during COVID-19?
3. Did COVID-19 increase burnout in people with lived experience, caregivers, and mental health care workers?
4. How did changes to the system impact the mental health of people with lived experience (harder to access, easier to access, more free services etc.) How could we improve this and what should we keep after COVID-19?

Quotes have been anonymized for member privacy. Figure 2.1 provides a summary infographic of major aims and conclusions.

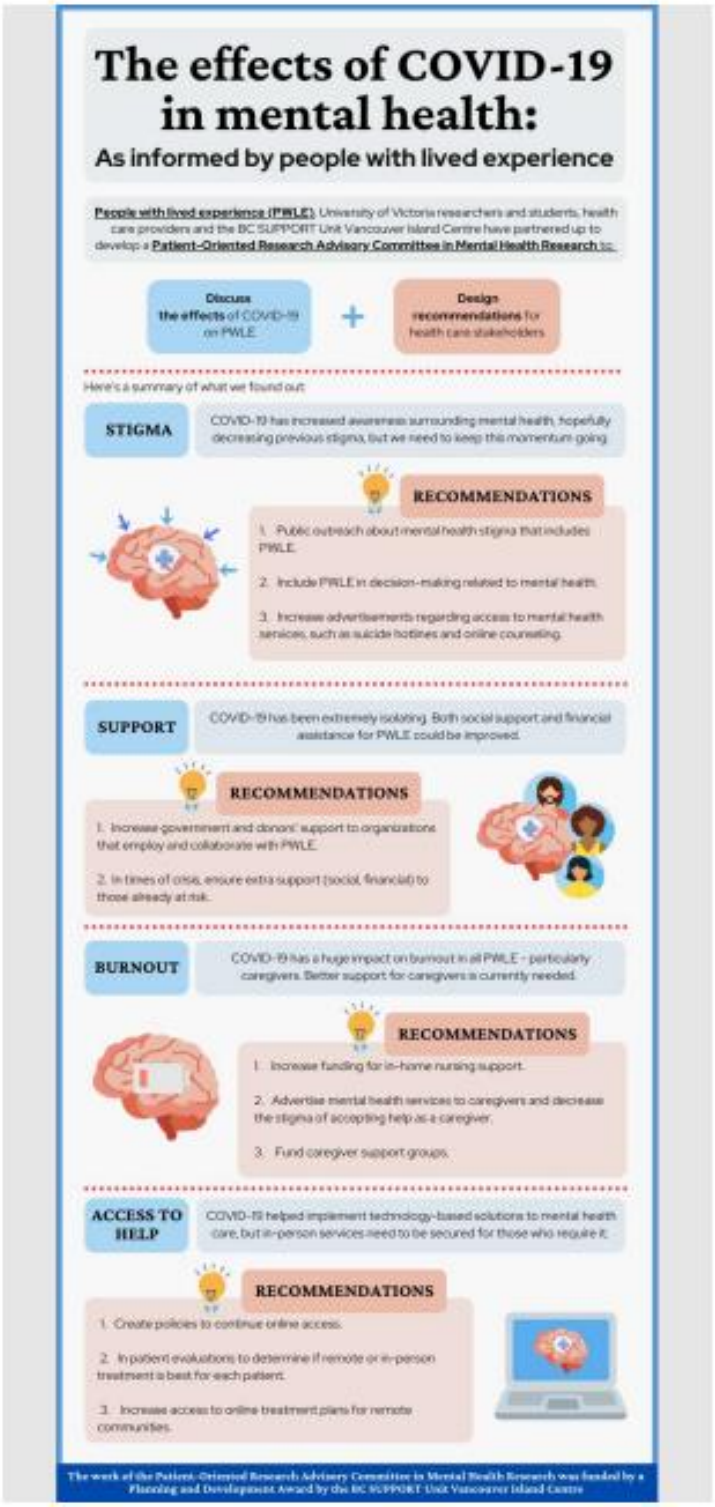


Figure 2.1. Summary infographic on the effects of COVID-19 in mental health as informed by people with lived experience. Four major areas that were impacted by COVID-19 were identified: stigma, support, burnout, and access to help. Recommendations for each priority area are identified, to later address through policy changes and future research. Created in collaboration with Elisa Gonçalves de Andrade and the POR-AC.

2.4.2.1 How has COVID-19 impacted the stigma surrounding mental health?

The purpose of this question was to determine whether the POR-AC thought that the impact of COVID-19 has decreased the stigma surrounding mental illness, as most people have, in one way or other, been impacted by high levels of stress and suffering during pandemic times. We wanted to investigate whether the increased prevalence of mental health issues in the general population would bring about a different view on mental illness and decrease the stigma surrounding it.

The members of the POR-AC agreed that awareness surrounding the continuum of "mental health to mental illness" has increased alongside the interest of the general population in discussions about mental health care.

"Right now the mental health question is the "next wave" of COVID – the crisis of the mind and how it has impacted everyone is at the forefront of many discussions."

- Member of POR-AC

The POR-AC believes that the onset of the pandemic has encouraged a shift in the way others view mental health issues, as many have experienced significant increases in stress. It is an important time to leverage this awareness and push towards novel policies that inform the general population of mental health issues, stigma, and resulting discrimination. Though awareness of mental health issues seem to be decreasing, POR-AC members suggest that stigma and discrimination in specific contexts (e.g. hiring practices) still occurs regularly, and may be important to target.

"Discrimination is also a large issue. For PWLE, it is hard to get help right now, when they are counting on help from government. My one thing is that just because you have a mental health diagnosis, does not mean you are less intelligent or an employment risk. People are very hesitant to hire people with pre-existing mental health conditions, which harms and pushes the stigma even more."

– Member of POR-AC

Recommendations developed by the committee were as follows: A shared sentiment was the need to leverage these current conversations surrounding mental health and mental illness. After the pandemic, the development of policies to ensure that the sympathy and understanding developed by the general population about mental health issues stay at the forefront of peoples' minds should

be prioritized. This new-found understanding and the dissemination of further information surrounding mental health could decrease the stigma associated with mental disorders. Public outreach and public awareness related to this issue can be carried out when the vaccination campaigns are successful and people begin to resume normal lives.

2.4.2.2 How important was support for mental health during COVID-19?

All members of the POR-AC agreed that familial, friendship and financial support were essential for mental health throughout the pandemic, however, the importance was higher for people with lived experience and caregivers. For those already struggling, the impact of isolation, loss of income, or loss of employment opportunities were substantial and often exacerbated existing issues.

"If I didn't have disability – it would be significantly more stressful, and I would not be able to get a job otherwise – recently I was discriminated against for disclosing my mental health at an interview with the salvation army. My counsellor was also cut, so having no family support would be very difficult and make everything a lot harder."

- Member of POR-AC

In certain situations, overreliance on technology has caused a loss of support:

"My connection to this stigma and mental health and jobs – we need to make sure HR is following through on workplace practices and how to chip away at these biases – nobody would discriminate against diabetes. For disability, my son had a technical glitch on the direct deposit of disability. If he has to cash a cheque, he has to acknowledge that he has a mental illness, and he is not willing to acknowledge this so he does not have money. I could cash the cheques, but this is going against his direct wishes and he has his own agency. There are no supports in place for issues like this."

- Member of POR-AC

The committee agrees that COVID-19 was a medical, social, and economic problem that had a very big effect on PWLE. It is essential to develop a framework to help those at risk not only during exceptional times, but normal times as well. Increasing the government and donors' support of organizations like "Mental Health Recovery Partners" would be a good starting point and directly benefit all parties in mental health care with a relatively modest investment effort.

2.4.2.3 Did COVID-19 increase burnout in PWLE, caregivers, and mental health workers?

"Who cares for the caregivers?" was a major point echoed in the discussion about COVID-19 and burnout.

"In the case of mental illness, we are not cared by the system, we are cared for by our families – day to day is family responsibilities, not healthcare systems of practitioners. When there is extra strain, it is almost impossible to cope."

- Member of POR-AC

"I have never experienced burnout this much – all of my normal supports are gone (my parents and my sister). You can't leave the house, and the stress of this is a lot. Our son is back in intensive care and we are lacking some form of liaison."

- Member of POR-AC

"…for caregivers who are at home with their loved ones, even accessing help online has been a struggle since they are at home all the time."

- Member of POR-AC

Certain members cited less stress when their loved ones were close to home, though stated some PWLE were reluctant to seek professional help due to fear of isolation which can increase burnout. With this in mind, increasing the level of support by the government to caregivers of those with mental health issues is critical. Increasing funded in-home nursing support or a shift towards emphasizing holistic approaches rather than the traditional medical model could also better help caregiver burnout.

2.4.2.4 How did changes to the system impact the mental health of people with lived experience?

Common themes of changes to the system were greater access to resources through technology such as video chats, telephones, and email.

"Within Island Health we are now able to share psychoeducation information through email with patients' consent-this could not happen easily years ago. Our new program is all virtual, or mostly virtually including filling out forms and having meetings-this has been really positive. The

restrictions on providing care have lessened and hopefully we will be able to continue this pattern to provide preventative care to more people."

- Member of POR-AC

"I found some of the systems were great to access – when we were in major crisis mode, I could talk to the psychiatrist directly over the phone which is a bridge we could not cross before. The online was also very helpful, as I did not have to leave the house to do that."

- Member of POR-AC

These sentiments were mirrored in preliminary qualitative findings from Dr. Paterson's study (unpublished) that suggests Canadians are enthusiastic about innovative approaches to access health care supports and services, with themes seen including need for increased availability of counselling services, increased service access methods including telephone, video, texting, online based, and 24/7 available service options, and a need for more transparency, easier access, and simplified application/referral processes for services. The continuation of easing access to mental health systems and support should be prioritized after COVID-19, as it benefits many different populations, such as those in remote communities, those unable to attend sessions in person, and more.

Moving forward, it will be critical to maintain the advantages of the new adaptations of technology-based systems used during the pandemic that have been deemed useful by people with lived experience. The possibilities of remote access need to be leveraged by taking advantage of the decrease of reliance on in-person care. This decrease may allow for the prioritization of people who are not well served by a remote approach and/or for people that clinicians deem to need in-person visits.

2.5 Discussion

To further promote inclusion of patient partners in laboratory-based research, multiple facets of translation need to be considered. First, experiential data from PWLE needs to be adapted into experiments that can be performed in a laboratory setting. Basic research often erases individual qualities to control for potential confounding variables, a practice in direct conflict with the individualized circumstances and questions of patient partners (e.g. "What is the right treatment

for me?", "How does my socioeconomic status impact treatment availability?", "What are the best treatments for different lifestyles?"). In addition, the equalization of language should be a priority in collaborations between researchers and patient partners – researchers need to place their ideas in lay terminology, and terms where that is not possible should be provided to patient partners beforehand, so they are able to familiarize themselves with the language beforehand.

It is also essential to emphasize that building strong relationships with patient partners takes time. The relationships mentioned in this chapter alongside patient partners have been built up gradually through talks over coffee, attending conferences together, and open lines of communication that allow for trust and comfort. The stronger the relationship between patient partner and researcher is, and with greater experience involving patient-oriented research into laboratory science, the more fruitful the collaboration will be. It also aids in future recruitment of more patient partners and caregivers who may be interested in engaging with basic scientific research.

The shift towards discussion regarding the impact of COVID-19 is a great example of how patient partner-led discussion can shift the dialogue and priorities of a group. The COVID-19 pandemic brought about additional burdens to PWLE of mental illness, including caregivers and family members. A series of six facilitated discussions amongst members with diverse backgrounds supported the necessity of addressing these unique burdens. Decreased access to traditional in-person treatments, increased isolation, and pandemic-related stress had increased impacts on those already struggling. However, the reflections of the POR-AC also found positives: increased awareness on mental health issues and the adaptability of the public mental health system. With the right policies implemented, we can learn from previous mistakes and leverage the positives to create better services for both dire circumstances and normal everyday life.

Future efforts to develop patient-oriented research within a laboratory setting should focus on further formatting of priority-setting partnerships (PSPs) in the context of a laboratory setting by defining research priorities and greater outreach of results. Social media is a powerful tool that can be used to disseminate results quickly to a large audience, whether through twitter posts, the creation of TikToks, or even short Youtube videos – all of which should be considered when discussing future translatability of the research. The continuation of the POR-AC has also focused on determining the most important areas of focus for disorders such as depression and psychosis, with two main topics addressed: resilience and recovery. Under this umbrella, the POR-AC hopes

to find the best research methodology and questions to tackle three main questions: (1) how to define recovery/resilience, (2) how to evaluate recovery/resilience, and (3) how to foster recovery/resilience. Particularly in the context of our laboratory, patient partners could combine personal investments in finding the best therapeutics with fewest side effects (under the recovery umbrella) through personal interests in working with animals by observing and classifying behaviours associated with side effects such as nausea, hyperlocomotion, and more. For assessment of resiliency, the use of animal models that display resilient phenotypes are now being considered (such as the unpredictable chronic mild stress model) to properly evaluate the underlying mechanisms of resiliency.

The necessity to prioritize active partnerships between patients and researchers are evident. By providing direct access to foundational scientists, the voices of patient partners shape research towards translational outcomes that greater benefit the patient population. This is especially important in mental health research, where the voices of those with lived experience are often downplayed or discredited. The exchange of knowledge between patients and researchers are advantageous on both sides. For patients, this can enhance confidence when approaching their healthcare practitioners or perhaps increase optimism in the work that is being conducted. For researchers, this generates ideas and input that benefit the group they are modeling, increases motivation towards their research, and provides a greater understanding of how this impacts individual patients. The fostering of a close relationship between patients and foundational researchers is essential to improving the future of translational science, mental health research, and patient-oriented research.

Chapter 3

Reelin and ketamine demonstrate parallel effects in synaptoneurosomes from CORT-treated animals, but differential effects on SERT clustering in peripheral lymphocytes.

3.1 Abstract

Determining the molecular mechanisms underlying current and potential fast-acting antidepressants is essential to the development of novel therapeutics. Ketamine's fast-acting antidepressant effects appear to be mediated through a fast and transient activation of mTORC1, a protein complex which increases excitatory synaptic signaling after phosphorylation through an increase in proteins such as PSD-95 and surface insertion of GluA1 AMPARs. Reelin, a neuromodulator expressed by GABAergic neurons, appears to similarly promote synaptogenesis and dendritogenesis to ketamine. Recent research suggests that it may also mediate these activities through a transient mTORC1 activation. This study aims to determine the impact of stress, ketamine and reelin on synaptic mTORC1. Rats underwent a 21-day chronic CORT-administration paradigm and then their hippocampi and cerebellums were micro-dissected out to determine region-specific differences. Synaptoneurosomes (isolated pre- and post-synaptic compartments) were then created from both the hippocampus and cerebellum and incubated with varying concentrations of reelin and ketamine. Results show that in the hippocampus CORT decreases PSD-95, mTORC1, and p-mTORC1 expression, which were similarly rescued by both reelin and ketamine. In contrast, there were no significant differences in PSD-95 across all treatment groups in the cerebellum. Levels of mTORC1 expression were not changed between CORT and vehicle groups, though both reelin and ketamine significantly increased expression after incubation. Levels of p-mTORC1 were significantly higher in the vehicle group than CORT-treated animals, and both reelin and ketamine rescued levels of p-mTOR after incubation. These findings display potential parallels between ketamine and reelin's molecular antidepressant-like effects and lend strength to further evaluation of the putative fast antidepressant-like actions of reelin.

3.2 Introduction

Major depressive disorder (MDD) is the leading cause of disability worldwide, with a lifetime prevalence rate of around 16% (Friedrich, 2017). Despite this prevalence, there have been many difficulties in developing effective therapeutics, in part due to the constellation of symptomatology that includes emotional, physical, and cognitive symptoms (Wong & Licinio, 2001). Unfortunately, initial therapeutics based off the monoaminergic hypothesis have demonstrated low efficacy, extrapyramidal side effects, and delays in therapeutic efficacy that do not mirror the biological effects (Cipriani et al., 2018; Rush et al., 2006). While these therapeutics are effective

for a certain percent of the population, it is clear from widespread use that a large portion of those diagnosed with MDD are non-responsive and require further treatment, which can worsen outcomes and increase risk for suicidality (McCormack & Korownyk, 2018; Shinohara et al., 2019).

Within the past two decades, researchers have observed that ketamine, a high-affinity N-methyl-D-aspartate receptor (NMDAR) antagonist, had fast-acting antidepressant effects in patients with treatment-resistant depression at subanesthetic doses (Berman et al., 2000; Ibrahim et al., 2011; Zarate et al., 2006). The rapid timescale of effects (within 24 hours in comparison to 2 weeks observed in monoaminergic antidepressants) has spurred a significant amount of research into ketamine's mechanism of action. In addition, ketamine has shown promise in treating symptoms that were initially difficult to target with monoaminergic antidepressants such as anhedonia, suicidality, and reduced motivation (Krystal et al., 2019). While ketamine's actions were initially thought to mediated through its NMDAR antagonism, recent research has suggested that its behavioral, electrophysiological, electroencephalographic, and cellular antidepressant effects are mediated through an increase in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor transmission (AMPAR). This increase in excitatory post-synaptic transmission leads to the activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling in the hippocampus and pre-frontal cortex, which in turn increases markers of excitatory synaptic transmission, synaptogenesis, and dendritogenesis (Zanos et al., 2016; Zanos & Gould, 2018). While ketamine is extremely promising as an antidepressant, certain side effects such as dissociation and hallucinations necessitate clinical observation for administration and make it undesirable for certain patient populations (Short et al., 2018). However, these extrapyramidal side effects are often associated with ketamine's NMDAR antagonism, not its mTORC1 mediation.

Reelin, an extracellular matrix glycoprotein, may work through similar cellular mechanisms to ketamine without antagonistic actions on NMDAR (Lee & D'Arcangelo, 2016). Shown to be decreased in the hippocampus of patients with depression (Fatemi et al., 2000; Guidotti et al., 2000; Impagnatiello et al., 1998), decreases in reelin parallel the progressive development of depressive-like behaviour in a chronic corticosterone model (Lussier et al., 2013). This downregulation of reelin can be rescued by both traditional (imipramine) and non-traditional antidepressants (etanercept, a TNF-α inhibitor) (Brymer et al., 2018; Fenton et al., 2015). Recent

research from our laboratory also demonstrates that both central and peripheral administration of reelin rescues depressive-like behaviour in a fast-acting manner (Allen et al., 2022; Brymer et al., 2020). Reelin has also been found to enhance dendritogenesis, dendritic maturation, and synaptogenesis (Ampuero et al., 2017; Faini et al., 2021; Wasser & Herz, 2017) in a similar manner to ketamine. However, it is yet unknown whether reelin may also exert therapeutic actions through the mTORC1 synaptic signaling pathway.

Lastly, the impact of depression is not exclusively on the central nervous system, with much research demonstrating changes in areas such as the gut microbiome and peripheral immune system (Capuco et al., 2020; Miller & Raison, 2015). Our lab has shown that in peripheral lymphocytes, membrane protein clustering (MPC) of the serotonin transporter (SERT) can indicate therapeutic response in treatment-naïve depression patients (Rivera-Baltanas et al., 2012, 2015). These changes in SERT clustering have been paralleled in our chronic corticosterone model for the study of depression and in heterozygous reeler mice (Lussier et al., 2011; Romay-Tallon et al., 2018), but thus far the effects of reelin and ketamine are unknown. In addition, the administration of rapamycin, an mTORC1 inhibitor, has recently been shown to prolong the antidepressant effects of ketamine (Abdallah et al., 2020) – however, this may be mediated by its strong peripheral anti-inflammatory effects, making the inflammatory system one of particular importance to research in the context of depression.

To determine potential parallel changes of ketamine and reelin on mTORC1 signaling after chronic stress exposure, I designed an experiment using synaptoneurosomes to capture the impact of both therapeutics at the synaptic-specific level. In addition, I used incubations of peripheral lymphocytes to analyze changes in SERT MPC to examine the effects that reelin and ketamine may have on the peripheral immune system.

3.3 Methodology

3.3.1 Animal husbandry

Male Long Evans rats (n = 8) were acquired from Charles River Laboratories (Montreal, Quebec, Canada) and were aged 6 weeks upon arrival, and weighed between 200 – 250 g. Animals were housed individually in clear polypropylene cages, with a wooden chew cube and red hut. Food (Purina rat chow) and water were provided ad libitum, while bedding was changed once per week.

The colony was thermal-controlled (21°C) and maintained on a 12-h light/dark cycle. All procedures were approved by the University of Victoria Animal Research Ethics Board and conducted in accordance with the Canadian Council on Animal Care.

3.3.2 Experimental procedures

Rats were habituated to the facility for one week after arrival, followed by another week of daily handling. Rats were weighed on the last day of handling and assigned randomly to two treatment groups: 21 days of vehicle (0.9% [w/v] sodium chloride and 2% [v/v] polysorbate-80 solution) or corticosterone (Steraloids) (40mg/kg suspended in vehicle solution) which was administered at a volume of 1ml/kg. All injections were administered subcutaneously between the hours of 08:00 and 11:00 a.m. Figure 3.1 shows the experimental timeline.

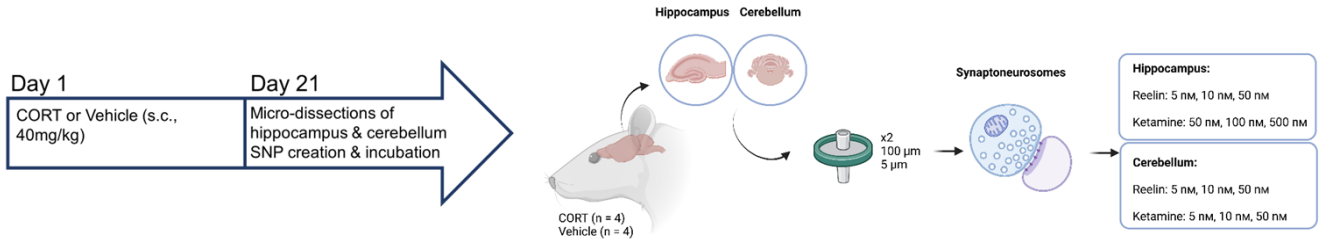


Figure 3.1. Experimental timeline and synaptoneurosome creation. CORT (40mg/kg) or vehicle was given over 21 days (s.c.) to create a model of chronic stress. After microdissection of the hippocampus and cerebellum, synaptoneurosomes were created through sequential filtrations (100 µm pore nylon filters, 5 µm nitrocellulose Durapore membrane filters) then centrifuged to obtain the synaptoneurosomes pellet. The pellet was then resuspended in warmed artificial cerebrospinal fluid and treated with varying concentrations of reelin (5 nM, 10 nM, 50 nM), ketamine (hippocampus: 50 nM, 100 nM, 500 nM; cerebellum: 5 nM, 10 nM, or 50 nM), or vehicle.

3.3.3 Tissue preparation

Corticosterone- (n = 4) and vehicle-treated (n = 4) animals were anesthetized with 5% isoflurane, then killed by decapitation. Following decapitation, the hippocampus and cerebellum were micro-dissected on ice and snap frozen in liquid nitrogen. Tissue was stored until use at −80˚C. Whole blood was collected at time of sacrifice to use for peripheral blood mononuclear cell (PBMC) isolation.

3.3.4 Synaptoneurosome creation and incubation protocol

Tissue was homogenized (Potter-Elvehjem homogenizer) on ice in a modified Krebs-Henseleit buffer (mKREBS) (in mM: 118.5 NaCl, 4.70 KCl, 1.18 MgCl2·6H2O, 2.50 CaCl2·2H2O, 1.18 KH2PO4, 24.90 NaHCO3, 10.00 glucose, pH adjusted to ~7.40 using 1.0 N HCl) supplemented with a protease inhibitor cocktail (#1860932, Thermoscientific, Waltham, MA). Following this, the homogenate was drawn into a 1 cc Luer lock syringe and passed through sequential filtrations. First, tissue was passed through 100 µm pore nylon filters (NY1H02500; EMD Millipore) followed by 5 µm nitrocellulose Durapore membrane filters (SBLP01300; Millipore). The filtered homogenate was centrifuged at 1000 × g for 15 min at 4 °C to collect the synaptoneurosomal pellet. After collection of the pellet, synaptoneurosomes were resuspended in pre-warmed artificial cerebrospinal fluid (ACSF) (32˚C) and divided evenly between drug experimental groups. Hippocampal SNPs were incubated with 5 nM, 10 nM, or 50 nM of the central fragment of reelin (used throughout the experiments conducted in this thesis), ketamine at a concentration of 50 nM, 100 nM, 500 nM, or ACSF alone (Fig. 3.1). Concentrations were based off of initial research from our lab on the effects of reelin (unpublished data) and previous *in vitro* ketamine research (Li et al., 2010). After adjustment, cerebellar SNPs were incubated with the same concentrations of reelin but reduced concentrations of ketamine (5 nM, 10 nM, or 50 nM). After a 30 min incubation, reactions were terminated with centrifugation in ice-cold mKREBS (3,490 x g for 15 min at 4˚C), then resuspended in 50 µl mKREBS that was supplemented with a protease and phosphatase inhibitor cocktail (78428, Thermoscientific, Waltham, MA). A portion of resuspended homogenate was used for detergent compatible (DC) protein assay analysis (#5000111, BioRad, Hercules, CA).

3.3.5 Lymphocyte isolation and incubation protocol

Peripheral blood mononuclear cells (PBMCs) were extracted from whole-blood of vehicle-treated animals following a previously published protocol (Romay-Tallon et al., 2017). After extraction, PBMCs were resuspended in RPMI 1640 medium (#11875093, Thermoscientific, Waltham, MA) diluted with 10% phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4; pH 7.4) [w/v] and 1% streptomycin. Cells were then incubated in either medium or 1 mM CORT or medium, followed by varying concentrations of reelin (0.5 nM, 1 nM, 5 nM), ketamine (10 nM, 50 nM, 100 nM, 250 nM), or medium. Concentrations of CORT and reelin were based off of previous research and equivalency with the chronic-CORT model for

depression. After a 1 hr incubation, cells were fixed for 5 min with 1% [w/v] paraformaldehyde, then centrifuged in 1:1 PBS (2 x 1000 x g, 10 min) for rinses.

3.3.6 SDS-PAGE and Western blotting protocols

Ten µg of protein of protein from the incubated SNPs was electrophoretically resolved at 200 V for 60 min in a 10% SDS-polyacrylamide gel. After proteins resolved through the gel, they were transferred onto polyvinylidene fluoride (PVDF) membranes (#IPVH00010, Millipore Sigma, Burlington, MA) via wet transfer (100 V on ice for 90 min). Following transfer, membranes were blocked for 1 hr at room temperature with 5% [w/v] bovine serum albumin (BSA). Primary antibodies diluted in blocking buffer were applied to probe for indicators of mTORC1 activity: post-synaptic density 95 (PSD-95) (P246, Millipore Sigma, Burlington, MA), total mTORC1 (#2983T, Cell Signaling Technology, Danvers, MA), and phosphorylated mTORC1 (p-mTORC1) (#2971S, Cell Signaling Technology, Danvers, MA). All membranes were incubated overnight in primary antibodies at 4˚C with gentle agitation. Blots were washed in tris-buffered saline with 1% [v/v] tween then incubated with horse radish peroxidase linked goat anti-mouse (ab97023, Abcam, Cambridge, UK) or goat anti-rabbit secondary (ab205718, Abcam, Cambridge, UK) antibody (1:5,000 in 5% [w/v] BSA) for 1 h at room temperature. Luminata Classico (for PSD-95, #WBLUR0500, Millipore Sigma, Burlington, MA) or Crescendo (for p-mTORC1 and mTORC1, # WBLUC0500, Millipore Sigma, Burlington, MA) were used for chemiluminescent detection. All images were captured using a SynGene imaging system and quantified with Fiji. Total protein normalization was conducted through a Ponceau stain (5% [v/v] Glacial Acetic Acid, 0.1% [w/v] Ponceau S). Total protein normalization was used due to an increased sensitization to loading differences across wells (Thacker et al., 2016).

3.3.7 Immunocytochemistry protocol

PBMCs were blocked for 10 min at room temperature (3% rat immunoglobulin, 1% [w/v] BSA in PBS), then incubated overnight at 4°C with rabbit anti-serotonin transporter (SERT) (1:100, #AB9322, Millipore Sigma, Burlington, MA). After rinses in PBS, secondary (1:250, goat anti-rabbit Alexa Fluor 568, (#ab175471, abcam, Cambridge, UK) was applied for 1 h at room temperature. To stain the nuclei and ascertain lymphocyte histology, Hoescht was applied for 10 min at room temperature (1:1000). After Hoescht staining, the samples were extended onto slides (Fisherbrand™ Superfrost™ Plus Microscope Slides) and cover-slipped with Citifluor-Mount

Solution (Electron Microscopy Science). Slides were stored at −20°C until imaging and analysis. A minimum of 50 lymphocytes were imaged per sample at 100x magnification on a Nikon Eclipse E800 microscope. Image analysis of the isolated lymphocytes was conducted in ImageJ using a previously described protocol (Romay-Tallon et al., 2017).

3.3.8 Statistical analyses

Statistical analyses were carried out using SPSS (IBM, USA). T-tests were used to analyze differences between CORT- and vehicle-treated animals for Western blotting. Assumptions of normality and homogeneity of variance were tested before conducting one-way ANOVAs. Within-subjects ANOVAs were used for the SNP data, as tissue from each animal was divided between all experimental groups. For lymphocyte analysis, a between subjects ANOVA was conducted. If a significant main effect was found, either a paired t-test (within subjects) or Tukey's post-hoc test (between subjects) was used to determine intergroup differences. Significance is defined as $p<0.05$. All data are expressed using mean ± standard error of mean (SEM).

3.4 Results

3.4.1 The effect of CORT, reelin, and ketamine on hippocampal synaptoneurosomes

Representative Western Blot images are shown in Figure 3.2A. PSD-95 expression in hippocampal synaptoneurosomes was significantly different amongst most treatment groups ($F = 4.634$, $p = 0.0029$). CORT-administration significantly decreased expression of PSD-95 from levels of vehicle ($p = 0.01$), a decrease which was rescued in a concentration-dependent manner by reelin (50 nM: $p = 0.0211$) and ketamine (50 nM: $p = 0.0191$; 500 nM: $p = 0.0237$) (Fig. 3.2B).

Phosphorylated mTORC1 was also significantly impacted by treatment ($F = 4.634$, $p = 0.0029$). Again, p-mTORC1 was decreased by chronic CORT administration ($p = 0.0185$ in comparison to vehicle) which was upregulated after administration of reelin (10 nM: $p = 0.0332$; 50 nM: $p = 0.001$). All concentrations of ketamine increased levels of p-mTORC1 from chronically stressed rats (50 nM: $p = 0.0024$; 100 nM: $p = 0.0025$; 500 nM: $p = 0.0018$) (Fig. 3.2C).

While mTORC1 was not significantly different between CORT-administered rats and vehicles, mTORC1 was decreased by nearly 76%. All concentrations of both reelin (5 nM: $p = 0.101$; 10 nM:

p = 0.0011; 50 nM: p < 0.0001) and ketamine (50 nM: p <0.0001; 100 nM: p<0.0001; 500 nM: p<0.001) were able to significantly increase mTORC1 expression (Fig. 3.2D).

The ratio of activity of mTORC1 (p-mTORC1/mTORC1) was affected by treatment (F = 4.104, p = 0.0055), but was interestingly upregulated (thought not significantly) by CORT-administration, though this may be due to the upregulation in general expression of both p-mTORC1 and mTORC1. Both reelin (5 nM: p = 0.0008; 10 nM: p = 0.0009; 50 nM: p = 0.0017) and ketamine (50 nM: p = 0.0025; 100 nM, p = 0.0013; 500 nM: p = 0.0013) decreased this ratio of activity from CORT, back to levels more similar to vehicle animals (Fig. 3.2E). Due to the high variability observed in this initial experiment, ketamine concentrations were adjusted for following analyses in the cerebellum.

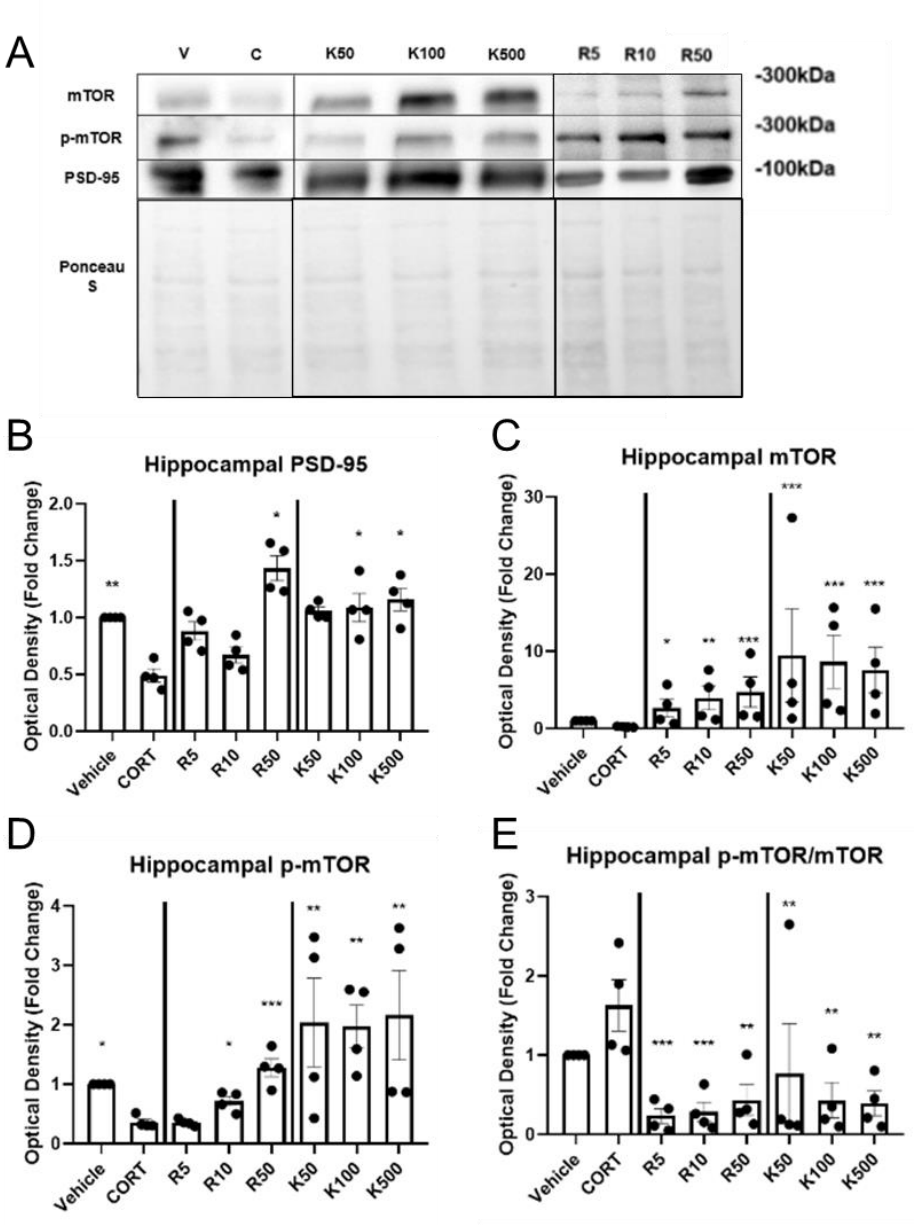


Figure 3.2. The effect of CORT, reelin, and ketamine on hippocampal synaptoneurosomes. (A) Representative Western blot images of synaptic protein expression from vehicle and CORT-adminstered animals with exposure to varying concentrations of ketamine and reelin. Ponceau S was used for total protein normalization. (B) Effects of ketamine and reelin on hippocampal post-synaptic density 95 (PSD-95) expression. Reelin rescued PSD-95 expression in a concentration-dependent manner. All concentrations of ketamine increased PSD-95 expression. (C) Hippocampal synaptic mTOR expression after treatment. CORT-administration decreased p-mTOR expression, which was increased by the all concentrations of reelin and. (D) Hippocampal synaptic p-mTOR expression after treatment. P-mTOR expression was decreased by CORT and was increased by the highest concentrations of reelin and the all concentrations of ketamine. (E) The ratio of active mTOR in hippocampal synaptoneurosomes after treatment. CORT increased the amount of p-mTOR activity, which was decreased by all concentrations of reelin and ketamine. All data are presented as mean ± SEM. R5, 5 nM reelin; R10, 10 nM reelin; R50, 50 nM reelin. K5, 5 nM ketamine; K10, 10 nM ketamine; K50, 50 nM ketamine. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. CORT.

3.4.2 The effect of CORT, reelin, and ketamine on cerebellar synaptoneurosomes

To determine whether the synaptic-level effects of reelin and ketamine in the hippocampus were region-specific, the cerebellum was micro-dissected and analyzed. In addition, reelin is highly expressed in the cerebellum and expressed from cerebellar granule cells throughout development to influence proper neuron migration, making this region of particular interest to analyze. No significant differences were found in PSD-95 expression between any subgroups (vehicle; CORT; reelin at 5 nM, 10 nM, 50 nM; ketamine at 5 nM, 10 nM, 50 nM), in sharp contrast to the previous hippocampal findings.

While there were no changes in PSD-95 (Fig. 3.3B), significant differences were found in expression of cerebellar p-mTORC1 [$F(1.289, 3.868) = 385.0$, $p < 0.0001$], mTORC1 [$F(1.257, 3.771) = 32.00$, $p = 0.0052$], and active ratio of mTORC1 (p-mTORC1/mTORC1) [$F(1.180, 3.539) = 20.99$, $p = 0.0128$] (Fig. 3.3A). Post-hoc analyses revealed that CORT-administered animals had significantly less p-mTORC1 than vehicles ($p = 0.0474$), an effect which was rescued by all concentrations of reelin (5 nM: $p < 0.0001$, 10 nM: $p = 0.0004$, 50 nM: $p = 0.015$) and the highest concentrations of ketamine (10 nM: $p < 0.0001$, 50 nM: $p = 0.0006$) (Fig. 3.3C).

Expression of total mTORC1 was not changed between vehicle- and CORT-administered animals, though 5 nM ($p < 0.0001$) and 50 nM (0.0122) of reelin as well as 5 nM ($p = 0.0003$) and 10 nM ($p = 0.0469$) of ketamine did still significantly increase mTORC1 expression. Interestingly, the highest concentration of ketamine (50 nM) significantly decreased expression of mTORC1 from levels of CORT ($p = 0.0319$) (Fig. 3.3D).

Lastly, in contrast to hippocampal synaptoneurosomes, the ratio of mTORC1 activity was downregulated with chronic stress in comparison to vehicles. However, treatment with reelin generally decreased this ratio of activity (5 nM: $p = 0.0016$, 50 nM: $p = 0.0447$). The effects of ketamine were more mixed, with the lowest concentration decreasing the active ratio of mTORC1 (5 nM: $p = 0.0078$) and the highest concentration increasing the ratio (50 nM: $p = 0.0072$) (Fig. 3.3E).

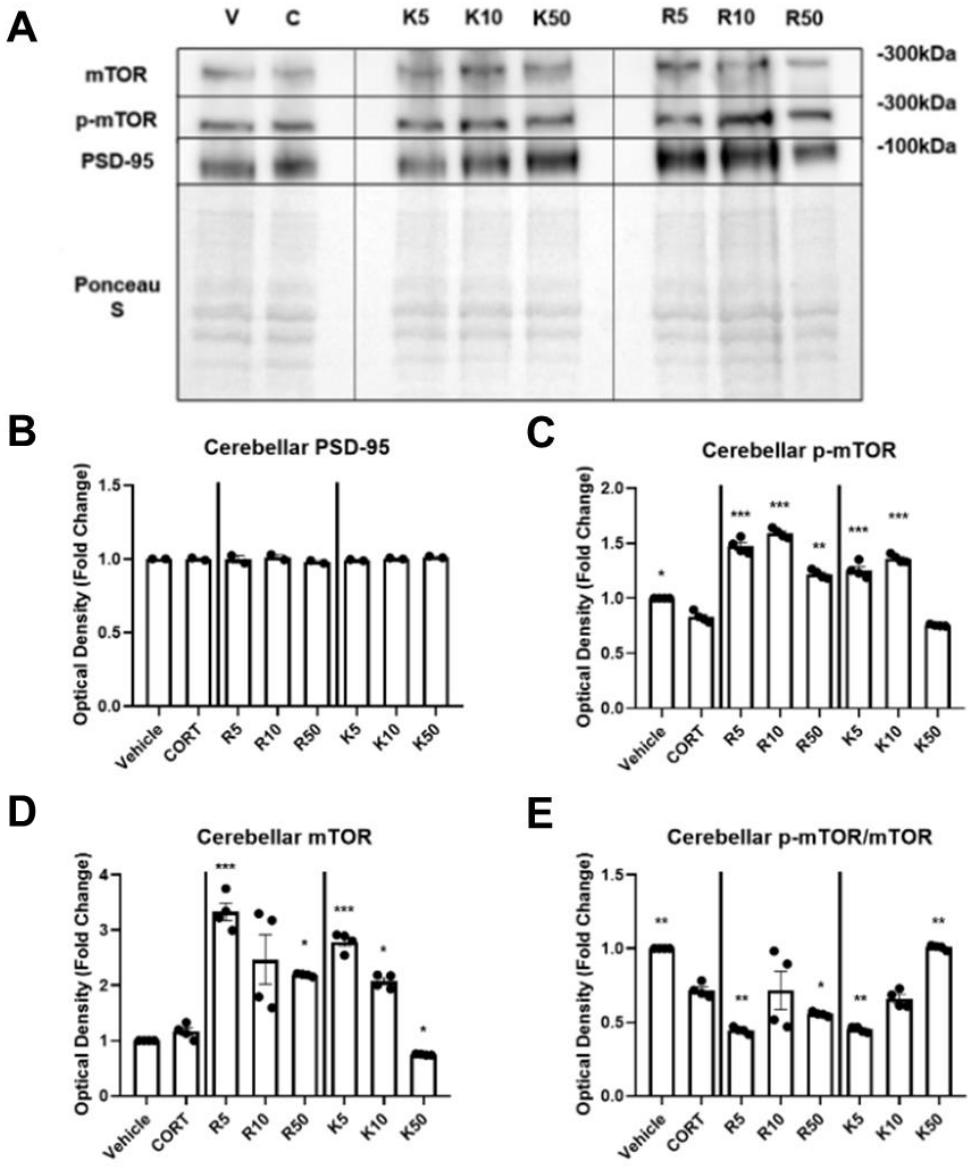


Figure 3.3. The effect of CORT, reelin, and ketamine on cerebellar synaptoneurosomes. (A) Representative Western blot images of synaptic protein expression from vehicle and CORT-adminstered animals with exposure to varying concentrations of ketamine and reelin. Ponceau S was used for total protein normalization. (B) Effects of ketamine and reelin on cerebellar post-synaptic density 95 (PSD-95) expression. No changes were observed across all conditions. (C) Cerebellar synaptic p-mTOR expression after treatment. CORT-administration decreased p-mTOR expression, which was increased by all concentrations of reelin and the lower concentrations of ketamine. (D) Cerebellar synaptic mTOR expression after treatment. mTOR expression was not changed by CORT but was increased by all concentrations of reelin and the lower concentrations of ketamine. The highest concentration of ketamine decreased mTOR expression below levels of CORT. (E) The ratio of active mTOR in cerebellar synaptoneurosomes after treatment. CORT decreased the amount of p-mTOR activity, which was increased by the highest concentration of ketamine. Decreases in this ratio were observed with two reelin concentrations (5 nM and 50 nM) and ketamine's lowest concentration. All data are presented as mean ± SEM. R5, 5 nM reelin; R10, 10 nM reelin; R50, 50 nM reelin. K5, 5 nM ketamine; K10, 10 nM ketamine; K50, 50 nM ketamine. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. CORT.

3.4.3 The effect of CORT, reelin, and ketamine on SERT clustering in lymphocytes

Previous research from our lab has found distinct changes in the clustering patterns of SERT in plasma membranes of lymphocytes in a chronic corticosterone model and treatment-naïve depression patients (Caruncho et al., 2019a). Given these changes, ascertaining the effects of potential novel therapeutics, such as ketamine and reelin, is of great interest. Figure 3.4 shows representative images of lymphocytes treated at varying concentrations of vehicle, CORT, reelin, and ketamine. A significant difference was found in size of SERT clusters across all groups [$F(7,16) = 8.047$, $p = 0.0003$]. Mirroring previous research, treatment with CORT increased the size of SERT clusters in comparison to vehicle ($p < 0.05$). The highest concentration of reelin decreased the size of the clusters after CORT incubation, shifting them back to levels of the vehicle ($p<0.05$). The lowest dose of ketamine (10 nM) had a similar impact to reelin; however this was not a significant rescue. Interestingly, higher doses of ketamine trended towards increasing increase the size of clusters from those of CORT-incubated lymphocytes. No differences were found in the number of SERT clusters across all experimental groups.

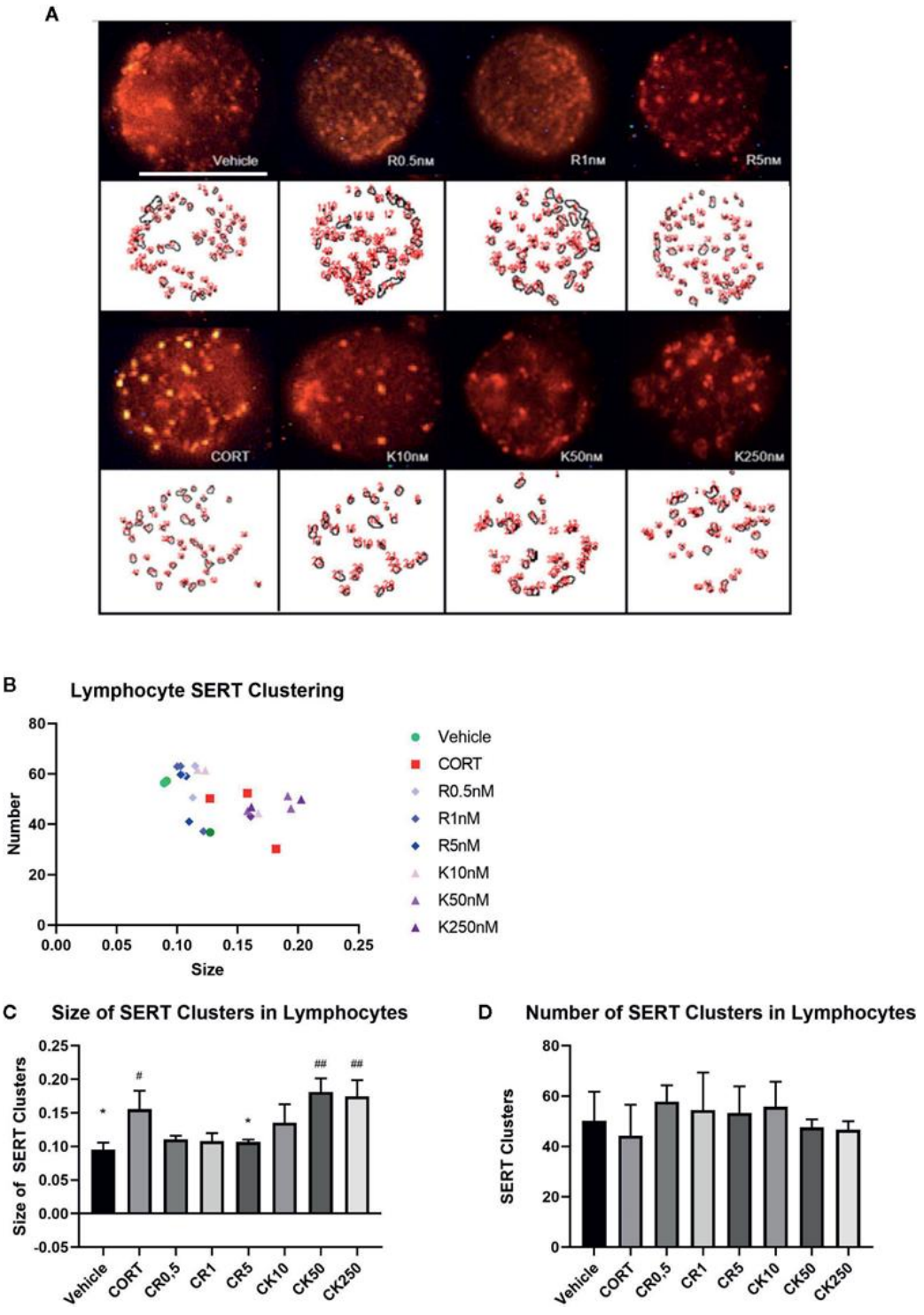


Figure 3.4 The effects of CORT, reelin, and ketamine on SERT clustering in peripheral lymphocytes. (A) Representative fluorescent microscopy images for each condition. (B) Distribution of clusters by number and size by treatment. (C) Average size of SERT clusters in each condition. CORT increased the average size of SERT clusters, which were rescued by reelin. (D) Average number of SERT clusters in each condition. No changes were found between all groups. All data expressed as mean ± SD. $^{\#}p < 0.05$ vs. vehicle, $^{*}p < 0.05$, $^{\#\#}p < 0.01$ vs. CORT. Figure adapted from: Johnston et al., 2021.

3.5 Discussion

To ascertain potential parallel mechanisms underlying the antidepressant-like effects of reelin and ketamine, synaptoneurosomes were isolated from hippocampal and cerebellar tissue from control and chronically stressed animals. After isolation, SNPs were divided into treatment groups of varying concentrations of reelin or ketamine, then examined for indicators of mTORC1 signaling. Synaptoneurosomes were used as they allowed us to measure synaptic-specific processes, which are hypothesized to underlie the fast-acting antidepressant effects of ketamine (Li et al., 2016; Zanos et al., 2016). Following previous research, CORT decreased PSD-95 expression in the hippocampus, an effect that was rescued in a concentration-dependent manner by both reelin and ketamine. However, in the cerebellum no changes were found across all experimental groups. This may be a reflection of the more stable nature of cerebellar synapses in adulthood, whereas hippocampal synapses are more sensitive to stress-induced and plastic changes (Tartt et al., 2022).

As mTORC1 activation is considered to be an essential molecular mechanism for fast-acting antidepressant effects (Zanos et al., 2016; Zanos & Gould, 2018; Zhou et al., 2014), the effect of chronic CORT-administration and reelin on mTORC1 was of great interest. Both mTORC1 and p-mTORC1 were reduced by CORT treatment in hippocampal and cerebellar synapses, reflecting the role that chronic stress can have in decreasing excitatory signaling (Pavlides et al., 2002). While both reelin and ketamine were effective in increasing expression of mTORC1 and p-mTORC1, ketamine appeared to have larger effects than those of reelin. However, recent reports finding that administration of rapamycin (an mTORC1 inhibitor) actually prolonged the antidepressant effects of ketamine, perhaps suggesting that a more moderate activation of mTORC1 could be effective (Abdallah et al., 2020). In addition, the high variability due to the concentrations of ketamine used in hippocampal synaptoneurosomes suggest that lower concentrations of ketamine may be effective when applied directly to the synapse, as confirmed by the lower variability observed in cerebellar SNP treatments.

Nevertheless, this research provides preliminary evidence that reelin can target mTORC1 expression and activity at the synaptic level, without the NMDAR antagonism associated with ketamine's psychotomimetic side effects. These psychotomimetic effects limit widespread use of ketamine, as they necessitate clinical observation and make it less desirable for certain clinical populations, such as those diagnosed with comorbid psychosis or schizophrenia. While this is quite

speculative, reelin may still be effective in those with comorbid neuropsychiatric disorders, as patients with schizophrenia have around 50% less reelin expression in the brain than healthy controls (Fatemi et al., 2000; Guidotti et al., 2000).

Quantification of SERT clustering in PBMCs revealed that chronic CORT-administration increased size of clusters, a parallel finding to previous research in animal models and patients with MDD (Caruncho et al., 2019). In addition, reelin was able to effectively rescue the size of SERT clusters in peripheral lymphocytes in a concentration-dependent manner, at a direct contrast to ketamine which increased cluster size after treatment. Alterations in SERT clustering have been associated with therapeutic efficacy of antidepressant medications (Rivera-Baltanas et al., 2012, 2015), and the effects of reelin on these clusters should be further explored. The resurgence of research related to the immune system and neuropsychiatric disorders has revealed that inflammation may play a particularly large role in treatment-resistant depression, the subset of patients which ketamine has been designated for (Johnston et al., 2023, *in press*). Also of interest is that while ketamine rapidly crosses the blood brain barrier due to its high lipid solubility, reelin is a high molecular weight protein (388 kDa) that may have more actions in the periphery, an idea which should be explored further using *in vivo* administration of both therapeutics.

Limitations in this study include small sample size, as it was primarily exploratory research. However, the division of the synaptoneurosomes for each rat between all experimental conditions to compare responses within-subjects allows for the elimination of many aspects of biological variability. Future research should also probe for more proteins associated with both the mTORC1 signaling pathway, and other pathways proposed to be hypothesized for its antidepressant effects such as ERK signaling (Ouyang et al., 2021; Yang et al., 2018; Yao et al., 2022).

In conclusion, the findings of the present study implicate chronic stress in the downregulation of mTORC1 signaling, and both reelin and ketamine as therapeutics that can upregulate this signaling at the synaptic level. In addition, it provides evidence to support the functioning of synaptoneurosomes *ex vivo*, which could be invaluable in future studies determining synaptic deficits and other potential therapeutics in neuropsychiatric disorders. Finally, the efficacy of reelin in reducing the size of SERT clustering on lymphocytes provides preliminary evidence towards reelin's mechanism of action in the periphery and lends strength to the role of inflammation in depressive pathophysiology.

Chapter 4

Ketamine rescues reelin expression in the repeated-CORT paradigm

4.1 Abstract

Depression is the leading cause of disability worldwide, with difficulties and barriers to treatment affecting a large proportion of the population. Chronic stress is a significant predisposition factor to depression and can be used to create animal models which reflect the human condition. Chronic CORT-administration results in a behavioral phenotype of depression that is associated with decreased hippocampal reelin levels and neurogenesis. Previous research has found that certain traditional antidepressants rescue reelin levels after chronic administration. However, the effect of an acute dose of ketamine has not yet been evaluated in a chronic CORT-administration paradigm. Rats received daily subcutaneous injections of CORT or vehicle for 21 days, then an acute dose of ketamine on day 22. After behavioral verification of a depressive-like phenotype, rats were sacrificed to perform immunohistochemical analyses of reelin-IR and GluA1-IR cells in the SGZ, as well as the number and dendritic complexity of new-born neurons in the DG GCL, measured through DCX-IR. The results demonstrated that an acute dose of ketamine was enough to rescue the number of reelin-IR cells that were decreased after chronic CORT-administration, but not enough to rescue the number or complexity of DCX+ cells. No significant differences in GluA1-IR cells in the SGZ were observed. These novel findings revealed for the first time that ketamine, a novel fast-acting antidepressant, was able to restore reelin in an animal model of depression.

4.2 Introduction

While initial pharmacological observations regarding the monoaminergic system's role in depression was an initial big step forward, decades have passed without much forward movement. There is still a scarcity in broadly efficacious treatments that do not have a substantial therapeutic time-lag and can generate sustained effects. Unfortunately, around 66% of patients respond inadequately to a first treatment trial, with 33% still remaining unresponsive after multiple trials with different pharmaceutical agents (Cipriani et al., 2018; Rush et al., 2006). Much research has been dedicated to uncovering the underlying etiology of depression, however new findings have done little to create novel therapeutics which could be more effective. As previously stated, ketamine, a non-competitive NMDAR antagonist, has been the most promising discovery for antidepressant therapeutics in decades. In subanesthetic doses, ketamine produces rapid and long-lasting antidepressant effects in patients with treatment-resistant depression (Berman et al., 2000; Ibrahim et al., 2011; Zarate et al., 2006). Traditionally hard to target symptomology such as

anhedonia, negative cognitive biases, and suicidal ideation have been successfully treated with this compound, making the unique mechanisms of ketamine a great interest to antidepressant researchers.

However, the underlying mechanisms of ketamine are still vague. Previous research has shown that the behavioral, electrophysiological, and electroencephalographic effects of ketamine were due to an increase in glutamatergic transmission which stimulates a fast, transient activation of the mechanistic target of rapamycin (mTOR) pathway in the prefrontal cortex and hippocampus (Li et al., 2010; Zanos et al., 2016, 2018). This upregulation of mTOR activity causes a sustained elevation of proteins related to excitatory synaptic transmission, such as postsynaptic density-95 protein (PSD95) and increased surface insertion of the Glutamate A1 (GluA1) subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Zanos & Gould, 2018).

A potential mediator of ketamine's effects may be reelin, a large extracellular glycoprotein expressed by GABAergic interneurons in adulthood (Lee & D'Arcangelo, 2016). Recent research has suggested that synaptic reelin signaling may me a key modulator of ketamine's antidepressant effects through Src kinase family downstream signaling (Kim et al., 2021). In the hippocampus, this glycoprotein plays many important roles in the hippocampus including promoting maturation of newborn granule cells (Bosch et al., 2016), enhancing dendritic spine development and maturation (Chameau et al., 2009; Hethorn et al., 2015; Niu et al., 2008), neurogenesis (Pujadas et al., 2010), and learning and memory (Weeber et al., 2002). Reelin was first found to be involved in neuropsychiatric disorders when a downregulation of reelin expression was observed in the hippocampus of schizophrenia, bipolar, and major depressive disorder patients (Fatemi et al., 2000; Guidotti et al., 2000; Impagnatiello et al., 1998). Heterozygous reeler mice, who express 40 – 60% less reelin, are also significantly more vulnerable to the depressionogenic effects of CORT (Lussier et al., 2011). Lastly, chronic CORT-administration causes a significant decrease in reelin expression in the hippocampus which parallels the development of depressive-like behavior (Lussier et al., 2013b).

The hippocampus is particularly susceptible to stress-induced impairments, due to the abundance in expression of glucocorticoid receptors (Herman et al., 2005). Patients diagnosed with MDD demonstrate large reduction in hippocampal volume which correlates with episode severity and age of onset (Bremner et al., 2000; Frodl et al., 2002; Lorenzetti et al., 2009; Sheline et al., 1996;

Videbech & Ravnkilde, 2004). These hippocampal volume reductions may be due, in part, to a decrease in new-born cell proliferation that is suppressed by stress, an effect that can be recapitulated in animal models for depression (Brummelte & Galea, 2010). Chronic administration of traditional, monoaminergic-based antidepressants has been somewhat successful in rescuing this decrease in neurogenesis (David et al., 2009; Fenton et al., 2015; Murray et al., 2008; Nandam et al., 2007), as well as administration of an anti-inflammatory agent (Brymer et al., 2018), ketamine (Ma et al., 2017; Soumier et al., 2016; Yamada & Jinno, 2019), and the administration of reelin itself (Allen et al., 2022; Brymer et al., 2020).

Considering the novelty of ketamine as a fast-acting antidepressant, I designed an experimental approach to evaluate the effect of ketamine on reelin-expressing cells in the hippocampus, as well as its impact on the glutamatergic system and hippocampal neurogenesis to determine how its fast-acting antidepressant effects may be mediated.

4.3 Methodology

4.3.1 Animal husbandry

32 adult male Long-Evans rats weighing 200-250 g upon arrival were purchased from Charles River Laboratories (Montreal, Quebec, Canada). Rats were housed individually in clear plastic cages with free access to food and water. The thermally controlled colony was maintained on a 12-h/12-h light/dark cycle, with lights turning on at 07:00am, at 21°C. Bedding was changed once per week and Purina rat chow was maintained at regular intervals. All procedures were approved by the University of Saskatchewan Animal Research Ethics Board and conducted in accordance with the Canadian Council on Animal Care.

4.3.2 Experimental procedures

After arrival to the facility, rats were given a week to habituate before another week of daily handling. Animals were weighed and assigned to the following treatment groups: 21 days of daily vehicle or CORT (40mg/kg) injections, and additionally a dose of vehicle or ketamine (15mg/kg) on day 22. Subcutaneous CORT or vehicle injections were administered between 08:00 and 11:00 a.m. Animals were weighed daily so that injections could be accurately administered at a volume of 1ml/kg suspended in 0.9% (w/v) sodium chloride and 2% (v/v) polysorbate-80 (Sigma Aldrich) solution. Ketamine hydrochloride (Vetalar; Bioniche Animal Health Canada Inc.; Belleville,

Ontario, Canada) or vehicle was suspended in saline and injected intraperitoneally on day 22 at a dose of 15 mg/kg at a volume of 1 ml/kg. The dose of ketamine was based off of previous pre-clinical research, which found that a dose of 10mg/kg – 15mg/kg is the most effective at rescuing depressive-like behavior. Behaviour was conducted as previously published in Kulyk, 2017. Briefly, rats underwent a fear conditioning and extinction based on a modified version of a protocol previously used (Deschaux et al., 2013) and further described (Kulyk, 2017). All behavioral testing procedures took place in standard operant chambers (VFC-008; Med Associates Inc., St. Albans, Vermont, United States) contained within sound-attenuating cubicles. Video Freeze Software (Med Associates Inc.) controlled the delivery of tone and shock stimuli, and recorded video from the camera at 30 frames per second. Behaviour will not be reported on in this thesis, but correlations between Reelin-IR, DCX-IR, and GluA1-IR and fear conditioning and extinction behaviour will be reported.

4.3.3 Tissue preparation

Animals were deeply anesthetized with 5% isofluorane (maintained through an isoflurane machine attached to a nosecone that was placed over the rat's nose) and transcardially perfused with ice-cold 4% (w/v) paraformaldehyde in 0.1M PB (pH 7.4). After removal, their brains were kept in the same paraformaldehyde fixative for 48 h at 4°C, then transferred to a 30% sucrose solution for 72 h before sectioning. They were then sectioned in the coronal plane at 30 µm on a cryostat (CM1850 UV, Leica Biosystems) at −20˚C. Sections were stored in cryoprotectant [30% (w/v) sucrose, 1% (w/v) polyvinylpyrrolidone, and 30% (v/v) ethylene glycol in 0.1 M PBS (pH = 7.4)] at -20°C until use.

4.3.4 Immunostaining

Every 6th section of the hippocampus was collected and placed in 6-well tissue culture plates as free-floating sections under gentle agitation. For staining, 5 sections containing the dorsal hippocampus were selected. When primary antibody was omitted from an additional well, no immunoreactive cells were detected. All rinses were conducted in tris-buffered saline (TBS) (50 mM Tris-Cl, 150 mM NaCl; pH 7.6). After initial rinses, sections were incubated in sodium citrate (pH6, 85 °C) for antigen retrieval. Next, primary antibodies were applied for 24 h at 4°C (mouse anti-reelin (EMD Millipore, MAB5364), rabbit anti-doublecortin (Cell Signaling Technologies, AB-561007), rabbit anti-GluA1 (EMD Millipore, AB1504); 1:1000) diluted in a blocking solution

(10% Triton X-100 [v/v], 15% normal goat serum (NGS) [w/v], and 1% bovine serum albumin (BSA) [w/v] dissolved in TBS). Sections were subsequently incubated in 10% hydrogen peroxide [v/v] for 30 mins, then incubated in appropriate secondary antibodies (biotinylated goat anti-mouse lgG and biotinylated goat anti-rabbit 1:500, Sigma-Aldrich, St. Louis, MO) diluted in above-described blocking buffer for 2 h at room temperature. The tissue was then incubated in an avidin-biotin complex for 1 h at room temperature (1:500, Vecta Stain Elite ABC reagent, Vector Labs). Each step was followed by 3 washes for 5 min in TBS. To visualize reelin, sections were stained using 0.002% 3'-diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) in TBS with 0.0078% hydrogen peroxide. Before visualizing DCX, sections were rinsed in sodium acetate before staining with 0.025% DAB [w/v] and 4.167% NiSO4 [w/v] dissolved in 0.002% H202 and sodium acetate. GluA1 was imaged using a chromagen of 0.05% DAB, 4.167% NiSO4, 0.002% ammonium chloride, 0.008% β-D glucose, and 0.003% glucose oxidase. This was followed by 2 rinses in sodium acetate and one rinse in TBS. The sections were mounted and coverslipped using Permount solution.

4.3.5 Imaging and cell counting for reelin-IR, DCX-IR, and GluA1-IR cells

Sections were imaged on a Nikon Eclipse E800 microscope. Using an unbiased optical fractionator method in Stereo Investigator (Version 8.0, MicroBrightField Inc.), immunoreactive cells in the SGZ were counted (for reelin, DCX, and GluA1) and categorized (for DCX). The SGZ was initially traced at 4x magnification, then 40x for stereological analyses with a field size of 3600 μm^2. Number estimates were gathered using this formula: Ntotal: ΣQ- × 1/ssf × A(x,y step)/a(frame) × t/h; where ΣQ- is the number of counted cells; ssf is the section sampling fraction (1/6); A is the area associated with each x,y movement (10,000 μm2); a(frame) is the area of the counting frame (3,600 μm2); t is the weighted average section thickness; and h is the height of the dissector P plane. A guard zone of 2 μm was used to prevent sectioning artifacts.

4.3.5.1 Categorization of DCX-IR cells

DCX-positive cells were categorized using a method previously described by Lussier and colleagues (2013a) and Allen and colleagues (2022). The effects of CORT and ketamine on the morphology of immature granule cells was evaluated through characterization of dendritic branching from DCX-IR cells. From the 5 sections of each animal, 100 DCX-IR cells that were evenly distributed across hemispheres were randomly selected for categorization. To avoid

potential bias, a meander scan method was employed to blind treatment groups. These 100 cells were sorted into 6 categories: 1 (no processes), 2 (one small process), 3 (medium process that reached the granule cell layer), 4 (process reaching the molecular layer), 5 (a major process which extended further into the molecular layer), and 6 (a defined dendritic tree that had branching into the granule cell layer). Categories 1 and 2 represent proliferative stages of development, whereas stages 3 and 4 represent a more intermediate developmental stage. Lastly, categories 5 and 6 represent mature stages of development, where the cell has started to integrate into existing circuitry. To analyze, the percentage of cells in each category was determined.

4.3.6 Statistical analyses

Statistical analyses were carried out using SPSS (IBM, USA). Assumptions of normality and homogeneity of variance were tested before conducting two-way ANOVAs. If a significant main effect was found of CORT or ketamine, Tukey's post-hoc tests were used to determine intergroup differences. Significance is defined as $p<0.05$, and trends are described as determined through percent rescue [100 – (CORT/ketamine mean – vehicle/vehicle mean) ÷ (CORT/vehicle mean – vehicle/vehicle mean) × 100]. All data are expressed using mean ± standard error of mean (SEM). Linear regressions were conducted on all correlational data.

4.4 Results

4.4.1 Reelin-IR cell counts

We have previously demonstrated that conventional and unconventional antidepressants (including Reelin itself) are able to protect from the CORT-induced downregulation of reelin the SGZ of the DG (Allen et al., 2022; Brymer et al., 2018; Fenton et al., 2015). In this cohort of rats, analyses of the dorsal SGZ showed CORT administration decreased reelin immunoreactive cells ($p = 0.0198$, $t = 2.902$, $df = 8$), which was rescued by ketamine administration ($p = 0.0034$, $t = 3.932$, $df = 9$). The percent rescue of reelin in the SGZ by ketamine administration was 87.21% (Figure 4.1).

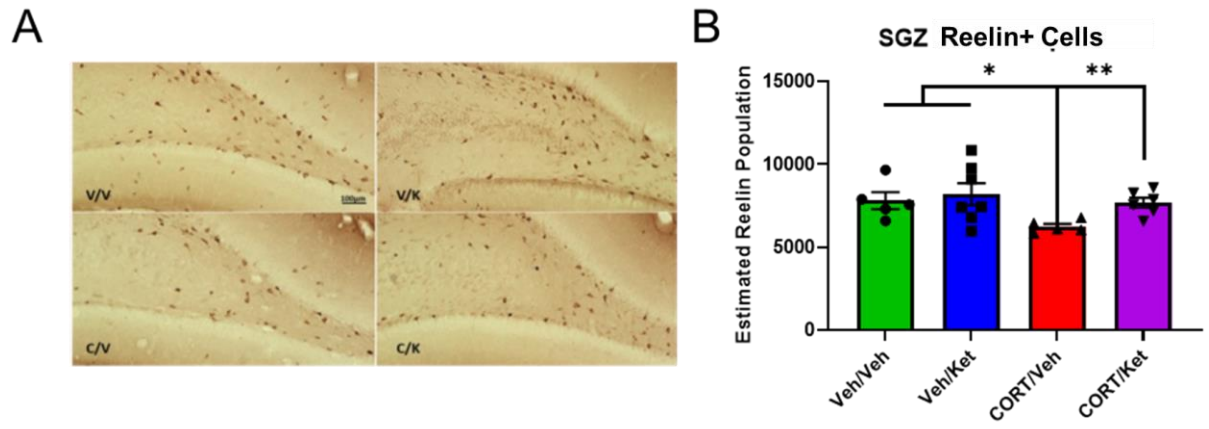


Figure 4.1. Reelin+ cells in the dentate gyrus subgranular zone. (A) Representative photomicrographs of reelin expression in the subgranular zone. (B) Estimated reelin population from unbiased stereology. Reelin expression was decreased by chronic CORT administration, but was rescued by an acute dose of ketamine. All data are expressed as mean ± SEM. Veh/Veh, vehicle/vehicle; Veh/Ket, vehicle/ketamine; CORT/Veh, CORT/vehicle; CORT/Ket, CORT/ketamine. *$p < 0.05$, **$p < 0.01$ vs. CORT/Veh.

4.4.2 DCX-IR cell counts and categorization

Doublecortin was used as a marker for neural maturation of newborn granule cells in the subgranular zone of the hippocampus, as it allows for both cell-counts and categorization of dendritic branching. Histological data showed large gaps of doublecortin positive cells in the upper blade of the dentate gyrus after CORT administration, an effect that was not rescued through ketamine administration (Fig. 4.2A). Significant differences were found between the controls and CORT-treated animals ($p = 0.0057$, $t = 3.735$, $df = 8$), but not any other subgroups, suggesting ketamine did not influence doublecortin levels in this sample (Figure 4.2B). Ketamine only rescued DCX-IR cells by 3.59%. No significant correlations were found between subgroups and complexity of dendritic branching on the newborn granule cells (Figure 4.2C).

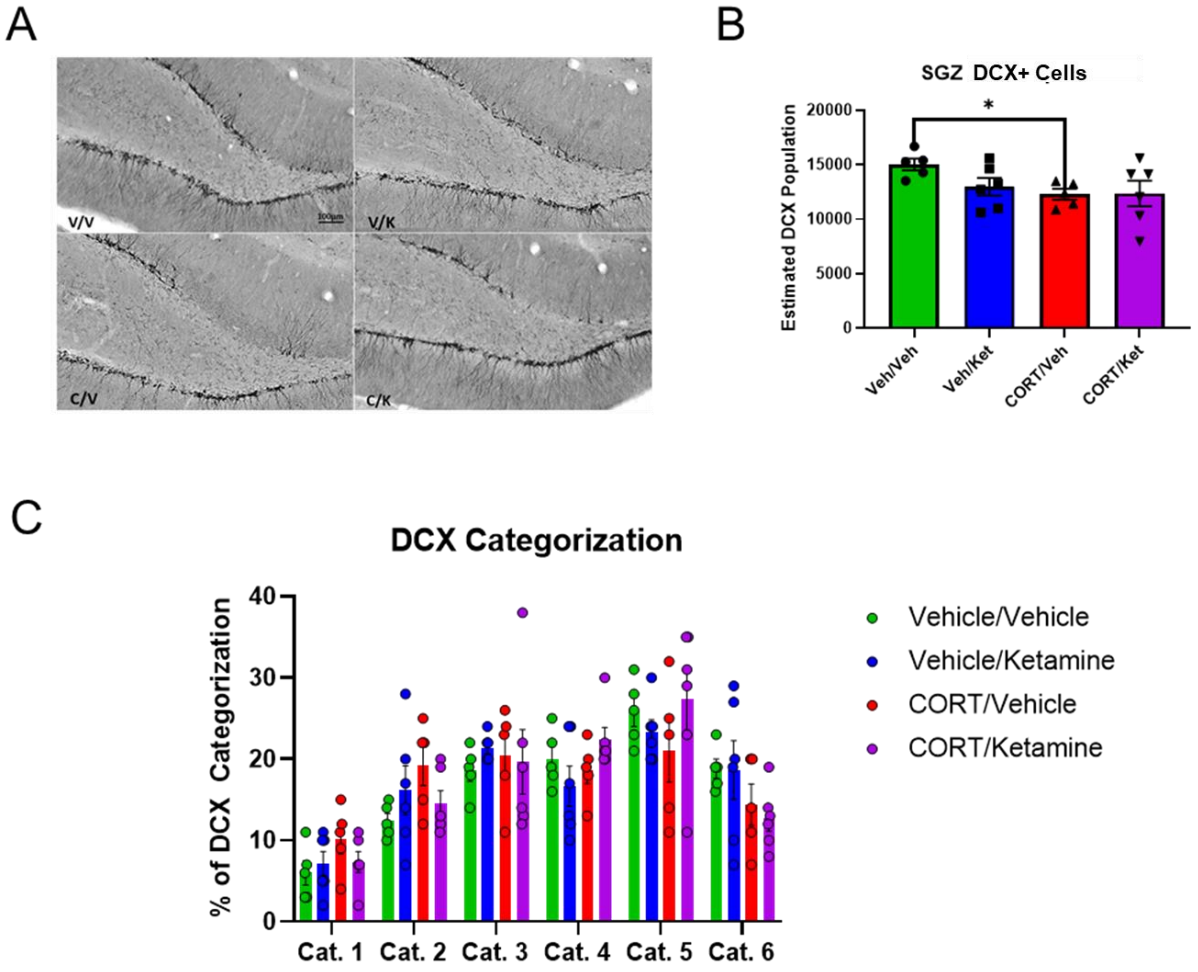


Figure 4.2. DCX+ cells in the dentate gyrus subgranular zone. (A) Representative photomicrographs of DCX expression in the subgranular zone. (B) Estimated DCX population from unbiased stereology. DCX expression was decreased by chronic CORT administration but was not rescued by an acute dose of ketamine. (C) Categorization of DCX-IR cells by maturity. No significant differences were found between groups. All data are expressed as mean ± SEM. Veh/Veh, vehicle/vehicle; Veh/Ket, vehicle/ketamine; CORT/Veh, CORT/vehicle; CORT/Ket, CORT/ketamine. *$p < 0.05$ vs. CORT/Veh.

4.4.3 GluA1-IR cell counts

No significant differences were found in counts of GluA1-IR cells in the SGZ, however there were trends towards CORT increasing GluA1-IR count and a partial rescue (at a decrease) by ketamine of 70.45%.

4.4.4 Correlations of cell counts with behaviour

In brief, Kulyk (2017) found results which suggest that a single subanesthetic dose of ketamine can restore the long-term expression of extinction behavior in animals with a history of chronic exogenous CORT exposure, and dramatically suppress the initial recall of conditioned fear irrespective of past CORT treatment. To determine any correlations between expression of reelin, DCX, and GluA1 in the SGZ and behaviour, I used the freezing behaviour from Brian Kulyk's thesis and correlated the fear re-conditioning and extinction test phases with reelin-IR, DCX-IR, and GluA1-IR cells. The percent of DCX-IR cells which were categorized as Category 6 were also correlated with behavioural measures. There were no significant correlations across all groups (Figure 4.3). When separated by treatment group, an increase of reelin ($Y = 133.6*X + 7016$, $p = 0.0258$) and GluA1($Y = 106.0*X + 6977$, $p = 0.0052$) were associated with an increase in freezing behaviour during the extinction test phase for the vehicle/ketamine subgroup (Figure 4.4).

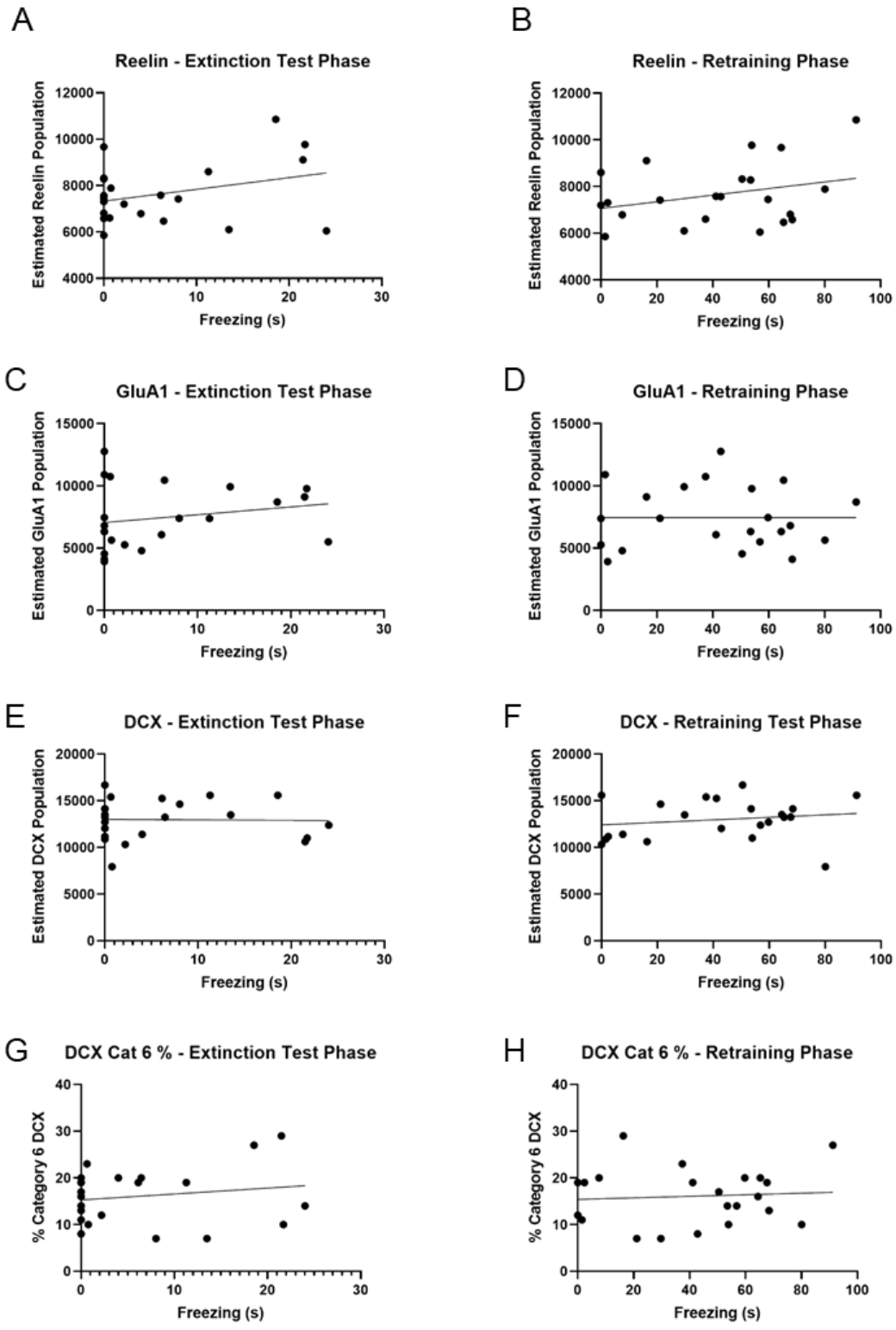


Figure 4.3 Correlations of reelin, DCX, and GluA1 all group cell counts with freezing behaviour. (A – H) No significant correlations were found across all groups.

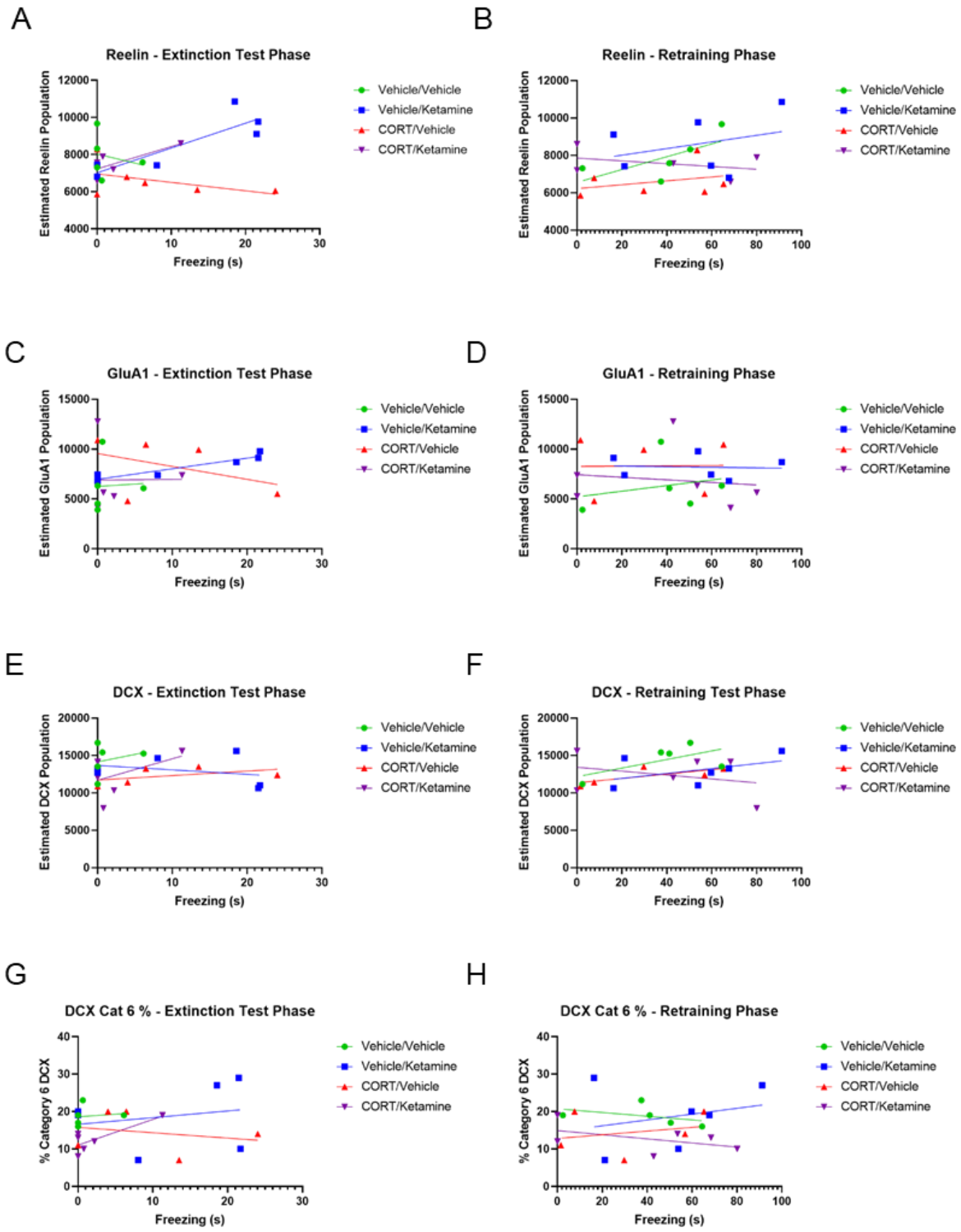


Figure 4.4 Correlations of reelin, DCX, and GluA1 by treatment group cell counts with freezing behaviour. (A) A significant correlation was found for the vehicle/ketamine treatment group between reelin cell population and extinction test phase freezing behaviour. (B) No significant correlations were found for any groups. (C) A significant correlation was found for the vehicle/ketamine treatment group between GluA1 cell population and extinction test phase freezing behaviour. (D – H) No significant correlations were found across all groups.

4.5 Discussion

This chapter provides evidence that ketamine is able to rescue the deficits of reelin-IR but not decreases of DCX-IR counts or complexity and induced by repeated CORT in the DG SGZ. No significant differences in GluA1 expression were found across any subgroup. Deficits in reelin expression in the dentate SGZ are rescued by both conventional (e.g. imipramine) and non-conventional (e.g. etanercept) drugs with antidepressant actions, which also reverse the increase in immobility in the forced swim test induced by CORT (Brymer et al., 2018; Fenton et al., 2015). Considering this, we expected ketamine to also be able to reverse the decrease in reelin+ cells in the SGZ in a fast-acting manner, which is consistent with our results. Recently, we demonstrated that intrahippocampal and peripheral infusions of recombinant reelin are able to also rescue the behavioral phenotype induced by repeated CORT and that they do so in a fast-acting manner (i.e. similar to ketamine) (Allen et al., 2022; Brymer et al., 2020). This brings about the possibility that rescuing hippocampal reelin may parallel ketamine's actions in its fast-acting antidepressant effects, similar to other potential fast-acting antidepressant molecules that have been studied (de Almeida et al., 2020; Hasegawa et al., 2019; Pazini et al., 2016). Further research should be conducted to determine if ketamine's antidepressant effects are augmented by the increase of reelin expression it stimulates in the hippocampus.

CORT had a significant impact on hippocampal neurogenesis, decreasing the populations of newborn and immature neurons in the subgranular zone. These data are supported by numerous other reports, which show consistent and significant decreases in hippocampal cell proliferation and survival in the adult rodent brain (Brummelte & Galea, 2010; Pham et al., 2003; Wong & Herbert, 2006). The administration of an acute dose of ketamine did not rescue adult neurogenesis in the chronic stress animal model in the same way that a single infusion of Reelin did not rescue DCX expression while repeated reelin infusions did (Brymer et al., 2020). However, mixed results regarding proliferation and maturation after a single dose of ketamine suggest that increased neurogenesis is not sufficient for the short term behavioral and biological antidepressant effects following acute administration (Soumier et al., 2016), but rather is potentially associated with the long-term antidepressant effects found after chronic ketamine administration (Clarke et al., 2017; Ma et al., 2017). Thereby, it seems that both acute ketamine and reelin do not have a fast effect in rescuing the effects on hippocampal neurogenesis (as ascertained by DCX labeling) but do so when administered repeatedly. It is therefore a possibility that the acute effects of reelin and ketamine

could reverse a subset of depressive symptoms, while chronic changes might be more effective in rescuing other symptoms (i.e. depression cognitive deficits). However, further experiments are necessary to elucidate this. Research in the ventral hippocampus would also be of interest, as it has been shown that the dorsal and ventral hippocampus are differentially affected by glucocorticoid administration and could have been disparately impacted by ketamine (Levone et al., 2021). It is important to note that researchers have recently demonstrated that DCX expression can increase in the absence of adult hippocampal neurogenesis, implicating that DCX does not always indicate the presence of neurogenesis (Mendez-David et al., 2020), so caution should be used when interpreting these results. An earlier marker of neurogenic activity, such as BrdU and NeuN, could also provide different results, due to staining earlier stages of neurogenesis (Rawat et al., 2022).

While there were no significant differences between GluA1 populations in the subgranular zone of the hippocampus, there were slight increases in expression in those who underwent chronic stress treatment. This is somewhat at odds with previous research studying patients with depression and chronic stress protocols (Duric et al., 2013; Kallarackal et al., 2013), however fear conditioning and extinction could interfere with these results. In contextual fear conditioning, GluA1 is often shown to be increased during the acquisition and retrieval phases, particularly in the dorsal hippocampus (Johansen et al., 2011; Sase et al., 2015). Chronically stressed animals had stronger retrieval of the fear conditioning, which would denote generally denote higher GluA1 levels. The decrease of GluA1 levels by ketamine closer to levels of vehicle and ketamine's success in decreasing fear recall and aiding extinction suggests that ketamine may be able to mediate homeostasis of GluA1 populations, given that in other animal models its antidepressant effects are associated with an increase in GluA1 expression (Yao et al., 2017; Zhang et al., 2016).

In conclusion, our research has shown that ketamine brings about a fast-rescuing effect of reelin expression in the hippocampal subgranular zone, and that reelin may perhaps work through similar synaptic mechanisms to ketamine that perhaps underlie the fast-acting antidepressant effects of this drug. Ketamine's rescue of reelin expression after CORT administration should drive further research into common signaling pathways and behavioural effects, as well as the efficacy of utilizing reelin to target negative emotional biases in memory. The findings of the present study could have implications for the treatment of human patients, providing new pathways to alter cognitive biases that are believed to support the development and maintenance of depression.

Chapter 5

In the repeated-CORT paradigm, reelin and ketamine have similar behavioural, biological, and electrophysiological effects

5.1 Abstract

Previous research has demonstrated that reelin, an endogenous glycoprotein, exerts putative fast-acting antidepressant-like effects in a chronic stress model. However, the electrophysiological and biological changes have not yet been measured within a 24-hour timeframe, often considered to be the time-course for "fast-acting" therapeutic effects. To address this paucity of research, this study aimed to measure the changed induced by reelin in parallel to ketamine within 24 hours after chronic corticosterone (CORT) administration. Male Long Evans rats were injected with 40 mg/kg of CORT (subcutaneously) or vehicle (saline) for 21 days. On the 21st day, an acute dose of reelin (3 µg, intravenously), ketamine (10 mg/kg, intraperitoneally), or vehicle (PBS) was administered. 24 hours later, animals underwent behavioural or electrophysiological testing followed immediately by sacrifice. Hippocampal long-term potentiation was measured after theta burst stimulation in the medial perforant path to determine the effects of reelin and ketamine on excitatory signaling. Behavioural changes on the forced swim test induced by CORT administration, such as increases in immobility and decreases in swimming, were rescued by both reelin and ketamine administration in a parallel manner. In addition, *in vivo* electrophysiology revealed decreases in hippocampal LTP after chronic stress that was able to be rescued within 24 hours by an acute dose of either reelin or ketamine. Parallel changes were observed for hippocampal reelin expression, however no major changes were observed in the expression of various synaptic-strength related proteins in synaptoneurosomes or peripheral serotonin transporter clustering, due to high variability between animals. In conclusion, this exploratory study provided a broad picture of the behavioural, electrophysiological, and molecular effects of reelin and ketamine within 24 hours and provides further support for reelin as a putative fast-acting antidepressant.

5.2 Introduction

Preliminary results in our lab have demonstrated that 3 µg of reelin administered peripherally (Allen et al., 2022) is sufficient to have antidepressant effects, however prolonged behavioural testing has not allowed us to measure the biological changes that happen within 24 hours of administration, an important period to determine the underpinnings of a fast-acting antidepressant response. Measuring parallels between ketamine and reelin is also essential, as it will give us an estimate of efficacy in comparison to a well-established antidepressant.

An area of priority in depression research is the hippocampus. The hippocampus is particularly susceptible to stress-induced impairments due to an abundance of glucocorticoid receptors in the limbic regions (Herman et al., 2005b), and patients with MDD often have reductions in hippocampal volume which can correlate with age of onset and episode severity (Bremner et al., 2000; Lorenzetti et al., 2009; Sheline et al., 2019; Videbech & Ravnkilde, 2004). As previously demonstrated, chronic CORT treatment is also capable of decreasing reelin expression in the SGZ of the hippocampus, a deficit that can be rescued by both ketamine and reelin (Allen et al., 2022; Johnston et al., 2020), as well as traditional antidepressants and some anti-inflammatory medications with antidepressant actions (Brymer et al., 2018; Fenton et al., 2015).

One of the main deficits observed in the hippocampus after chronic stress is a decrease in long-term potentiation (LTP), significantly impacting learning and memory (Kizilbash et al., 2002; Lynch, 2004; Strömgren, 1977; Weingartner et al., 1981). While ketamine has been found to improve LTP in pre-clinical models of chronic stress (Gilbert & Zarate, 2020; Sumner et al., 2020b), significantly less is known about the impact reelin has on hippocampal LTP. While the previous studies delineated in this thesis have found an increase in molecular markers of synaptic plasticity after reelin administration, electrophysiological measures are required to provide confirmation of increased excitatory signaling.

In addition, preliminary research presented in this thesis suggests that both ketamine and reelin have synaptic-specific effects *in vitro* on proteins indicative of increases in excitatory signaling, such as PSD-95 and p-mTOR expression. *In vivo* comparisons of excitatory-signaling related proteins between synaptoneurosomes and whole homogenate could provide further information into synaptic-specific processes that underlie depression and fast-acting antidepressants. Peripheral measurements of serotonin transporter (SERT) clustering are also changed after reelin and ketamine application *in vitro*, but no studies have researched changes in membrane protein clustering after fast-acting antidepressant-like effects *in vivo.*

This study aimed to provide a full exploratory picture of both reelin and ketamine as putative fast-acting antidepressants, and to provide insight into behavioural, circuit, molecular, and peripheral changes that occur within 24 hours after drug administration.

5.3 Methodology

5.3.1 Animal husbandry

72 male Long-Evans rats weighing 200-250 g and aged 6 weeks upon arrival were purchased from Charles River Laboratories (Montreal, Quebec, Canada). Rats were housed individually in clear polypropylene cages with access to food and water ad libitum, with a wooden chew cube and a red hut. The thermally controlled colony was maintained on a 12-h/12-h light/dark cycle, with lights turning on at 07:00am, at 21°C. Bedding was changed once per week and Purina rat chow was maintained at regular intervals. All procedures were approved by the University of Victoria Animal Research Ethics Board and conducted in accordance with the Canadian Council on Animal Care.

5.3.2 Experimental procedures

After arrival to the facility, rats were given a week to habituate before another week of daily handling. Animals were weighed and assigned to the following treatment groups: 21 days of daily vehicle or CORT (40mg/kg) injections, and additionally a dose of vehicle (PBS), reelin (3 µg) or ketamine (15mg/kg) on day 22. Subcutaneous CORT or vehicle injections were administered between 08:00 and 11:00 a.m. Animals were weighed daily so that injections could be accurately administered at a volume of 1ml/kg suspended in 0.9% (w/v) sodium chloride and 2% (v/v) polysorbate-80 (Sigma Aldrich) solution. 3 µg of recombinant reelin (R&D systems, 3820-MR-025; composed of RR3-6 and having a predicted molecular weight of 180kDa by SDSPAGE using reducing conditions) was based off of previous effective dose (Allen et al., 2022) and suspended in 0.5ml of 0.1M phosphate buffered saline (PBS, pH=7.4). Reelin was administered intravenously into the lateral tail vein on day 21 between the hours of 08:00am and 11:00am. Ketamine hydrochloride (Narketan; Vetoquinol; Lavaltrie, Quebec, Canada) or vehicle was suspended in saline and injected intraperitoneally between the hours of 08:00am and 11:00am on day 21 at a dose of 10 mg/kg at a volume of 1 ml/kg. The dose of ketamine was based off of previous pre-clinical research, which found that a dose of 10mg/kg – 15mg/kg is the most effective at rescuing depressive-like behavior. Experimental calendar can be viewed in Figure 5.1.

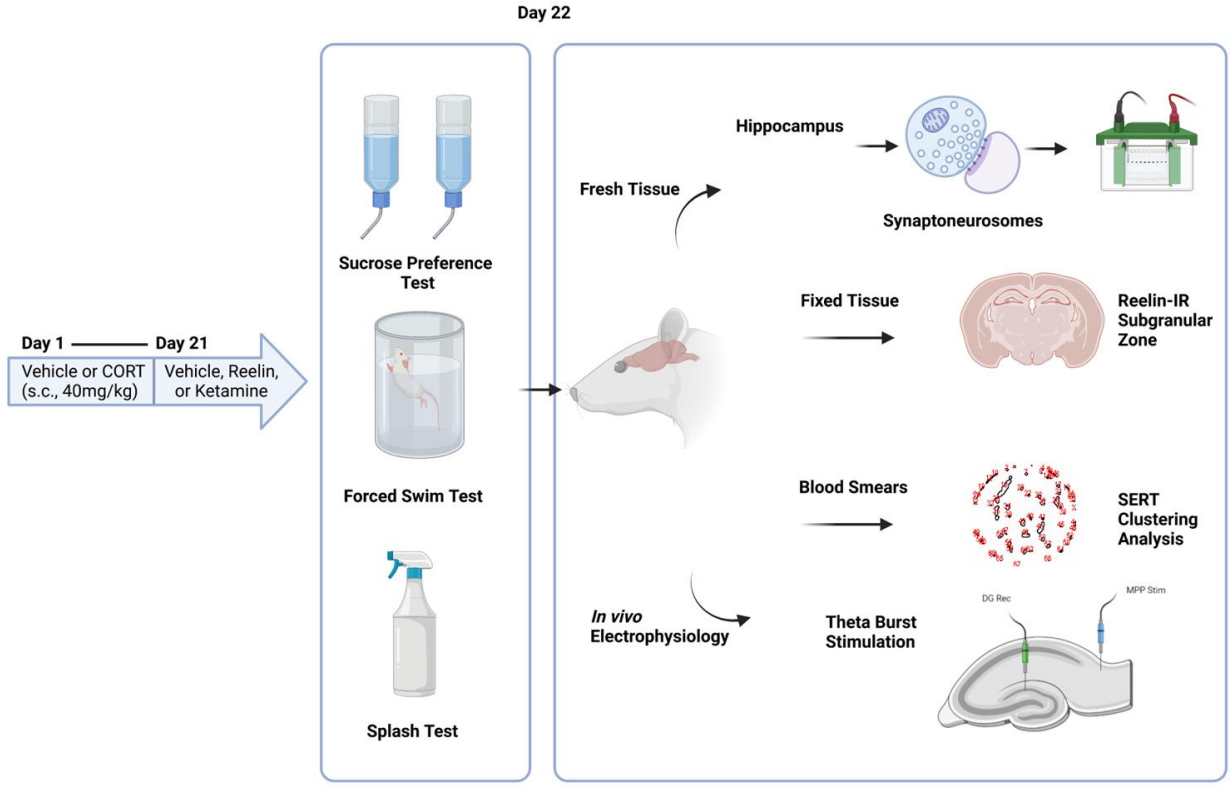


Figure 5.1. Experimental timeline and analyses. Male Long Evans rats received daily injections of CORT (40 mg/kg) or vehicle (saline) for 21 days (subcutaneously). On the 21st day, they were administered an injection of reelin (3 µg, intravenously), ketamine (10 mg/kg, intraperitoneally), or vehicle (PBS). 24 hours later, they underwent behavioural testing, then blood collection and sacrifice for fresh or fixed tissue. The animals who underwent *in vivo* electrophysiology underwent a theta burst stimulation protocol to induce LTP, which was recorded from the dentate gyrus. S.c. – subcutaneous, MPP – medial perforant path, DG – dentate gyrus.

5.3.3 Behavioural tests

5.3.3.1 Forced swim test

Animals who were not undergoing *in vivo* electrophysiology (n = 48) underwent a modified one-day version of the Porsolt FST (Porsolt, 1978). This test was originally designed to serve as a preclinical assay to assess the efficacy of antidepressant drugs (Marks et al., 2009). The purpose of the one-day protocol is to abolish any potential confounding effects of memory that may occur throughout a two-day protocol. Rats were placed for 10 mins in a Plexiglas swim tank (25cm wide × 25cm long × 60cm high) and filled with water (27±2°C) to a depth of approximately 30cm. The amount of time swimming, climbing, immobility, and latency to immobility were manually scored

over the 10 min test time. Immobility was defined by the rat floating or moving just enough to keep afloat in order to interpret their coping response to inescapable stress (Commons et al., 2017), an indicator of despair-like behaviour.

5.3.3.2 Sucrose preference test

The sucrose preference test (SPT) is a commonly used model of anhedonia, a loss of pleasure in activities that used to be pleasurable. On day 20, rats were habituated to having access to two water bottles for one day, then on day 21 to two bottles of a 1% sucrose solution. For the test on day 22, rats had access to one bottle of water and one bottle of sucrose solution, with sides being swapped at the halfway point (12 h) to control for preferences in bottle placement. Both bottles were weighed before and after consumption to determine amount drank, and differences in consumption between sucrose and water were calculated to determine preference.

5.3.3.3 Sucrose splash test

In rats that underwent electrophysiology (n = 24), the sucrose splash test (SST) was used to measure grooming behaviour, a proxy indicator of behaviour such as motivation and self-care. Rats were sprayed with a 10% sucrose solution on their dorsal coat. Due to viscosity of the solution, animals will initiate grooming behavior. Rats displaying depressive-like behaviour will often delay initiation of grooming and spend less time grooming. Rats were recorded and scored for 10 min on “grooming” and “non-grooming” behaviour, as well as latency to grooming.

5.3.4 Tissue preparation

5.3.4.1 Perfusions

Half of the animals not used for *in vivo* electrophysiology (n = 24) were deeply anesthetized with 5% isofluorane (maintained through an isoflurane machine attached to a nosecone that was placed over the rat’s nose) and transcardially perfused with ice-cold 4% (w/v) paraformaldehyde in 0.1M PB (pH 7.4). After removal, their brains were kept in the same paraformaldehyde fixative for 48 h at 4°C, then transferred to a 30% sucrose solution for 72 h before sectioning. They were then sectioned in the coronal plane at 30 μm on a cryostat (CM1850 UV, Leica Biosystems) at −20˚C. Sections were stored in cryoprotectant [30% (w/v) sucrose, 1% (w/v) polyvinylpyrrolidone, and 30% (v/v) ethylene glycol in 0.1 M PBS (pH = 7.4)] at -20°C until use.

5.3.4.2 Microdissections and SNP creation

The remaining half of animals not used for *in vivo* electrophysiology (n = 24) were anesthetized with 5% isofluorane and killed by decapitation. Immediately after sacrifice, the hippocampus was micro-dissected on ice and snap frozen in liquid nitrogen then stored until use at -80˚C. Tissue was thawed on ice prior to homogenization in ice using a Potter-Elvehjem homogenized containing chilled modified Krebs-Henseleit buffer (mKREBS) (in mM: 118.5 NaCl, 4.70 KCl, 1.18 MgCl2·6H2O, 2.50 CaCl2·2H2O, 1.18 KH2PO4, 24.90 NaHCO3, 10.00 glucose, pH adjusted to ~7.40 using 1.0 N HCl) supplemented with a protease inhibitor cocktail (#1860932, Thermoscientific, Waltham, MA). A portion of the whole homogenate was stored for later analysis. The remaining homogenate was drawn into a 1 cc Luer lock syringe and passed through sequential filtrations [100 μm pore nylon filters (NY1H02500; EMD Millipore); 5 μm nitrocellulose Durapore membrane filters (SBLP01300; Millipore)], and was centrifuged at 1000 × g for 15 min at 4 °C. After centrifugation, the pellet was resuspended in the mKREBs buffer containing protease and phosphatase inhibitors for analysis with Western blotting. The DC Protein Assay (Biorad) was used to quantify total protein in each sample.

5.3.5 *In vivo* electrophysiology

On the day of the experiment, animals (n = 24) were weighed and then anesthetized deeply (1.5g/kg of urethane, intraperitoneally, for non-recovery experiments). Supplemental doses of anesthesia were used when necessary (0.3ml at a time) until rats were fully unconscious, as determined by respiratory rate and withdrawal reflex. Rats were placed on a stereotaxic frame (Kopf Instruments) and had gel tears applied to their eyes. Body temperature was kept steady at 37°C using a regulated homeothermic control unit (Harvard Instruments, MA, USA). Following sterile protocols, an incision was made to expose the dorsal surface of skull and expose the bregma landmark. Two holes were drilled using a stereotaxic drill for the recording (3.5 mm posterior and 2.4 mm lateral to bregma) and stimulating (7.4 mm posterior and 3.4 mm lateral to bregma) electrodes. Another two holes were drilled anterior and lateral to the bregma for grounding the recording electrode.

Baseline recordings were obtained using a 0.12 ms pulse at 0.0067 Hz. A stable baseline was required for at least 30 min before a theta burst stimulation protocol. This theta burst protocol consisted of 10 bursts of 5 pulses (0.25 ms duration) at 400 Hz with a 200 ms inter-burst interval, which was repeated 4 times in 30 sec intervals. After the TBS protocol, baseline levels of

stimulation were continued for 1 hour. The slope of the rising phase (10 – 90%) of field EPSP immediately after stimulation was used to determine PTP. For analysis of LTP and synaptic efficacy, the slope of the rising phase of the fEPSP was measured 55 - 60min post stimulation. Input/output (I/O) function was used to determine baseline excitatory transmission. All slope data are presented as mean percent change from pre-stimulation baseline. To confirm electrode placement, brains were post-fixed in 4% paraformaldehyde, sectioned at 50µm, then stained in Cresyl Violet to verify electrode location.

5.3.6 Immunohistochemistry protocol

Reelin-IR cells were visualized through the immunohistochemical procedures previously described in this thesis. In brief, every 6th section of the hippocampus was collected and rinsed in TBS, then incubated in sodium citrate (pH 6; 85 °C) for antigen retrieval. Sections were then blocked (TBS, 10% Triton X-100 [v/v], 15% Normal Goat Serum[v/v]) for 30 min at room temperature before being placed in primary (mouse anti-reelin, 1:1000; EMD Millipore, Burlington, MA) diluted in blocking solution overnight at 4°C. After primary incubation ,sections were incubated in 10% hydrogen peroxide in TBS for 30 min, followed by incubation in the secondary antibody (biotinylated goat anti-mouse IgG, 1:500, Sigma-Aldrich, St. Louis, MO) diluted in blocking buffer for 1 h at room temperature. Finally, tissue was incubated for 1 h in avidin-biotin complex (1:500, Vecta Stain Elite ABC reagent, Vector Labs), then visualized using 0.002% [w/v] DAB (Sigma-Aldrich, St. Louis, MO) and 0.0078% [v/v] H_2O_2 in TBS before being mounted on polarized glass slides. After drying overnight, sections were dehydrated using increasing concentrations of ethanol and cleared in xylenes, then coverslipped using Permount mounting medium (Thermo Fisher Scientific, Waltham, MA).

Reelin-IR cells were counted in the subgranular zone of the hippocampus using a Zeiss Axioimager M.2. An unbiased optical fractionator method was used for stereological estimates (Stereo Investigator, 2022.2.1, MBF Bioscience). The area of interest was traced at 2.5x magnification, with stereological analyses undertaken at 20x magnification. Equations for number estimates have been previously described in Chapter 4.

5.3.7 SDS-PAGE and Western blotting

For both WH and SNPs, 10 µg of protein was electrophoretically resolved in 10% TGX Stain-Free™ FastCast™ Acrylamide Solutions (BioRad, Hercules, CA) at 200 V for 60 min. After the run, protein was then transferred in a semi-dry method onto 0.2 µm PVDF membranes (#1704272, BioRad, Hercules, CA) using the Trans-Blot Turbo Transfer System (BioRad, Hercules, CA). Membranes were blocked with 5% (w/v) BSA for 1 h at room temperature. Primary antibodies were applied overnight at 4°C at a 1:1000 concentration, diluted in 5% (w/v) BSA. Proteins measured in the hippocampus were mTOR (#2972S, CST), p-mTOR (#2971S, CST), Synapsin I (#6710, CST), PSD-95 (#2507S, CST), GluA1 (#13185S, CST), p-GluA1 (#75574S, CST), p-Erk1/2 (#9101S, CST), CREB (#4820S, CST), p-CREB (#9198S, CST), and GluN2b (UC Davis). After primary incubation, blots were washed in tris-buffered saline with 1% [v/v] tween (TBST), then incubated for 1 h at room temperature with a horseradish peroxidase-linked goat anti-mouse or goat anti-rabbit secondary diluted at 1:5000 in blocking buffer. After washes, Luminata Crescendo or Classico (#WBLUR0500 and # WBLUC0500, Millipore Sigma, Burlington, MA) were used for chemiluminescent detection. Technical duplicates or triplicates were analyzed for every protein from each animal to ensure proper representation of relative protein amounts. The SynGene imaging system was used to capture all images, and FIJI was used for quantification of Western blot bands. Ponceau staining was used as a normalization standard to avoid changes in house-keeping proteins that may occur in the model of depressive-like behaviour.

5.3.8 Immunocytochemistry protocol

Blood smears were collected on slides (Fisherbrand, Superfrost Plus), from the animals at time of sacrifice that did not undergo electrophysiology. Following a previously described protocol (Romay-Tallon et al., 2017b), slides were fixed in 1% paraformaldehyde for 10mins, then rinsed with PBS. After rinses, slides were blocked for an hour at room temperature (3% rat immunoglobulin, 1% [w/v] BSA in PBS), then incubated with primary (rabbit anti-serotonin transporter, 1:100, #AB9322, Millipore Sigma) overnight at 4°C. Secondary was applied for 1 hour at room temperature after rinses in PBS (1:250, goat anti-rabbit Alexa Fluor 568, #ab175471, abcam, Cambridge, UK diluted in PBS). Hoescht (1:1000) was applied for 10 min at room temperature to ascertain lymphocyte histology. Slides were then cover-slipped with AF1 Citifluor-Mountant Solution (Electron Microscopy Sciences) and stored at −20°C until imaging. A minimum of 50 lymphocytes were imaged per sample at 63x magnification on a Zeiss Axioimager

M.2 microscope. A Fiji macro created by Brady Reive, a PhD candidate in our lab, was used to analyze the lymphocytes based on previous principles of cluster analysis (Romay-Tallon et al., 2017). Quantification of number and size of SERT clusters was used for statistical analyses.

5.3.9 Statistical analyses

Two-way ANOVAs were used for all experiments to assess differences between condition (vehicle or CORT) and treatment group (vehicle, reelin, or ketamine). Statistics were conducted on SPSS (v27, IBM). If significance was detected in the ANOVA, Tukey's post-hoc tests were used for multiple comparisons between conditions and treatments.

5.4 Results

5.4.1 Behaviour

5.4.1.1 Forced swim test

Significant group differences were found for swimming [$F(2, 41) = 6.384$, $p = 0.0039$], immobility [$F(2, 41) = 5.184$, $p = 0.0098$], and latency to immobility [$F(1, 41) = 7.670$, $p = 0.0084$] in the forced swim test (Fig. 5.2A – D). Rats treated with CORT/vehicle had significantly higher time spent immobile than vehicle groups ($p = 0.0003$), an effect which was rescued by both reelin ($p = 0.0049$) and ketamine ($p = 0.0047$). Time spent swimming was also significantly decreased compared to the vehicle subgroups in the CORT/vehicle group ($p = 0.0002$), an effect that was upregulated by reelin ($p = 0.0028$) and ketamine ($p = 0.0034$). Lastly, rats treated with C/V had a shorter latency to immobility (time until immobile) than the vehicle group ($p = 0.017$), but this was only significantly rescued by reelin treatment ($p = 0.0423$), not ketamine. No significant effect on climbing was found.

5.4.1.2 Sucrose preference and splash tests

Sucrose consumption during the habituation (day before test, with two bottles of sucrose) and test phase (one bottle of water, one bottle of sucrose) were measured to determine anhedonic-like behaviour. Throughout the test phase, the side of the cage the bottle was placed on was switched at 12 h (halfway through) to avoid any confounding effects of location preference. No significant differences were found between any groups on the habituation phase (Fig. 5.2E), however during the test phase [$F(2, 56) = 5.984$, $p = 0.0044$] animals in the CORT/Vehicle subgroup consumed

significantly less sucrose (as measured by % sucrose consumption) than vehicle/vehicles ($p = 0.0088$) (Fig. 5.2F). However, neither reelin nor ketamine had any effect on increasing sucrose consumption. The sucrose splash test was used on the animals who underwent electrophysiology to determine grooming behaviour. No significant differences were found between any of the subgroups (Fig. 5.2G-H).

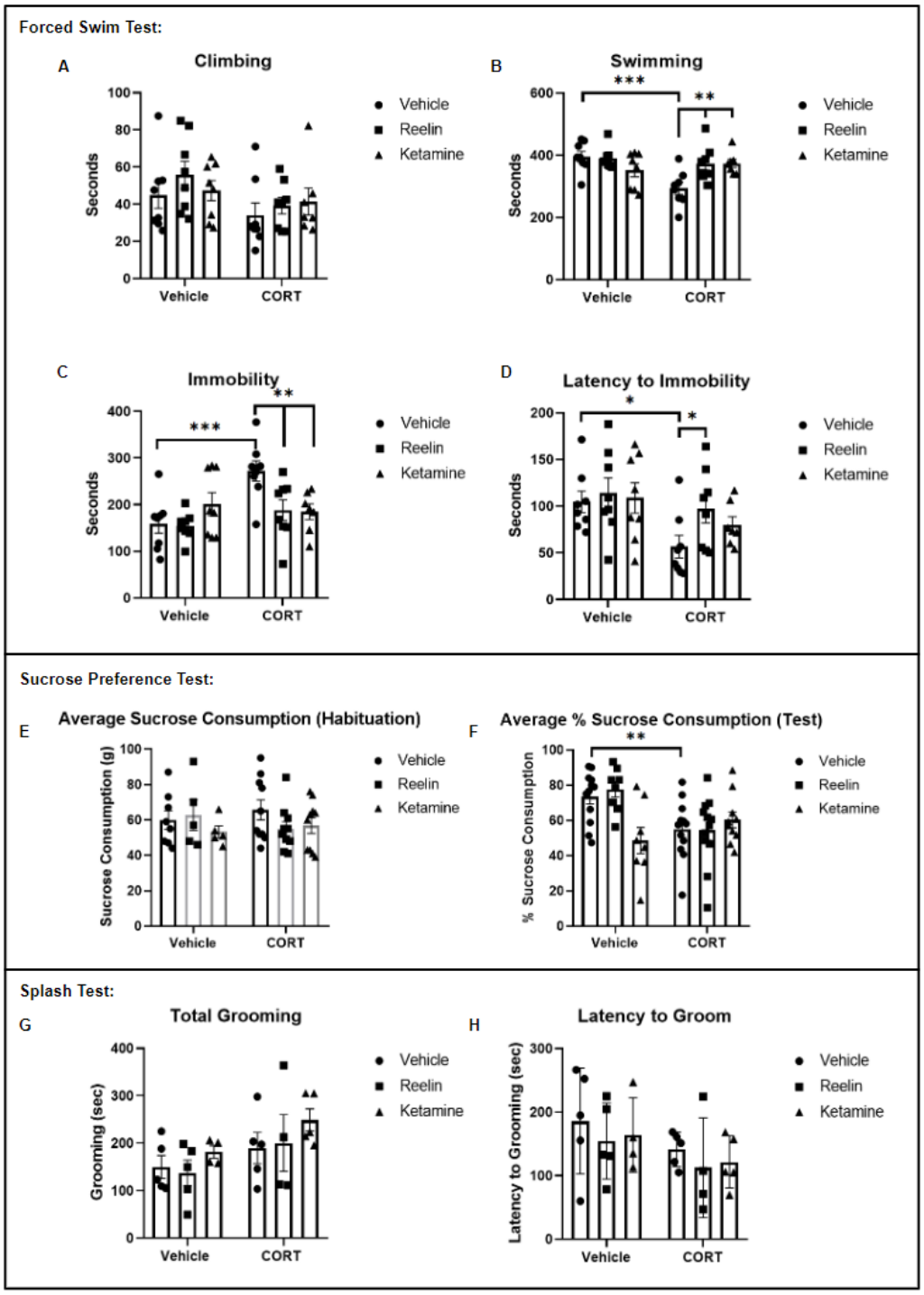


Figure 5.2. Fast-acting behavioural effects of reelin and ketamine. (A) No significant differences were found across groups in FST climbing. (B) CORT administration significantly decreased swimming in the FST, which was rescued by both reelin and ketamine. (C) CORT administration significantly increased immobility in the FST, which was rescued by both reelin and ketamine. (D) Latency to immobility in the FST was decreased by CORT but was rescued only by reelin administration. (E) No changes were found in habituation-phase sucrose consumption. (F) Sucrose consumption was significantly decreased by CORT, but not changed by reelin or ketamine. (G-H) No changes were found in the sucrose splash test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

5.4.2 *In vivo* electrophysiology

5.4.2.1 I/O curve

Increasing pulse widths (0.04mA, 0.08mA, 0.12mA, 0.16mA, 0.2mA, and 0.24mA) were measured after a stable signal was present and before baseline measurements were taken to determine input/output (I/O) function. In all animals, the fEPSP slope was significantly greater with increased stimulation. No significant differences were found in fEPSP slopes between any experimental subgroups, suggesting baseline synaptic excitability was similar (Fig. 5.4A).

5.4.2.2 PTP and LTP

A stable baseline was collected for at least 30min, then the TBS protocol was applied. The baseline stimulation protocol was then continued for 1 hr. Post-tetanic potentiation (PTP) was defined as 1min after TBS protocol and long-term potentiation (LTP) was defined as 55min – 60min after the TBS protocol. In both PTP [$F(2, 18) = 7.179$, $p = 0.0051$] and LTP [$F(2, 18) = 3.924$, $p = 0.0385$], significant effects of condition and treatment were found. Vehicle/vehicle demonstrated significantly higher PTP than CORT/vehicle ($p = 0.0008$), and both reelin and ketamine trended towards rescue (Fig. 5.4C). LTP was decreased by CORT ($p = 0.0045$), and this effect was significantly rescued by both reelin ($p = 0.0342$) and ketamine ($p = 0.0493$) (Fig. 5.4D). Individual traces (Fig. 5.3) and means are graphed (Fig. 5.4B)

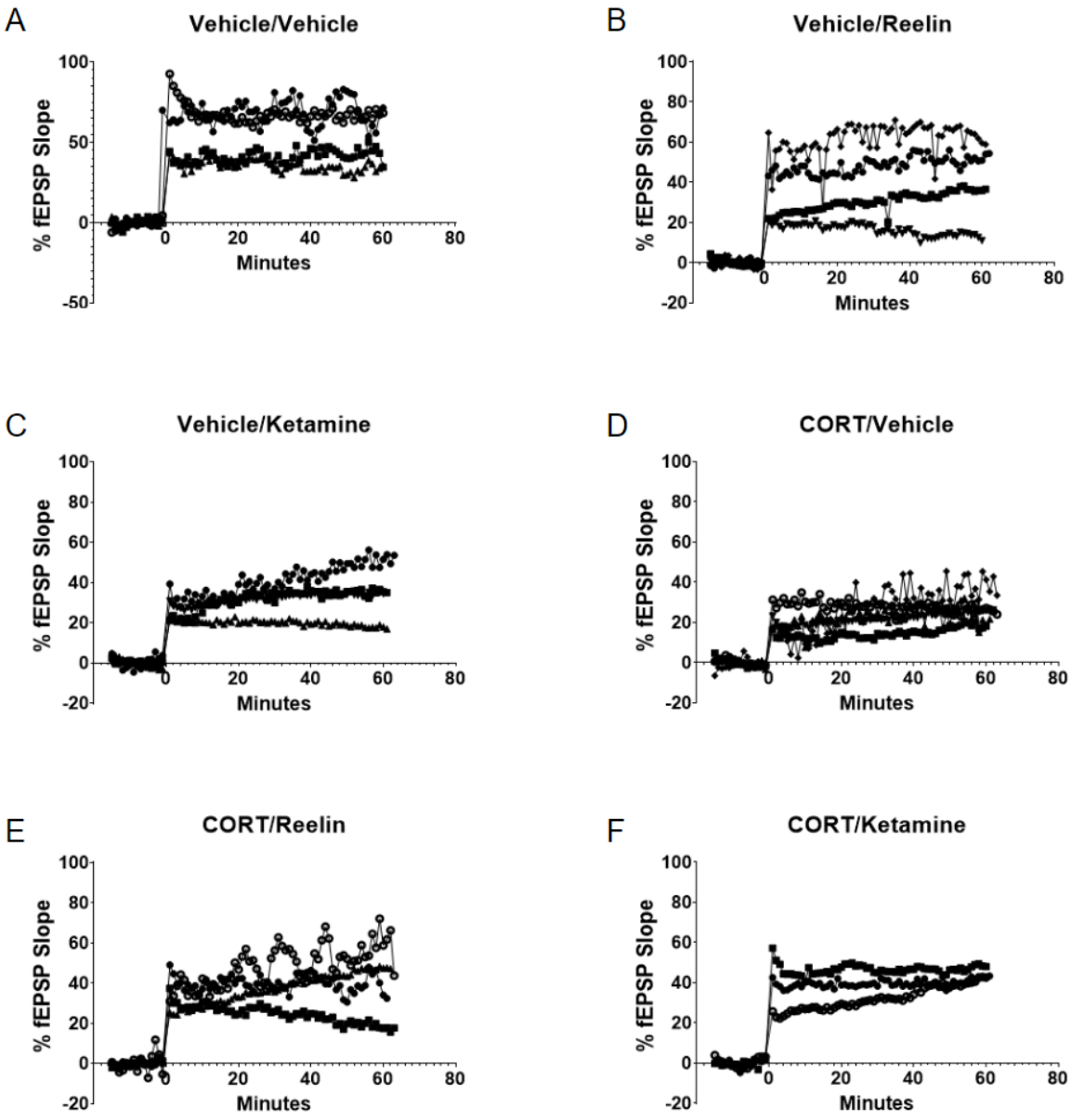


Figure 5.3. ***In vivo*** **electrophysiology individual traces for each treatment group.** (A-F) Individual (animal) traces for each treatment group. TBS stimulation was applied at 0 minutes. PTP was measured at 1 minute and LTP was measured between 55 min – 60 min.

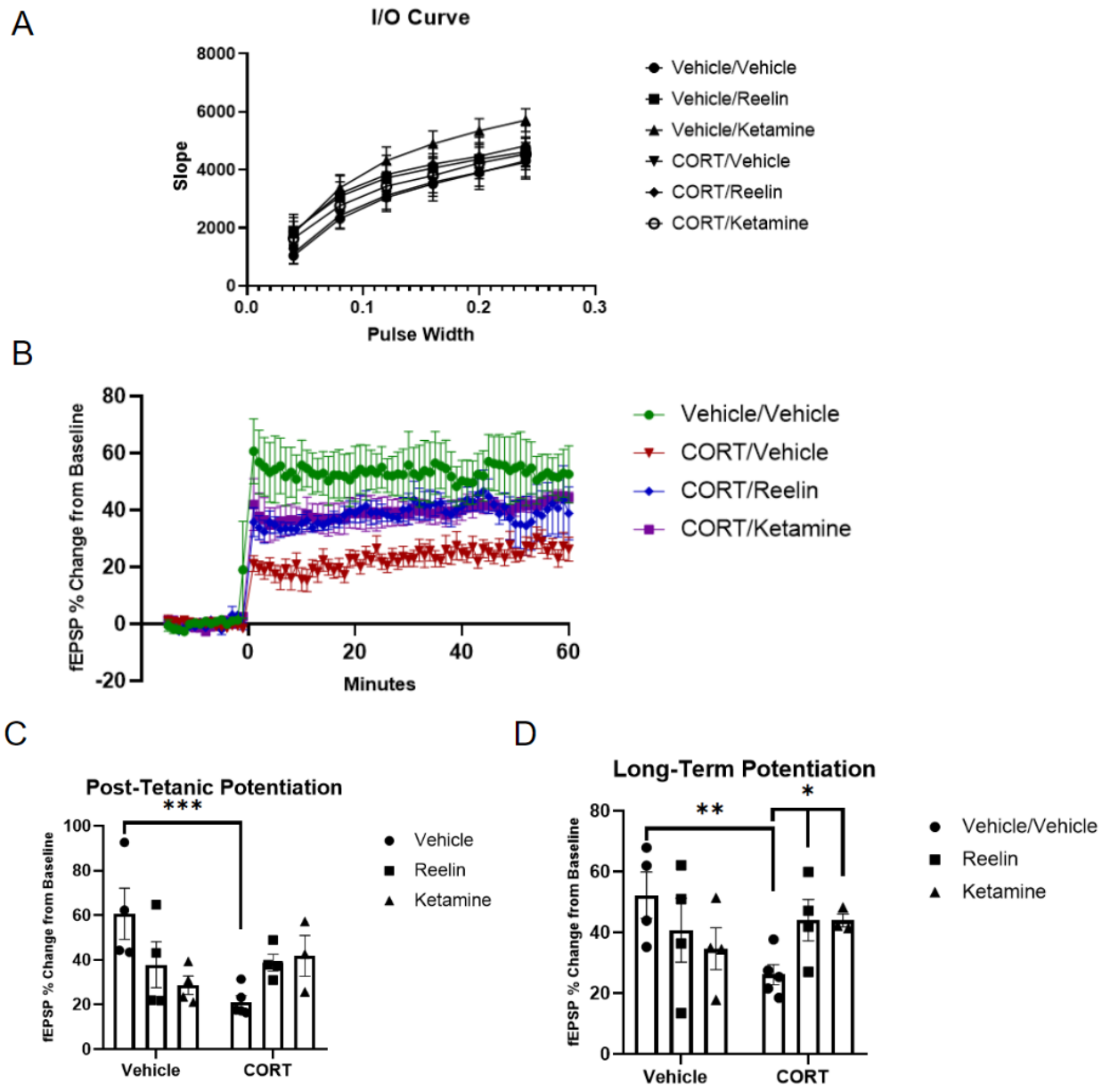


Figure 5.4. *In vivo* electrophysiological recordings from vehicle, CORT, reelin, and ketamine administered rats. (A) I/O function was measured using increasing pulse widths to determine baseline excitability. No differences were found between treatment groups. (B) Average fEPSP slope as a % change from baseline for vehicle/vehicle, CORT/vehicle, CORT/reelin, and CORT/ketamine experimental groups. A stable baseline was recorded for 30 min. Theta burst stimulation (TBS) was applied at 0 min. Post-tetanic potentiation (PTP) was measured at 1 min after theta burst stimulation (TBS) and long-term potentiation (LTP) was measured 55 – 60 min after TBS. (C) Changes in PTP potentiation after TBS. CORT significantly decreased PTP, which was not rescued by reelin or ketamine. (D) Changes in LTP after TBS. CORT administration significantly decreased LTP, which was recued by both reelin and ketamine. All data expressed as mean ± SEM *p < 0.05, **p < 0.01, ***p <0.001

5.4.3. Reelin expression in the subgranular zone

Previous research has determined that both reelin and ketamine are able to rescue reelin expression after a few days (Allen et al., 2022; Brymer et al., 2020; Johnston et al., 2020), however this has not previously been assessed within 24 hrs to determine whether the fast-acting effects of both therapeutics may be related to an upregulation in reelin expression. Expression of reelin in the subgranular zone of the dorsal hippocampus was affected by both condition and treatment [F (2, 18) = 3.732, p = 0.0441]. As expected, chronic CORT treatment significantly decreased reelin expression in the SGZ of the hippocampus (p = 0.0129), an effect that was significantly rescued by both reelin (p = 0.0421) and ketamine (p = 0.0443) (Fig. 5.5A-B).

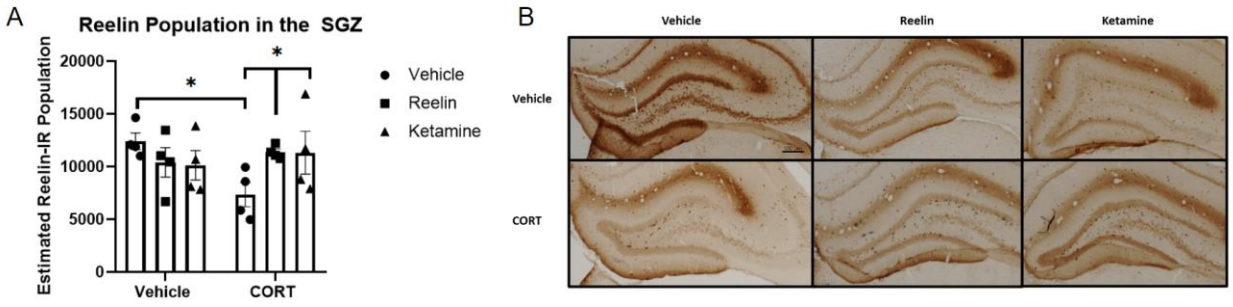


Figure 5.5. Reelin expression in the subgranular zone after reelin and ketamine administration. (A) Unbiased stereology estimates of reelin cell population in the dentate gyrus subgranular zone. CORT administration significantly decreased reelin expression, which was rescued by an acute dose of reelin and ketamine. (B) Representative photomicrographs of reelin expression in the subgranular zone. All data expressed as mean ± SEM *p < 0.05.

5.4.4 Western blotting analyses

Western blotting analyses looked at condition and treatment differences in both WH and SNP tissue for p-mTOR, mTOR, ratio of active mTOR, p-GluA1, GluA1, ratio of active GluA1, p-CREB, CREB, ratio of active CREB, GluN2b, PSD-95, Synapsin I, and p-ERK1/2 (Fig. 5.6 and 5.7). However, significant differences were only found in p-mTOR expression [F (3, 84) = 3.638, p = 0.016], where ketamine treatment significantly increased levels of WH p-mTOR after chronic CORT administration (p = 0.0161) and p-ERK expression [F (3, 72) = 4.868, p = 0.0039], though post-hoc analyses revealed no specific inter-group differences.

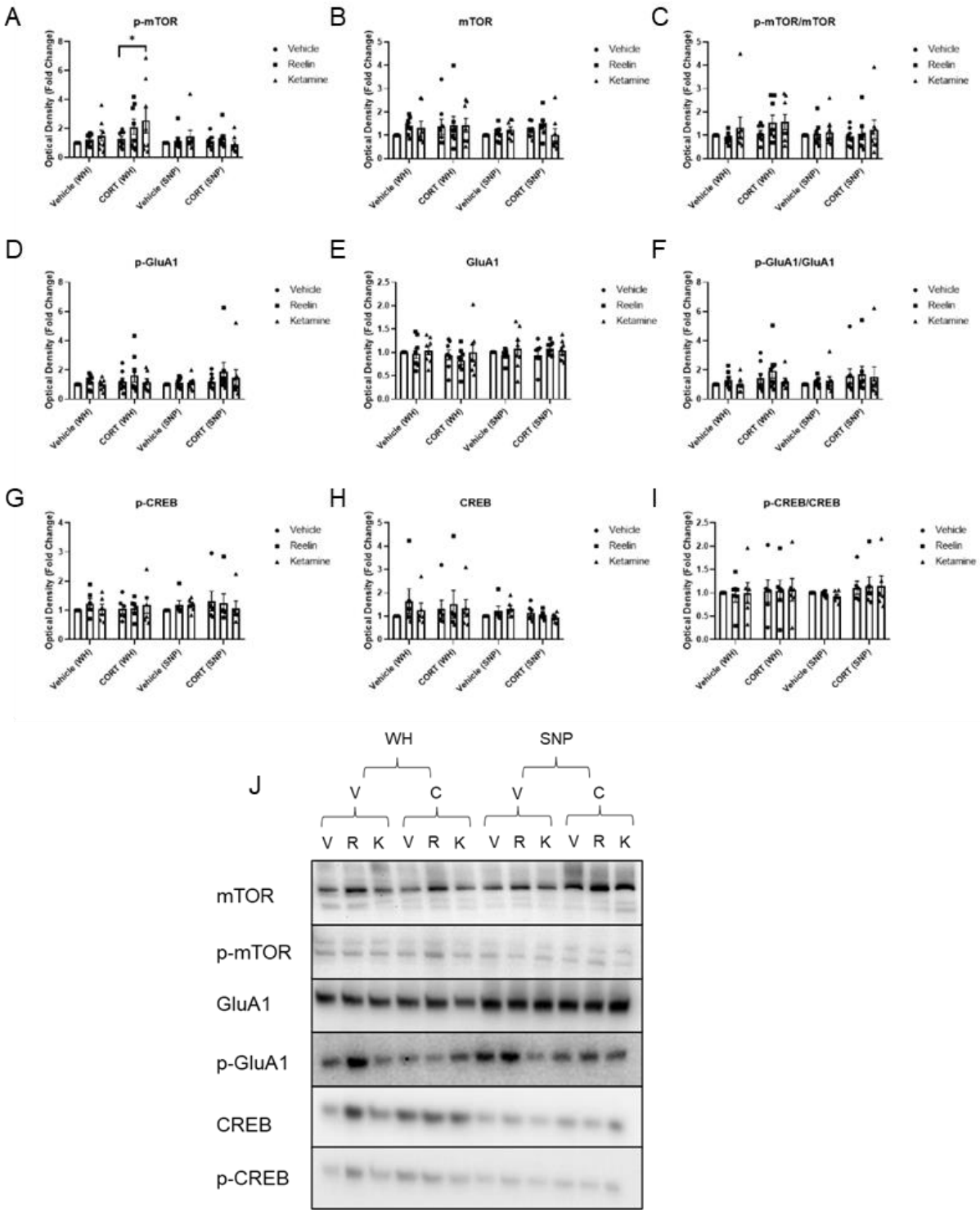


Figure 5.6. The effect of CORT, reelin, and ketamine treatment on whole cell and synaptic proteins (part I). (A) Whole cell p-mTOR expression was increased after ketamine adminstration. (B-I) No significant differences were found between any treatment groups for all proteins and activity ratios measured. All bands were normalized to total protein expression as indicated by Ponceau S. V = vehicle, R = reelin, K = ketamine, C = CORT, WH = whole homogenate, SNP = synaptoneurosomes. All data is expressed as mean ± SEM. *$p < 0.05$

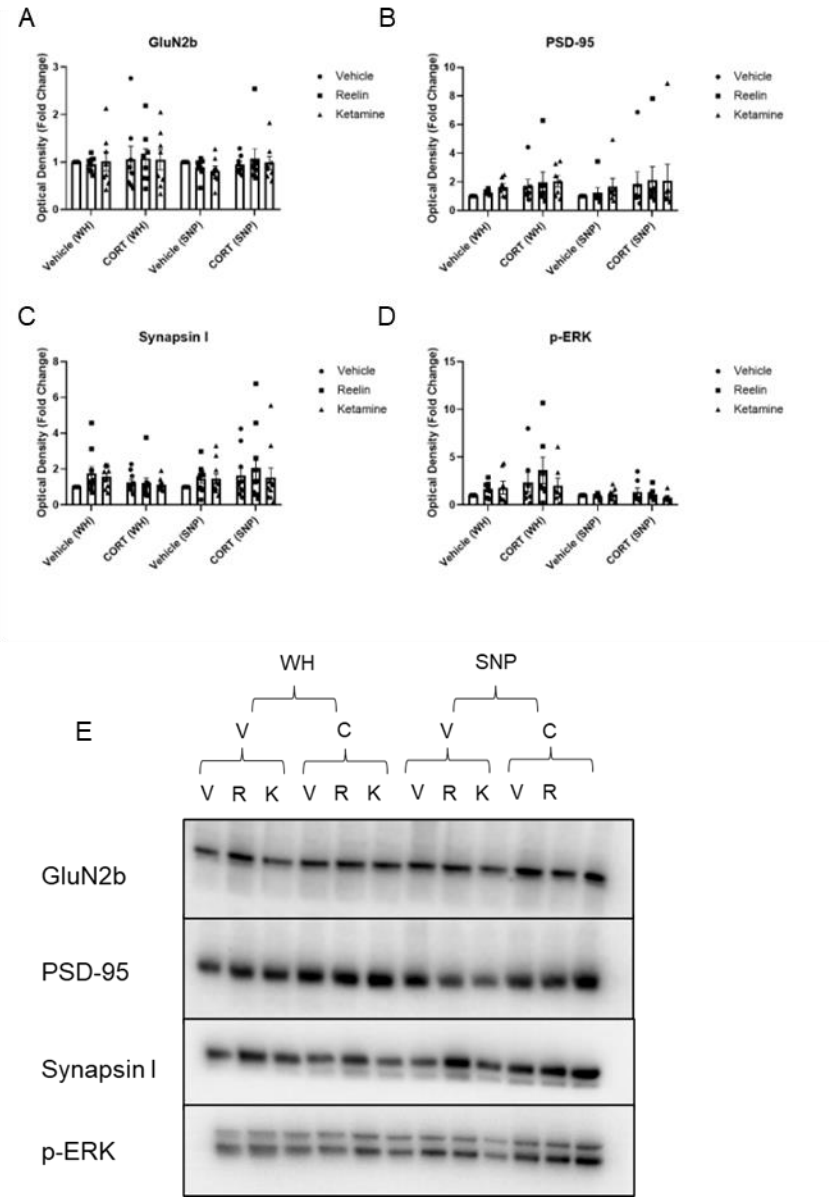


Figure 5.7. The effect of CORT, reelin, and ketamine treatment on whole cell and synaptic proteins (part II). (A-I) No significant differences were found between any treatment groups for all proteins measured. All bands were normalized to total protein expression as indicated by Ponceau S. V = vehicle, R = reelin, K = ketamine, C = CORT, WH = whole homogenate, SNP = synaptoneurosomes. All data is expressed as mean ± SEM. *$p < 0.05$

5.4.5 SERT clustering on PBMCs

No significant differences were observed in either size or number of SERT clusters in peripheral lymphocytes, suggesting that 24 hours after peripheral administration may not be enough time to demonstrate large differences in patterns of SERT clustering (Fig. 5.8A-C).

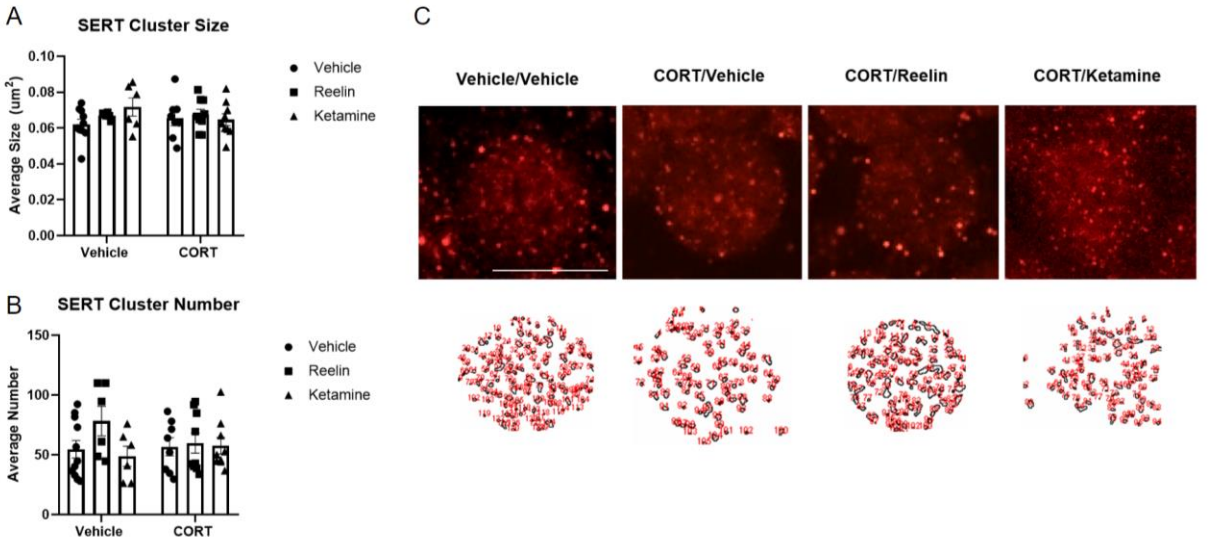


Figure 5.8. SERT clustering in peripheral lymphocytes after treatment. (A – B) No significant differences were found in SERT cluster size or number after treatment. (C) Representative fluorescent microscopy images from vehicle/vehicle, CORT/vehicle, CORT/reelin, and CORT/ketamine subgroups. All data are expressed as mean ± SEM.

5.5 Discussion

Evidently, both ketamine and reelin are able to rescue behavioural, electrophysiological and molecular indicators of chronic corticosterone administration within 24 hours. In the FST, a proxy measure for despair-like behaviour, peripheral ketamine and reelin administration were able to rescue the increase in immobility and decrease in swimming that was observed after CORT treatment. This finding is supported by multiple studies which have demonstrated reelin and ketamine's ability to rescue FST-related behaviour (Allen et al., 2022; Brymer et al., 2020; Fitzgerald et al., 2019), a common measure for antidepressant efficacy in animal models. The SPT was used to assess anhedonic-like behaviour, a hallmark symptom of treatment-resistant depression which has been effectively targeted by ketamine in a clinical setting (Nogo et al., 2022). While CORT-administration increased anhedonia, this was not rescued by administration of either

reelin or ketamine. However, anhedonia is typically difficult to treat or define clinically and is often one of the most stubborn symptoms after treatment (Rizvi et al., 2016; Treadway & Zald, 2011). Future research should determine the impact that reelin or ketamine may have after the 24-hour period of this study. No differences across groups were found in the sucrose splash test, an indicator of grooming. However, the splash test has had mixed results in chronic stress models (Hu et al., 2017) and may demnstrate a symptom category in depression that is not represented by a chronic corticosterone model.

To my knowledge, this is the first study which demonstrates reelin's ability to rescue hippocampal LTP in a model for the study of depression. Hippocampal LTP is essential for learning and memory, processes known to be heavily impacted in depression (Kizilbash et al., 2002; Lynch, 2004; Strömgren, 1977; Weingartner et al., 1981). The medial perforant path in particular is important for context discrimination and fear learning (Ferbinteanu et al., 1999), which are both affected in the chronic corticosterone model and patients with depression (Britton et al., 2011; Camp et al., 2012; Wurst et al., 2021). Increases in excitatory transmission are often associated with ketamine's efficacy as a fast-acting antidepressant (Gilbert & Zarate, 2020), and the effectiveness of reelin in increasing LTP after TBS, even above that of ketamine in this study, adds another facet to the potential of reelin as a putative fast-acting therapeutic. Interestingly, the vehicle/ketamine group also showed decreased LTP, which is in line with previous research suggesting that ketamine may have deleterious effects in healthy controls. The observed parallels between pre-clinical and clinical research are important for the future translation of this work.

Previous research, some of it presented earlier in this thesis, has demonstrated that both ketamine and reelin administration are able to rescue reelin expression in the subgranular zone of the dentate gyrus (Allen et al., 2022; Brymer et al., 2020; Johnston et al., 2020). However, these measurements have been taken days after therapeutic administration, and may not reflect their rapid antidepressant-like responses. I hoped to investigate whether an upregulation of endogenous reelin would coincide with the short-term behavioural effects of ketamine and exogenous reelin. It is unlikely that reelin is able to cross the blood-brain barrier given its large size (410 kda for full-length reelin), suggesting that peripheral administration may be able to increase reelin expression through other mechanisms. Future research should determine the exact mechanisms by which both

ketamine and exogenous reelin administration are able to increase hippocampal reelin administration.

Despite changes found after *in vitro* treatment of synaptoneurosomes (Chapter 3), no significant changes were found in the proteins measured here, aside from an increase in p-mTOR after ketamine administration. Greater variability found between animals, in contrast to the initial synaptoneurosome study, decreased the possibility of finding significant changes between groups. however future research should expand on the changes observed in synaptoneurosomes, given the role that synaptic-specific transcription changes appear to mediate fast-acting antidepressant-like effects (Li et al., 2010).

In contrast to previous research, no differences in SERT cluster size were found in chronic CORT-administered rats (Caruncho et al., 2019), most likely due to variability between samples. Parallel to previous work, no changes in number of SERT clusters were found. The earlier investigation of the effects of reelin and ketamine on lymphocytes *in vitro* found significant changes, however this was not replicated *in vivo*. This may be due to the short time course of measurements, and a future study should determine whether changes in peripheral SERT clustering happen longer than 24 hours after therapeutic administration. Interestingly, SERT clustering on lymphocytes after ketamine administration appeared to be more diffuse in comparison to all other groups but this was not able to be directly quantified.

As the goal of this study was to provide a more exploratory, but broad, look at the changes 24 hours after ketamine and reelin administration, low sample sizes may have limited statistical analyses. In addition, chronic CORT-administration provides a reliable and validated model for the study of depression, it is unable to completely mimic every facet of clinical depression. In particular, the unique characteristics of treatment-resistant depression (such as anhedonia and suicidal ideation) are difficult to model and may present unique results when studying putative fast-acting antidepressants. Future research should expand on these preliminary findings, as well as utilize varying models for the study of depression to increase the translatability of results. In conclusion, this study provides multiple perspectives of how reelin and ketamine exert their antidepressant-like effects in a chronic corticosterone model, including behavioural, electrophysiological, and molecular changes which are known to be impacted in depression.

Chapter 6

Investigating the response of iPSC-derived neurons from participants with TRD to (2R,6R)-HNK and reelin

6.1 Abstract

Treatment-resistant depression, or failure to respond to first-line treatments, is associated with worse clinical outcomes and longer course of illness. However, treatment-resistant depression is more difficult to model in an animal phenotype, and other routes must be considered to properly address and research novel therapeutics for treatment-resistant depression. To this end, we have begun to explore an iPSC-derived model from treatment-resistant depression participants which could provide a better indication of what is happening inside of the human brain. Ketamine, a known effective therapeutic for treatment-resistant depression, has been shown throughout previous chapters to parallel the effects of reelin, an endogenous glycoprotein that is downregulated in depression. However, the role of reelin in treatment-resistant depression is yet unknown. Activity of the mechanistic target of rapamycin complex 1 (mTORC1) and related downstream signalers has been attributed to the antidepressant effects of ketamine and appears to be associated with reelin's antidepressant-like actions. (2R,6R)-hydroxynorketamine (HNK) is a major metabolite of ketamine which, at therapeutic levels, appears to activate mTORC1 without antagonizing NMDARs. To determine the effects of (2R,6R)-HNK and reelin on cortical activity, iPSCs were reprogrammed from peripheral blood mononuclear cells collected from treatment-resistant depression participants and healthy controls, then cultured into cortical neurons. After neurons reached maturity, reelin and (2R,6R)-HNK were applied at varying concentrations and time courses. Protein expression was measured through Western blotting and immunocytochemistry, and gene expression was indicated through RNA sequencing. Baseline protein differences were found between controls and TRD neurons (mTOR, GluA1, TrkB, p-ERK, and ERK). While reelin and (2R,6R)-HNK had parallel effects on protein expression, opposing treatment effects were observed between 1-hour and 24-hour timepoints in TRD neurons. No treatment effects were observed across all proteins in control cell lines. This study, while exploratory, provides evidence towards the molecular actions of (2R,6R)-HNK and reelin in treatment-resistant depression. The differences between control and TRD cell lines warrant further investigation. While still preliminary, iPSC-derived neurons could provide a valuable *in vitro* model for the study of TRD and hold promise for the evaluation of novel therapeutics such as (2R,6R)-HNK and reelin.

6.2 Introduction

The findings presented previously in this thesis find behavioural, electrophysiological, and molecular changes in *in vitro* and *in vivo* models for the study of depression. However, there are severe limitations to the use of animal models, which are often homogenous in direct contrast the heterogeneity of the human experience of depression. In addition, animal models cannot exactly mimic certain aspects of behaviour and biology that are present in humans, such as suicidal ideation (Comai & Gobbi, 2016). However, we are not yet able to study brain tissue from living humans, which limit the molecular changes which can be observed in depression or as a response to treatment. With this information, the question becomes how to create more translational models that could help the difficulties experienced taking novel therapeutics from bench to bedside.

Of particular interest is developing therapeutics that are useful in treatment-resistant depression (TRD), a condition which is particularly difficult to replicate in animal models (Willner & Belzung, 2015). Generally, treatment-resistant depression (TRD) is defined inadequate response to one or more class of antidepressants; however this definition does vary (Fava, 2003). Those who do not improve, or show a partial response, may be faced with additional challenges including functional impairment, lowered quality of life, high relapse rates, and increased risk of suicidality (Shinohara et al., 2019). Given these challenges, it is essential to develop therapeutics that are effective for TRD.

As a fast-acting antidepressant, ketamine has been primarily used to target TRD (Kishimoto et al., 2016), an effect which appears to be mediated through mTORC1 activation and related downstream signaling in the prefrontal cortex and hippocampus (Duman et al., 2019; Zanos & Gould, 2018). As shown in previous research presented in this thesis, the upregulation of proteins such as phosphorylated-mTORC1 (p-mTORC1), postsynaptic density-95 protein (PSD95) and surface insertion of Glutamate A1 (GluA1) subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors often (though not always) accompany ketamine's antidepressant-like effects. Metabolites of ketamine may also have disparate antidepressant effects. For example, (2R,6R)-hydroxynorketamine (HNK) is a major metabolite of ketamine that appears to produce similar rapid antidepressant effects in animal models without the associated side effects of ketamine administration. Interestingly, (2R,6R)-HNK does not appear to antagonize NMDARs at therapeutic doses, further cementing the role of mTOR activation in ketamine's antidepressant effects (Zanos et al., 2018a).

Reelin has demonstrated significant parallels to ketamine's fast-acting antidepressant-like effects throughout this dissertation but has not yet been investigated in the context of TRD. As reelin signaling may mediate some of ketamine's antidepressant actions (Kim et al., 2021), exogenous reelin administration could be useful to address a TRD phenotype which is characterized most often by worse symptomatology and a longer disease course. Reelin's documented abilities to increase synaptogenesis, synaptic potentiation, and dendritogenesis (Wasser & Herz, 2017) could prove effective at targeting hallmarks of TRD.

As mentioned previously, molecular research of depression (including TRD) has been limited by the inability to directly investigate human neurons. However, the development of human induced pluripotent stem cells (iPSCs) through the reprogramming of human fibroblasts, keratinocytes, and lymphocytes has opened up investigations into the cellular mechanisms of various disorders and therapeutics in humans (Doss & Sachinidis, 2019; Engle et al., 2018; Li et al., 2018; Soldner & Jaenisch, 2012).

iPSC modeling has been used previously for the study of psychiatric disorders such as bipolar disorder, schizophrenia, autism spectrum disorders, and depression (McNeill et al., 2020). To our knowledge, only two previous reports have been published of iPSC-modeling from TRD patients, which focused on the serotonergic system (Vadodaria et al., 2019a,b).

The purpose of the current study was to determine the impact of (2R,6R)-HNK and reelin on iPSC-derived neurons from TRD patients. In addition, baseline differences between iPSC derived neurons between healthy controls and TRD patients will be analyzed to further characterize the use of iPSCs in the development of novel depression therapeutics.

6.3 Methodology

6.3.1 Collection of iPSCs

In accordance with NIH ethics, peripheral mononuclear blood cells (PMBCs) were collected from 5 TRD patients and 2 healthy controls. Generation of human iPSCs from PBMCs and fibroblast cells were conducted at National Heart, Lung, and Blood Institute (NHLBI/NIH) iPSC Core Facility using non-integration methods.

6.3.2 Differentiation and growth of cell cultures

The STEMdiff™ embryoid body protocol was followed for 19 days to develop single-cell neural progenitor cells (NPCs). Neural induction was confirmed through visual inspection, and neural rosettes were selected. Cells were seeded onto PLO/laminin-coated dishes (half with poly-L-lysine coated coverslips) at a density of 1.5 x 10^4 – 3 x 10^4 cells/cm2. The cells were then incubated at 37°C with 5% circulating CO2 and supplemented with BrainPhys™ Neuronal Medium, 1% N2 Supplement-A, 2% Neurocult™ SM1 Neuronal Supplement, 20ng/ml GDNF, 20ng/ml BDNF, 1mM Dibutyryl-cAMP, and 200nM Ascorbic Acid for 10 weeks with half-medium changes every two days. A CCK-8 cell counting kit was used as a viability assay.

6.3.3 Treatment of cultures

After 10 weeks, cells were divided into 5 different conditions (vehicle + DMSO; 5nM, 10nM, and 50nM reelin; 1μM (2R,6R)-HNK) at 2 timepoints (1 hour; 24 hours) to assess short and long-term effects of reelin and ketamine metabolite application. Each cell line was cultured in enough wells to allow for multiples of every treatment concentration, for a total of 40 wells/cell line, to allow for duplicates in Western blot and immunocytochemical (ICC) analyses. Half of the wells were rinsed and scraped for Western blot analysis and the other half were rinsed with PBS and fixed with paraformaldehyde (PFA) (4%) for 10 min for ICC.

6.3.4 SDS-PAGE and Western blotting

For Western blotting, cells were homogenized in cold lysis buffer. 10 μg of protein were electrophoretically resolved in Mini-PROTEAN® TGX Precast 4 – 12% gels (BioRad, Hercules, CA) then transferred onto 0.2 μm polyvinylidene fluoride (PVDF) membranes via a semi-dry transfer method in the Trans-Blot Turbo Transfer System (BioRad, Hercules, CA). Membranes were blocked using 5% (w/v) milk for unphosphorylated proteins, and 5% (w/v) BSA for phosphorylated proteins for 1 h at room temperature. After measurement of mTOR (#2972S, CST) or ERK (#5376S, CST), the same blot was stripped and analyzed for p-mTOR (#2971S, CST) or p-ERK (#9101S, CST) to provide individual ratios of activity. Other proteins analyzed were PSD-95 (#2507S, CST), Synapsin I (#6710, CST), GluA1 (#13185S, CST), Dab1 (#3328S, CST), and GluN2b (UC Davis). Immunocytochemical protocols and analyses can be found in Appendix A.

6.3.5 Statistical analyses

Statistics were conducted using SPSS (v20.0, IBM). Baseline differences between TRD and HC cell lines were compared with independent t-tests. To determine the impact of Reelin and (2R,6R)-HNK on iPSCs, the Kruskal-Wallis test was used to correct for non-parametric data. Dunn's multiple comparison test was used for post-hoc comparisons.

6.4 Results

6.4.1 Western blotting

Significant baseline differences were found between the TRD and HC cell lines (Fig. 6.2). TRD cells had significantly higher baseline expression of GluA1 ($p = 0.016$), TrkB ($p = 0.031$), ratio of mTOR activity ($p = 0.011$), p-ERK ($p = 0.001$), and ERK ($p = 0.006$) at 1 hour. At 24 hours, levels of PSD-95 ($p = 0.005$), GluA1 ($p = 0.007$), and TrkB ($p = 0.007$) were significantly higher and levels of mTOR ($p = 0.039$), p-ERK ($p = 0.007$), and ratio of ERK activity ($p = 0.004$) were significantly lower.

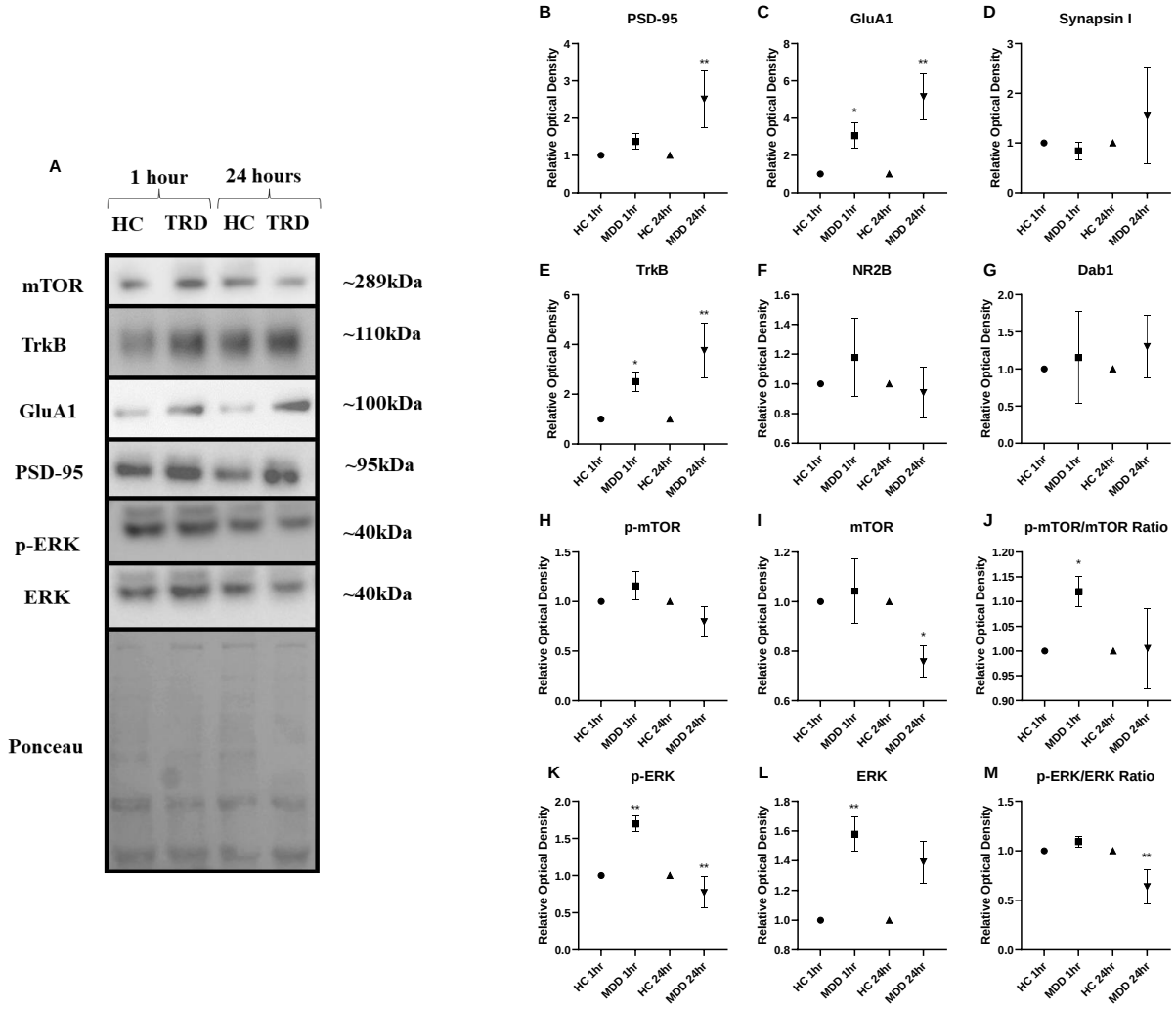


Fig. 6.1. Baseline differences in HC and TRD iPSC-derived neurons. (A) Representative Western Blot images of iPSC-derived neurons from HC and TRD participants at 1 hour and 24 hours with significant differences. All proteins were normalized to Ponceau (total protein normalization) (B – M) The relative differences in expression of PSD-95, GluA1, Synapsin I, TrkB, NR2B, Dab1, p-mTOR, mTOR, ratio of mTOR activity, p-ERK, ERK, and ratio of ERK activity between HC and TRD iPSC-derived neurons at 1 hour and 24 hours. All data are expressed as mean ± SEM. HC, healthy control; TRD, treatment-resistant depression; *p<0.05, **p<0.01 vs. HC.

In iPSC-derived neurons from TRD patients, both Reelin and (2R,6R)-HNK demonstrated parallel effects at both 1 hour and 24 hours (Fig. 3). PSD-95 showed significant changes after both 1-hour treatment ($H(4) = 13.68$, $p = 0.0078$) and 24-hour treatment ($H(4) = 9.979$, $p = 0.0408$). Post-hoc tests revealed PSD-95 was significantly increased with 50nM of Reelin ($p = 0.032$) and 1μM of (2R,6R)-HNK ($p = 0.0021$). In contrast, PSD-95 was downregulated after 24 hours with 50nM of Reelin ($p = 0.0162$). While GluA1 was not up-regulated with any treatment at 1 hour, the AMPAR

subunit was significantly down-regulated at 24 hours ($H(4) = 15.42$, $p = 0.0039$) with all concentrations of Reelin (5nM, p = 0.0311; 10nM, p = 0.0068; 50nM, p = 0.0015). Synapsin I showed concentration-dependent changes at both 1 hour ($H(4) = 17.51$, $p = 0.0015$) and 24 hours ($H(4) = 22.98$, $p = 0.0001$), with post-hoc tests revealing the consistent up-regulation of expression at 1 hour (Reelin 10nM, p = 0.0064; Reelin 50nM, p = 0.0016; HNK 1µM, p = 0.0032) and down-regulation at 24 hours (Reelin 10nM, p = 0.0009; Reelin 50nM, p = 0.0002; HNK 1µM, p = 0.0004). All treatment increased levels of Dab1 after 1 hour ($H(4) = 0.0011$, $p = 0.0011$; Reelin 5nM, p = 0.0331; Reelin 10nM, p = 0.032; Reelin 50nM, p = 0.0009; HNK 1µM, p = 0.0009). This was paralleled by a decrease in expression of Dab1 at 24 hours ($H(4) = 16.55$, $p = 0.0024$; Reelin 10nM, p = 0.0014; Reelin 50nM, p = 0.0037; HNK 1µM, p = 0.0217). No significant results were found in expression of TrkB and NR2B.

Activity indicators of the major cellular signaling pathways, mTOR and ERK, had mixed results. p-mTOR, mTOR, and the ratio of mTOR activity had no significant differences at either timepoint except for a slight downregulation of p-mTOR at 24 hours with (2R,6R)-HNK application ($H(4) = 11.77$, $p = 0.0191$; HNK 1µM, p = 0.0197). However, an upregulation of p-ERK was found at 1 hour ($H(4) = 19.51$, $p = 0.0006$; Reelin 50nM, p = 0.0016; HNK 1µM, p = 0.0014). After 24 hours, it was also significantly decreased ($H(4) = 13.35$, $p = 0.0097$) with (2R,6R)-HNK treatment (p = 0.0072). Significant differences were not found in expression of total ERK, or the ratio of ERK activity.

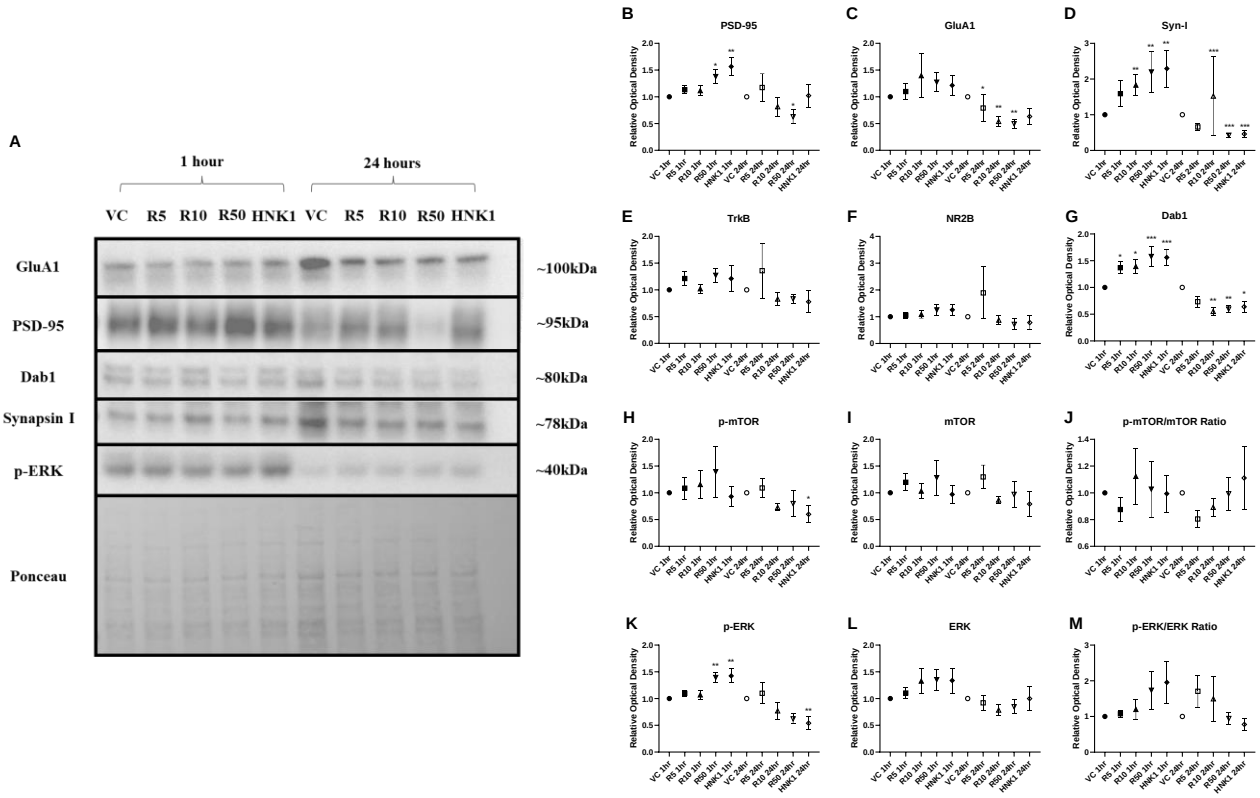


Fig. 6.2. The impact of Reelin and (2R,6R)-HNK on TRD iPSC-derived neurons. (A) Representative Western Blot images of the impact of Reelin and (2R,6R)-HNK on iPSC-derived neurons from TRD participants at 1 hour and 24 hours. All proteins were normalized to Ponceau (total protein expression). Images shown for proteins with significant differences in expression. (B-M) Graphs showing the expression levels of PSD-95, GluA1, Synapsin I, TrkB, NR2B, Dab1, p-mTOR, mTOR, ratio of mTOR activity, p-ERK, ERK, and ratio of ERK activity after treatment. VC, vehicle control; R5, Reelin 5nM; R10, Reelin 10nM; R50, Reelin 50nM; HNK1, (2R,6R)-HNK 1µM; *p<0.05, **p<0.01, ***p<0.001 vs. VC.

No significant differences were found at either 1 hour or 24 hours in the HC cell lines that were treated with Reelin (50nM) or (2R,6R)-HNK (1µM) (Fig. 6.4).

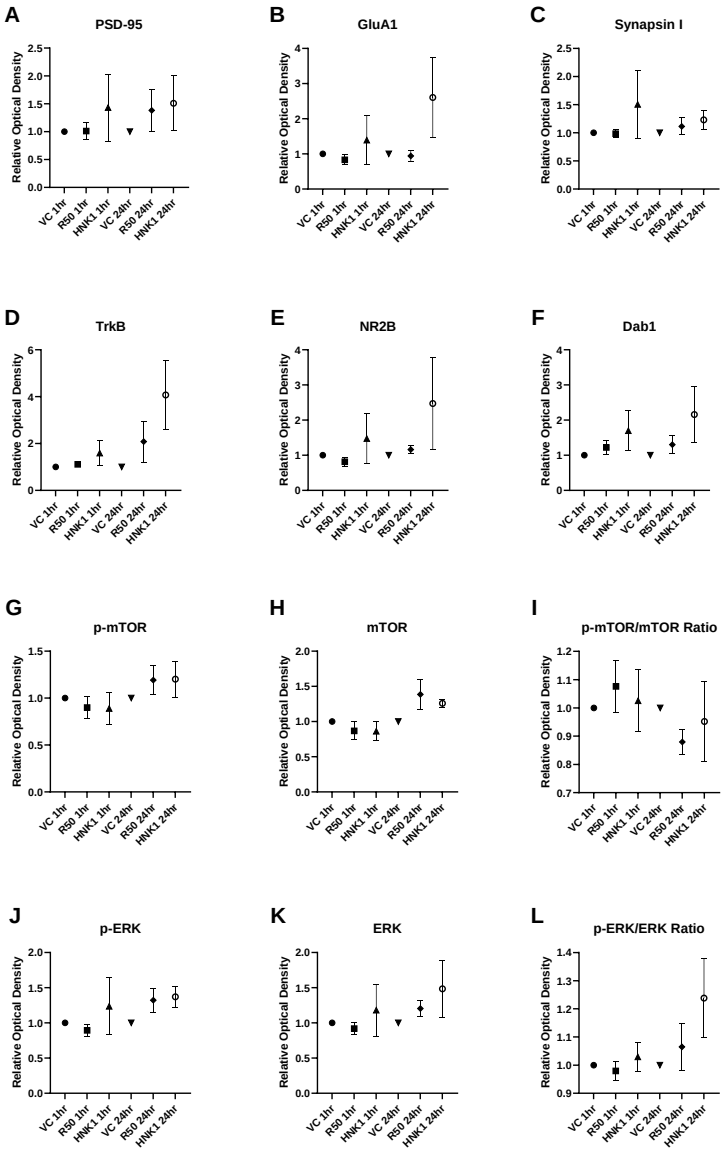


Fig. 6.3. The impact of reelin and (2R,6R)-HNK on iPSC-derived neurons from healthy controls. (A – L) Relative optical density amounts from Western blotting protein analyses. All proteins were normalized to Ponceau. No significant differences were found across any treatment groups.

6.5 Discussion

The use of iPSC-derived neurons from TRD patients can provide a unique *in vitro* model for the screening of novel therapeutics. Many reviews have previously discussed both the limitations and merits of iPSC modeling for neurological diseases, emphasizing the need for in-depth characterization of the *in vitro* model (Doss & Sachinidis, 2019; Engle et al., 2018; Li et al., 2018; McNeill et al., 2020; Soldner & Jaenisch, 2012). Modeling depression using iPSCs has an added

level of difficulty due to lower levels of genetic heritability (~30% - 50%) and heterogeneity in genetic differences (Kendall et al., 2021). Previous literature on iPSC-derived neurons from TRD patients has focused on the serotonergic system, demonstrating distinct differences between non-responders, responders, and controls (Vadodaria, et al., 2019a, b). I hope to add to the literature of by demonstrating both baseline differences in iPSC-derived neurons from TRD and HC participants, as well as responsivity to both novel and better-characterized therapeutics.

Significant differences appeared between iPSC-derived neurons from TRD patients and HCs. Unexpectedly, the expression of GluA1, TrkB, and ERK were significantly higher in TRD cell lines at both 1 hour and 24 hours. While an up-regulation of GluA1 is typically associated with antidepressant effects of ketamine (Duman et al., 2019), there are mixed results in the literature regarding the role of GluA1 in depression. For example, a reduction in Gria1 mRNA (which encodes for GluA1) has been found in hippocampal tissue of depressed patients and in those of chronically stressed rats (Duric et al., 2013; Toth et al., 2008). It also appears that high GluA1 expression may be associated with cognitive impairment in certain animal models (Gross et al., 2015). TrkB has similar mixed results, with some finding enhanced TrkB signaling associated with certain aspects of depressive-like behaviour, such as fear conditioning in animal models (Takei et al., 2011)or having no effect on depressive-like behaviour at all (Zörner et al., 2003), in contrast to others who found TrkB heavily involved in depressive-like behaviour (Chen-Tsai et al., 2004; JiaWen et al., 2018; Koike et al., 2013).

There was an evident time and concentration-dependent impact of reelin that paralleled the effects of (2R,6R)-HNK on the TRD cell lines. PSD-95, Synapsin I, Dab1, and p-ERK were all significantly increased at one hour with higher concentrations of reelin and (2R,6R)-HNK, demonstrating parallel effects between the two treatments. Interestingly, these effects were reversed at the 24-hour mark, suggesting that prolonged exposure to reelin or (2R,6R)-HNK may have deleterious effects in cell cultures. The increase in Synapsin I suggests that both reelin and (2R,6R)-HNK have an affect at the pre-synaptic level and may increase neurotransmitter release which supports previous research on ketamine's pre-synaptic mechanisms (Müller et al., 2013). The parallels between (2R,6R)-HNK and reelin suggest that they may work on overlapping or parallel mechanisms, which has been supported by previous research in animal models (Johnston et al., 2020; Kim et al., 2021).

No changes in p-mTORC1 or mTORC1 expression were observed, however this may be due to a more transient effect than could be measured at a 1-hour time-point and prompts further investigation. Previous research in animal models found that p-mTORC1 expression peaked between 30min – 1hr after ketamine administration in synaptoneurosomes (Li et al., 2010). Of note, rapamycin (an mTORC1 inhibitor) prolonged ketamine's antidepressant effects in a clinical setting, prompting a re-evaluation of the necessity of mTOR for ketamine's antidepressant-like effects (Abdallah et al., 2020). The up-regulation of p-ERK expression parallels previous literature that has demonstrated ERK signaling, an important regulator of glucose metabolism, is rescued by ketamine in a depressive-like animal model (Ouyang et al., 2021). Previous research has also demonstrated a down-regulation of ERK in pre-frontal cortices of depression patients (Dwivedi et al., 2001).

In contrast to the TRD-derived cell lines, those collected from controls had no significant differences across all conditions. While 1-hour of administration did not increase protein expression across all measures, prolonged exposure to any treatment did not decrease expression. Therefore, healthy control-derived cells may have more resilience against external challenges in comparison to those from TRD patients. This is similar to previous findings in other iPSC-derived models, such as those for bipolar disorder which have found bipolar disorder cell-line specific responses to lithium that did not appear in healthy controls (Mertens et al., 2015).

As previously mentioned, there are limits to sampling at 1 hour and 24 hours. Future studies should determine if changes in mTORC1 activity and expression are observed at earlier timepoints and determine whether shorter exposures could have long-term effects. iPSC-derived neurons, particularly for depression modeling, is also still in its infancy (Engle et al., 2018). Further characterization of iPSC-derived neurons from treatment-resistant depression patients could increase the translatability of results. From this research, we have shown that there are baseline differences in iPSC-derived neurons between HC and MDD participants. In addition, these cell lines respond differently to application of Reelin and (2R,6R)-HNK, particularly after 24 hours of application. These results further the characterization of iPSC-derived neurons from TRD participants, as well as further elucidating the parallel mechanisms between a ketamine metabolite and reelin.

Chapter 7

General Discussion

7.1 Summary of main findings

There were two main aims of this dissertation: *(1)* to increase the translatability of laboratory-based science with the inclusion of patient-oriented research and the utilization of varying techniques to present findings cross-species and cross-techniques, and *(2)* to ascertain the molecular changes that accompany the actions of reelin as a potential fast-acting antidepressants in comparison to ketamine, a well-established novel therapeutic. To our knowledge, the molecular and electrophysiological effects of peripheral reelin administration have not yet been measured within 24 hours, the typical time course for a rapid-acting antidepressant action. This dissertation expanded on the findings of intrahippocampal infusions of reelin to normalize behavioural deficits (Brymer et al., 2020), and the longer-term actions of peripheral reelin administration (Allen et al., 2022).

Chapter 2: Despite recent advances on the inclusion of patient voices in clinical and public health research, there is still an evident paucity of patient input in early stages of medical research (Hollis et al., 2018; Kelly et al., 2015; Knight et al., 2016; Madden & Morley, 2016). To address this, the first focus of this thesis was the creation of guidelines which would allow for better preparation of researchers and patient partners to engage in laboratory-based research. Initial recruitment should focus on local outreach groups and areas where people with lived experience have equal footing with researchers. Following this, building the partnership should take place in a neutral setting in order to avoid unequal dynamics between patient partner and researcher. Inclusion of family members or caregivers can help make the situation comfortable and provide valuable input themselves. We found that prioritizing comfort of the patient partners in the beginning led to greater involvement in the research later. Researchers need to prioritize analyzing their own potential biases and stigma, particularly when studying neuropsychiatric disorders, which diagnoses carry stigma higher than other medical diagnoses. Specialized language should also be avoided when possible and described in depth if necessary to use. In addition, unique issues such as discussions on animal research need to be handled in a sensitive manner, including a focus on harm reduction. Discussions such as these with our patient partners informed later decisions in this dissertation, such as the focus on iPSC-derived models which can be tested without harm to humans or animals.

As a few years of this dissertation and the formation of the Patient-Oriented Research Advisory Committee (POR-AC) have taken place during the COVID-19 pandemic, a significant amount of discussion went into the unique impact of COVID-19 on people with lived experience in mental health disorders. From these discussions, four priority areas emerged:

1. How has COVID-19 impacted the stigma surrounding mental health?
2. How important was support (familial, financial, etc.) for mental health during COVID-19?
3. Did COVID-19 increase burnout in people with lived experience, caregivers, and mental health care workers?
4. How did changes to the system impact the mental health of people with lived experience (harder to access, easier to access, more free services etc.) How could we improve this and what should we keep after COVID-19?

Addressing these through multifaceted viewpoints from patient partners, researchers, administrators, and clinicians, recommendations related to each priority themes were developed for policy makers and future research. Main themes in these recommendations include increasing public outreach to combat mental health stigma, involvement of people with lived experience in policy decision-making, increases in funding for support and care, continuing online access to mental health services, and prioritizing in-person care where necessary.

Future research is necessary to translate patient priorities into direct research questions that can be addressed in a laboratory-based setting. However, it is evident that patient engagement provides innumerable benefits to both researchers and patients and should be prioritized throughout the next decade of mental health research.

Chapter 3: As previously described, traditional antidepressants have low remission rates and delays in therapeutic efficacy that do not mirror their biological changes. Ketamine has demonstrated clinical fast-acting antidepressant effects, that are hypothesized to mediated by synaptic mTORC1 activation (Li et al., 2010; Zanos et al., 2016; Zanos & Gould, 2018). To assess the molecular changes induced by reelin, a putative rapid-acting antidepressant, synaptic-specific changes were measured in parallel to ketamine in synaptoneurosomes after chronic-CORT administration. In the hippocampus, an area greatly impacted in depression, CORT administration decreased levels of PSD-95, p-mTORC1, and mTORC1 expression. Reelin induced concentration-

specific increases in all measured proteins, which are associated with upregulations in synaptic strength and therapeutic efficacy. Ketamine also increased these synaptic-specific protein levels in a parallel manner.

In the cerebellum, an area with high endogenous reelin expression, CORT decreased levels of p-mTORC1, which was rescued by all concentrations of reelin and the highest concentrations of ketamine. While not decreased by CORT, mTORC1 expression was also increased in a concentration-dependent manner by reelin. Interestingly, the highest concentration of ketamine decreased mTORC1 expression. These results suggest that reelin and ketamine's ability to rescue mTORC1 signaling is not limited to the hippocampus and may have some therapeutic effects on cerebellar functions impaired in depression such as emotional experience (Minichino et al., 2014)

Changes in peripheral SERT clustering on lymphocytes were also analyzed, as they are known to be impacted both in depression and chronic stress paradigms (Caruncho et al., 2019). Parallel to previous research, CORT-treatment increased average SERT cluster size, which was rescued in a concentration-dependent manner by reelin. Interestingly, ketamine was not able to rescue SERT cluster size, and appeared to increase average cluster size at the highest concentration, perhaps suggesting divergent mechanisms of reelin and ketamine on peripheral immune signaling.

Chapter 4: With the observed parallel effects of reelin and ketamine on synaptic protein expression in Chapter 3, I aimed to determine whether ketamine may be able to mediate endogenous reelin expression in order to exert its rapid-acting antidepressant effects, alongside other markers associated with antidepressant efficacy. Male Long Evans rats underwent a chronic CORT-administration paradigm and were administered an acute dose of ketamine the day after CORT treatment ended (15 mg/kg). After behavioural verification of a depressive-like phenotype using a fear conditioning and extinction model, immunohistochemical analyses ascertained subgranular cell populations expressing reelin, GluA1, and DCX. An acute dose of ketamine was able to rescue reelin expression that was decreased after chronic CORT. In support of this finding, recent research suggested that reelin signaling is necessary for ketamine's antidepressant-like effects (Kim et al., 2021) and an increase in endogenous reelin expression could be a primary mediator of this mechanism.

DCX, a marker used as a proxy for neurogenesis, was decreased after chronic CORT-administration, paralleling previous research (Allen et al., 2022; Brymer et al., 2020; Lebedeva et

al., 2020). However, an acute dose of ketamine was unable to rescue either count or complexity of DCX-IR cells. Previous research has demonstrated mixed results, but most shows that repeated administration of ketamine more reliably increases hippocampal neurogenesis. In addition, DCX is a marker for later neurogenic stages, and the time course of measurement may not have captured an increase after ketamine administration. Future research should look at earlier markers of neurogenesis, such as BrdU and NeuN. No differences were observed in GluA1 expression across all groups, but immunohistochemical measures used here are unable to capture surface insertion of GluA1 subunits which may provide a fuller picture of decreased or increased excitatory transmission (Zhang et al., 2016).

To determine whether fear extinction behaviour was associated with any changes in protein expression in the subgranular zone, I correlated the behavioural tests conducted by Brian Kulyk (2017) with the biological measurements presented in this dissertation. However, no significant changes were found outside of correlations of the vehicle/ketamine subgroup between fear extinction, GluA1, and reelin expression.

Chapter 5: Following the observation that ketamine administration can increase endogenous reelin, the next aim was to ascertain the parallels between ketamine and exogenous reelin administration in a chronic stress model for the study of depression. 3 µg of exogenous reelin was administered intravenously, as determined by previous research demonstrating the greatest efficacy in antidepressant-like effects (Allen et al., 2022). Ketamine (10 mg/kg, intraperitoneally) was administered in parallel to provide a comparison to a well-validated rapid-acting antidepressant and to determine whether reelin could more efficiently induce rapid-acting antidepressant-like effects. To provide a broad picture of the impact of reelin and ketamine, behavioural, electrophysiological, and molecular measurements were taken within 24 hours after administration. Both reelin and ketamine rescued the depressive-like effects of CORT on swimming and immobility on the FST, a screening tool for antidepressant efficacy, despair-like behaviour and stress coping mechanisms (Commons et al., 2017; Fenton et al., 2015; Hibicke et al., 2020). Interestingly, only reelin was able to rescue the decreased latency to immobility caused by CORT administration. On the sucrose preference test, an indicator of anhedonia, neither reelin nor ketamine were able to rescue the anhedonic-like effects of CORT.

In the dentate gyrus, both reelin and ketamine significantly increased long-term potentiation, an essential process for learning and memory which is heavily dysregulated in depression (Gilbert & Zarate, 2020). In parallel to Chapter 4, hippocampal reelin expression was increased after an acute dose of both reelin and ketamine. Given its large molecular weight, it is unlikely that exogenous reelin administered peripherally would be able to cross the blood-brain barrier, suggesting that a peripheral increase in reelin is able to rescue expression of reelin in the brain through a different mechanism. In contrast to the findings of Chapter 3, no major changes in protein expression were found in hippocampal synaptoneurosomes or peripheral SERT clustering, however high variability between animals and low sample sizes may account for the lack of significance.

Chapter 6: Following the first major aim of this thesis and guidance from our patient partners, the final experiment of this dissertation attempted to increase the translatability of previous findings by assessing the impact of reelin and (2R,6R)-HNK, a ketamine metabolite, in iPSC-derived neurons from participants diagnosed with treatment-resistant depression and healthy controls. Treatment-resistant depression (TRD), the failure to respond to first-line antidepressant treatment, is associated with worse long-term outcomes, more concomitant disorders, and a longer course of illness (Fava, 2003; Papakostas et al., 2003). In addition, animal models often fail to replicate this specific phenotype (Willner & Belzung, 2015), further complicating the development of novel therapeutics. To help address some of these challenges, we reprogrammed peripheral blood mononuclear cells to iPSCs from participants diagnosed with TRD and healthy controls. iPSCs were then differentiated to cortical neurons to assess the reactions of mature neurons to the application of reelin and (2R,6R)-HNK.

Multiple baseline differences were observed between TRD-derived cell lines and healthy controls, with an increased expression of total mTORC1, GluA1, TrkB, p-ERK, and ERK in TRD cell lines. This contrasts with previous research in post-mortem tissue of depression patients (Duric et al., 2013) but warrants further investigation to determine why these baseline differences occur given the preliminary nature of this research. At 1 hour, reelin induced concentration-dependent changes in multiple proteins associated with fast-acting antidepressant response and in parallel to previous chapters of this dissertation such as PSD-95, Synapsin I, Dab1, and p-ERK. At 24 hours however, these same proteins decreased in a concentration-dependent manner. Both the increases at 1 hour and decreases at 24 hours were paralleled by (2R,6R)-HNK, further supporting reelin's putative

actions as a rapid-acting antidepressant. The stark differences in protein expression after treatment suggest that TRD cell lines may have difficulties mediating homeostatic balance, or that negative feedback loop mechanisms may be activated by prolonged exposure to different therapeutics. Interestingly, no significant differences were found across treatment groups or timepoints in healthy control cells. The differences in response to reelin and (2R,6R)-HNK between participants with TRD and healthy controls parallel previous research, which found that only iPSCs from patients diagnosed with bipolar disorder responded to application of lithium (Mertens et al., 2015).

While still extremely preliminary, iPSC-derived neurons from participants with TRD have the potential to be a high-throughput method to study individual response to traditional and novel antidepressants, which could prove invaluable to the future of medicine. Challenges still include the proportion of depression and erasure of epigenetic changes, which will be addressed in further detail later in this discussion.

7.2 Underlying mechanisms of fast-acting antidepressants

Results from this dissertation suggest that reelin may be a promising putative fast-acting antidepressant. A multitude of previous research has demonstrated that exogenous reelin application is able to rescue deficits associated with depression including dendritic spine outgrowth and density, long-term potentiation, and deficits in learning and memory (Hethorn et al., 2015; Qiu,et al., 2006; Rogers et al., 2011, 2013; Weeber et al., 2002). Research from our laboratory has expanded this work in a chronic stress model, demonstrating that peripheral and acute intrahippocampal infusions of reelin are able to rescue behavioural and biological deficits (Brymer et al., 2020). Following this research, the effects of repeated and acute doses of peripheral reelin were confirmed, demonstrating short-term behavioural and biological effects (Allen et al., 2022).

However, this dissertation is the first to my knowledge that demonstrates that an acute dose of exogenous reelin has fast-acting behavioural, electrophysiological, and molecular antidepressant-like effects in a variety of models for the study of depression. Many of these changes appear to be mediated by synaptic changes in protein expression that cause an upregulation of excitatory signaling between neurons. In each study presented, reelin was compared against ketamine – the gold standard for fast-acting antidepressants – to determine any potential parallels. Despite having a variety of approaches (Western blotting, immunostaining, behavioural measures, *in vivo*

electrophysiology) and models (synaptoneurosomes, lymphocytes, chronic corticosterone treatment, iPSCs from participants with TRD), reelin and ketamine often had parallel effects.

This is particularly important as reelin appears to activate parallel signaling pathways to ketamine without antagonizing NMDAR, which suggests it may not have similar psychotomimetic effects that limit the use of ketamine as an antidepressant (Bonaventura et al., 2021; Morgan & Curran, 2012; Sassano-Higgins et al., 2016). However, there are evidently downstream signaling mechanisms which contribute to rescuing depressive behaviours and depressive-like phenotypes. The focus of most fast-acting antidepressant research has been the importance of excitatory transmission and balance between excitation and inhibition (Duman et al., 2019). Across experiments, I found that reelin was able to increase synaptic indicators of increased excitatory signaling in both *in vitro* and *in vivo*, as well as hippocampal LTP. Increases in AMPAR transmission have been repeatedly associated with the upregulation of TrkB and mTORC1 signaling, leading to downstream events such as surface insertion of GluA1 subunits and increases in PSD-95 expression which anchor glutamatergic receptors to the synapse (Cavalleri et al., 2018; Li et al., 2010; Ly et al., 2018; Zanos & Gould, 2018; Zhou et al., 2014). In addition, previous research from our laboratory has found that the rapid-acting effects of intrahippocampal reelin administration is blocked by CNQX, a competitive AMPAR antagonist (Brymer et al., 2020). Normalization of GluA1 expression after CORT exposure has also been observed after intrahippocampal and peripheral administration of reelin (Allen et al., 2022; Brymer et al., 2020).

Other antidepressants, or potential antidepressants, also appear to mediate AMPAR transmission. The behavioural changes induced by fluoxetine and imipramine also rely on AMPAR-mediated transmission (Aleksandrova et al., 2017; Koike & Chaki, 2014). mGluR antagonists, which enhance excitatory transmission through blocking G_i signaling, demonstrate fast-acting antidepressant-like effects without the associated side effects of ketamine (Aleksandrova et al., 2017; Dogra & Conn, 2021; Koike et al., 2013). The antidepressant effects of ketamine appear to be primarily mediated through increased AMPAR transmission (Duman et al., 2019b) or preferential antagonism of NMDARs on GABAergic interneurons to disinhibit excitatory neurons (Miller et al., 2016). The parallels observed between reelin and ketamine (or (2R,6R)-HNK) throughout this dissertation further support the role that reelin may have in strengthening excitatory transmission.

However, there is still a significant amount of debate on the role that increasing excitatory neurotransmission plays in eliminating depressive symptomatology. Not all evidence supports increases in AMPAR transmission as the mechanism underlying fast-acting antidepressants. Ketamine has a multitude of other targets, such as the opioid system and anti-inflammatory effects which could contribute to its antidepressant effects (Johnston et al., 2023, *in press*). In addition, AMPAR modulators such as AMPAkines (otherwise known as "CX compounds") have repeatedly failed in later-phase clinical trials, despite early pre-clinical promise (Kadriu et al., 2021). Excitotoxicity has also been implicated in depression (Olloquequi et al., 2018), as excessive glutamate release can lead to a dysregulation of Ca^{2+} homeostasis. This contradiction may explain why ketamine has been uniquely effective in clinical trials, as NMDAR overactivity is most often associated with excitotoxicity in depression (Liu et al., 2007; Olloquequi et al., 2018). The changes observed in transmission also appear to be highly region-specific, with increases in amygdalar excitatory neurotransmission often associated with depressive-like behaviour (Mitra & Sapolsky, 2008; Myers & Greenwood-Van Meerveld, 2007).

Given all of this information, it is likely that at least a portion of reelin's observed rapid-acting antidepressant-like effects are mediated through an increase in AMPAR transmission, leading to an increase in mTORC1 signaling which causes downstream increases in synaptic proteins in the hippocampus and cortex. However, future research should determine the effects that increasing reelin expression has on other downstream signaling pathways and the necessity of AMPAR transmission for reelin's therapeutic actions.

7.3 A note on the importance of increasing the translation of basic research

As discussed throughout this thesis, one of the major challenges in basic, or foundational, research is the translation of findings from bench to bedside. Interpreting and extrapolating findings from animals or cell cultures to the complexity of human beings is extremely difficult and often fails, leading to the widely known "valleys of death" associated with research translation. Despite early antidepressants being some of the first target-informed drugs developed, a lack of understanding of the mechanisms which bring about an anti-depressant effect has limited the discovery of effective novel therapeutics and the ability for many people to receive sufficient treatment. I hope to have convinced you throughout this dissertation of the need to consider how best to translate

findings from pre-clinical research to those that are useful for patients who have been diagnosed with any neuropsychiatric disorder, not just depression.

The pathophysiology of depression is still poorly understood, and a significant amount of research is still needed to determine the underlying mechanisms and changes that occur in states such as chronic stress. This is further complicated by the disparities between animal models and the human experience of depression, which are often not directly related. For example, suicidal ideation, a hallmark of TRD, is extremely difficult to model in a species which does not have naturally occurring death by suicide. Combinations of different animal models which demonstrate different facets of each disorder are still necessary to translate pre-clinical findings into clinical therapeutics.

It is also essential to begin taking patient priorities into account, such as limiting side effects and length of time on the medication. For example, extreme weight gain is a side effect of many antipsychotics and often leads to low treatment adherence rates, even if it effectively targets symptoms associated with psychosis or schizophrenia. In addition, individuals diagnosed with neuropsychiatric disorders will often have different symptom domains they wish to prioritize treatment for, depending on their lifestyles and personal goals.

Lastly, shifting to models which consider the heterogeneity of neuropsychiatric disorders and treatment responses are essential for the development of personalized medicine, hopefully the next big step in psychiatry. Models such as, but not limited to, iPSC-derived cell cultures and organoids can provide insight into neuronal mechanisms that we are not yet able to observe *in vivo.* Improvements in technology such as neuroimaging will also play a large role in the personalization of medicine over the following decades.

7.4 Limitations

7.4.1 Sex differences

One of the major limitations of this thesis was the exclusion of females from the *in vitro* and *in vivo* work. Women are significantly more likely to experience depression, with lifetime prevalence rates that are double what are reported for men (Noble, 2005). This sexual dimorphism could arise from a multitude of causes, including symptom reporting, stress reactivity, and treatment responsiveness (Altemus et al., 2014; Eid et al., 2019). Various chronic stress models also demonstrate sex differences, which is not surprising given the ease with which estrous hormones

are able to cross the blood-brain barrier and alter glucocorticoid receptor activity (Bourke et al., 2012; Guo et al., 2018). However, these estrous hormones have been found to have neuroprotective effects, which can result in females having less sensitivity to the depressogenic effects of chronic unpredictable mild stress (Dalla et al., 2005). The disparities between human observations and the pre-clinical literature emphasize the need for more translational models that can address both the potential neuroprotective effects of estrous hormones with the increased prevalence rates of depression in women.

Interestingly, most clinical work regarding the antidepressant effects of ketamine have found no sex differences, however much of this research fails to disaggregate findings between males and females (Ponton et al., 2022). Pre-clinical models have found that females are more sensitive to dose and initial magnitude of response when administered ketamine, but males have prolonged responses (Franceschelli et al., 2015; Okine et al., 2020; Saland et al., 2017; Sarkar & Kabbaj, 2016). Ovarectomized females do not respond to low-dose ketamine, but this can be rectified with hormone replacement therapy (Carrier & Kabbaj, 2013; Saland et al., 2017). mTORC1 activation after ketamine administration is also varied amongst males, diestrus and proestrus females (Dossat et al., 2018). While most clinical studies find no sex differences in treatment response to ketamine, the psychotomimetic side effects are reported to be stronger in men, including stronger dissociation and memory loss (Derntl et al., 2019; Morgan et al., 2006; Zhang et al., 2013). Women report more physiological symptoms of ketamine administration such as headaches and nausea (Freeman et al., 2019; Zhang et al., 2013).

Sex hormones are known to affect reelin expression, with administration of high levels of testosterone decreasing expression of reelin in male starlings (Absil et al., 2003). In addition, data from our lab has shown that females have lower baseline levels of reelin in the paraventricular nucleus of the hypothalamus which are unaffected by corticosterone administration. In addition, the colocalization of reelin and oxytocin in the paraventricular nucleus was also greater in males (Sánchez-Lafuente et al., 2022). Despite these changes in the hypothalamus, research from our lab has revealed minimal to no sex differences in response to peripheral reelin administration. No differences in hippocampal reelin, GluA1, or $GABA_AR$ expression were observed at baseline, after chronic CORT administration, or with peripheral reelin administration. Peripherally, small differences were observed in changes of SERT cluster size, where females had a full recovery and

males only a partial rescue. The lack of differences observed was paralleled by behavioural tests, which found no sex differences after peripheral reelin administration aside from small changes in movement that could be attributed to bodyweight differences (Allen et al., 2022). In my own research, while both males and females were included for the iPSC-derived neurons, low sample size precluded any conclusions that could have been made based on sex. This does not mean however that different models for the study of depression would not reveal more striking sex differences, or that other behavioural and biological analyses could reveal strong sex differences.

7.4.2 Mechanistic-based studies

While this thesis work revealed molecular, electrophysiological, and behavioural changes in response to reelin and ketamine administration, no inhibitors, or other methods to manipulate activity, were used to probe for necessary proteins and pathways involved in reelin's or ketamine's antidepressant-like effects. As reelin was able to upregulate proteins such as GluA1, PSD-95, TrkB, and mTORC1 activity, we hypothesize that reelin's antidepressant-like actions are primarily mediated through mTORC1 signaling. However, other pathways that reelin is known to act on such as Dab1-mediated NMDAR regulation and Rap1 signaling could contribute to these effects, and the use of inhibitors such as rapamycin (an mTORC1 inhibitor) and GGTI (a Rap1 inhibitor) could provide extremely valuable insight into reelin's actions (Bock & May, 2016). Our lab has shown that inhibition of AMPAR signaling was able to abolish reelin's antidepressant-like effects after direct infusion into the hippocampus (Brymer et al., 2020), however there are a multitude of downstream signalers from AMPARs that could mediate reelin's antidepressant effects.

Translation from pre-clinical mechanistic studies do not always directly apply to clinical research. For instance, repeated pre-clinical studies found that inhibition of mTORC1 signaling through rapamycin administration blocked ketamine's antidepressant-like effects (Cavalleri et al., 2018; Li et al., 2010; Zanos et al., 2016). In contrast, the administration of rapamycin in participants with TRD actually prolonged the antidepressant effects of ketamine (Abdallah et al., 2020; Averill et al., 2022), contradicting the pre-clinical research. This may be due to difficulties of rapamycin entering the brain, leaving ketamine to upregulate neuronal mTOR signaling while rapamycin had anti-inflammatory effects in the periphery that could complement ketamine's therapeutic effects (Attur et al., 2000; Chen et al., 2013). Interestingly, recent research found that ketamine's

antidepressant-like actions could be blocked by inhibition of ApoER2, SRK, or PI3k signaling (Kim et al., 2021).

7.4.3 Methodological variety

Lastly, a limitation of this thesis work was the variety of methodologies used to examine the effects of both reelin and ketamine. While there were many parallel findings (increases in PSD-95, Synapsin I, etc.), certain disparities between experiments were also observed (increases in mTOR and p-mTOR expression) which could be attributed to differences in methodology. For instance, synaptoneurosomes were treated for 30 minutes in contrast to one hour used for the iPSC-derived neurons. In addition, the use of (2R,6R)-HNK, a ketamine metabolite that does not appear to bind to NMDARs with much efficacy, could produce varied results to the use of racemic ketamine.

However, the variety of methodologies used could also be considered a strength of this thesis. Finding consistencies amongst such varied techniques helps confirm that even between different models, time courses, and procedures, similar proteins were still upregulated. Evidently, mechanistic studies in each of these models would provide even greater insight into the underlying mechanisms of reelin and ketamine as rapid-acting antidepressants.

7.5 Future directions

7.5.1 Translation of patient priorities into research questions

An important next step in the incorporation of patient-oriented research is translating patient priorities into research questions that can be tackled by pre-clinical research. In the patient-oriented research advisory committee, we have begun these first steps. After preliminary discussions between patient partners, researchers, administrators, and clinicians, two major topic areas were prioritized: recovery and resilience. Both topics are difficult to define, particularly when considering subjective vs. objective opinions on what “recovery” and “resilience” mean to each individual. Objective viewpoints may define recovery as decreases on many of the scales I have mentioned earlier in this dissertation (MADRS, HAMD, BDI), ability to hold a job, or a variety of clinical factors considered in diagnosis. However, subjective definitions can vary greatly between individuals and often are not aligned with objective scales or measures. An example of the translation of this disparity in definitions is the Canadian-Personal Recovery Outcome Measure (C-PROM). In Canada, the C-PROM has been developed in conjunction with patient partners in

order to promote new ways of looking at recovery. The C-PROM is based around six dimensions of recovery: *(1)* creating a culture and language of hope, *(2)* recovery is individual, *(3)* recovery is contextual, *(4)* each person involved has diverse needs that should be addressed, *(5)* working with First Nations, Inuit, and Métis cultures is essential, and *(6)* the transformation of services and systems is important for recovery.

While this is a wonderful example of translation from patient priorities to clinical and public health practice, significantly more work is needed in the scope of pre-clinical, or foundational, research. For this, we have first focused on the topic of resilience. A major question arose from this discussion: Are there biomarkers which can predict resilience to stress exposure? To address this in future research, our laboratory aims to begin using a chronic unpredictable mild stress model, which results in "resilient" vs. "non-resilient" groups (Hu et al., 2017; Zhao et al., 2019). From consecutive blood draws, we could analyze various proteins in the periphery, such as pro-inflammatory cytokines that have been previously linked to resilience and later treatment-response (Majd et al., 2020; Strawbridge et al., 2015; Su et al., 2020).

7.5.2 Importance of reelin signaling for ketamine's antidepressant effects

Research presented in this thesis demonstrates that an acute dose of ketamine is able to rescue reelin expression in the hippocampus. Interestingly, recent research found that inhibition of reelin signaling through genetic deletion of ApoER2 or inhibition of SFKs or PI3k was able to abolish the antidepressant-like effects of ketamine. Specifically, blockade of these key mediators of reelin signaling was sufficient to disrupt ketamine's behavioural effects as well as hippocampal NMDAR-mediated synaptic plasticity (Kim et al., 2021). Cytoplasmic Dab1 signaling, which was increased by (2R,6R)-HNK in the iPSC-derived neurons from participants with TRD, would also be an area of great interest given that it is hypothesized to be necessary for reelin's effects on dendritic outgrowth and synaptic signaling (Bosch et al., 2016; Niu et al., 2008). Interestingly, *in vivo* administration of ketamine did not affect tyrosine phosphorylation of Dab1 (Kim et al., 2021), but future research should still determine the necessity of intracellular Dab1 signaling for the antidepressant-like effects of both ketamine and reelin.

The method by which ketamine increases reelin expression should also be extensively examined. Reelin expression is decreased through hypermethylation of the *RELN* promoter (Chen et al.,

2002), which can be reversed through histone deacetylase inhibitors such as trichostatin A and valproic acid (Mitchell et al., 2005). Histone deacetylation is able to promote the formation of heterochromatin, which blocks access to transcription factor binding sites in promoter regions, therefore repressing transcription of protein. Multiple lines of evidence have now suggested that ketamine may able to decrease histone deacetylase 5 (HDAC5) activity via phosphorylation (Choi et al., 2015, 2017). This HDAC5 phosphorylation has also been shown to increase BDNF levels in rats, further contributing to ketamine's antidepressant-like effects (Choi et al., 2017). Evidently, there are still many known steps missing between administration of ketamine and upregulation of reelin expression that deserve to be explored further.

7.5.3 Time-course of reelin's antidepressant-like effects

The time-course of reelin's rapid-acting antidepressant-like effects has not yet been fully realized. In this dissertation, I describe the biological changes after reelin administration that can take place from 30 min – 1 hr *in vitro* and the behavioural and biological changes observed after 24 hours in a chronic stress model. However, it is known that both clinically and pre-clinically ketamine is able to exert antidepressant actions within hours (Krystal et al., 2019), an effect that may be parallel with reelin administration. Assessment of behaviour, such as the FST, and biological changes, such as increases in p-GluA1 and p-mTORC1 should be evaluated at varying early timepoints such as 1 – 12 hrs after administration. Preliminary work from our lab has demonstrated that both reelin and ketamine were able to rescue immobility in the FST 1 hr, 6 hrs, and 12 hrs after peripheral administration demonstrating strong rapid-acting antidepressant-like effects (unpublished data).

Other potential fast-acting depressants, such as serotonergic psychedelics, have been shown to exert long-term effects months to years after an acute administration (Agin-Liebes et al., 2020; Carhart-Harris & Goodwin, 2017; Hibicke et al., 2020), demonstrating that repeated administration may not be necessary for certain therapeutics. Even if multiple administrations are necessary, long time periods between doses would benefit many patients. Decreases in amount of time spent with therapeutics, money spent, and increasing accessibility would open up fast-acting therapeutics to more diverse populations.

In contrast, an acute dose of ketamine is only able to lower depression scores for an average of two weeks, necessitating repeated administration for more long-term effects (Murrough et al., 2013) which could present challenges with substance use and issues like ulcerative cystitis (Short

et al., 2018). It is unknown how long reelin can exert its antidepressant-like effects, but preliminary data from our lab has shown a decrease in immobility one week after ketamine and/or reelin administration, with the strongest decreases appearing in those administered both reelin and ketamine together (unpublished data), which opens discussion on the efficacy of reelin as a potential adjunctive treatment.

7.5.4 Reelin-altering compounds as adjunctive therapeutics

Novel drug development and approval takes years of research and billions of dollars in costs, which limits the production of novel therapeutics. In addition, due to previous high rates of failure, therapeutics for neuropsychiatric disorders are often prioritized to a lesser extent than for other indications. However, knowledge on the effects of reelin and its antidepressant-like actions can inform research on already approved compounds which mediate reelin signaling and expression. For example, research has demonstrated that Furin Inhibitor I is able to strongly inhibit reelin cleavage, increasing efficacy of reelin signaling (Kohno et al., 2009). Multiple medications may have furin-inhibition capabilities, such as Cepharantine, used in Japan for alopecia but which may hold antiviral properties (Ginex et al., 2021). Sulconazole, used primarily to treat skin infections, is also able to inhibit furin signaling (Villoutreix et al., 2020).

Proteases which belong to a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family have also been shown to cleave reelin (Lambert De Rouvroit et al., 1999). ADAMTS-4/5 inhibitors have already been FDA approved for osteoarthritis treatments and could a useful adjunct to depression therapeutics. Further pre-clinical and clinical research should determine the impact that these medications have in conjunction with therapeutics such as SSRIs and ketamine, and whether the improvement of reelin signaling could better therapeutic efficacy.

7.5.5 Reelin in other neuropsychiatric disorders

The focus of this thesis was the role that reelin may have in ameliorating depressive-like symptoms and pathology, but reelin has been implicated in a multitude of neuropsychiatric disorders including schizophrenia, bipolar disorder, autism spectrum disorders, temporal lobe epilepsy, and Alzheimer's disease (Fatemi et al., 2000, 2001; Guidotti et al., 2000, 2016; Impagnatiello et al., 1998; Knable et al., 2004). Some research suggests that decreases in reelin may confer a vulnerability to certain neuropsychiatric disorders (Fatemi, 2011), as observed in heterozygous

reeler mice who are more sensitive to stress-induced impairments (Lussier et al., 2011; Notaras et al., 2020; Schroeder et al., 2015). In fact, mice which overexpress reelin are protected from behavioural phenotypes related to bipolar disorder and schizophrenia (Teixeira et al., 2011). Impairments in social behaviours after chronic exposure to tetrahydrocannabinol are also more severe in reelin-deficient mice (Iemolo et al., 2021).

In a social isolation animal model for the study of post-traumatic stress disorder (PTSD), mRNA levels of reelin were decreased significantly in the hippocampus, frontal cortex, and basolateral amygdala (Nin et al., 2011). These decreases and associated anxiety-like and aggressive behaviours were able to be rescued from a single bilateral infusion of reelin into the amygdala. Our lab has found no changes in anxiety-like behaviour from chronic CORT or reelin treatment (Allen et al., 2022), however others have found that reelin supplementation was able to reduce anxiety-like behaviour after exposure to prenatal inflammation (Ibi et al., 2020). Deficiencies in reelin expression also exacerbates hyperlocomotion after cocaine exposure, an effect which may be mediated by the co-expression of *RELN* in the dorsomedial striatum to *DRD1*, which encodes for the D1 dopamine receptor (Guglielmo et al., 2022). This finding implicates the role which reelin may play in certain substance use disorders, and once again how low levels of reelin can confer vulnerability to a variety of psychiatric disorders.

Reelin has also been implicated in neurological disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Botella-López et al., 2006; Cho et al., 2022; Jesse et al., 2009; Sáez-Valero et al., 2003). In a model of PD, environmental enrichment increased reelin levels in the striatum, which had a protective effect on the local dopaminergic neurons. In addition, striatal reelin levels were varied dependent on disease stage. An *in vitro* model of PD also found that blocking reelin with CR-50 increased α-Synuclein accumulation and neuronal dopamine degeneration (Cho et al., 2022). In an animal model for AD, reelin was able to attenuate hyperphosphorylation of Tau protein and amyloid β-protein aggregation, leading to improvements in cognition (Cuchillo-Ibañez et al., 2016; Pujadas et al., 2014). The pathology of AD has been consistently linked with an early down-regulation in reelin expression, before any aggregation of amyloid-β protein aggregation (Herring et al., 2012). In addition, reelin appears to slow plaque accumulation through activation of its canonical receptors (VLDLR and ApoER2) which interact

with soluble amyloid-β species and decrease phosphorylated Tau through GSK-3β inhibition (Beffert et al., 2002; Jossin & Goffinet, 2007; Pujadas et al., 2014; Toral-Rios et al., 2020).

Evidently, reelin expression changes in a multitude of psychiatric and neurological disorders, and may be an avenue to rescue behaviours and cognitive deficits in these disorders. Certain commonalities of these disorders include deficits in synaptic plasticity, which reelin has been demonstrated to rescue in this thesis and other research (Hethorn et al., 2015; Rogers et al., 2011, 2013), as well as cognitive deficits in various models rescued by reelin supplementation (Allen et al., 2022; Brymer et al., 2020; Hethorn et al., 2015; Ibi et al., 2020; Rogers et al., 2011, 2013).

7.5.6 Characterization and development of iPSCs from participants with TRD

Lastly, an area of great interest would be the further characterization of iPSCs from participants with MDD and TRD. iPSC models provide great promise to the future of individualized medicine, allowing for assessment of each participant's unique genetic background (McNeill et al., 2020). In this thesis, I demonstrated that iPSC-derived neurons from TRD participants and healthy controls have significant baseline differences and disparate responses to treatment with reelin or (2R,6R)-HNK. This finding prompts further description of these cell lines, as well as an attempt to increase accuracy of this *in vitro* model.

As mentioned previously, there have been many challenges in properly translating pre-clinical research to clinical results, particularly for neuropsychiatric disorders such as depression. Due to a lack of suitable pre-clinical models, failure rates for novel psychiatric therapeutics are extremely high (Arnerić et al., 2018; Kinch, 2015). While various *in vitro* and *in vivo* models are still extremely useful for uncovering mechanistic data and assessing specific symptom pathologies, neuropsychiatric disorders are extremely heterogenous and difficult to model within a single construct (Goldberg, 2011; Nestler et al., 2002). The development of pluripotent stem cells by Takahashi and Yamanaka (Takahashi et al., 2007) has paved the way for the future of *in vitro* modeling and individualized medicine. Not only are iPSCs expandable and self-renewable cultures (Kammers et al., 2017; Koch et al., 2009; Papapetrou et al., 2009), they are also able to be differentiated into a variety of cell types that can be used to model specific CNS regions (Muratore et al., 2014; Paes et al., 2017; Silva et al., 2021).

Initial studies have focused more on diseases that have strong underlying genetic factors or more easily defined biological changes, such as Parkinson's Disease (PD) (Parmar & Björklund, 2020), AD (Essayan-Perez et al., 2019), Huntington's disease (Smith-Geater et al., 2020), or autism spectrum disorders such as Fragile X Syndrome (Lee et al., 2022; Liu et al., 2012). Schizophrenia and bipolar disorder, which have stronger genetic components than depression, have also been investigated (O'Shea & McInnis, 2015; Räsänen et al., 2022). Similar to the reported results in this thesis, iPSC-derived neurons from those diagnosed with bipolar disorder respond to therapeutics differently than controls. In this study, lithium treatment *in vitro* significantly changed calcium signaling and electrophysiological signals from bipolar disorder participants but not healthy controls (Chen et al., 2014). Differences in *in vitro* lithium response have also been observed between iPSC-derived neurons from clinical lithium-responders and non-responders (Mertens et al., 2015), which suggests that iPSC models could provide a valuable model to determine therapeutic response. The differences in therapeutic response between iPSC-dervied neurons has also been observed in schizophrenia, where multiple studies have been able to differentiate between responders and non-responders (Grunwald, 2018; Nakazawa et al., 2017; Paulsen et al., 2014). Evidently, the translational applicability of iPSC models could be invaluable to the treatment of various neuropsychiatric disorders, including depression.

However, a recent meta-analysis on iPSC-models for neuropsychiatric disorders was able to include only 2 studies related to major depressive disorder, in comparison to those for autism spectrum disorder (n = 6), bipolar disorder (n = 11), and schizophrenia (n = 23) (McNeill et al., 2020). In these two studies, forebrain neurons were differentiated from iPSCs collected from SSRI-responders, non-responders, and healthy controls. *In vitro* application of 5-HT to these neurons resulted in different responses, where non-responders had a significantly higher calcium response to responders and healthy controls, as well as dysregulated protocadherin alpha gene expression (Vadodaria et al., 2019a,b). While demonstrating initial promise in differentiation between therapeutic response, further characterization and use of iPSC-derived neurons from treatment-resistant depression patients is desperately needed.

However, there are still challenges related to the use of iPSC models for depression. Early research has shown that the reprogramming of cells erases epigenetic markers (Hafner et al., 1994). This erasure presents barriers to fully capturing the complement of markers involved in each

individual's depression, which are known to be affected by environmental factors such as stress, heavily discussed earlier in this thesis. Transdifferentiation, the method that eliminates intermediary iPSCs to generate neurons directly from fibroblasts, may be a solution to keep epigenetic markers that are playing a role in disease experience and progression (Mertens et al., 2018; Pfisterer et al., 2011). Another challenge is the lack of region-specific representation and representative connectivity in 2D cultures (Duval et al., 2017; Nakazawa et al., 2019). While 2D cultures are more easily reproduced and allow for higher throughput, these disadvantages cannot be ignored.

Due to these challenges, the next step in iPSC-derived cell culture research is the use of 3D brain organoids or spheroids and the development of region-specific cell expression and organization, which could allow for observations on regional connectivity differences and larger-scale interactions that are ignored in 2D neuronal cells (Ho et al., 2018). Preliminary work has begun in both region-specificity and 3D-modeling for various disorders, such as schizophrenia and substance use disorder (Ho et al., 2022). In fact, protocols now exist for generating nearly 50 different nervous system cell fates, which can be co-cultured in a manner that best represents different brain regions. Recent research demonstrated disparate single-cell transcriptional responses to buprenorphine and oxycodone in iPSC-derived brain organoids from participants with opioid-use disorder (Ho et al., 2022).

3D neural aggregates, made up of neurons, astrocytes, and oligodendroglial cells from human tissue are also able to create synchronous neuronal activity within 3 weeks, a hallmark of functional assembly (Izsak et al., 2020). Evidently, the ability to mimic complex circuitry needs to be further developed, such as the Default Mode Network, which has been shown to play a role in depression and therapeutic response (Marchetti et al., 2012). In addition, CRISPR/cas9 technology is being considered to genetically edit various iPSC-derived models (Tian et al., 2020) to provide stronger models for disorders that are more polygenic as well as the ability to reinstate epigenetic markers which may have been erased in the reprogramming process (Xie et al., 2018). These exciting new avenues should be applied to the future of iPSC technology and provide better insight and further development of *in vitro* modeling for depression research.

7.6 Importance of this work and closing remarks

The prevalence rates of depression have been steadily increasing, but current therapeutics have been unable to keep up. It is estimated that only 2/3 of those with depression will respond to first-line treatments (Cipriani et al., 2018; Rush et al., 2006; Shinohara et al., 2019), a response rate which is compounded mainly by placebo effects (Kirsch, 2019). In addition, treatment adherence to typical antidepressants is fairly low (Rush et al., 2006; Warden et al., 2007), affected by side effects, patient priorities, and various social and biological factors for patients that are often not considered in biomedical sciences.

Ketamine has been the first breakthrough in decades, demonstrating fast-acting antidepressant effects that can treat typically stubborn symptoms in treatment-resistant populations within hours. However, ketamine can induce psychotomimetic side effects which make it undesirable for certain patient populations. In addition, its potential for abuse necessitates supervised administration, which can limit the generalizability of this therapeutic to those who do not have ease of access to transport, limited time, or other difficulties that can present barriers to effective treatment.

A better understanding of the underlying mechanisms of ketamine has demonstrated the importance of AMPAR transmission in its antidepressant, but not psychotomimetic effects, has spurred research to determine novel compounds which can work effectively. While direct modulation of AMPAR transmission has proven somewhat unsuccessful in clinical trials (Kadriu et al., 2021), indirect actions to increase this transmission and activate certain downstream signalers of ketamine's actions, such as mTORC1, hold great promise. Reelin, as demonstrated here, may act in a parallel manner to ketamine as demonstrated by both *in vitro* and *in vivo* approaches, including novel approaches such as the development of iPSC-derived neurons from patients with treatment-resistant depression.

Further research on reelin, and its signaling pathway, provides a promising future approach for novel fast-acting antidepressants and the treatment of mood disorders. Increasing the translatability of research, from early stages which include patient partner input to later research which attempts to encompass the individuality and heterogeneity of depression is essential for the future development of depression therapeutic research.

References

Aasen, T., Raya, A., Barrero, M. J., Garreta, E., Consiglio, A., Gonzalez, F., Vassena, R., Bilić, J., Pekarik, V., Tiscornia, G., Edel, M., Boué, S., & Belmonte, J. C. I. (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nature Biotechnology*, *26*(11), 1276–1284. https://doi.org/10.1038/nbt.1503

Abdallah, C. G., Averill, L. A., Collins, K. A., Geha, P., Schwartz, J., Averill, C., Dewilde, K. E., Wong, E., Anticevic, A., Tang, C. Y., Iosifescu, D. v., Charney, D. S., & Murrough, J. W. (2016). Ketamine Treatment and Global Brain Connectivity in Major Depression. *Neuropsychopharmacology 2017 42:6*, *42*(6), 1210–1219. https://doi.org/10.1038/npp.2016.186

Abdallah, C. G., Averill, L. A., Gueorguieva, R., Goktas, S., Purohit, P., Ranganathan, M., Sherif, M., Ahn, K. H., D'Souza, D. C., Formica, R., Southwick, S. M., Duman, R. S., Sanacora, G., & Krystal, J. H. (2020). Modulation of the antidepressant effects of ketamine by the mTORC1 inhibitor rapamycin. *Neuropsychopharmacology*, *45*(6), 990–997. https://doi.org/10.1038/s41386-020-0644-9

Abdallah, C. G., Jackowski, A., Salas, R., Gupta, S., Sato, J. R., Mao, X., Coplan, J. D., Shungu, Di. C., & Mathew, S. J. (2017). The Nucleus Accumbens and Ketamine Treatment in Major Depressive Disorder. *Neuropsychopharmacology 2017 42:8*, *42*(8), 1739–1746. https://doi.org/10.1038/npp.2017.49

Abdallah, C. G., Sanacora, G., Duman, R. S., & Krystal, J. H. (2018). The neurobiology of depression, ketamine and rapid-acting antidepressants: Is it glutamate inhibition or activation? *Pharmacology & Therapeutics*, *190*, 148–158. https://doi.org/10.1016/J.PHARMTHERA.2018.05.010

Abdolmaleky, H. M., Cheng, K. H., Russo, A., Smith, C. L., Faraone, S. v., Wilcox, M., Shafa, R., Glatt, S. J., Nguyen, G., Ponte, J. F., Thiagalingam, S., & Tsuang, M. T. (2005). Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: A preliminary report. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *134B*(1), 60–66. https://doi.org/10.1002/AJMG.B.30140

Abelaira, H. M., Rosa, T., de Moura, A. B., Andrade, N. M., Martinello, N. S., Maciel, L. R., Botelho, M. E. M., Borba, L. A., Chede, B. C., Arent, C. O., Joaquim, L., Bonfante, S., Danielski, L. G., Tuon, T., Petronilho, F., Quevedo, J., & Réus, G. Z. (2022). Combination of electroconvulsive stimulation with ketamine or escitalopram protects the brain against inflammation and oxidative stress induced by maternal deprivation and is critical for associated behaviors in male and female rats. *Molecular Neurobiology*, *59*(3), 1452–1475. https://doi.org/10.1007/S12035-021-02718-X

Absil, P., Pinxten, R., Balthazart, J., & Eens, M. (2003). Effects of testosterone on Reelin expression in the brain of male European starlings. *Cell and Tissue Research*, *312*(1), 81–93. https://doi.org/10.1007/S00441-003-0701-9/FIGURES/8

Acevedo-Diaz, E. E., Cavanaugh, G. W., Greenstein, D., Kraus, C., Kadriu, B., Zarate, C. A., & Park, L. T. (2020). Comprehensive assessment of side effects associated with a single dose of ketamine in treatment-resistant depression. *Journal of Affective Disorders*, *263*, 568–575. https://doi.org/10.1016/J.JAD.2019.11.028

Adam, E. K., Doane, L. D., Zinbarg, R. E., Mineka, S., Craske, M. G., & Griffith, J. W. (2010). Prospective prediction of major depressive disorder from cortisol awakening responses in adolescence. *Psychoneuroendocrinology*, *35*(6), 921–931. https://doi.org/10.1016/J.PSYNEUEN.2009.12.007

Agin-Liebes, G. I., Malone, T., Yalch, M. M., Mennenga, S. E., Ponté, K. L., Guss, J., Bossis, A. P., Grigsby, J., Fischer, S., & Ross, S. (2020). Long-term follow-up of psilocybin-assisted psychotherapy for psychiatric and existential distress in patients with life-threatening cancer. *Journal of Psychopharmacology*, *34*(2), 155–166. https://doi.org/10.1177/0269881119897615

Ago, Y., Tanabe, W., Higuchi, M., Tsukada, S., Tanaka, T., Yamaguchi, T., Igarashi, H., Yokoyama, R., Seiriki, K., Kasai, A., Nakazawa, T., Nakagawa, S., Hashimoto, K., & Hashimoto, H. (2019). (R)-Ketamine Induces a Greater Increase in Prefrontal 5-HT Release Than (S)-Ketamine and Ketamine Metabolites via an AMPA Receptor-Independent Mechanism. *International Journal of Neuropsychopharmacology*, *22*(10), 665–674. https://doi.org/10.1093/IJNP/PYZ041

Aguilar-Valles, A., de Gregorio, D., Matta-Camacho, E., Eslamizade, M. J., Khlaifia, A., Skaleka, A., Lopez-Canul, M., Torres-Berrio, A., Bermudez, S., Rurak, G. M., Simard, S., Salmaso, N., Gobbi, G., Lacaille, J. C., & Sonenberg, N. (2021). Antidepressant actions of ketamine engage cell-specific translation via eIF4E. *Nature*, *590*(7845), 315–319. https://doi.org/10.1038/s41586-020-03047-0

Aklillu, E., Karlsson, S., Zachrisson, O. O., Ozdemir, V., & Agren, H. (2009). Association of MAOA gene functional promoter polymorphism with CSF dopamine turnover and atypical depression. *Pharmacogenetics and Genomics*, *19*(4), 267–275. https://doi.org/10.1097/FPC.0B013E328328D4D3

Alasaari, J. S., Lagus, M., Ollila, H. M., Toivola, A., Kivimäki, M., Vahtera, J., Kronholm, E., Härmä, M., Puttonen, S., & Paunio, T. (2012). Environmental Stress Affects DNA Methylation of a CpG Rich Promoter Region of Serotonin Transporter Gene in a Nurse Cohort. *PLoS ONE*, *7*(9), e45813. https://doi.org/10.1371/journal.pone.0045813

Aleksandrova, L. R., & Phillips, A. G. (2021). Neuroplasticity as a convergent mechanism of ketamine and classical psychedelics. *Trends in Pharmacological Sciences*, *42*(11), 929–942. https://doi.org/10.1016/J.TIPS.2021.08.003

Aleksandrova, L. R., Phillips, A. G., & Wang, Y. T. (2017). Antidepressant effects of ketamine and the roles of AMPA glutamate receptors and other mechanisms beyond NMDA receptor antagonism. *Journal of Psychiatry and Neuroscience*, *42*(4), 222–229. https://doi.org/10.1503/JPN.160175

Ali, S. H., Madhana, R. M., Athira, K. v., Kasala, E. R., Bodduluru, L. N., Pitta, S., Mahareddy, J. R., & Lahkar, M. (2015). Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. *Steroids*, *101*, 37–42. https://doi.org/10.1016/J.STEROIDS.2015.05.010

Allen, J. A., Halverson-Tamboli, R. A., & Rasenick, M. M. (2006). Lipid raft microdomains and neurotransmitter signalling. *Nature Reviews Neuroscience 2007 8:2*, *8*(2), 128–140. https://doi.org/10.1038/nrn2059

Allen, J., Romay-Tallon, R., Mitchell, M. A., Brymer, K. J., Johnston, J., Sánchez-Lafuente, C. L., Pinna, G., Kalynchuk, L. E., & Caruncho, H. J. (2022). Reelin has antidepressant-like effects after repeated or singular peripheral injections. *Neuropharmacology*, *211*, 109043. https://doi.org/10.1016/J.NEUROPHARM.2022.109043

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics 17*(8), 487-500. https://doi.org/10.1038/nrg.2016.59

Alnefeesi, Y., Chen-Li, D., Krane, E., Jawad, M. Y., Rodrigues, N. B., Ceban, F., di Vincenzo, J. D., Meshkat, S., Ho, R. C. M., Gill, H., Teopiz, K. M., Cao, B., Lee, Y., McIntyre, R. S., & Rosenblat, J. D. (2022). Real-world effectiveness of ketamine in treatment-resistant depression: A systematic review & meta-analysis. *Journal of Psychiatric Research*, *151*, 693–709. https://doi.org/10.1016/J.JPSYCHIRES.2022.04.037

Altemus, M., Sarvaiya, N., & Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology*, *35*(3), 320–330. https://doi.org/10.1016/J.YFRNE.2014.05.004

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience*, *31*(3), 571–591. https://doi.org/10.1016/0306-4522(89)90424-7

Ampuero, E., Jury, N., Härtel, S., Marzolo, M. P., & van Zundert, B. (2017). Interfering of the Reelin/ApoER2/PSD95 Signaling Axis Reactivates Dendritogenesis of Mature Hippocampal Neurons. *Journal of Cellular Physiology*, *232*(5), 1187–1199. https://doi.org/10.1002/JCP.25605

Anderson, M. C., Hasan, F., McCrodden, J. M., & Tipton, K. F. (1993). Monoamine oxidase inhibitors and the cheese effect. *Neurochemical Research*, *18*(11), 1145–1149. https://doi.org/10.1007/BF00978365/METRICS

Andrashko, V., Novak, T., Brunovsky, M., Klirova, M., Sos, P., & Horacek, J. (2020). The Antidepressant Effect of Ketamine Is Dampened by Concomitant Benzodiazepine Medication. *Frontiers in Psychiatry*, *11*, 844. https://doi.org/10.3389/FPSYT.2020.00844

Anttila, S., Huuhka, K., Huuhka, M., Rontu, R., Hurme, M., Leinonen, E., & Lehtimäki, T. (2007). Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. *Journal of Neural Transmission*, *114*(8), 1065–1068. https://doi.org/10.1007/S00702-007-0705-9

Arimitsu, N., Mizukami, Y., Shimizu, J., Takai, K., Suzuki, T., & Suzuki, N. (2021). Defective Reelin/Dab1 signaling pathways associated with disturbed hippocampus development of homozygous yotari mice. *Molecular and Cellular Neuroscience*, *112*, 103614.

Arnerić, S. P., Kern, V. D., & Stephenson, D. T. (2018). Regulatory-accepted drug development tools are needed to accelerate innovative CNS disease treatments. *Biochemical Pharmacology*, *151*, 291–306. https://doi.org/10.1016/J.BCP.2018.01.043

Attur, M. G., Patel, R., Thakker, G., Vyas, P., Levartovsky, D., Patel, P., Naqvi, S., Raza, R., Patel, K., Abramson, D., Bruno, G., Abramson, S. B., & Amin, A. R. (2000). Differential anti-inflammatory effects of immunosuppressive drugs: Cyclosporin, rapamycin and FK-506 on inducible nitric oxide synthase, nitric oxide, cyclooxygenase-2 and PGE2 production. *Inflammation Research*, *49*(1), 20–26. https://doi.org/10.1007/PL00000199

Auer, R. N., Jensen, M. L., & Whishaw, I. Q. (1989). Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 sector of the hippocampus. *Journal of Neuroscience*, *9*(5), 1641–1647. https://doi.org/10.1523/JNEUROSCI.09-05-01641.1989

Austin, M. P., Mitchell, P., & Goodwin, G. M. (2001). Cognitive deficits in depression: Possible implications for functional neuropathology. *The British Journal of Psychiatry*, *178*(3), 200–206. https://doi.org/10.1192/BJP.178.3.200

Auta, J., Smith, R. C., Dong, E., Tueting, P., Sershen, H., Boules, S., Lajtha, A., Davis, J., & Guidotti, A. (2013). DNA-methylation gene network dysregulation in peripheral blood lymphocytes of schizophrenia patients. *Schizophrenia Research*, *150*(1), 312–318. https://doi.org/10.1016/J.SCHRES.2013.07.030

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., Kavalali, E. T., & Monteggia, L. M. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, *475*(7354), 91–96. https://doi.org/10.1038/nature10130

Averill, L. A., Averill, C. L., Gueorguieva, R., Fouda, S., Sherif, M., Ahn, K. H., Ranganathan, M., D'Souza, D. C., Southwick, S. M., Sanacora, G., Duman, R. S., Krystal, J. H., & Abdallah, C. G. (2022). mTORC1 inhibitor effects on rapid ketamine-induced reductions in suicidal ideation in patients with treatment-resistant depression. *Journal of Affective Disorders*, *303*, 91–97. https://doi.org/10.1016/J.JAD.2022.01.104

Ayhan, C. H. B., Bilgin, H., Uluman, O. T., Sukut, O., Yilmaz, S., & Buzlu, S. (2020). A Systematic Review of the Discrimination Against Sexual and Gender Minority in Health Care Settings. *International Journal of Health Services*, *50*(1), 44–61. https://doi.org/10.1177/0020731419885093

Bagby, R. M., Quilty, L. C., & Ryder, A. C. (2008). Personality and depression. *Canadian Journal of Psychiatry, 53*(1), 14-25. https://doi.org/10.1177/070674370805300104

Baj, G., Carlino, D., Gardossi, L., & Tongiorgi, E. (2013). Toward a unified biological hypothesis for the BDNF Val66Met-associated memory deficits in humans: A model of

impaired dendritic mRNA trafficking. *Frontiers in Neuroscience*, *7*(OCT), 188. https://doi.org/10.3389/FNINS.2013.00188

Ballard, E. D., & Zarate, C. A. (2020). The role of dissociation in ketamine's antidepressant effects. *Nature Communications*, *11*(1), 1–7. https://doi.org/10.1038/s41467-020-20190-4

Balmaceda, V., Cuchillo-Ibáñez, I., Pujadas, L., García-Ayllón, M. S., Saura, C. A., Nimpf, J., Soriano, E., & Sáez-Valero, J. (2014). ApoER2 processing by presenilin-1 modulates reelin expression. *The FASEB Journal*, *28*(4), 1543–1554. https://doi.org/10.1096/FJ.13-239350

Banke, T. G., Bowie, D., Lee, H. K., Huganir, R. L., Schousboe, A., & Traynelis, S. F. (2000). Control of GluR1 AMPA Receptor Function by cAMP-Dependent Protein Kinase. *Journal of Neuroscience*, *20*(1), 89–102. https://doi.org/10.1523/JNEUROSCI.20-01-00089.2000

Banke, T. G., Dravid, S. M., & Traynelis, S. F. (2005). Protons Trap NR1/NR2B NMDA Receptors in a Nonconducting State. *Journal of Neuroscience*, *25*(1), 42–51. https://doi.org/10.1523/JNEUROSCI.3154-04.2005

Banki, C. M., Bissette, G., Arato, M., O'Connor, L., & Nemeroff, C. B. (1987). CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *American Journal of Psychiatry*, *144*(7), 873–877. https://doi.org/10.1176/AJP.144.7.873

Banks, W. A., Kastin, A. J., & Broadwell, R. D. (1995). Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation*, *2*(4), 241–248. https://doi.org/10.1159/000097202

Bartsch, T., Döhring, J., Rohr, A., Jansen, O., & Deuschl, G. (2011). CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. *Proceedings of the National Academy of Sciences*, *108*(42), 17562–17567. https://doi.org/10.1073/PNAS.1110266108

Basu, J., & Siegelbaum, S. A. (2015). The Corticohippocampal Circuit, Synaptic Plasticity, and Memory. *Cold Spring Harbor Perspectives in Biology*, *7*(11), a021733. https://doi.org/10.1101/CSHPERSPECT.A021733

Bats, C., Groc, L., & Choquet, D. (2007). The Interaction between Stargazin and PSD-95 Regulates AMPA Receptor Surface Trafficking. *Neuron*, *53*(5), 719–734. https://doi.org/10.1016/J.NEURON.2007.01.030

Baxter, L. R., Schwartz, J. M., Phelps, M. E., Mazziotta, J. C., Guze, B. H., Selin, C. E., Gerner, R. H., & Sumida, R. M. (1989). Reduction of Prefrontal Cortex Glucose Metabolism Common to Three Types of Depression. *Archives of General Psychiatry*, *46*(3), 243–250. https://doi.org/10.1001/ARCHPSYC.1989.01810030049007

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An Inventory for Measuring Depression. *Archives of General Psychiatry*, *4*(6), 561–571. https://doi.org/10.1001/ARCHPSYC.1961.01710120031004

Becker, S., & Wojtowicz, J. M. (2007). A model of hippocampal neurogenesis in memory and mood disorders. *Trends in Cognitive Sciences*, *11*(2), 70–76. https://doi.org/10.1016/J.TICS.2006.10.013

Beffert, U., Durudas, A., Weeber, E. J., Stolt, P. C., Giehl, K. M., Sweatt, J. D., Hammer, R. E., & Herz, J. (2006). Functional Dissection of Reelin Signaling by Site-Directed Disruption of Disabled-1 Adaptor Binding to Apolipoprotein E Receptor 2: Distinct Roles in Development and Synaptic Plasticity. *Journal of Neuroscience*, *26*(7), 2041–2052. https://doi.org/10.1523/JNEUROSCI.4566-05.2006

Beffert, U., Morfini, G., Bock, H. H., Reyna, H., Brady, S. T., & Herz, J. (2002). Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3β. *Journal of Biological Chemistry*, *277*(51), 49958–49964. https://doi.org/10.1074/jbc.M209205200

Beffert, U., Weeber, E. J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J. D., Li, W. P., Adelmann, G., Frotscher, M., Hammer, R. E., & Herz, J. (2005). Modulation of Synaptic Plasticity and Memory by Reelin Involves Differential Splicing of the Lipoprotein Receptor Apoer2. *Neuron*, *47*(4), 567–579. https://doi.org/10.1016/J.NEURON.2005.07.007

Belzung, C., & Lemoine, M. (2011). Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biology of Mood & Anxiety Disorders*, *1*(1), 1–14. https://doi.org/10.1186/2045-5380-1-9

Benhayon, D., Magdaleno, S., & Curran, T. (2003). Binding of purified Reelin to ApoER2 and VLDLR mediates tyrosine phosphorylation of Disabled-1. *Molecular Brain Research*, *112*(1–2), 33–45. https://doi.org/10.1016/S0169-328X(03)00032-9

Berger, S., Gureczny, S., Reisinger, S. N., Horvath, O., & Pollak, D. D. (2019). Effect of chronic corticosterone treatment on depression-like behavior and sociability in female and male c57bl/6n mice. *Cells*, *8*(9), 1018. https://doi.org/10.3390/cells8091018

Berger, T., Lee, H., Young, A. H., Aarsland, D., & Thuret, S. (2020). Adult Hippocampal Neurogenesis in Major Depressive Disorder and Alzheimer's Disease. *Trends in Molecular Medicine*, *26*(9), 803–818. https://doi.org/10.1016/J.MOLMED.2020.03.010

Berlim, M. T., & Turecki, G. (2007). What is the meaning of treatment resistant/refractory major depression (TRD)? A systematic review of current randomized trials. *European Neuropsychopharmacology*, *17*(11), 696–707. https://doi.org/10.1016/J.EURONEURO.2007.03.009

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., & Krystal, J. H. (2000a). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351–354. https://doi.org/10.1016/S0006-3223(99)00230-9

Besnier, E., Clavier, T., Tonon, M. C., Selim, J., Lefevre-Scelles, A., Morin, F., Tamion, F., Dureuil, B., Castel, H., & Compere, V. (2017). Ketamine and Etomidate Down-regulate the

Hypothalamic–Pituitary–Adrenal Axis in an Endotoxemic Mouse Model. *Anesthesiology*, *127*(2), 347–354. https://doi.org/10.1097/ALN.0000000000001704

Bibikova, M., Barnes, B., Tsan, C., Ho, V., Klotzle, B., Le, J. M., Delano, D., Zhang, L., Schroth, G. P., Gunderson, K. L., Fan, J. B., & Shen, R. (2011). High density DNA methylation array with single CpG site resolution. *Genomics*, *98*(4), 288–295. https://doi.org/10.1016/J.YGENO.2011.07.007

Birkmayer, W., Knoll, J., Riederer, P., Youdim, M. B. H., Hars, V., & Marton, J. (1985). Increased life expectancy resulting from addition of l-deprenyl to Madopar® treatment in Parkinson's disease: A longterm study. *Journal of Neural Transmission*, *64*(2), 113–127. https://doi.org/10.1007/BF01245973

Boaz, A., Biri, D., & Mckevitt, C. (2016). Rethinking the relationship between science and society: Has there been a shift in attitudes to Patient and Public Involvement and Public Engagement in Science in the United Kingdom? *Health Expectations*, *19*(3), 592–601. https://doi.org/10.1111/hex.12295

Bock, H. H., & May, P. (2016). Canonical and non-canonical reelin signaling. *Frontiers in Cellular Neuroscience*, *10*(Jun), 166. https://doi.org/10.3389/fncel.2016.00166

Bok, E., Cho, E. J., Chung, E. S., Shin, W. H., & Jin, B. K. (2018). Interleukin-4 contributes to degeneration of dopamine neurons in the lipopolysaccharide treated substantia nigra in vivo. *Experimental Neurobiology*, *27*(4), 309–319. https://doi.org/10.5607/EN.2018.27.4.309

Bomholt, S. F., Harbuz, M. S., Blackburn-Munro, G., & Blackburn-Munro, R. E. (2004). Involvement and Role of the Hypothalamo-pituitary-adrenal (HPA) Stress Axis in Animal Models of Chronic Pain and Inflammation. *Stress*, *7*(1), 1-14. https://doi.org/10.1080/10253890310001650268

Bonaventura, J., Lam, S., Carlton, M., Boehm, M. A., Gomez, J. L., Solís, O., Sánchez-Soto, M., Morris, P. J., Fredriksson, I., Thomas, C. J., Sibley, D. R., Shaham, Y., Zarate, C. A., & Michaelides, M. (2021). Pharmacological and behavioral divergence of ketamine enantiomers: implications for abuse liability. *Molecular Psychiatry*, *26*(11). https://doi.org/10.1038/s41380-021-01093-2

Bosch, C., Masachs, N., Exposito-Alonso, D., Martínez, A., Teixeira, C. M., Fernaud, I., Pujadas, L., Ulloa, F., Comella, J. X., Defelipe, J., Merchán-Ṕrez, A., & Soriano, E. (2016). Reelin Regulates the Maturation of Dendritic Spines, Synaptogenesis and Glial Ensheathment of Newborn Granule Cells. *Cerebral Cortex*, *26*(11), 4282–4298. https://doi.org/10.1093/CERCOR/BHW216

Botella-López, A., Burgaya, F., Gavín, R., García-Ayllón, M. S., Gómez-Tortosa, E., Peña-Casanova, J., Ureña, J. M., del Río, J. A., Blesa, R., Soriano, E., & Sáez-Valero, J. (2006). Reelin expression and glycosylation patterns are altered in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(14), 5573–5578. https://doi.org/10.1073/PNAS.0601279103

Bourke, C. H., Harrell, C. S., & Neigh, G. N. (2012). Stress-induced sex differences: Adaptations mediated by the glucocorticoid receptor. *Hormones and Behavior*, *62*(3), 210–218. https://doi.org/10.1016/J.YHBEH.2012.02.024

Brachman, R. A., McGowan, J. C., Perusini, J. N., Lim, S. C., Pham, T. H., Faye, C., Gardier, A. M., Mendez-David, I., David, D. J., Hen, R., & Denny, C. A. (2016). Ketamine as a Prophylactic Against Stress-Induced Depressive-like Behavior. *Biological Psychiatry*, *79*(9), 776–786. https://doi.org/10.1016/J.BIOPSYCH.2015.04.022

Bravo, J. A., Díaz-Veliz, G., Mora, S., Ulloa, J. L., Berthoud, V. M., Morales, P., Arancibia, S., & Fiedler, J. L. (2009). Desipramine prevents stress-induced changes in depressive-like behavior and hippocampal markers of neuroprotection. *Behavioural Pharmacology*, *20*(3), 273–285. https://doi.org/10.1097/FBP.0B013E32832C70D9

Breault, L. J., Rittenbach, K., Hartle, K., Abins-Wagner, R. B., Beaudrap, C. de, Jasaui, Y., Ardell, E., Purdon, S. E., Michael, A., Sullivan, G., Unger, A. S. R., Vandall-Walker, L., Necyk, B., Krawec, K., Manafò, E., & Mason-Lai, P. (2018). People with lived experience (PWLE) of depression: Describing and reflecting on an explicit patient engagement process within depression research priority setting in Alberta, Canada. *Research Involvement and Engagement*, *4*(1), 1–10. https://doi.org/10.1186/S40900-018-0115-1

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000). Hippocampal volume reduction in major depression. *American Journal of Psychiatry*, *157*(1), 115–117. https://doi.org/10.1176/AJP.157.1.115

Brennand, K. J., Landek-Salgado, M. A., & Sawa, A. (2014). Modeling Heterogeneous Patients With a Clinical Diagnosis of Schizophrenia With Induced Pluripotent Stem Cells. *Biological Psychiatry*, *75*(12), 936–944. https://doi.org/10.1016/J.BIOPSYCH.2013.10.025

Brennand, K., Savas, J. N., Kim, Y., Tran, N., Simone, A., Hashimoto-Torii, K., Beaumont, K. G., Kim, H. J., Topol, A., Ladran, I., Abdelrahim, M., Matikainen-Ankney, B., Chao, S. H., Mrksich, M., Rakic, P., Fang, G., Zhang, B., Yates, J. R., & Gage, F. H. (2015). Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. *Molecular Psychiatry*, *20*(3), 361–368. https://doi.org/10.1038/mp.2014.22

Britton, J. C., Lissek, S., Grillon, C., Norcross, M. A., & Pine, D. S. (2011). Development of anxiety: the role of threat appraisal and fear learning. *Depression and Anxiety*, *28*(1), 5–17. https://doi.org/10.1002/DA.20733

Brodie, D. A. (1966). A Comparison of Anticholinergic Drugs on Gastric Secretion, Gastric Emptying, and Pupil Diameter in the Rat. *Gastroenterology*, *50*(1), 45–50. https://doi.org/10.1016/S0016-5085(66)80099-9

Brotto, L. A., Gorzalka, B. B., & Barr, A. M. (2001). Paradoxical effects of chronic corticosterone on forced swim behaviours in aged male and female rats. *European Journal of Pharmacology*, *424*(3), 203–209. https://doi.org/10.1016/S0014-2999(01)01148-7

Browne, C. A., & Lucki, I. (2013). Antidepressant effects of ketamine: Mechanisms underlying fast-acting novel antidepressants. *Frontiers in Pharmacology*, *4*(DEC), 161. https://doi.org/10.3389/FPHAR.2013.00161

Brummelte, S., & Galea, L. A. M. (2010). Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience*, *168*(3), 680–690. https://doi.org/10.1016/j.neuroscience.2010.04.023

Brymer, K. J., Fenton, E. Y., Kalynchuk, L. E., & Caruncho, H. J. (2018). Peripheral etanercept administration normalizes behavior, hippocampal neurogenesis, and hippocampal reelin and GABAA receptor expression in a preclinical model of depression. *Frontiers in Pharmacology*, *9*(FEB), 121. https://doi.org/10.3389/FPHAR.2018.00121

Brymer, K. J., Johnston, J., Botterill, J. J., Romay-Tallon, R., Mitchell, M. A., Allen, J., Pinna, G., Caruncho, H. J., & Kalynchuk, L. E. (2020). Fast-acting antidepressant-like effects of Reelin evaluated in the repeated-corticosterone chronic stress paradigm. *Neuropsychopharmacology 2020 45:10*, *45*(10), 1707–1716. https://doi.org/10.1038/s41386-020-0609-z

Buganim, Y., Markoulaki, S., van Wietmarschen, N., Hoke, H., Wu, T., Ganz, K., Akhtar-Zaidi, B., He, Y., Abraham, B. J., Porubsky, D., Kulenkampff, E., Faddah, D. A., Shi, L., Gao, Q., Sarkar, S., Cohen, M., Goldmann, J., Nery, J. R., Schultz, M. D., … Jaenisch, R. (2014). The Developmental Potential of iPSCs Is Greatly Influenced by Reprogramming Factor Selection. *Cell Stem Cell*, *15*(3), 295–309. https://doi.org/10.1016/J.STEM.2014.07.003

Burcusa, S. L., & Iacono, W. G. (2007). Risk for recurrence in depression. *Clinical Psychology Review*, *27*(8), 959–985. https://doi.org/10.1016/J.CPR.2007.02.005

Burt, D., Zembar, M., & Niederehe, G. (1995). Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Psychological Bulletin*, *117*(2), 285. https://psycnet.apa.org/buy/1995-21418-001

Butler, D. (2008). Translational research: Crossing the valley of death. *Nature*, *453*(7197), 840-842. https://doi.org/10.1038/453840a

Buzśaki, G., & Wang, X. J. (2012). Mechanisms of Gamma Oscillations. *Annual Review of Neuroscience*, *35*, 203. https://doi.org/10.1146/ANNUREV-NEURO-062111-150444

Calvier, L., Demuth, G., Manouchehri, N., Wong, C., Sacharidou, A., Mineo, C., Shaul, P. W., Monson, N. L., Kounnas, M. Z., Stüve, O., & Herz, J. (2020). Reelin depletion protects against autoimmune encephalomyelitis by decreasing vascular adhesion of leukocytes. *Science Translational Medicine*, *12*(556). https://doi.org/10.1126/SCITRANSLMED.AAY7675

Calvier, L., Xian, X., Lee, R. G., Sacharidou, A., Mineo, C., Shaul, P. W., Kounnas, M. Z., Tsai, S., & Herz, J. (2021). Reelin Depletion Protects Against Atherosclerosis by Decreasing Vascular Adhesion of Leukocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *41*(4), 1309–1318. https://doi.org/10.1161/ATVBAHA.121.316000

Camargo, A., Dalmagro, A. P., Wolin, I. A. V., Kaster, M. P., & Rodrigues, A. L. S. (2021). The resilient phenotype elicited by ketamine against inflammatory stressors-induced depressive-like behavior is associated with NLRP3-driven signaling pathway. *Journal of Psychiatric Research*, *144*, 118–128. https://doi.org/10.1016/J.JPSYCHIRES.2021.09.057

Camp, R. M., Remus, J. L., Kalburgi, S. N., Porterfield, V. M., & Johnson, J. D. (2012). Fear conditioning can contribute to behavioral changes observed in a repeated stress model. *Behavioural Brain Research*, *233*(2), 536–544. https://doi.org/10.1016/J.BBR.2012.05.040

Capuco, A., Urits, I., Hasoon, J., Chun, R., Gerald, B., Wang, J. K., Kassem, H., Ngo, A. L., Abd-Elsayed, A., Simopoulos, T., Kaye, A. D., & Viswanath, O. (2020). Current Perspectives on Gut Microbiome Dysbiosis and Depression. *Advances in Therapy*, *37*(4), 1328-1346. https://doi.org/10.1007/s12325-020-01272-7

Carhart-Harris, R. L., & Goodwin, G. M. (2017). The Therapeutic Potential of Psychedelic Drugs: Past, Present, and Future. *Neuropsychopharmacology 2017 42:11*, *42*(11), 2105–2113. https://doi.org/10.1038/npp.2017.84

Caron-Flinterman, J. F., Broerse, J. E. W., & Bunders, J. F. G. (2005). The experiential knowledge of patients: A new resource for biomedical research? *Social Science and Medicine*, *60*(11), 2575–2584. https://doi.org/10.1016/j.socscimed.2004.11.023

Carrier, N., & Kabbaj, M. (2013). Sex differences in the antidepressant-like effects of ketamine. *Neuropharmacology*, *70*, 27–34. https://doi.org/10.1016/J.NEUROPHARM.2012.12.009

Carroll, B. J., Cassidy, F., Naftolowitz, D., Tatham, N. E., Wilson, W. H., Iranmanesh, A., Liu, P. Y., & Veldhuis, J. D. (2007). Pathophysiology of hypercortisolism in depression. *Acta Psychiatrica Scandinavica*, *115*(SUPPL. 433), 90–103. https://doi.org/10.1111/J.1600-0447.2007.00967.X

Caruncho, H. J., Brymer, K., Romay-Tallón, R., Mitchell, M. A., Rivera-Baltanás, T., Botterill, J., Olivares, J. M., & Kalynchuk, L. E. (2016). Reelin-related disturbances in depression: Implications for translational studies. *Frontiers in Cellular Neuroscience*, *10*(FEB), 48. https://doi.org/10.3389/fncel.2016.00048

Caruncho, H. J., Rivera-Baltanas, T., Romay-Tallon, R., Kalynchuk, L. E., & Olivares, J. M. (2019). Patterns of Membrane Protein Clustering in Peripheral Lymphocytes as Predictors of Therapeutic Outcomes in Major Depressive Disorder. *Frontiers in Pharmacology*, *10*, 190. https://doi.org/10.3389/FPHAR.2019.00190

Caruncho, H. J., Romay-Tallón, R., Dopeso-Reyes, I. G., Lussier, A. L., & Kalynchuk, L. E. (2010). The coexpression of reelin and neuronal nitric oxide synthase in a subpopulation of dentate gyrus neurons is downregulated in heterozygous reeler mice. *Neural Plasticity*, *2010*. https://doi.org/10.1155/2010/130429

Casey, L. S., Reisner, S. L., Findling, M. G., Blendon, R. J., Benson, J. M., Sayde, J. M., & Miller, C. (2019). Discrimination in the United States: Experiences of lesbian, gay,

bisexual, transgender, and queer Americans. *Health Services Research*, *54*(S2), 1454–1466. https://doi.org/10.1111/1475-6773.13229

Cattaneo, A., Ferrari, C., Turner, L., Mariani, N., Enache, D., Hastings, C., Kose, M., Lombardo, G., McLaughlin, A. P., Nettis, M. A., Nikkheslat, N., Sforzini, L., Worrell, C., Zajkowska, Z., Cattane, N., Lopizzo, N., Mazzelli, M., Pointon, L., Cowen, P. J., … Pariante, C. M. (2020). Whole-blood expression of inflammasome- and glucocorticoid-related mRNAs correctly separates treatment-resistant depressed patients from drug-free and responsive patients in the BIODEP study. *Translational Psychiatry*, *10*(1), 1–14. https://doi.org/10.1038/s41398-020-00874-7

Cavalleri, L., Merlo Pich, E., Millan, M. J., Chiamulera, C., Kunath, T., Spano, P. F., & Collo, G. (2018). Ketamine enhances structural plasticity in mouse mesencephalic and human iPSC-derived dopaminergic neurons via AMPAR-driven BDNF and mTOR signaling. *Molecular Psychiatry*, *23*(4), 812–823. https://doi.org/10.1038/mp.2017.241

Chai, X., Förster, E., Zhao, S., Bock, H. H., & Frotscher, M. (2009). Reelin Stabilizes the Actin Cytoskeleton of Neuronal Processes by Inducing n-Cofilin Phosphorylation at Serine3. *Journal of Neuroscience*, *29*(1), 288–299. https://doi.org/10.1523/JNEUROSCI.2934-08.2009

Chaki, S. (2021). mGlu2/3 Receptor Antagonists as Rapid-Acting Antidepressants. *Contemporary Clinical Neuroscience*, 111–126. https://doi.org/10.1007/978-3-030-79790-4_7

Chameau, P., Inta, D., Vitalis, T., Monyer, H., Wadman, W. J., & van Hooft, J. A. (2009). The N-terminal region of reelin regulates postnatal dendritic maturation of cortical pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(17), 7227–7232. https://doi.org/10.1073/PNAS.0810764106

Chan, C. S., Weeber, E. J., Kurup, S., Sweatt, J. D., & Davis, R. L. (2003). Integrin Requirement for Hippocampal Synaptic Plasticity and Spatial Memory. *Journal of Neuroscience*, *23*(18), 7107–7116. https://doi.org/10.1523/JNEUROSCI.23-18-07107.2003

Chan, J. N. M., Lee, J. C. D., Lee, S. S. P., Hui, K. K. Y., Chan, A. H. L., Fung, T. K. H., Sánchez-Vidaña, D. I., Lau, B. W. M., & Ngai, S. P. C. (2017). Interaction effect of social isolation and high dose corticosteroid on neurogenesis and emotional behavior. *Frontiers in Behavioral Neuroscience*, *11*, 18. https://doi.org/10.3389/FNBEH.2017.00018

Chang, H. H., Lee, I. H., Gean, P. W., Lee, S. Y., Chi, M. H., Yang, Y. K., Lu, R. B., & Chen, P. S. (2012). Treatment response and cognitive impairment in major depression: Association with C-reactive protein. *Brain, Behavior, and Immunity*, *26*(1), 90–95. https://doi.org/10.1016/J.BBI.2011.07.239

Charney, D. S. (2004). Psychobiological Mechanism of Resilience and Vulnerability: Implications for Successful Adaptation to Extreme Stress. *American Journal of Psychiatry*, *161*(2), 195–216. https://doi.org/10.1176/APPI.AJP.161.2.195

Cheasty, M., Clare, A. W., & Collins, C. (1998). Relation between sexual abuse in childhood and adult depression: case-control study. *BMJ*, *316*(7126), 198–201. https://doi.org/10.1136/BMJ.316.7126.198

Chen, H. C., Fong, T. H., Hsu, P. W., & Chiu, W. T. (2013). Multifaceted effects of rapamycin on functional recovery after spinal cord injury in rats through autophagy promotion, anti-inflammation, and neuroprotection. *Journal of Surgical Research*, *179*(1), e203–e210. https://doi.org/10.1016/J.JSS.2012.02.023

Chen, H. M., DeLong, C. J., Bame, M., Rajapakse, I., Herron, T. J., McInnis, M. G., & O'Shea, K. S. (2014). Transcripts involved in calcium signaling and telencephalic neuronal fate are altered in induced pluripotent stem cells from bipolar disorder patients. *Translational Psychiatry*, *4*(3), e375–e375. https://doi.org/10.1038/tp.2014.12

Chen, J., Olsen, R. K., Preston, A. R., Glover, G. H., & Wagner, A. D. (2011). Associative retrieval processes in the human medial temporal lobe: Hippocampal retrieval success and CA1 mismatch detection. *Learning & Memory*, *18*(8), 523–528. https://doi.org/10.1101/LM.2135211

Chen, M. H., Li, C. T., Lin, W. C., Hong, C. J., Tu, P. C., Bai, Y. M., Cheng, C. M., & Su, T. P. (2018). Rapid inflammation modulation and antidepressant efficacy of a low-dose ketamine infusion in treatment-resistant depression: A randomized, double-blind control study. *Psychiatry Research*, *269*, 207–211. https://doi.org/10.1016/J.PSYCHRES.2018.08.078

Chen, Y., Beffert, U., Ertunc, M., Tang, T. S., Kavalali, E. T., Bezprozvanny, I., & Herz, J. (2005). Reelin Modulates NMDA Receptor Activity in Cortical Neurons. *Journal of Neuroscience*, *25*(36), 8209–8216. https://doi.org/10.1523/JNEUROSCI.1951-05.2005

Chen, Y., & Guillemin, G. J. (2009). Kynurenine pathway metabolites in humans: Disease and healthy states. *International Journal of Tryptophan Research*, *2*(1), 1–19.

Chen, Y., Sharma, R. P., Costa, R. H., Costa, E., & Grayson, D. R. (2002). On the epigenetic regulation of the human reelin promoter. *Nucleic Acids Research*, *30*(13), 2930–2939. https://doi.org/10.1093/NAR/GKF401

Chen, Z. Y., Patel, P. D., Sant, G., Meng, C. X., Teng, K. K., Hempstead, B. L., & Lee, F. S. (2004). Variant Brain-Derived Neurotrophic Factor (BDNF) (Met66) Alters the Intracellular Trafficking and Activity-Dependent Secretion of Wild-Type BDNF in Neurosecretory Cells and Cortical Neurons. *Journal of Neuroscience*, *24*(18), 4401–4411. https://doi.org/10.1523/JNEUROSCI.0348-04.2004

Chen-Tsai, C. P., Colome-Grimmer, M., & Wagner, R. F. (2004). Correlations among Neural Cell Adhesion Molecule, Nerve Growth Factor, and Its Receptors, TrkA, TrkB, TrkC, and p75NGFR, in Perineural Invasion by Basal Cell and Cutaneous Squamous Cell Carcinomas. *Dermatologic Surgery*, *30*(7), 1009–1016. https://doi.org/10.1111/J.1524-4725.2004.30306.X

Cho, E., Kim, K., Kim, H., & Cho, S. R. (2022). Reelin protects against pathological α-synuclein accumulation and dopaminergic neurodegeneration after environmental enrichment in Parkinson's disease. *Neurobiology of Disease*, *175*, 105898. https://doi.org/10.1016/J.NBD.2022.105898

Choi, M., Lee, S. H., Park, M. H., Kim, Y. S., & Son, H. (2017). Ketamine induces brain-derived neurotrophic factor expression via phosphorylation of histone deacetylase 5 in rats. *Biochemical and Biophysical Research Communications*, *489*(4), 420–425. https://doi.org/10.1016/J.BBRC.2017.05.157

Choi, M., Lee, S. H., Wang, S. E., Ko, S. Y., Song, M., Choi, J. S., Kim, Y. S., Duman, R. S., & Son, H. (2015). Ketamine produces antidepressant-like effects through phosphorylation-dependent nuclear export of histone deacetylase 5 (HDAC5) in rats. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(51), 15755–15760. https://doi.org/10.1073/PNAS.1513913112

Christensen, H., Hadzi-Pavlovic, D., Andrews, G., & Mattick, R. (1987). Behavior Therapy and Tricyclic Medication in the Treatment of Obsessive-Compulsive Disorder: A Quantitative Review. *Journal of Consulting and Clinical Psychology*, *55*(5), 701–711. https://doi.org/10.1037/0022-006X.55.5.701

CIHR. (2019). *Foundations of SPOR*. https://cihr-irsc.gc.ca/e/51039.html

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., Leucht, S., Ruhe, H. G., Turner, E. H., Higgins, J. P. T., Egger, M., Takeshima, N., Hayasaka, Y., Imai, H., Shinohara, K., Tajika, A., Ioannidis, J. P. A., & Geddes, J. R. (2018). Comparative Efficacy and Acceptability of 21 Antidepressant Drugs for the Acute Treatment of Adults With Major Depressive Disorder: A Systematic Review and Network Meta-Analysis. *Focus*, *16*(4), 420–429.

Citri, A., & Malenka, R. C. (2008). Synaptic plasticity: Multiple forms, functions, and mechanisms. *Neuropsychopharmacology*, *33*(1), 18-41. https://doi.org/10.1038/sj.npp.1301559

Clark, R. E., & Squirea, L. R. (2013). Similarity in form and function of the hippocampus in rodents, monkeys, and humans. *Proceedings of the National Academy of Sciences*, *110*(supplement_2), 10365–10370. https://doi.org/10.1073/PNAS.1301225110

Clarke, M., Razmjou, S., Prowse, N., Dwyer, Z., Litteljohn, D., Pentz, R., Anisman, H., & Hayley, S. (2017). Ketamine modulates hippocampal neurogenesis and pro-inflammatory cytokines but not stressor induced neurochemical changes. *Neuropharmacology*, *112*, 210–220. https://doi.org/10.1016/J.NEUROPHARM.2016.04.021

Colbran, R. J., & Brown, A. M. (2004). Calcium/calmodulin-dependent protein kinase II and synaptic plasticity. *Current Opinion in Neurobiology*, *14*(3), 318–327. https://doi.org/10.1016/J.CONB.2004.05.008

Collingridge, G. L., & Bliss, T. V. P. (1987). NMDA receptors - their role in long-term potentiation. *Trends in Neurosciences*, *10*(7), 288–293. https://doi.org/10.1016/0166-2236(87)90175-5

Comai, S., & Gobbi, G. (2016). Translational research in suicide: Is it possible to study suicide in animal models? *Understanding Suicide: From Diagnosis to Personalized Treatment*, 177–188. https://doi.org/10.1007/978-3-319-26282-6_14

Commons, K. G., Cholanians, A. B., Babb, J. A., & Ehlinger, D. G. (2017). The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior. *ACS Chemical Neuroscience*, *8*(5), 955–960.

Contractor, A., Mulle, C., & Swanson, G. T. (2011). Kainate receptors coming of age: milestones of two decades of research. *Trends in Neurosciences*, *34*(3), 154–163. https://doi.org/10.1016/J.TINS.2010.12.002

Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of Neurobiology*, *60*(2), 236–248. https://doi.org/10.1002/NEU.20025

Cooper, J. A. (2008). A mechanism for inside-out lamination in the neocortex. *Trends in Neurosciences*, *31*(3), 113–119. https://doi.org/10.1016/J.TINS.2007.12.003

Coppen, A., Shaw, D. M., Herzberg, B., & Maggs, R. (1967). Tryptophan in the treatment of depression. *Lancet*, *2*(7527), 1178–1180. https://doi.org/10.1016/s0140-6736(67)91894-6

Costi, S., Evers, A., Jha, M. K., Klein, M., Overbey, J. R., Goosens, K. A., Burgess, J., Alvarez, K., Feder, A., Charney, D. S., & Murrough, J. W. (2023). A randomized pilot study of the prophylactic effect of ketamine on laboratory-induced stress in healthy adults. *Neurobiology of Stress*, *22*, 100505. https://doi.org/10.1016/J.YNSTR.2022.100505

Cotter, P. A., Mulligan, O. F., Landau, S., Papadopoulos, A., Lightman, S. L., & Checkley, S. A. (2002). Vasoconstrictor response to topical beclomethasone in major depression. *Psychoneuroendocrinology*, *27*(4), 475–487. https://doi.org/10.1016/S0306-4530(01)00065-8

Cozanitis, D. A. (2016). Daniel Bovet, Nobelist: muscle relaxants in anaesthesia. *Wiener Medizinische Wochenschrift*, *166*(15), 487-499.

Cuchillo-Ibañez, I., Balmaceda, V., Mata-Balaguer, T., Lopez-Font, I., & Sáez-Valero, J. (2016). Reelin in Alzheimer's Disease, Increased Levels but Impaired Signaling: When More is Less. *Journal of Alzheimer's Disease*, *52*(2), 403–416. https://doi.org/10.3233/JAD-151193

Cui, Z., Feng, R., Jacobs, S., Duan, Y., Wang, H., Cao, X., & Tsien, J. Z. (2013). Increased NR2A:NR2B ratio compresses long-term depression range and constrains long-term memory. *Scientific Reports*, *3*(1), 1–10. https://doi.org/10.1038/srep01036

Cuijpers, P., Karyotaki, E., Weitz, E., Andersson, G., Hollon, S. D., & van Straten, A. (2014a). The effects of psychotherapies for major depression in adults on remission, recovery and

improvement: A meta-analysis. *Journal of Affective Disorders*, *159*, 118–126. https://doi.org/10.1016/J.JAD.2014.02.026

Cuijpers, P., Sijbrandij, M., Koole, S. L., Andersson, G., Beekman, A. T., & Reynolds, C. F. (2014b). Adding Psychotherapy to Antidepressant Medication in Depression and Anxiety Disorders: a Meta-Analysis. *Focus*, *12*(3), 347–358. https://doi.org/10.1176/appi.focus.12.3.347

Culbertson, V. L., Rahman, S. E., Bosen, G. C., Caylor, M. L., Echevarria, M. M., & Xu, D. (2018). Implications of off-target serotoninergic drug activity: an analysis of serotonin syndrome reports using a systematic bioinformatics approach. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *38*(9), 888-898.

Cuthbert, B. N., & Insel, T. R. (2013). Toward the future of psychiatric diagnosis: The seven pillars of RDoC. *BMC Medicine*, *11*(1), 1–8. https://doi.org/10.1186/1741-7015-11-126

Czysz, A. H., Schappi, J. M., & Rasenick, M. M. (2014). Lateral Diffusion of Gαs in the Plasma Membrane Is Decreased after Chronic but not Acute Antidepressant Treatment: Role of Lipid Raft and Non-Raft Membrane Microdomains. *Neuropsychopharmacology 2015 40:3*, *40*(3), 766–773. https://doi.org/10.1038/npp.2014.256

da Prada, M., Zürcher, G., Wüthrich, I., & Haefely, W. E. (1988). On tyramine, food, beverages and the reversible MAO inhibitor moclobemide. *Journal of neural transmission, 26*, 31-56. https://europepmc.org/article/med/3283290

Dahlström, M., Ahonen, A., Ebeling, H., Torniainen, P., Heikkilä, J., & Moilanen, I. (2000). Elevated hypothalamic/midbrain serotonin (monoamine) transporter availability in depressive drug-naive children and adolescents. *Molecular Psychiatry*, *5*(5), 514–522. https://doi.org/10.1038/sj.mp.4000766

Dai, D., Miller, C., Valdivia, V., Boyle, B., Bolton, P., Li, S., Seiner, S., & Meisner, R. (2022). Neurocognitive effects of repeated ketamine infusion treatments in patients with treatment resistant depression: a retrospective chart review. *BMC Psychiatry*, *22*(1), 1–8. https://doi.org/10.1186/S12888-022-03789-3

Dal Pozzo, V., Crowell, B., Briski, N., Crockett, D. P., & D'arcangelo, G. (2020). Reduced Reelin Expression in the Hippocampus after Traumatic Brain Injury. *Biomolecules*, *10*(7), 975. https://doi.org/10.3390/BIOM10070975

Dalgleish, T., & Werner-Seidler, A. (2014). Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics. *Trends in Cognitive Sciences*, *18*(11), 596–604. https://doi.org/10.1016/J.TICS.2014.06.010

Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., Sfikakis, A., & Papadopoulou-Daifoti, Z. (2005). Chronic mild stress impact: Are females more vulnerable? *Neuroscience*, *135*(3), 703–714. https://doi.org/10.1016/J.NEUROSCIENCE.2005.06.068

Danysz, W., Wroblewski, J. T., & Costa, E. (1988). Learning impairment in rats by N-methyl-D-aspartate receptor antagonists. *Neuropharmacology*, *27*(6), 653–656. https://doi.org/10.1016/0028-3908(88)90189-X

D'Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M., & Curran, T. (1999). Reelin is a ligand for lipoprotein receptors. *Neuron*, *24*(2), 471-479.

D'Arcangelo, G., Miao, G. G., Chen, S. C., Scares, H. D., Morgan, J. I., & Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature*, *374*(6524), 719–723. https://doi.org/10.1038/374719a0

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A. M., Guilloux, J. P., & David, D. J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Frontiers in Behavioral Neuroscience*, *8*(MAY), 136. https://doi.org/10.3389/fnbeh.2014.00136

David, D. J., & Gourion, D. (2016). Antidepressant and tolerance: Determinants and management of major side effects. *L'encephale*, *42*(6), 553–561. https://doi.org/10.1016/J.ENCEP.2016.05.006

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., Drew, M., Craig, D. A., Guiard, B. P., Guilloux, J. P., Artymyshyn, R. P., Gardier, A. M., Gerald, C., Antonijevic, I. A., Leonardo, E. D., & Hen, R. (2009). Neurogenesis-Dependent and -Independent Effects of Fluoxetine in an Animal Model of Anxiety/Depression. *Neuron*, *62*(4), 479–493. https://doi.org/10.1016/J.NEURON.2009.04.017

Davis, M. A., Lin, L. A., Liu, H., & Sites, B. D. (2017). Prescription Opioid Use among Adults with Mental Health Disorders in the United States. *The Journal of the American Board of Family Medicine*, *30*(4), 407–417. https://doi.org/10.3122/JABFM.2017.04.170112

de Almeida, R. F., Pocharski, C. B., Rodrigues, A. L. S., Elisabetsky, E., & Souza, D. O. (2020). Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-65300-w

de Bergeyck, V., Naerhuyzen, B., Goffinet, A. M., & Lambert de Rouvroit, C. (1998). A panel of monoclonal antibodies against reelin, the extracellular matrix protein defective in reeler mutant mice. *Journal of Neuroscience Methods*, *82*(1), 17–24. https://doi.org/10.1016/S0165-0270(98)00024-7

de Carlo, V., Calati, R., & Serretti, A. (2016). Socio-demographic and clinical predictors of non-response/non-remission in treatment resistant depressed patients: A systematic review. *Psychiatry Research*, *240*, 421–430. https://doi.org/10.1016/J.PSYCHRES.2016.04.034

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463-475. https://doi.org/10.1038/nrn1683

de Kloet, E. R., Reul, J. M. H. M., & Sutanto, W. (1990). Corticosteroids and the brain. *The Journal of Steroid Biochemistry and Molecular Biology*, *37*(3), 387–394. https://doi.org/10.1016/0960-0760(90)90489-8

de Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain Corticosteroid Receptor Balance in Health and Disease. *Endocrine Reviews*, *19*(3), 269–301. https://doi.org/10.1210/EDRV.19.3.0331

de La Gándara, J., Agüera, L., Rojo, J. E., Ros, S., & de Pedro, J. M. (2005). Use of antidepressant combinations: which, when and why? Results of a Spanish survey. *Acta Psychiatrica Scandinavica*, *112*(428), 32–35. https://doi.org/10.1111/J.1600-0447.2005.00678.X

de la Salle, S., Phillips, J. L., Blier, P., & Knott, V. (2022). Electrophysiological correlates and predictors of the antidepressant response to repeated ketamine infusions in treatment-resistant depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *115*, 110507. https://doi.org/10.1016/J.PNPBP.2021.110507

Derkach, V. A., Oh, M. C., Guire, E. S., & Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature Reviews Neuroscience*, *8*(2), 101-113. https://doi.org/10.1038/nrn2055

Derntl, B., Hornung, J., Sen, Z. D., Colic, L., Li, M., & Walter, M. (2019). Interaction of sex and age on the dissociative effects of ketamine action in young healthy participants. *Frontiers in Neuroscience*, *13*(JUN), 616. https://doi.org/10.3389/FNINS.2019.00616

Deschaux, O., Zheng, X., Lavigne, J., Nachon, O., Cleren, C., Moreau, J. L., & Garcia, R. (2013). Post-extinction fluoxetine treatment prevents stress-induced reemergence of extinguished fear. *Psychopharmacology*, *225*(1), 209–216. https://doi.org/10.1007/S00213-012-2806-X

DeSilva, U., D'Arcangelo, G., Braden, V. v., Chen, J., Miao, G. G., Curran, T., & Green, E. D. (1997). The human reelin gene: isolation, sequencing, and mapping on chromosome 7. *Genome Research*, *7*(2), 157–164. https://doi.org/10.1101/GR.7.2.157

Diazgranados, N., Ibrahim, L., Brutsche, N. E., Newberg, A., Kronstein, P., Khalife, S., Kammerer, W. A., Quezado, Z., Luckenbaugh, D. A., Salvadore, G., Machado-Vieira, R., Manji, H. K., & Zarate, C. A. (2010). A Randomized Add-on Trial of an N-methyl-D-aspartate Antagonist in Treatment-Resistant Bipolar Depression. *Archives of General Psychiatry*, *67*(8), 793–802. https://doi.org/10.1001/ARCHGENPSYCHIATRY.2010.90

Dieterich, D. C., Karpova, A., Mikhaylova, M., Zdobnova, I., König, I., Landwehr, M., Kreutz, M., Smalla, K. H., Richter, K., Landgraf, P., Reissner, C., Boeckers, T. M., Zuschratter, W., Spilker, C., Seidenbecher, C. I., Garner, C. C., Gundelfinger, E. D., & Kreutz, M. R. (2008). Caldendrin-Jacob: A protein liaison that couples NMDA receptor signalling to the nucleus. *PLoS Biology*, *6*(2), 0286–0306. https://doi.org/10.1371/journal.pbio.0060034

Dimoula, A., Fotellis, D., Aivalioti, E., Delialis, D., Polissidis, A., Patras, R., ... & Stamatelopoulos, K. (2022). Off-Target Effects of Antidepressants on Vascular Function and Structure. *Biomedicines*, *10*(1), 56.

do Vale, E. M., Xavier, C. C., Nogueira, B. G., Campos, B. C., de Aquino, P. E. A., da Costa, R. O., Leal, L. K. A. M., de Vasconcelos, S. M. M., Neves, K. R. T., & de Barros Viana, G. S. (2016). Antinociceptive and Anti-Inflammatory Effects of Ketamine and the Relationship to Its Antidepressant Action and GSK3 Inhibition. *Basic & Clinical Pharmacology & Toxicology*, *119*(6), 562–573. https://doi.org/10.1111/BCPT.12637

Dogra, S., & Conn, P. J. (2021). Targeting metabotropic glutamate receptors for the treatment of depression and other stress-related disorders. *Neuropharmacology*, *196*, 108687. https://doi.org/10.1016/J.NEUROPHARM.2021.108687

Domino, E. F. (1999). History of modern psychopharmacology: A personal view with an emphasis on antidepressants. *Psychosomatic Medicine*, *61*(5), 591–598. https://doi.org/10.1097/00006842-199909000-00002

Domino, E. F., Domino, S. E., Smith, R. E., Domino, L. E., Goulet, J. R., Domino, K. E., & Zsigmond, E. K. (1984). Ketamine kinetics in unmedicated and diazepam-premedicated subjects. *Clinical Pharmacology & Therapeutics*, *36*(5), 645–653. https://doi.org/10.1038/CLPT.1984.235

Domino, E. F., & Warner, D. S. (2010). Taming the Ketamine Tiger. *Anesthesiology*, *113*(3), 678–684. https://doi.org/10.1097/ALN.0B013E3181ED09A2

Dong, E., Caruncho, H., Liu, W. S., Smalheiser, N. R., Grayson, D. R., Costa, E., & Guidotti, A. (2003). A reelin–integrin receptor interaction regulates Arc mRNA translation in synaptoneurosomes. *Proceedings of the National Academy of Sciences*, *100*(9), 5479–5484. https://doi.org/10.1073/PNAS.1031602100

Dong, J., Zhou, Q., Wei, Z., Yan, S., Sun, F., & Cai, X. (2018). Protein kinase A mediates scopolamine-induced mTOR activation and an antidepressant response. *Journal of Affective Disorders*, *227*, 633–642. https://doi.org/10.1016/J.JAD.2017.11.041

Doolin, K., Allers, K. A., Pleiner, S., Liesener, A., Farrell, C., Tozzi, L., O'Hanlon, E., Roddy, D., Frodl, T., Harkin, A., & O'Keane, V. (2018). Altered tryptophan catabolite concentrations in major depressive disorder and associated changes in hippocampal subfield volumes. *Psychoneuroendocrinology*, *95*, 8–17. https://doi.org/10.1016/J.PSYNEUEN.2018.05.019

Doss, M. X., & Sachinidis, A. (2019). Current challenges of iPSC-based disease modeling and therapeutic implications. *Cells*, *8*(5), 403. https://doi.org/10.3390/cells8050403

Dossat, A. M., Wright, K. N., Strong, C. E., & Kabbaj, M. (2018). Behavioral and biochemical sensitivity to low doses of ketamine: Influence of estrous cycle in C57BL/6 mice. *Neuropharmacology*, *130*, 30–41. https://doi.org/10.1016/J.NEUROPHARM.2017.11.022

Dowlatshahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (1998). Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet*, *352*(9142), 1754–1755. https://doi.org/10.1016/s0140-6736(05)79827-5

Dravid, S. M., Erreger, K., Yuan, H., Nicholson, K., Le, P., Lyuboslavsky, P., Almonte, A., Murray, E., Mosely, C., Barber, J., French, A., Balster, R., Murray, T. F., & Traynelis, S. F. (2007). Subunit-specific mechanisms and proton sensitivity of NMDA receptor channel block. *The Journal of Physiology*, *581*(1), 107–128. https://doi.org/10.1113/JPHYSIOL.2006.124958

du Jardin, K. G., Liebenberg, N., Cajina, M., Müller, H. K., Elfving, B., Sanchez, C., & Wegener, G. (2018). S-ketamine mediates its acute and sustained antidepressant-like activity through a 5-HT1B receptor dependent mechanism in a genetic rat model of depression. *Frontiers in Pharmacology*, *8*(JAN), 978. https://doi.org/10.3389/FPHAR.2017.00978

Du, X., & Pang, T. Y. (2015). Is dysregulation of the HPA-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Frontiers in Psychiatry*, *6*(MAR), 32. https://doi.org/10.3389/FPSYT.2015.00032

Dudek, K. A., Dion-Albert, L., Lebel, M., LeClair, K., Labrecque, S., Tuck, E., Perez, C. F., Golden, S. A., Tamminga, C., Turecki, G., Mechawar, N., Russo, S. J., & Menard, C. (2020). Molecular adaptations of the blood–brain barrier promote stress resilience vs. Depression. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(6), 3326–3336. https://doi.org/10.1073/PNAS.1914655117

Dulabon, L., Olson, E. C., Taglienti, M. G., Eisenhuth, S., McGrath, B., Walsh, C. A., Kreidberg, J. A., & Anton, E. S. (2000). Reelin Binds α3β1 Integrin and Inhibits Neuronal Migration. *Neuron*, *27*(1), 33–44. https://doi.org/10.1016/S0896-6273(00)00007-6

Duman, R. S., Nakagawa, S., & Malberg, J. (2001). Regulation of adult neurogenesis by antidepressant treatment. *Neuropsychopharmacology*, *25*(6), 836–844. https://doi.org/10.1016/s0893-133x(01)00358-x

Duman, R. S., Sanacora, G., & Krystal, J. H. (2019). Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron*, *102*(1), 75–90. https://doi.org/10.1016/J.NEURON.2019.03.013

Duman, R. S., Shinohara, R., Fogaça, M. v., & Hare, B. (2019). Neurobiology of rapid-acting antidepressants: convergent effects on GluA1-synaptic function. *Molecular Psychiatry 2019 24:12*, *24*(12), 1816–1832. https://doi.org/10.1038/s41380-019-0400-x

Durakoglugil, M. S., Chen, Y., White, C. L., Kavalali, E. T., & Herz, J. (2009). Reelin signaling antagonizes β-amyloid at the synapse. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(37), 15938–15943. https://doi.org/10.1073/PNAS.0908176106

Duric, V., Banasr, M., Stockmeier, C. A., Simen, A. A., Newton, S. S., Overholser, J. C., Jurjus, G. J., Dieter, L., & Duman, R. S. (2013). Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. *International Journal of Neuropsychopharmacology*, *16*(1), 69–82. https://doi.org/10.1017/S1461145712000016

Duval, K., Grover, H., Han, L. H., Mou, Y., Pegoraro, A. F., Fredberg, J., & Chen, Z. (2017). Modeling physiological events in 2D vs. 3D cell culture. *Physiology*, *32*(4), 266–277. https://doi.org/10.1152/PHYSIOL.00036.2016

Dwivedi, Y., Rizavi, H. S., Roberts, R. C., Conley, R. C., Tamminga, C. A., & Pandey, G. N. (2001). Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. *Journal of Neurochemistry*, *77*(3), 916–928. https://doi.org/10.1046/J.1471-4159.2001.00300.X

Eaton, W. W., Shao, H., Nestadt, G., Lee, B. H., Bienvenu, O. J., & Zandi, P. (2008). Population-Based Study of First Onset and Chronicity in Major Depressive Disorder. *Archives of General Psychiatry*, *65*(5), 513–520. https://doi.org/10.1001/ARCHPSYC.65.5.513

Edelstein, S. B., & Breakefield, X. O. (1986). Monoamine oxidases A and B are differentially regulated by glucocorticoids and 'aging' in human skin fibroblasts. *Cellular and Molecular Neurobiology*, *6*(2), 121–150. https://doi.org/10.1007/BF00711066/METRICS

Edinoff, A. N., Akuly, H. A., Hanna, T. A., Ochoa, C. O., Patti, S. J., Ghaffar, Y. A., Kaye, A. D., Viswanath, O., Urits, I., Boyer, A. G., Cornett, E. M., & Kaye, A. M. (2021). Selective serotonin reuptake inhibitors and adverse effects: A narrative review. *Neurology International*, *13*(3), 387-401. https://doi.org/10.3390/neurolint13030038

Eid, R. S., Gobinath, A. R., & Galea, L. A. M. (2019a). Sex differences in depression: Insights from clinical and preclinical studies. *Progress in Neurobiology*, *176*, 86–102. https://doi.org/10.1016/J.PNEUROBIO.2019.01.006

Eisch, A. J., & Petrik, D. (2012). Depression and hippocampal neurogenesis: A road to remission? *Science*, *338*(6103), 72–75.

Eisenmann, J. (2017). Translational Gap between Laboratory and Playing Field: New Era to Solve Old Problems in Sports Science. *Translational Journal of the American College of Sports Medicine*, *2*(8), 37–43.

Elberse, J. E., Caron-Flinterman, J. F., & Broerse, J. E. W. (2011). Patient–expert partnerships in research: how to stimulate inclusion of patient perspectives. *Health Expectations*, *14*(3), 225–239. https://doi.org/10.1111/J.1369-7625.2010.00647.X

Elhussiny, M. E. A., Carini, G., Mingardi, J., Tornese, P., Sala, N., Bono, F., Fiorentini, C., la Via, L., Popoli, M., Musazzi, L., & Barbon, A. (2021). Modulation by chronic stress and ketamine of ionotropic AMPA/NMDA and metabotropic glutamate receptors in the rat hippocampus. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *104*, 110033. https://doi.org/10.1016/J.PNPBP.2020.110033

Enache, D., Pariante, C. M., & Mondelli, V. (2019). Markers of central inflammation in major depressive disorder: A systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. *Brain, Behavior, and Immunity*, *81*, 24–40. https://doi.org/10.1016/J.BBI.2019.06.015

Engle, S. J., Blaha, L., & Kleiman, R. J. (2018). Best Practices for Translational Disease Modeling Using Human iPSC-Derived Neurons. *Neuron*, *100*(4), 783–797. https://doi.org/10.1016/J.NEURON.2018.10.033

Erb, S. J., Schappi, J. M., & Rasenick, M. M. (2016). Antidepressants accumulate in lipid rafts independent of monoamine transporters to modulate redistribution of the G protein, Goαs. *Journal of Biological Chemistry*, *291*(38), 19725–19733. https://doi.org/10.1074/jbc.M116.727263

Erickson, M. A., Morofuji, Y., Owen, J. B., & Banks, W. A. (2014). Rapid Transport of CCL11 across the Blood-Brain Barrier: Regional Variation and Importance of Blood Cells. *The Journal of Pharmacology and Experimental Therapeutics*, *349*(3), 497. https://doi.org/10.1124/JPET.114.213074

Essayan-Perez, S., Zhou, B., Nabet, A. M., Wernig, M., & Huang, Y. W. A. (2019). Modeling Alzheimer's disease with human iPS cells: advancements, lessons, and applications. *Neurobiology of Disease*, *130*, 104503. https://doi.org/10.1016/J.NBD.2019.104503

Evans, J. W., Szczepanik, J., Brutsché, N., Park, L. T., Nugent, A. C., & Zarate, C. A. (2018). Default Mode Connectivity in Major Depressive Disorder Measured Up to 10 Days After Ketamine Administration. *Biological Psychiatry*, *84*(8), 582–590. https://doi.org/10.1016/J.BIOPSYCH.2018.01.027

Faini, G., del Bene, F., & Albadri, S. (2021). Reelin functions beyond neuronal migration: from synaptogenesis to network activity modulation. *Current Opinion in Neurobiology*, *66*, 135–143. https://doi.org/10.1016/J.CONB.2020.10.009

Falconer, D. S. (1951). Two new mutants, 'trembler' and 'reeler', with neurological actions in the house mouse (Mus musculus L.). *Journal of Genetics*, *50*(2), 192–205. https://doi.org/10.1007/BF02996215

Fangmann, P., Assion, H. J., Juckel, G., González, C. Á., & López-Muñoz, F. (2008). Half a century of antidepressant drugs: On the clinical introduction of monoamine oxidase inhibitors, tricyclics, and tetracyclics. Part II: Tricyclics and tetracyclics. *Journal of Clinical Psychopharmacology*, *28*(1), 1–4. https://doi.org/10.1097/JCP.0B013E3181627B60

Farmer, C. A., Gilbert, J. R., Moaddel, R., George, J., Adeojo, L., Lovett, J., Nugent, A. C., Kadriu, B., Yuan, P., Gould, T. D., Park, L. T., & Zarate, C. A. (2020). Ketamine metabolites, clinical response, and gamma power in a randomized, placebo-controlled, crossover trial for treatment-resistant major depression. *Neuropsychopharmacology*, *45*(8), 1398–1404. https://doi.org/10.1038/s41386-020-0663-6

Fatemi, S. H. (2011). Reelin, a Marker of Stress Resilience in Depression and Psychosis. *Neuropsychopharmacology 2011 36:12*, *36*(12), 2371–2372. https://doi.org/10.1038/npp.2011.169

Fatemi, S. H., Earle, J. A., & McMenomy, T. (2000). Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. *Molecular Psychiatry*, *5*(6), 654–663. https://doi.org/10.1038/sj.mp.4000783

Fatemi, S. H., Kroll, J. L., & Stary, J. M. (2001). Altered levels of Reelin and its isoforms in schizophrenia and mood disorders. *NeuroReport*, *12*(15), 3209–3215. https://doi.org/10.1097/00001756-200110290-00014

Fava, M. (2003). Diagnosis and definition of treatment-resistant depression. *Biological Psychiatry*, *53*(8), 649–659. https://doi.org/10.1016/S0006-3223(03)00231-2

Fava, M., & Davidson, K. G. (1996). Definition and epidemiology of treatment-resistant depression. *Psychiatric Clinics of North America*, *19*(2), 179–200. https://doi.org/10.1016/S0193-953X(05)70283-5

Fenton, E. Y., Fournier, N. M., Lussier, A. L., Romay-Tallon, R., Caruncho, H. J., & Kalynchuk, L. E. (2015). Imipramine protects against the deleterious effects of chronic corticosterone on depression-like behavior, hippocampal reelin expression, and neuronal maturation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *60*, 52–59. https://doi.org/10.1016/J.PNPBP.2015.02.001

Ferbinteanu, J., Holsinger, R. M. D., & McDonald, R. J. (1999). Lesions of the medial or lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context. *Behavioural Brain Research*, *101*(1), 65–84. https://doi.org/10.1016/S0166-4328(98)00144-2

Fernandes, B., Gama, C. S., Massuda, R., Torres, M., Camargo, D., Kunz, M., Belmonte-de-Abreu, P. S., Kapczinski, F., de Almeida Fleck, M. P., & Inês Lobato, M. (2009). Serum brain-derived neurotrophic factor (BDNF) is not associated with response to electroconvulsive therapy (ECT): A pilot study in drug resistant depressed patients. *Neuroscience Letters*, *453*(3), 195–198. https://doi.org/10.1016/J.NEULET.2009.02.032

Fischell, J., van Dyke, A. M., Kvarta, M. D., Legates, T. A., & Thompson, S. M. (2015). Rapid Antidepressant Action and Restoration of Excitatory Synaptic Strength After Chronic Stress by Negative Modulators of Alpha5-Containing GABAA Receptors. *Neuropsychopharmacology 2015 40:11*, *40*(11), 2499–2509. https://doi.org/10.1038/npp.2015.112

Fitzgerald, P. J., & Watson, B. O. (2018). Gamma oscillations as a biomarker for major depression: an emerging topic. *Translational Psychiatry*, *8*(1), 1–7. https://doi.org/10.1038/s41398-018-0239-y

Fitzgerald, P. J., Yen, J. Y., & Watson, B. O. (2019). Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test. *PLoS ONE*, *14*(4), e0215554. https://doi.org/10.1371/journal.pone.0215554

Foerschner, A. M. (2010). The History of Mental Illness: From 'Skull Drills' to 'Happy Pills'. *Inquiries Journal*, *2*(09). http://www.inquiriesjournal.com/articles/1673/the-history-of-mental-illness-from-skull-drills-to-happy-pills

Förster, E., Tielsch, A., Saum, B., Weiss, K. H., Johanssen, C., Graus-Porta, D., Müller, U., & Frotscher, M. (2002). Reelin, Disabled 1, and β1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proceedings of the National Academy of Sciences*, *99*(20), 13178–13183. https://doi.org/10.1073/PNAS.202035899

Foster, A. C., Vezzani, A., French, E. D., & Schwarcz, R. (1984). Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neuroscience Letters*, *48*(3), 273–278. https://doi.org/10.1016/0304-3940(84)90050-8

Fraga, D. B., Camargo, A., Olescowicz, G., Padilha, D. A., Mina, F., Budni, J., Brocardo, P. S., & Rodrigues, A. L. S. (2021). Ketamine, but not fluoxetine, rapidly rescues corticosterone-induced impairments on glucocorticoid receptor and dendritic branching in the hippocampus of mice. *Metabolic Brain Disease*, *36*(8), 2223–2233. https://doi.org/10.1007/S11011-021-00743-2

Franceschelli, A., Sens, J., Herchick, S., Thelen, C., & Pitychoutis, P. M. (2015). Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naïve and "depressed" mice exposed to chronic mild stress. *Neuroscience*, *290*, 49–60. https://doi.org/10.1016/J.NEUROSCIENCE.2015.01.008

Freeman, M. P., Papakostas, G. I., Hoeppner, B., Mazzone, E., Judge, H., Cusin, C., Mathew, S., Sanacora, G., Iosifescu, D., DeBattista, C., Trivedi, M. H., & Fava, M. (2019). Sex differences in response to ketamine as a rapidly acting intervention for treatment resistant depression. *Journal of Psychiatric Research*, *110*, 166–171. https://doi.org/10.1016/J.JPSYCHIRES.2019.01.010

Freis, E. D. (1954). Mental Depression in Hypertensive Patients Treated for Long Periods with Large Doses of Reserpine. *New England Journal of Medicine*, *251*(25), 1006–1008. https://doi.org/10.1056/nejm195412162512504

Friedrich, M. J. (2017). Depression Is the Leading Cause of Disability Around the World. *JAMA*, *317*(15), 1517–1517. https://doi.org/10.1001/JAMA.2017.3826

Frodl, T., Meisenzahl, E. M., Zetzsche, T., Born, C., Groll, C., Jäger, M., Leinsinger, G., Bottlender, R., Hahn, K., & Möller, H. J. (2002). Hippocampal changes in patients with a first episode of major depression. *American Journal of Psychiatry*, *159*(7), 1112–1118.

Frye, M. A., Blier, P., & Tye, S. J. (2015). Implications for large scale study design and clinical development. *Journal of Clinical Psychopharmacology*, *35*(3), 334–336. https://doi.org/10.1097/JCP.0000000000000316

Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., Ozaki, N., Inoue, T., Kusumi, I., Koyama, T., Tsuchiyama, K., & Terao, T. (2011). DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS ONE*, *6*(8), e23881. https://doi.org/10.1371/journal.pone.0023881

Fujita, Y., Hashimoto, Y., Hashimoto, H., Chang, L., & Hashimoto, K. (2021). Dextran sulfate sodium-induced inflammation and colitis in mice are ameliorated by (R)-ketamine, but not (S)-ketamine: A role of TrkB signaling. *European Journal of Pharmacology*, *897*, 173954. https://doi.org/10.1016/J.EJPHAR.2021.173954

Funk, K. A., & Bostwick, J. R. (2013). A comparison of the risk of QT prolongation among SSRIs. *Annals of Pharmacotherapy*, *47*(10), 1330-1341.

Fusaki, N., Ban, H., Nishiyama, A., Saeki, K., & Hasegawa, M. (2009). Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proceedings of the Japan Academy, Series B*, *85*(8), 348–362. https://doi.org/10.2183/PJAB.85.348

Gareri, P., Falconi, U., de Fazio, P., & de Sarro, G. (2000). Conventional and new antidepressant drugs in the elderly. *Progress in Neurobiology*, *61*(4), 353–396. https://doi.org/10.1016/S0301-0082(99)00050-7

Gershon, E. S., Hamovit, J., Guroff, J. J., Dibble, E., Leckman, J. F., Sceery, W., Targum, S. D., Nurnberger, J. I., Goldin, L. R., & Bunney, W. E. (1982). A Family Study of Schizoaffective, Bipolar I, Bipolar II, Unipolar, and Normal Control Probands. *Archives of General Psychiatry*, *39*(10), 1157–1167. https://doi.org/10.1001/ARCHPSYC.1982.04290100031006

Giatti, S., Di Domizio, A., Diviccaro, S., Cioffi, L., Marmorini, I., Falvo, E., ... & Melcangi, R. C. (2022). Identification of a novel off-target of paroxetine: Possible role in sexual dysfunction induced by this SSRI antidepressant drug. *Journal of Molecular Structure*, 1268, 133690.

Gibbons, J. L., & McHugh, P. R. (1962). Plasma cortisol in depressive illness. *Journal of Psychiatric Research*, *1*(2), 162–171. https://doi.org/10.1016/0022-3956(62)90006-7

Gilbert, J. R., & Zarate, C. A. (2020). Electrophysiological biomarkers of antidepressant response to ketamine in treatment-resistant depression: Gamma power and long-term potentiation. *Pharmacology Biochemistry and Behavior*, *189*, 172856. https://doi.org/10.1016/J.PBB.2020.172856

Gilbert, P. E., & Brushfield, A. M. (2009). The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(5), 774–781. https://doi.org/10.1016/J.PNPBP.2009.03.037

Gilmore, E. C., & Herrup, K. (2000). Cortical development: Receiving Reelin. *Current Biology*, *10*(4), R162–R166. https://doi.org/10.1016/S0960-9822(00)00332-8

Ginex, T., Garaigorta, U., Ramírez, D., Castro, V., Nozal, V., Maestro, I., García-Cárceles, J., Campillo, N. E., Martinez, A., Gastaminza, P., & Gil, C. (2021). Host-directed FDA-approved drugs with antiviral activity against SARS-CoV-2 identified by hierarchical in silico/in vitro screening methods. *Pharmaceuticals*, *14*(4), 332. https://doi.org/10.3390/PH14040332/S1

Gjerstad, J. K., Lightman, S. L., & Spiga, F. (2018). Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress*, *21*(5), 403–416. https://doi.org/10.1080/10253890.2018.1470238

Goldberg, D. (2011). The heterogeneity of "major depression". *World Psychiatry*, *10*(3), 226. https://doi.org/10.1002/J.2051-5545.2011.TB00061.X

Goodman, J., & McIntyre, C. K. (2017). Impaired spatial memory and enhanced habit memory in a rat model of post-traumatic stress disorder. *Frontiers in Pharmacology*, *8*(SEP), 663. https://doi.org/10.3389/FPHAR.2017.00663

Gorzalka, B. B., & Hanson, L. A. (1998). Sexual behavior and wet dog shakes in the male rat: regulation by corticosterone. *Behavioural Brain Research*, *97*(1–2), 143–151. https://doi.org/10.1016/S0166-4328(98)00035-7

Gorzalka, B. B., Hanson, L. A., & Hong, J. J. (2001). Ketanserin attenuates the behavioural effects of corticosterone: Implications for 5-HT2A receptor regulation. *European Journal of Pharmacology*, *428*(2), 235–240. https://doi.org/10.1016/S0014-2999(01)01353-X

Gorzalka, B., Hanson, L., Harrington, J., Killam, S., & Campbell-Meiklejohn, D. (2003). Conditioned taste aversion: Modulation by 5-HT receptor activity and corticosterone. *European Journal of Pharmacology*, *471*(2), 129–134. https://doi.org/10.1016/S0014-2999(03)01790-4

Gotthardt, M., Trommsdorff, M., Nevitt, M. F., Shelton, J., Richardson, J. A., Stockinger, W., Nimpf, J., & Herz, J. (2000). Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *Journal of Biological Chemistry*, *275*(33), 25616–25624. https://doi.org/10.1074/jbc.M000955200

Gould, E., & Tanapat, P. (1999). Stress and hippocampal neurogenesis. *Biological Psychiatry*, *46*(11), 1472–1479. https://doi.org/10.1016/S0006-3223(99)00247-4

Gourley, S. L., Kiraly, D. D., Howell, J. L., Olausson, P., & Taylor, J. R. (2008). Acute Hippocampal Brain-Derived Neurotrophic Factor Restores Motivational and Forced Swim Performance After Corticosterone. *Biological Psychiatry*, *64*(10), 884–890. https://doi.org/10.1016/j.biopsych.2008.06.016

Gourley, S. L., & Taylor, J. R. (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodents. *Current Protocols in Neuroscience*, *49*(SUPPL), 9.32.1-9.32.11. https://doi.org/10.1002/0471142301.ns0932s49

Greenwald, M., Greenstein, D., Park, L., Acevedo-Diaz, E., Cavanaugh, G., & Zarate, C. A. (2020). Do Acute Experiences During Ketamine Infusions Influence its Antidepressant Effects? *Biological Psychiatry*, *87*(9), S276. https://doi.org/10.1016/j.biopsych.2020.02.713

Gregus, A., Wintink, A. J., Davis, A. C., & Kalynchuk, L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behavioural Brain Research*, *156*(1), 105–114. https://doi.org/10.1016/J.BBR.2004.05.013

Groc, L., Choquet, D., Stephenson, F. A., Verrier, D., Manzoni, O. J., & Chavis, P. (2007). NMDA Receptor Surface Trafficking and Synaptic Subunit Composition Are Developmentally Regulated by the Extracellular Matrix Protein Reelin. *Journal of Neuroscience*, *27*(38), 10165–10175. https://doi.org/10.1523/JNEUROSCI.1772-07.2007

Gross, M., Sheinin, A., Nesher, E., Tikhonov, T., Baranes, D., Pinhasov, A., & Michaelevski, I. (2015). Early onset of cognitive impairment is associated with altered synaptic plasticity and enhanced hippocampal GluA1 expression in a mouse model of depression. *Neurobiology of Aging*, *36*(5), 1938–1952. https://doi.org/10.1016/J.NEUROBIOLAGING.2015.02.015

Grossert, A., Mehrjardi, N. Z., Bailey, S. J., Lindsay, M. A., Hescheler, J., Šarić, T., & Teusch, N. (2019). Ketamine increases proliferation of human iPSC-derived neuronal progenitor cells via insulin-like growth factor 2 and independent of the NMDA receptor. *Cells*, *8*(10), 1139. https://doi.org/10.3390/cells8101139

Grunebaum, M. F., Ellis, S. P., Li, S., Oquendo, M. A., & Mann, J. J. (2004). Antidepressants and Suicide Risk in the United States, 1985-1999. *The Journal of Clinical Psychiatry*, *65*(11), 1017. https://dev.psychiatrist.com/jcp/depression/suicide/antidepressants-suicide-risk-united-states

Grunwald, L.-M. (2018). iPSC-derived cortical neurons from patients with schizophrenia exhibit changes in early neuronal development. *Dissertation - Der Eberhard Karls Universität Tübingen*.

Guglielmo, G. de, Iemolo, A., Nur, A., Turner, A., Montilla-Perez, P., & Telese, F. (2022). Reelin deficiency exacerbates cocaine-induced hyperlocomotion by enhancing neuronal activity in the dorsomedial striatum. *BioRxiv*, 2022.04.12.488105. https://doi.org/10.1101/2022.04.12.488105

Guidotti, A., Auta, J., Davis, J. M., Gerevini, V. D., Dwivedi, Y., Grayson, D. R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., & Costa, E. (2000). Decrease in Reelin and Glutamic Acid Decarboxylase67 (GAD67) Expression in Schizophrenia and Bipolar Disorder: A Postmortem Brain Study. *Archives of General Psychiatry*, *57*(11), 1061–1069. https://doi.org/10.1001/ARCHPSYC.57.11.1061

Guidotti, A., Grayson, D. R., & Caruncho, H. J. (2016). Epigenetic RELN dysfunction in schizophrenia and related neuropsychiatric disorders. *Frontiers in Cellular Neuroscience*, *10*(APR), 89. https://doi.org/10.3389/fncel.2016.00089

Guo, L., Chen, Y. X., Hu, Y. T., Wu, X. Y., He, Y., Wu, J. L., Huang, M. L., Mason, M., & Bao, A. M. (2018). Sex hormones affect acute and chronic stress responses in sexually dimorphic patterns: Consequences for depression models. *Psychoneuroendocrinology*, *95*, 34–42. https://doi.org/10.1016/J.PSYNEUEN.2018.05.016

Gururajan, A., Clarke, G., Dinan, T. G., & Cryan, J. F. (2016). Molecular biomarkers of depression. *Neuroscience & Biobehavioral Reviews*, *64*, 101–133. https://doi.org/10.1016/J.NEUBIOREV.2016.02.011

Hack, I., Hellwig, S., Junghans, D., Brunne, B., Bock, H. H., Zhao, S., & Frotscher, M. (2007). Divergent roles of ApoER2 and Vldlr in the migration of cortical neurons. *Development*, *134*(21), 3883–3891. https://doi.org/10.1242/DEV.005447

Hall, O. T., Jordan, A., Teater, J., Dixon-Shambley, K., McKiever, M. E., Baek, M., Garcia, S., Rood, K. M., & Fielin, D. A. (2022). Experiences of racial discrimination in the medical setting and associations with medical mistrust and expectations of care among black patients seeking addiction treatment. *Journal of Substance Abuse Treatment*, *133*, 108551. https://doi.org/10.1016/J.JSAT.2021.108551

Hamann, J., Cohen, R., Leucht, S., Busch, R., & Kissling, W. (2007). Shared Decision Making and Long-Term Outcome in Schizophrenia Treatment. *The Journal of Clinical Psychiatry*, *68*(7), 19493. https://www.psychiatrist.com/jcp/schizophrenia/shared-decision-making-long-term-outcome-schizophrenia

Hamann, J., Langer, B., Winkler, V., Busch, R., Cohen, R., Leucht, S., & Kissling, W. (2006). Shared decision making for in-patients with schizophrenia. *Acta Psychiatrica Scandinavica*, *114*(4), 265–273. https://doi.org/10.1111/J.1600-0447.2006.00798.X

Hamburgh, M. (1963). Analysis of the postnatal developmental effects of "reeler," a neurological mutation in mice. A study in developmental genetics. *Developmental Biology*, *8*(2), 165–185. https://doi.org/10.1016/0012-1606(63)90040-X

Hamed, M. G. M., & Hagag, R. S. (2020). The possible immunoregulatory and anti-inflammatory effects of selective serotonin reuptake inhibitors in coronavirus disease patients. *Medical Hypotheses*, *144*, 110140. https://doi.org/10.1016/J.MEHY.2020.110140

Handelmann, G. E., & Olton, D. S. (1981). Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. *Brain Research*, *217*(1), 41–58. https://doi.org/10.1016/0006-8993(81)90183-9

Hanson, L. A., & Gorzalka, B. B. (1999). The influence of corticosterone on serotonergic stereotypy and sexual behavior in the female rat. *Behavioural Brain Research*, *104*(1–2), 27–35. https://doi.org/10.1016/S0166-4328(99)00046-7

Hardingham, G. E., & Bading, H. (2002). Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1600*(1–2), 148–153. https://doi.org/10.1016/S1570-9639(02)00455-7

Hardingham, G. E., & Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: Implications for neurodegenerative disorders. *Nature Reviews Neuroscience*, *11*(10), 682-696. https://doi.org/10.1038/nrn2911

Hare, B. D., Shinohara, R., Liu, R. J., Pothula, S., DiLeone, R. J., & Duman, R. S. (2019). Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. *Nature Communications*, *10*(1), 1–12. https://doi.org/10.1038/s41467-018-08168-9

Harvey, L., & Boksa, P. (2012). A stereological comparison of GAD67 and reelin expression in the hippocampal stratum oriens of offspring from two mouse models of maternal inflammation during pregnancy. *Neuropharmacology*, *62*(4), 1767–1776. https://doi.org/10.1016/J.NEUROPHARM.2011.11.022

Harvey, P. O., Fossati, P., Pochon, J. B., Levy, R., LeBastard, G., Lehéricy, S., Allilaire, J. F., & Dubois, B. (2005). Cognitive control and brain resources in major depression: An fMRI study using the n-back task. *NeuroImage*, *26*(3), 860–869. https://doi.org/10.1016/J.NEUROIMAGE.2005.02.048

Hasegawa, Y., Zhu, X., & Kamiya, A. (2019). NV-5138 as a fast-acting antidepressant via direct activation of mTORC1 signaling. *The Journal of Clinical Investigation*, *129*(6), 2207–2209. https://doi.org/10.1172/JCI129702

Hashimoto, K., Sawa, A., & Iyo, M. (2007). Increased Levels of Glutamate in Brains from Patients with Mood Disorders. *Biological Psychiatry*, *62*(11), 1310–1316. https://doi.org/10.1016/J.BIOPSYCH.2007.03.017

Hayashi, Y., Nabeshima, Y., Kobayashi, K., Miyakawa, T., Tanda, K., Takao, K., Suzuki, H., Esumi, E., Noguchi, S., Matsuda, Y., Sasaoka, T., Noda, T., Miyazaki, J. I., Mishina, M., Funabiki, K., & Nabeshima, Y. I. (2014). Enhanced stability of hippocampal place representation caused by reduced magnesium block of NMDA receptors in the dentate gyrus. *Molecular Brain*, *7*(1), 1–17. https://doi.org/10.1186/1756-6606-7-44

He, T., Wang, D., Wu, Z., Huang, C., Xu, X., Xu, X., Liu, C., Hashimoto, K., & Yang, C. (2022). A bibliometric analysis of research on (R)-ketamine from 2002 to 2021. *Neuropharmacology*, *218*, 109207. https://doi.org/10.1016/J.NEUROPHARM.2022.109207

Hebb, D. O. (1949). The first stage of perception: growth of the assembly. *The Organization of Behavior*, *4*, 60–78. https://doi.org/10.1016/0301-0082(84)90021-2

Heils, A., Teufel, A., Petri, S., Seemann, M., Bengel, D., Balling, U., Riederer, P., & Lesch, K. P. (1995). Functional promoter and polyadenylation site mapping of the human serotonin

(5-HT) transporter gene. *Journal of Neural Transmission*, *102*(3), 247–254. https://doi.org/10.1007/BF01281159

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: Insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710. https://doi.org/10.1016/J.PSYNEUEN.2008.03.008

Heiske, A., Jesberg, J., Krieg, J. C., & Vedder, H. (2003). Differential effects of antidepressants on glucocorticoid receptors in human primary blood cells and human monocytic U-937 cells. *Neuropsychopharmacology*, *28*(4), 807–817. https://doi.org/10.1038/sj.npp.1300056

Hellwig, S., Hack, I., Kowalski, J., Brunne, B., Jarowyj, J., Unger, A., Bock, H. H., Junghans, D., & Frotscher, M. (2011). Role for Reelin in Neurotransmitter Release. *Journal of Neuroscience*, *31*(7), 2352–2360. https://doi.org/10.1523/JNEUROSCI.3984-10.2011

Herd, D. W., Anderson, B. J., Keene, N. A., & Holford, N. H. G. (2008). Investigating the pharmacodynamics of ketamine in children. *Pediatric Anesthesia*, *18*(1), 36–42. https://doi.org/10.1111/J.1460-9592.2007.02384.X

Hergovich, N., Singer, E., Agneter, E., Eichler, H. G., Graselli, U., Simhandl, C., & Jilma, B. (2001). Comparison of the Effects of Ketamine and Memantine on Prolactin and Cortisol Release in Men: A Randomized, Double-blind, Placebo-controlled Trial. *Neuropsychopharmacology*, *24*(5), 590–593. https://doi.org/10.1016/s0893-133x(00)00194-9

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in Neurosciences*, *20*(2), 78–84. https://doi.org/10.1016/S0166-2236(96)10069-2

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201–1213. https://doi.org/10.1016/J.PNPBP.2005.08.006

Herring, A., Donath, A., Steiner, K. M., Widera, M. P., Hamzehian, S., Kanakis, D., Kölble, K., Elali, A., Hermann, D. M., Paulus, W., & Keyvani, K. (2012). Reelin Depletion is an Early Phenomenon of Alzheimer's Pathology. *Journal of Alzheimer's Disease*, *30*(4), 963–979. https://doi.org/10.3233/JAD-2012-112069

Hesselgrave, N., Troppoli, T. A., Wulff, A. B., Cole, A. B., & Thompson, S. M. (2021). Harnessing psilocybin: Antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(17), e2022489118.

Hethorn, W. R., Ciarlone, S. L., Filonova, I., Rogers, J. T., Aguirre, D., Ramirez, R. A., Grieco, J. C., Peters, M. M., Gulick, D., Anderson, A. E., L. Banko, J., Lussier, A. L., & Weeber, E. J. (2015). Reelin supplementation recovers synaptic plasticity and cognitive deficits in a

mouse model for Angelman syndrome. *European Journal of Neuroscience*, *41*(10), 1372–1380. https://doi.org/10.1111/EJN.12893

Hibi, T., & Hattori, M. (2009). The N-terminal fragment of Reelin is generated after endocytosis and released through the pathway regulated by Rab11. *FEBS Letters*, *583*(8), 1299–1303. https://doi.org/10.1016/J.FEBSLET.2009.03.024

Hibicke, M., Landry, A. N., Kramer, H. M., Talman, Z. K., & Nichols, C. D. (2020). Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression. *ACS Applied Materials and Interfaces*.

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A., Mumby, M. C., Cooper, J. A., & Herz, J. (1999). Direct Binding of Reelin to VLDL Receptor and ApoE Receptor 2 Induces Tyrosine Phosphorylation of Disabled-1 and Modulates Tau Phosphorylation. *Neuron*, *24*(2), 481–489. https://doi.org/10.1016/S0896-6273(00)80861-2

Hill, R. A., Klug, M., Kiss Von Soly, S., Binder, M. D., Hannan, A. J., & van den Buuse, M. (2014). Sex-specific disruptions in spatial memory and anhedonia in a 'two hit' rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus*, *24*(10), 1197–1211. https://doi.org/10.1002/hipo.22302

Hirota, K., & Lambert, D. G. (2022). Ketamine; history and role in anesthetic pharmacology. *Neuropharmacology*, *216*, 109171. https://doi.org/10.1016/J.NEUROPHARM.2022.109171

Hirschfeld, R. M. A. (2001). The comorbidity of major depression and anxiety disorders: Recognition and management in primary care. *Primary Care Companion to the Journal of Clinical Psychiatry*, *3*(6), 244–254. https://doi.org/10.4088/pcc.v03n0609

Hitti, F. L., & Siegelbaum, S. A. (2014). The hippocampal CA2 region is essential for social memory. *Nature*, *508*(1), 88–92. https://doi.org/10.1038/nature13028

Hliák, Z., & Krejčí, I. (2002). MK-801 induced amnesia for the elevated plus-maze in mice. *Behavioural Brain Research*, *131*(1–2), 221–225. https://doi.org/10.1016/S0166-4328(01)00347-3

Ho, B. X., Pek, N. M. Q., & Soh, B. S. (2018). Disease Modeling Using 3D Organoids Derived from Human Induced Pluripotent Stem Cells. *International Journal of Molecular Sciences*, *19*(4), 936. https://doi.org/10.3390/IJMS19040936

Ho, M. F., Zhang, C., Moon, I., Zhu, X., Coombes, B. J., Biernacka, J., Skime, M., Oesterle, T. S., Karpyak, V. M., Schmidt, K., Gliske, K., Ngo, Q., Skillon, C., Seppala, M. D., Li, H., & Weinshilboum, R. M. (2022). Single cell transcriptomics reveals distinct transcriptional responses to oxycodone and buprenorphine by iPSC-derived brain organoids from patients with opioid use disorder. *Molecular Psychiatry*, 1–11. https://doi.org/10.1038/s41380-022-01837-8

Hoffman, A. N., Paode, P. R., May, H. G., Ortiz, J. B., Kemmou, S., Lifshitz, J., Conrad, C. D., & Currier Thomas, T. (2017). Early and persistent dendritic hypertrophy in the basolateral

amygdala following experimental diffuse traumatic brain injury. *Journal of Neurotrauma*, *34*(1), 213–219. https://doi.org/10.1089/NEU.2015.4339

Holick, K. A., Lee, D. C., Hen, R., & Dulawa, S. C. (2007). Behavioral Effects of Chronic Fluoxetine in BALB/cJ Mice Do Not Require Adult Hippocampal Neurogenesis or the Serotonin 1A Receptor. *Neuropsychopharmacology 2008 33:2*, *33*(2), 406–417. https://doi.org/10.1038/sj.npp.1301399

Hollis, C., Sampson, S., Simons, L., Davies, E. B., Churchill, R., Betton, V., Butler, D., Chapman, K., Easton, K., Gronlund, T. A., Kabir, T., Rawsthorne, M., Rye, E., & Tomlin, A. (2018). Identifying research priorities for digital technology in mental health care: results of the James Lind Alliance Priority Setting Partnership. *The Lancet Psychiatry*, *5*(10), 845–854. https://doi.org/10.1016/S2215-0366(18)30296-7

Hollon, S. D., Stewart, M. O., & Strunk, D. (2006). Enduring effects for cognitive behavior therapy in the treatment of depression and anxiety. *Annual Review of Psychology, 57*, 285-315. https://doi.org/10.1146/annurev.psych.57.102904.190044

Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implicatons for therapy. *Journal of Affective Disorders*, *62*(1–2), 77–91. https://doi.org/10.1016/S0165-0327(00)00352-9

Howland, J. G., & Cazakoff, B. N. (2010). Effects of acute stress and GluN2B-containing NMDA receptor antagonism on object and object–place recognition memory. *Neurobiology of Learning and Memory*, *93*(2), 261–267. https://doi.org/10.1016/J.NLM.2009.10.006

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., ... & Humphrey, P. P. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacological reviews*, *46*(2), 157-203.

Hoyo-Becerra, C., Schlaak, J. F., & Hermann, D. M. (2014). Insights from interferon-α-related depression for the pathogenesis of depression associated with inflammation. *Brain, Behavior, and Immunity*, *42*, 222–231. https://doi.org/10.1016/J.BBI.2014.06.200

Hu, C., Luo, Y., Wang, H., Kuang, S., Liang, G., Yang, Y., Mai, S., & Yang, J. (2017). Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress. *PLoS ONE*, *12*(9), e0185129. https://doi.org/10.1371/JOURNAL.PONE.0185129

Huang, C. Y., Liu, C. L., Ting, C. Y., Chiu, Y. T., Cheng, Y. C., Nicholson, M. W., & Hsieh, P. C. H. (2019). Human iPSC banking: barriers and opportunities. *Journal of Biomedical Science 2019 26:1*, *26*(1), 1–14. https://doi.org/10.1186/S12929-019-0578-X

Huang, H., Winter, E. E., Wang, H., Weinstock, K. G., Xing, H., Goodstadt, L., Stenson, P. D., Cooper, D. N., Smith, D., Albà, M. M., Ponting, C. P., & Fechtel, K. (2004). Evolutionary conservation and selection of human disease gene orthologs in the rat and mouse genomes. *Genome Biology*, *5*(7), 1–15. https://doi.org/10.1186/GB-2004-5-7-R47

Huang, T., Balasubramanian, R., Yao, Y., Clish, C. B., Shadyab, A. H., Liu, B., ... & Hankinson, S. E. (2021). Associations of depression status with plasma levels of candidate lipid and amino acid metabolites: a meta-analysis of individual data from three independent samples of US postmenopausal women. *Molecular psychiatry*, 26(7), 3315-3327.

Huettner, J. E., & Bean, B. P. (1988). Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proceedings of the National Academy of Sciences*, *85*(4), 1307–1311. https://doi.org/10.1073/PNAS.85.4.1307

Huynh, N. N., & McIntyre, R. S. (2008). What are the implications of the STAR*D trial for primary care? A review and synthesis. *Primary Care Companion to the Journal of Clinical Psychiatry*, *10*(2), 91–96. https://doi.org/10.4088/pcc.v10n0201

Ibi, D., Nakasai, G., Koide, N., Sawahata, M., Kohno, T., Takaba, R., Nagai, T., Hattori, M., Nabeshima, T., Yamada, K., & Hiramatsu, M. (2020). Reelin Supplementation Into the Hippocampus Rescues Abnormal Behavior in a Mouse Model of Neurodevelopmental Disorders. *Frontiers in Cellular Neuroscience*, *14*, 285. https://doi.org/10.3389/FNCEL.2020.00285

Ibrahim, L., Diazgranados, N., Luckenbaugh, D. A., Machado-Vieira, R., Baumann, J., Mallinger, A. G., & Zarate, C. A. (2011). Rapid decrease in depressive symptoms with an N-methyl-d-aspartate antagonist in ECT-resistant major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(4), 1155–1159. https://doi.org/10.1016/j.pnpbp.2011.03.019

Ichihara, H., Jingami, H., & Toh, H. (2001). Three novel repetitive units of reelin. *Molecular Brain Research*, *97*(2), 190–193. https://doi.org/10.1016/S0169-328X(01)00307-2

Iemolo, A., Montilla-Perez, P., Nguyen, J., Risbrough, V. B., Taffe, M. A., & Telese, F. (2021). Reelin deficiency contributes to long-term behavioral abnormalities induced by chronic adolescent exposure to Δ9-tetrahydrocannabinol in mice. *Neuropharmacology*, *187*, 108495. https://doi.org/10.1016/J.NEUROPHARM.2021.108495

Ilango, A., Shumake, J., Wetzel, W., Scheich, H., & Ohl, F. W. (2013). Electrical Stimulation of Lateral Habenula during Learning: Frequency-Dependent Effects on Acquisition but Not Retrieval of a Two-Way Active Avoidance Response. *PLoS ONE*, *8*(6), e65684. https://doi.org/10.1371/journal.pone.0065684

Impagnatiello, F., Guidotti, A. R., Pesold, C., Dwivedi, Y., Caruncho, H., Pisu, M. G., Uzunov, D. P., Smalheiser, N. R., Davis, J. M., Pandey, G. N., Pappas, G. D., Tueting, P., Sharma, R. P., & Costa, E. (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proceedings of the National Academy of Sciences*, *95*(26), 15718–15723. https://doi.org/10.1073/PNAS.95.26.15718

Insausti, R., & Amaral, D. G. (2003). Hippocampal Formation. *The Human Nervous System: Second Edition*, 871-914. https://doi.org/10.1016/B978-012547626-3/50024-7

Iob, E., Kirschbaum, C., & Steptoe, A. (2019). Persistent depressive symptoms, HPA-axis hyperactivity, and inflammation: the role of cognitive-affective and somatic symptoms. *Molecular Psychiatry*, *25*(5), 1130–1140. https://doi.org/10.1038/s41380-019-0501-6

Iro, C. M., Hamati, R., el Mansari, M., & Blier, P. (2021). Repeated but Not Single Administration of Ketamine Prolongs Increases of the Firing Activity of Norepinephrine and Dopamine Neurons. *International Journal of Neuropsychopharmacology*, *24*(7), 570–579. https://doi.org/10.1093/IJNP/PYAB010

Isosaka, T., Hattori, K., & Yagi, T. (2006). NMDA-receptor proteins are upregulated in the hippocampus of postnatal heterozygous reeler mice. *Brain Research*, *1073–1074*(1), 11–19. https://doi.org/10.1016/J.BRAINRES.2005.12.049

Ivy, A. S., Brunson, K. L., Sandman, C., & Baram, T. Z. (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: A clinically relevant model for early-life stress. *Neuroscience*, *154*(3), 1132–1142. https://doi.org/10.1016/J.NEUROSCIENCE.2008.04.019

Izsak, J., Seth, H., Theiss, S., Hanse, E., & Illes, S. (2020). Human Cerebrospinal Fluid Promotes Neuronal Circuit Maturation of Human Induced Pluripotent Stem Cell-Derived 3D Neural Aggregates. *Stem Cell Reports*, *14*(6), 1044–1059. https://doi.org/10.1016/J.STEMCR.2020.05.006

Jaako, K., Aonurm-Helm, A., Kalda, A., Anier, K., Zharkovsky, T., Shastin, D., & Zharkovsky, A. (2011). Repeated citalopram administration counteracts kainic acid-induced spreading of PSA-NCAM-immunoreactive cells and loss of reelin in the adult mouse hippocampus. *European Journal of Pharmacology*, *666*(1–3), 61–71. https://doi.org/10.1016/J.EJPHAR.2011.05.008

Jackson, J. L., Shimeall, W., Sessums, L., DeZee, K. J., Becher, D., Diemer, M., Berbano, E., & O'Malley, P. G. (2010). Tricyclic antidepressants and headaches: systematic review and meta-analysis. *BMJ*, *341*(7778), 869. https://doi.org/10.1136/BMJ.C5222

Jaiswal, M., Zech, W. D., Goos, M., Leutbecher, C., Ferri, A., Zippelius, A., Carrì, M. T., Nau, R., & Keller, B. U. (2009). Impairment of mitochondrial calcium handling in a mtSOD1 cell culture model of motoneuron disease. *BMC Neuroscience*, *10*(1), 1–16. https://doi.org/10.1186/1471-2202-10-64/FIGURES/7

James Lind Alliance (2020). *About Priority Setting Partnerships | James Lind Alliance*. National Institute for Health Research. https://www.jla.nihr.ac.uk/about-the-james-lind-alliance/about-psps.htm

Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. *Behavioral Neuroscience*, *97*(6), 873–889. https://doi.org/10.1037/0735-7044.97.6.873

Jesse, S., Steinacker, P., Lehnert, S., Gillardon, F., Hengerer, B., & Otto, M. (2009). Neurochemical Approaches in the Laboratory Diagnosis of Parkinson and Parkinson

Dementia Syndromes: A Review. *CNS Neuroscience & Therapeutics*, *15*(2), 157–182. https://doi.org/10.1111/J.1755-5949.2008.00064.X

JiaWen, W., Hong, S., ShengXiang, X., & Jing, L. (2018). Depression- and anxiety-like behaviour is related to BDNF/TrkB signalling in a mouse model of psoriasis. *Clinical and Experimental Dermatology*, *43*(3), 254–261. https://doi.org/10.1111/CED.13378

Johansen, J. P., Cain, C. K., Ostroff, L. E., & Ledoux, J. E. (2011). Molecular Mechanisms of Fear Learning and Memory. *Cell*, *147*(3), 509–524. https://doi.org/10.1016/J.CELL.2011.10.009

Johnson, S. A., Fournier, N. M., & Kalynchuk, L. E. (2006). Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. *Behavioural Brain Research*, *168*(2), 280–288. https://doi.org/10.1016/j.bbr.2005.11.019

Johnston, J. N., Campbell, D., Caruncho, H. J., Henter, I. D., Ballard, E. D., & Zarate, C. A. (2022). Suicide Biomarkers to Predict Risk, Classify Diagnostic Subtypes, and Identify Novel Therapeutic Targets: 5 Years of Promising Research. *International Journal of Neuropsychopharmacology*, *25*(3), 197-214. https://doi.org/10.1093/ijnp/pyab083

Johnston, J.N., Greenwald, M.S., Henter, I.D., Kraus, C., Mkrtchian, A., Clark, N.G., Park, L.T., Gold, P., Zarate, C.A., Kadriu, B. (2023). Inflammation, Stress, and Depression: An Exploration of Ketamine's Therapeutic Profile. *Drug Discovery Today, in press.*

Johnston, J. N., Ridgway, L., Cary-Barnard, S., Allen, J., Sanchez-Lafuente, C. L., Reive, B., ... & Caruncho, H. J. (2021). Patient oriented research in mental health: matching laboratory to life and beyond in Canada. *Research Involvement and Engagement*, *7*(1), 1-11.

Johnston, J. N., Thacker, J. S., Desjardins, C., Kulyk, B. D., Romay-Tallon, R., Kalynchuk, L. E., & Caruncho, H. J. (2020). Ketamine Rescues Hippocampal Reelin Expression and Synaptic Markers in the Repeated-Corticosterone Chronic Stress Paradigm. *Frontiers in Pharmacology*, *11*, 1387. https://doi.org/10.3389/fphar.2020.559627

Jossin, Y. (2020). Reelin functions, mechanisms of action and signaling pathways during brain development and maturation. *Biomolecules* 10(6), 964. https://doi.org/10.3390/biom10060964

Jossin, Y., & Goffinet, A. M. (2007). Reelin Signals through Phosphatidylinositol 3-Kinase and Akt To Control Cortical Development and through mTor To Regulate Dendritic Growth. *Molecular and Cellular Biology*, *27*(20), 7113–7124.

Jossin, Y., Ignatova, N., Hiesberger, T., Herz, J., Lambert De Rouvroit, C., & Goffinet, A. M. (2004a). The Central Fragment of Reelin, Generated by Proteolytic Processing In Vivo, Is Critical to Its Function during Cortical Plate Development. *Journal of Neuroscience*, *24*(2), 514–521. https://doi.org/10.1523/JNEUROSCI.3408-03.2004

Kadriu, B., Greenwald, M., Henter, I. D., Gilbert, J. R., Kraus, C., Park, L. T., & Zarate, C. A. (2021). Ketamine and Serotonergic Psychedelics: Common Mechanisms Underlying the

Effects of Rapid-Acting Antidepressants. *International Journal of Neuropsychopharmacology*, *24*(1), 8–21. https://doi.org/10.1093/IJNP/PYAA087

Kadriu, B., Musazzi, L., Johnston, J. N., Kalynchuk, L. E., Caruncho, H. J., Popoli, M., & Zarate, C. A. (2021). Positive AMPA receptor modulation in the treatment of neuropsychiatric disorders: A long and winding road. *Drug Discovery Today*, *26*(12), 2816-2838. https://doi.org/10.1016/j.drudis.2021.07.027

Kallarackal, A. J., Kvarta, M. D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A. M., & Thompson, S. M. (2013). Chronic Stress Induces a Selective Decrease in AMPA Receptor-Mediated Synaptic Excitation at Hippocampal Temporoammonic-CA1 Synapses. *Journal of Neuroscience*, *33*(40), 15669–15674. https://doi.org/10.1523/JNEUROSCI.2588-13.2013

Kalynchuk, L. E., Gregus, A., Boudreau, D., & Perrot-Sinal, T. S. (2004). Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behavioral Neuroscience*, *118*(6), 1365–1377. https://doi.org/10.1037/0735-7044.118.6.1365

Kambeitz, J. P., & Howes, O. D. (2015). The serotonin transporter in depression: Meta-analysis of in vivo and post mortem findings and implications for understanding and treating depression. *Journal of Affective Disorders*, *186*, 358–366. https://doi.org/10.1016/J.JAD.2015.07.034

Kammers, K., Taub, M. A., Ruczinski, I., Martin, J., Yanek, L. R., Frazee, A., Gao, Y., Hoyle, D., Faraday, N., Becker, D. M., Cheng, L., Wang, Z. Z., Leek, J. T., Becker, L. C., & Mathias, R. A. (2017). Integrity of Induced Pluripotent Stem Cell (iPSC) Derived Megakaryocytes as Assessed by Genetic and Transcriptomic Analysis. *PLoS ONE*, *12*(1), e0167794. https://doi.org/10.1371/JOURNAL.PONE.0167794

Kandel, E. (2013). The New Science of Mind and the Future of Knowledge. *Neuron*, *80*(3), 546–560. https://doi.org/10.1016/J.NEURON.2013.10.039

Kantrowitz, J. T., Dong, Z., Milak, M. S., Rashid, R., Kegeles, L. S., Javitt, D. C., ... & John Mann, J. (2021). Ventromedial prefrontal cortex/anterior cingulate cortex Glx, glutamate, and GABA levels in medication-free major depressive disorder. *Translational psychiatry*, 11(1), 419.

Kato, M., & Dobyns, W. B. (2003). Lissencephaly and the molecular basis of neuronal migration. *Human Molecular Genetics*, *12*(suppl_1), R89–R96. https://doi.org/10.1093/HMG/DDG086

Katz, R. J., Roth, K. A., & Carroll, B. J. (1981). Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience & Biobehavioral Reviews*, *5*(2), 247–251. https://doi.org/10.1016/0149-7634(81)90005-1

Kavalali, E. T., & Monteggia, L. M. (2015). How does ketamine elicit a rapid antidepressant response? *Current Opinion in Pharmacology*, *20*, 35–39. https://doi.org/10.1016/J.COPH.2014.11.005

Kawasaki, T., Ogata, M., Kawasaki, C., Ogata, J., Inoue, Y., & Shigematsu, A. (1999). Ketamine Suppresses Proinflammatory Cytokine Production in Human Whole Blood In Vitro. *Anesthesia & Analgesia*, *89*(3), 665. https://doi.org/10.1213/00000539-199909000-00024

Kelly, S., Lafortune, L., Hart, N., Cowan, K., Fenton, M., & Brayne, C. (2015). Dementia priority setting partnership with the James Lind Alliance: Using patient and public involvement and the evidence base to inform the research agenda. *Age and Ageing*, *44*(6), 985–993. https://doi.org/10.1093/ageing/afv143

Kelly, W. F., Checkley, S. A., Bender, D. A., & Mashiter, K. (1983). Cushing's Syndrome and Depression—A Prospective Study of 26 Patients. *The British Journal of Psychiatry*, *142*(1), 16–19. https://doi.org/10.1192/BJP.142.1.16

Kendall, K. M., van Assche, E., Andlauer, T. F. M., Choi, K. W., Luykx, J. J., Schulte, E. C., & Lu, Y. (2021). The genetic basis of major depression. *Psychological Medicine*, *51*(13), 2217–2230. https://doi.org/10.1017/S0033291721000441

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1999). Causal relationship between stressful life events and the onset of major depression. *American Journal of Psychiatry*, *156*(6), 837–841.

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2000). Stressful life events and previous episodes in the etiology of major depression in women: An evaluation of the 'kindling' hypothesis. *American Journal of Psychiatry*, *157*(8), 1243–1251.

Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *American Journal of Psychiatry*, *158*(4), 582–586.

Kendler, K. S., Thornton, L. M., & Prescott, C. A. (2001). Gender differences in the rates of exposure to stressful life events and sensitivity to their depressogenic effects. *American Journal of Psychiatry*, *158*(4), 587–593.

Kennedy, S. H., Javanmard, M., & Vaccarino, F. J. (1997). A review of functional neuroimaging in mood disorders: Positron emission tomography and depression. *Canadian Journal of Psychiatry*, *42*(5), 467-475. https://doi.org/10.1177/070674379704200502

Kesner, R. P. (2013). An analysis of the dentate gyrus function. *Behavioural Brain Research*, *254*, 1–7. https://doi.org/10.1016/J.BBR.2013.01.012

Kessing, L. V. (2004). Endogenous, Reactive and Neurotic Depression – Diagnostic Stability and Long-Term Outcome. *Psychopathology*, *37*(3), 124–130. https://doi.org/10.1159/000078611

Kessler, D. S., MacNeill, S. J., Tallon, D., Lewis, G., Peters, T. J., Hollingworth, W., Round, J., Burns, A., Chew-Graham, C. A., Anderson, I. M., Shepherd, T., Campbell, J., Dickens, C. M., Carter, M., Jenkinson, C., Macleod, U., Gibson, H., Davies, S., & Wiles, N. J. (2018). Mirtazapine added to SSRIs or SNRIs for treatment resistant depression in primary care:

phase III randomised placebo controlled trial (MIR). *BMJ*, *363*. https://doi.org/10.1136/BMJ.K4218

Kessler, R. C., & Bromet, E. J. (2013). The epidemiology of depression across cultures. *Annual Review of Public Health*, *34*, 119. https://doi.org/10.1146/ANNUREV-PUBLHEALTH-031912-114409

Kessler, R. C., McGonagle, K. A., Swartz, M., Blazer, D. G., & Nelson, C. B. (1993). Sex and depression in the National Comorbidity Survey I: Lifetime prevalence, chronicity and recurrence. *Journal of Affective Disorders*, *29*(2–3), 85–96. https://doi.org/10.1016/0165-0327(93)90026-G

Kessler, R. C., Nelson, C. B., Mcgonagle, K. A., Liu, J., Swartz, M., & Blazer, D. G. (1996). Comorbidity of DSM–III–R Major Depressive Disorder in the General Population: Results from the US National Comorbidity Survey. *The British Journal of Psychiatry*, *168*(S30), 17–30. https://doi.org/10.1192/S0007125000298371

Kessler, R. C., Zhao, S., Blazer, D. G., & Swartz, M. (1997). Prevalence, correlates, and course of minor depression and major depression in the national comorbidity survey. *Journal of Affective Disorders*, *45*(1–2), 19–30. https://doi.org/10.1016/S0165-0327(97)00056-6

Khalili-Mahani, N., Martini, C. H., Olofsen, E., Dahan, A., & Niesters, M. (2015). Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *BJA: British Journal of Anaesthesia*, *115*(1), 68–75. https://doi.org/10.1093/BJA/AEV135

Kim, D., Kim, C. H., Moon, J. il, Chung, Y. G., Chang, M. Y., Han, B. S., Ko, S., Yang, E., Cha, K. Y., Lanza, R., & Kim, K. S. (2009). Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. *Cell Stem Cell*, *4*(6), 472-476. https://doi.org/10.1016/j.stem.2009.05.005

Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Reviews Neuroscience*, *3*(6), 453–462. https://doi.org/10.1038/nrn849

Kim, J.W., Herz, J., Kavalali, E. T., & Monteggia, L. M. (2021). A key requirement for synaptic Reelin signaling in ketamine-mediated behavioral and synaptic action. *Proceedings of the National Academy of Sciences*, *118*(20), e2103079118. https://doi.org/10.1073/pnas.2103079118

Kinch, M. S. (2015). An overview of FDA-approved biologics medicines. *Drug Discovery Today*, *20*(4), 393–398. https://doi.org/10.1016/J.DRUDIS.2014.09.003

Kirsch, I. (2019). Placebo effect in the treatment of depression and anxiety. *Frontiers in Psychiatry*, *10*(JUN), 407. https://doi.org/10.3389/FPSYT.2019.00407/BIBTEX

Kishimoto, T., Chawla, J. M., Hagi, K., Zarate, C. A., Kane, J. M., Bauer, M., & Correll, C. U. (2016). Single-dose infusion ketamine and non-ketamine N-methyl-d-aspartate receptor antagonists for unipolar and bipolar depression: a meta-analysis of efficacy, safety and time

trajectories. *Psychological Medicine*, *46*(7), 1459–1472. https://doi.org/10.1017/S0033291716000064

Kitada, Y., Miyauchi, T., Satoh, A., & Satoh, S. (1981). Effects of antidepressants in the rat forced swimming test. *European Journal of Pharmacology*, *72*(2–3), 145–152. https://doi.org/10.1016/0014-2999(81)90269-7

Kizilbash, A. H., Vanderploeg, R. D., & Curtiss, G. (2002). The effects of depression and anxiety on memory performance. *Archives of Clinical Neuropsychology*, *17*(1), 57–67. https://doi.org/10.1093/ARCLIN/17.1.57

Kleen, J. K., Sitomer, M. T., Killeen, P. R., & Conrad, C. D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience*, *120*(4), 842–851. https://doi.org/10.1037/0735-7044.120.4.842

Klein, D. N., Kotov, R., & Bufferd, S. J. (2011). Personality and depression: Explanatory models and review of the evidence. *Annual Review of Clinical Psychology*, *7*(1), 269-295. https://doi.org/10.1146/annurev-clinpsy-032210-104540

Klein, M. E., Chandra, J., Sheriff, S., & Malinow, R. (2020). Opioid system is necessary but not sufficient for antidepressive actions of ketamine in rodents. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(5), 2656–2662. https://doi.org/10.1073/PNAS.1916570117

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C. M., Pace, T. W. W., Mercer, K. B., Mayberg, H. S., Bradley, B., Nemeroff, C. B., Holsboer, F., Heim, C. M., Ressler, K. J., Rein, T., & Binder, E. B. (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature Neuroscience*, *16*(1), 33–41. https://doi.org/10.1038/nn.3275

Klengel, T., Pape, J., Binder, E. B., & Mehta, D. (2014). The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology*, *80*, 115–132. https://doi.org/10.1016/J.NEUROPHARM.2014.01.013

Klok, M. D., Alt, S. R., Irurzun Lafitte, A. J. M., Turner, J. D., Lakke, E. A. J. F., Huitinga, I., Muller, C. P., Zitman, F. G., Ronald de Kloet, E., & DeRijk, R. H. (2011). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of Psychiatric Research*, *45*(7), 871–878. https://doi.org/10.1016/J.JPSYCHIRES.2010.12.002

Knable, M. B., Barci, B. M., Webster, M. J., Meador-Woodruff, J., & Torrey, E. F. (2004). Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium. *Molecular Psychiatry 2004 9:6*, *9*(6), 609–620. https://doi.org/10.1038/sj.mp.4001471

Knierim, J. J. (2015). The hippocampus. *Current Biology*, *25*(23), R1116–R1121. https://doi.org/10.1016/J.CUB.2015.10.049

Knight, S. R., Metcalfe, L., O'Donoghue, K., Ball, S. T., Beale, A., Beale, W., Hilton, R., Hodkinson, K., Lipkin, G. W., Loud, F., Marson, L. P., & Morris, P. J. (2016). Defining priorities for future research: Results of the UK Kidney transplant priority setting partnership. *PLoS ONE*, *11*(10), e0162136. https://doi.org/10.1371/journal.pone.0162136

Knuesel, I. (2010). Reelin-mediated signaling in neuropsychiatric and neurodegenerative diseases. *Progress in Neurobiology*, *91*(4), 257–274. https://doi.org/10.1016/J.PNEUROBIO.2010.04.002

Knuesel, I., Nyffeler, M., Mormède, C., Muhia, M., Meyer, U., Pietropaolo, S., Yee, B. K., Pryce, C. R., LaFerla, F. M., Marighetto, A., & Feldon, J. (2009). Age-related accumulation of Reelin in amyloid-like deposits. *Neurobiology of Aging*, *30*(5), 697–716. https://doi.org/10.1016/J.NEUROBIOLAGING.2007.08.011

Koch, P., Kokaia, Z., Lindvall, O., & Brüstle, O. (2009). Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling. *The Lancet Neurology*, *8*(9), 819–829. https://doi.org/10.1016/S1474-4422(09)70202-9

Koch, S., Strasser, V., Hauser, C., Fasching, D., Brandes, C., Bajari, T. M., Schneider, W. J., & Nimpf, J. (2002). A secreted soluble form of ApoE receptor 2 acts as a dominant-negative receptor and inhibits Reelin signaling. *The EMBO Journal*, *21*(22), 5996–6004. https://doi.org/10.1093/EMBOJ/CDF599

Köhler-Forsberg, O., Buttenschøn, H. N., Tansey, K. E., Maier, W., Hauser, J., Dernovsek, M. Z., Henigsberg, N., Souery, D., Farmer, A., Rietschel, M., McGuffin, P., Aitchison, K. J., Uher, R., & Mors, O. (2017). Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. *Brain, Behavior, and Immunity*, *62*, 344–350. https://doi.org/10.1016/J.BBI.2017.02.020

Kohno, S., Kohno, T., Nakano, Y., Suzuki, K., Ishii, M., Tagami, H., Baba, A., & Hattori, M. (2009). Mechanism and significance of specific proteolytic cleavage of Reelin. *Biochemical and Biophysical Research Communications*, *380*(1), 93–97. https://doi.org/10.1016/J.BBRC.2009.01.039

Koie, M., Okumura, K., Hisanaga, A., Kamei, T., Sasaki, K., Deng, M., Baba, A., Kohno, T., & Hattori, M. (2014). Cleavage within reelin repeat 3 regulates the duration and range of the signaling activity of reelin protein. *Journal of Biological Chemistry*, *289*(18), 12922–12930. https://doi.org/10.1074/jbc.M113.536326

Koike, H., & Chaki, S. (2014). Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. *Behavioural Brain Research*, *271*, 111–115. https://doi.org/10.1016/J.BBR.2014.05.065

Koike, H., Fukumoto, K., Iijima, M., & Chaki, S. (2013). Role of BDNF/TrkB signaling in antidepressant-like effects of a group II metabotropic glutamate receptor antagonist in animal models of depression. *Behavioural Brain Research*, *238*(1), 48–52. https://doi.org/10.1016/J.BBR.2012.10.023

Koike, H., Iijima, M., & Chaki, S. (2011). Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behavioural Brain Research*, *224*(1), 107–111. https://doi.org/10.1016/J.BBR.2011.05.035

Kokane, S. S., Armant, R. J., Bolaños-Guzmán, C. A., & Perrotti, L. I. (2020). Overlap in the neural circuitry and molecular mechanisms underlying ketamine abuse and its use as an antidepressant. *Behavioural Brain Research*, *384*, 112548. https://doi.org/10.1016/J.BBR.2020.112548

Koning, A. S. C. A. M., Buurstede, J. C., van Weert, L. T. C. M., & Meijer, O. C. (2019). Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective. *Journal of the Endocrine Society*, *3*(10), 1917–1930. https://doi.org/10.1210/JS.2019-00158

Kowalczyk, M., Szemraj, J., Bliźniewska, K., Maes, M., Berk, M., Su, K. P., & Gałecki, P. (2019). An immune gate of depression–Early neuroimmune development in the formation of the underlying depressive disorder. *Pharmacological reports, 71*(6), 1299-1307.

Kraft, J. B., Slager, S. L., McGrath, P. J., & Hamilton, S. P. (2005). Sequence Analysis of the Serotonin Transporter and Associations with Antidepressant Response. *Biological Psychiatry*, *58*(5), 374–381. https://doi.org/10.1016/J.BIOPSYCH.2005.04.048

Krahn, M., & Naglie, G. (2008). The next step in guideline development: Incorporating patient preferences. *Journal of the American Medical Association, 300*(4), 436-438. https://doi.org/10.1001/jama.300.4.436

Kramár, E. A., Bernard, J. A., Gall, C. M., & Lynch, G. (2002). Alpha3 integrin receptors contribute to the consolidation of long-term potentiation. *Neuroscience*, *110*(1), 29–39. https://doi.org/10.1016/S0306-4522(01)00540-1

Kraus, C., Mkrtchian, A., Kadriu, B., Nugent, A. C., Zarate, C. A., & Evans, J. W. (2020). Evaluating global brain connectivity as an imaging marker for depression: influence of preprocessing strategies and placebo-controlled ketamine treatment. *Neuropsychopharmacology, 45*(6), 982–989. https://doi.org/10.1038/s41386-020-0624-0

Kraus, C., Rabl, U., Vanicek, T., Carlberg, L., Popovic, A., Spies, M., Bartova, L., Gryglewski, G., Papageorgiou, K., Lanzenberger, R., Willeit, M., Winkler, D., Rybakowski, J. K., & Kasper, S. (2017). Administration of ketamine for unipolar and bipolar depression. *International Journal of Psychiatry in Clinical Practice, 21*(1), 2-12. https://doi.org/10.1080/13651501.2016.1254802

Krishnan, K. R. R., Delong, M., Kraemer, H., Carney, R., Spiegel, D., Gordon, C., McDonald, W., Dew, M. A., Alexopoulos, G., Buckwalter, K., Cohen, P. D., Evans, D., Kaufmann, P. G., Olin, J., Otey, E., & Wainscott, C. (2002). Comorbidity of depression with other medical diseases in the elderly. *Biological Psychiatry, 52*(6), 559–588. https://doi.org/10.1016/S0006-3223(02)01472-5

Kritchevsky, M., Chang, J., & Squire, L. R. (2004). Functional Amnesia: Clinical Description and Neuropsychological Profile of 10 Cases. *Learning & Memory*, *11*(2), 213–226. https://doi.org/10.1101/LM.71404

Krugers, H. J., Hoogenraad, C. C., & Groc, L. (2010). Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. *Nature Reviews Neuroscience*, *11*(10), 675-681. https://doi.org/10.1038/nrn2913

Krystal, J. H., Abdallah, C. G., Sanacora, G., Charney, D. S., & Duman, R. S. (2019). Ketamine: A Paradigm Shift for Depression Research and Treatment. *Neuron*, *101*(5), 774–778. https://doi.org/10.1016/J.NEURON.2019.02.005

Kubera, M., Obuchowicz, E., Goehler, L., Brzeszcz, J., & Maes, M. (2011). In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(3), 744–759. https://doi.org/10.1016/J.PNPBP.2010.08.026

Kubo, K. I., Mikoshiba, K., & Nakajima, K. (2002). Secreted Reelin molecules form homodimers. *Neuroscience Research*, *43*(4), 381–388. https://doi.org/10.1016/S0168-0102(02)00068-8

Kuhn, R. (1958). The treatment of depressive states with G 22355 (imipramine hydrochloride). *The American Journal of Psychiatry*, *115*(5), 459–464. https://doi.org/10.1176/ajp.115.5.459

Kulyk, B. D. (2017). Antidepressant-Like Effects of Ketamine on Fear Conditioning and Extinction. (Dissertation, University of Saskatchewan). https://harvest.usask.ca/handle/10388/7664

Kuo, D. C., Tran, M., Shah, A. A., & Matorin, A. (2015). Depression and the Suicidal Patient. *Emergency Medicine Clinics of North America*, *33*(4), 765–778. https://doi.org/10.1016/j.emc.2015.07.005

Kurdi, M. S., Theerth, K. A., & Deva, R. S. (2014). Ketamine: Current applications in anesthesia, pain, and critical care. *Anesthesia, Essays and Researches*, *8*(3), 283. https://doi.org/10.4103/0259-1162.143110

Kvarta, M. D., Bradbrook, K. E., Dantrassy, H. M., Bailey, A. M., & Thompson, S. M. (2015). Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses. *Journal of Neurophysiology*, *114*(3), 1713–1724. https://doi.org/10.1152/JN.00359.2015

Labaka, A., Goñi-Balentziaga, O., Lebeña, A., & Pérez-Tejada, J. (2018). Biological Sex Differences in Depression: A Systematic Review. *Biological Research for Nursing*, *20*(4), 383–392.

Labonté, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., Bureau, A., Mechawar, N., Szyf, M., Meaney, M. J., & Turecki, G. (2012). Genome-wide Epigenetic

Regulation by Early-Life Trauma. *Archives of General Psychiatry*, *69*(7), 722–731. https://doi.org/10.1001/ARCHGENPSYCHIATRY.2011.2287

Lacor, P. N., Grayson, D. R., Auta, J., Sugaya, I., Costa, E., & Guidotti, A. (2000). Reelin secretion from glutamatergic neurons in culture is independent from neurotransmitter regulation. *Proceedings of the National Academy of Sciences*, *97*(7), 3556–3561. https://doi.org/10.1073/PNAS.97.7.3556

Lally, N., Nugent, A. C., Luckenbaugh, D. A., Ameli, R., Roiser, J. P., & Zarate, C. A. (2014). Anti-anhedonic effect of ketamine and its neural correlates in treatment-resistant bipolar depression. *Translational Psychiatry*, *4*(10), e469. https://doi.org/10.1038/tp.2014.105

LaMarca, E. A., Powell, S. K., Akbarian, S., & Brennand, K. J. (2018). Modeling neuropsychiatric and neurodegenerative diseases with induced pluripotent stem cells. *Frontiers in Pediatrics*, *6*, 82. https://doi.org/10.3389/FPED.2018.00082

Lambert De Rouvroit, C., de Bergeyck, V., Cortvrindt, C., Bar, I., Eeckhout, Y., & Goffinet, A. M. (1999). Reelin, the Extracellular Matrix Protein Deficient in Reeler Mutant Mice, Is Processed by a Metalloproteinase. *Experimental Neurology*, *156*(1), 214–217. https://doi.org/10.1006/EXNR.1998.7007

Lebedeva, K. A., Allen, J., Kulhawy, E. Y., Caruncho, H. J., & Kalynchuk, L. E. (2020). Cyclical administration of corticosterone results in aggravation of depression-like behaviors and accompanying downregulations in reelin in an animal model of chronic stress relevant to human recurrent depression. *Physiology & Behavior*, *224*, 113070. https://doi.org/10.1016/J.PHYSBEH.2020.113070

Lebedeva, K. A., Caruncho, H. J., & Kalynchuk, L. E. (2017). Cyclical corticosterone administration sensitizes depression-like behavior in rats. *Neuroscience Letters*, *650*, 45–51. https://doi.org/10.1016/J.NEULET.2017.04.023

Lee, A., Xu, J., Wen, Z., & Jin, P. (2022). Across Dimensions: Developing 2D and 3D Human iPSC-Based Models of Fragile X Syndrome. *Cells*, *11*(11), 1725. https://doi.org/10.3390/CELLS11111725

Lee, G. H., & D'Arcangelo, G. (2016). New insights into reelin-mediated signaling pathways. *Frontiers in Cellular Neuroscience*, *10*, 122. https://doi.org/10.3389/FNCEL.2016.00122

Lee, J. W., & Jung, M. W. (2017). Separation or binding? Role of the dentate gyrus in hippocampal mnemonic processing. *Neuroscience & Biobehavioral Reviews*, *75*, 183–194. https://doi.org/10.1016/J.NEUBIOREV.2017.01.049

Lee, T., Jarome, T., Li, S. J., Kim, J. J., & Helmstetter, F. J. (2009). Chronic stress selectively reduces hippocampal volume in rats: A longitudinal magnetic resonance imaging study. *NeuroReport*, *20*(17), 1554–1558. https://doi.org/10.1097/WNR.0b013e328332bb09

Lehmann, H. E., & Ban, T. A. (1997). The history of the psychopharmacology of schizophrenia. *Canadian Journal of Psychiatry*, *42*(2), 152-163. https://doi.org/10.1177/070674379704200205

Lehr, A. B., Kumar, A., Tetzlaff, C., Hafting, T., Fyhn, M., & Stöber, T. M. (2021). CA2 beyond social memory: Evidence for a fundamental role in hippocampal information processing. *Neuroscience & Biobehavioral Reviews*, *126*, 398–412. https://doi.org/10.1016/J.NEUBIOREV.2021.03.020

Levone, B. R., Codagnone, M. G., Moloney, G. M., Nolan, Y. M., Cryan, J. F., & O' Leary, O. F. (2021). Adult-born neurons from the dorsal, intermediate, and ventral regions of the longitudinal axis of the hippocampus exhibit differential sensitivity to glucocorticoids. *Molecular Psychiatry*, *26*(7), 3240–3252. https://doi.org/10.1038/s41380-020-0848-8

Li, J., Xie, X., Li, Y., Liu, X., Liao, X., Su, Y. A., & Si, T. (2017). Differential behavioral and neurobiological effects of chronic corticosterone treatment in adolescent and adult rats. *Frontiers in Molecular Neuroscience*, *10*, 25. https://doi.org/10.3389/FNMOL.2017.00025

Li, L., Chao, J., & Shi, Y. (2018). Modeling neurological diseases using iPSC-derived neural cells: iPSC modeling of neurological diseases. *Cell and Tissue Research*, *371*(1), 143–151. https://doi.org/10.1007/S00441-017-2713-X

Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., Li, X. Y., Aghajanian, G., & Duman, R. S. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, *329*(5994), 959–964. https://doi.org/10.1126/science.1190287

Li, Y., Mu, Y., & Gage, F. H. (2009). Chapter 5 Development of Neural Circuits in the Adult Hippocampus. *Current Topics in Developmental Biology*, *87*, 149–174. https://doi.org/10.1016/S0070-2153(09)01205-8

Li, Y., Shen, R., Wen, G., Ding, R., Du, A., Zhou, J., Dong, Z., Ren, X., Yao, H., Zhao, R., Zhang, G., Lu, Y., & Wu, X. (2017). Effects of ketamine on levels of inflammatory cytokines IL-6, IL-1β, and TNF-α in the hippocampus of mice following acute or chronic administration. *Frontiers in Pharmacology*, *8*(Mar), 139. https://doi.org/10.3389/FPHAR.2017.00139/

Lim, G. Y., Tam, W. W., Lu, Y., Ho, C. S., Zhang, M. W., & Ho, R. C. (2018). Prevalence of Depression in the Community from 30 Countries between 1994 and 2014 /692/699/476/1414 /692/499 article. *Scientific Reports*, *8*(1), 1–10. https://doi.org/10.1038/s41598-018-21243-x

Lindert, J., von Ehrenstein, O. S., Grashow, R., Gal, G., Braehler, E., & Weisskopf, M. G. (2014). Sexual and physical abuse in childhood is associated with depression and anxiety over the life course: Systematic review and meta-analysis. *International Journal of Public Health*, *59*(2), 359–372.

Lisman, J., Buzsáki, G., Eichenbaum, H., Nadel, L., Rangananth, C., & Redish, A. D. (2017). Viewpoints: How the hippocampus contributes to memory, navigation and cognition. *Nature Neuroscience, 20*(11), 1434-1447. https://doi.org/10.1038/nn.4661

Liu, B., Liu, J., Wang, M., Zhang, Y., & Li, L. (2017). From serotonin to neuroplasticity: Evolvement of theories for major depressive disorder. *Frontiers in Cellular Neuroscience, 11*, 305. https://doi.org/10.3389/FNCEL.2017.00305/

Liu, J., Kościelska, K. A., Cao, Z., Hulsizer, S., Grace, N., Mitchell, G., Nacey, C., Githinji, J., McGee, J., Garcia-Arocena, D., Hagerman, R. J., Nolta, J., Pessah, I. N., & Hagerman, P. J. (2012). Signaling defects in iPSC-derived fragile X premutation neurons. *Human Molecular Genetics*, *21*(17), 3795–3805. https://doi.org/10.1093/HMG/DDS207

Liu, J., Xu, X., Luo, Q., Luo, Y., Chen, Y., Lui, S., Wu, M., Zhu, H., Kemp, G. J., & Gong, Q. (2017). Brain grey matter volume alterations associated with antidepressant response in major depressive disorder. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/s41598-017-10676-5

Liu, S. J., & Zukin, R. S. (2007). Ca2+-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends in Neurosciences*, *30*(3), 126–134. https://doi.org/10.1016/J.TINS.2007.01.006

Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B. G., Guidotti, A., & Costa, E. (2001). Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *Proceedings of the National Academy of Sciences*, *98*(6), 3477–3482. https://doi.org/10.1073/PNAS.051614698

Liu, X., Xu, Y., Jiang, S., Cui, D., Qian, Y., & Jiang, K. (2009). Family-based association study between brain-derived neurotrophic factor gene and major depressive disorder of Chinese descent. *Psychiatry Research*, *169*(2), 169–172. https://doi.org/10.1016/J.PSYCHRES.2008.06.014

Liu, Y., Tak, P. W., Aarts, M., Rooyakkers, A., Liu, L., Ted, W. L., Dong, C. W., Lu, J., Tymianski, M., Craig, A. M., & Yu, T. W. (2007). NMDA Receptor Subunits Have Differential Roles in Mediating Excitotoxic Neuronal Death Both In Vitro and In Vivo. *Journal of Neuroscience*, *27*(11), 2846–2857. https://doi.org/10.1523/JNEUROSCI.0116-07.2007

Liu, Z., Zhu, F., Wang, G., Xiao, Z., Wang, H., Tang, J., Wang, X., Qiu, D., Liu, W., Cao, Z., & Li, W. (2006). Association of corticotropin-releasing hormone receptor1 gene SNP and haplotype with major depression. *Neuroscience Letters*, *404*(3), 358–362. https://doi.org/10.1016/J.NEULET.2006.06.016

López-Gil, X., Jiménez-Sánchez, L., Campa, L., Castro, E., Frago, C., & Adell, A. (2019). Role of Serotonin and Noradrenaline in the Rapid Antidepressant Action of Ketamine. *ACS Chemical Neuroscience*, *10*(7), 3318–3326.

López-Giménez, J. F., & González-Maeso, J. (2018). Hallucinogens and serotonin 5-HT2A receptor-mediated signaling pathways. *Current Topics in Behavioral Neurosciences*, *36*, 45–73.

Lòpez-Muñoz, F., Álamo, C., Rubio, G., García-García, P., & Pardo, A. (2007). Reboxetine combination in treatment-resistant depression to selective serotonin reuptake inhibitors. *Pharmacopsychiatry*, *40*(1), 14–19. https://doi.org/10.1055/S-2007-958523/ID/31

Lophatananon, A., Tyndale-Biscoe, S., Malcolm, E., Rippon, H. J., Holmes, K., Firkins, L. A., Fenton, M., Crowe, S., Stewart-Brown, S., Gnanapragasam, V. J., & Muir, K. R. (2011). The James Lind alliance approach to priority setting for prostate cancer research: AN integrative methodology based on patient and clinician participation. *BJU International*, *108*(7), 1040-1043. https://doi.org/10.1111/j.1464-410X.2011.10609.x

Lopresti, A. L. (2017). Cognitive behaviour therapy and inflammation: A systematic review of its relationship and the potential implications for the treatment of depression. *Australian and New Zealand Journal of Psychiatry*, *51*(6), 565–582.

Lorenzetti, V., Allen, N. B., Fornito, A., & Yücel, M. (2009). Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. *Journal of Affective Disorders*, *117*(1–2), 1–17. https://doi.org/10.1016/J.JAD.2008.11.021

Lu, W. Y., Man, H. Y., Ju, W., Trimble, W. S., MacDonald, J. F., & Wang, Y. T. (2001). Activation of Synaptic NMDA Receptors Induces Membrane Insertion of New AMPA Receptors and LTP in Cultured Hippocampal Neurons. *Neuron*, *29*(1), 243–254. https://doi.org/10.1016/S0896-6273(01)00194-5

Lugo-Huitrón, R., Ugalde Muñiz, P., Pineda, B., Pedraza-Chaverrí, J., Ríos, C., & Pérez-De La Cruz, V. (2013). Quinolinic acid: An endogenous neurotoxin with multiple targets. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2013/104024

Lumsden, E. W., Troppoli, T. A., Myers, S. J., Zanos, P., Aracava, Y., Kehr, J., Lovett, J., Kim, S., Wang, F. H., Schmidt, S., Jenne, C. E., Yuan, P., Morris, P. J., Thomas, C. J., Zarate, C. A., Moaddel, R., Traynelis, S. F., Pereira, E. F. R., Thompson, S. M., … Gould, T. D. (2019). Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5160–5169. https://doi.org/10.1073/pnas.1816071116

Luo, C., & Ecker, J. R. (2015). Exceptional epigenetics in the brain. *Science*, *348*(6239), 1094–1095.

Luo, G. Q., Liu, L., Gao, Q. W., Wu, X. N., Xiang, W., & Deng, W. T. (2017). Mangiferin prevents corticosterone-induced behavioural deficits via alleviation of oxido-nitrosative stress and down-regulation of indoleamine 2,3-dioxygenase (IDO) activity. *Neurological Research*, *39*(8), 709–718. https://doi.org/10.1080/01616412.2017.1310705

Lüscher, B., & Möhler, H. (2019). Brexanolone, a neurosteroid antidepressant, vindicates the gabaergic deficit hypothesis of depression and may foster. *F1000Research*, *8*(1). https://doi.org/10.12688/f1000research.18758.1

Lüscher, C., Malenka, R. C., Sheng, M., Sabatini, B., & Sü, T. C. (2012). NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). *Cold Spring Harbor Perspectives in Biology*, *4*(6), a005710. https://doi.org/10.1101/CSHPERSPECT.A005710

Lussier, A. L., Lebedeva, K., Fenton, E. Y., Guskjolen, A., Caruncho, H. J., & Kalynchuk, L. E. (2013a). The progressive development of depression-like behavior in corticosterone-treated rats is paralleled by slowed granule cell maturation and decreased reelin expression in the adult dentate gyrus. *Neuropharmacology*, *71*, 174–183. https://doi.org/10.1016/J.NEUROPHARM.2013.04.012

Lussier, A. L., Romay-Tallón, R., Caruncho, H. J., & Kalynchuk, L. E. (2013b). Altered GABAergic and glutamatergic activity within the rat hippocampus and amygdala in rats subjected to repeated corticosterone administration but not restraint stress. *Neuroscience*, *231*, 38–48. https://doi.org/10.1016/J.NEUROSCIENCE.2012.11.037

Lussier, A. L., Romay-Tallón, R., Kalynchuk, L. E., & Caruncho, H. J. (2011). Reelin as a putative vulnerability factor for depression: Examining the depressogenic effects of repeated corticosterone in heterozygous reeler mice. *Neuropharmacology*, *60*(7–8), 1064–1074. https://doi.org/10.1016/J.NEUROPHARM.2010.09.007

Ly, C., Greb, A. C., Cameron, L. P., Wong, J. M., Barragan, E. v., Wilson, P. C., Burbach, K. F., Soltanzadeh Zarandi, S., Sood, A., Paddy, M. R., Duim, W. C., Dennis, M. Y., McAllister, A. K., Ori-McKenney, K. M., Gray, J. A., & Olson, D. E. (2018). Psychedelics Promote Structural and Functional Neural Plasticity. *Cell Reports*, *23*(11), 3170–3182. https://doi.org/10.1016/J.CELREP.2018.05.022

Lynch, M. A. (2004). Long-Term Potentiation and Memory. *Physiological Reviews*, *84*(1), 87–136.

Ma, K., Zhang, H., & Baloch, Z. (2016). Pathogenetic and Therapeutic Applications of Tumor Necrosis Factor-α (TNF-α) in Major Depressive Disorder: A Systematic Review. *International Journal of Molecular Sciences*, *17*(5), 733. https://doi.org/10.3390/IJMS17050733

Ma, L., Shen, Q., Yang, S., Xie, X., Xiao, Q., Yu, C., Cao, L., & Fu, Z. (2018). Effect of chronic corticosterone-induced depression on circadian rhythms and age-related phenotypes in mice. *Acta Biochimica et Biophysica Sinica*, *50*(12), 1236–1246. https://doi.org/10.1093/ABBS/GMY132

Ma, L., Xu, Y., Wang, G., & Li, R. (2019). What do we know about sex differences in depression: A review of animal models and potential mechanisms. *Progress in Neuro-*

Psychopharmacology and Biological Psychiatry, *89*, 48–56. https://doi.org/10.1016/J.PNPBP.2018.08.026

Ma, Z., Zang, T., Birnbaum, S. G., Wang, Z., Johnson, J. E., Zhang, C. L., & Parada, L. F. (2017). TrkB dependent adult hippocampal progenitor differentiation mediates sustained ketamine antidepressant response. *Nature Communications*, *8*(1), 1–14. https://doi.org/10.1038/s41467-017-01709-8

MacDonald, J. F., Miljkovic, Z., & Pennefather. (1987). Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. *Journal of Neurophysiology*, *58*(2), 251–266. https://doi.org/10.1152/jn.1987.58.2.251

Macosko, E. Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A. R., Kamitaki, N., Martersteck, E. M., Trombetta, J. J., Weitz, D. A., Sanes, J. R., Shalek, A. K., Regev, A., & McCarroll, S. A. (2015). Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell*, *161*(5), 1202–1214. https://doi.org/10.1016/J.CELL.2015.05.002

MacQueen, G., & Frodl, T. (2011). The hippocampus in major depression: Evidence for the convergence of the bench and bedside in psychiatric research. *Molecular Psychiatry*, *16*(3), 252–264. https://doi.org/10.1038/mp.2010.80

MacQueen, G. M., Campbell, S., McEwen, B. S., Macdonald, K., Amano, S., Joffe, R. T., Nahmias, C., & Trevor Young, L. (2003). Course of illness, hippocampal function, and hippocampal volume in major depression. *Proceedings of the National Academy of Sciences*, *100*(3), 1387–1392. https://doi.org/10.1073/PNAS.0337481100

Madden, M., & Morley, R. (2016). Exploring the challenge of health research priority setting in partnership: Reflections on the methodology used by the james lind alliance pressure ulcer priority setting partnership. *Research Involvement and Engagement*, *2*(1), 1–20. https://doi.org/10.1186/S40900-016-0026-Y/TABLES/1

Magnani, F., Tatell, C. G., Wynne, S., Williams, C., & Haase, J. (2004). Partitioning of the serotonin transporter into lipid microdomains modulates transport of serotonin. *Journal of Biological Chemistry*, *279*(37), 38770–38778. https://doi.org/10.1074/jbc.M400831200

Maier, W., Lichtermann, D., Minges, J., Heun, R., Hallmayer, J., & Benkert, O. (1992). Schizoaffective disorder and affective disorders with mood-incongruent psychotic features: Keep separate or combine? Evidence from a family study. *American Journal of Psychiatry*, *149*(12), 1666–1673. https://doi.org/10.1176/AJP.149.12.1666

Majd, M., Saunders, E. F. H., & Engeland, C. G. (2020). Inflammation and the dimensions of depression: A review. *Frontiers in Neuroendocrinology*, *56*, 100800. https://doi.org/10.1016/J.YFRNE.2019.100800

Malenka, R. C., & Nicoll, R. A. (1997). Silent Synapses Speak Up Minireview. *Neuron*, *19*, 473–476.

Malik, N., & Rao, M. S. (2013). A review of the methods for human iPSC derivation. *Methods in Molecular Biology*, *997*, 23–33.

Malison, R. T., Price, L. H., Berman, R., van Dyck, C. H., Pelton, G. H., Carpenter, L., Sanacora, G., Owens, M. J., Nemeroff, C. B., Rajeevan, N., Baldwin, R. M., Seibyl, J. P., Innis, R. B., & Charney, D. S. (1998). Reduced brain serotonin transporter availability in major depression as measured by [123I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane and single photon emission computed tomography. *Biological Psychiatry*, *44*(11), 1090–1098. https://doi.org/10.1016/S0006-3223(98)00272-8

Marazita, M. L., Neiswanger, K., Cooper, M., Zubenko, G. S., Giles, D. E., Frank, E., Kupfer, D. J., & Kaplan, B. B. (1997). Genetic Segregation Analysis of Early-Onset Recurrent Unipolar Depression. *The American Journal of Human Genetics*, *61*(6), 1370–1378. https://doi.org/10.1086/301627

Marazziti, D., Consoli, G., Masala, I., Catena Dell'Osso, M., & Baroni, S. (2010). Latest advancements on serotonin and dopamine transporters in lymphocytes. *Mini Reviews in Medicinal Chemistry*, 10(1), 32-40.

Marchetti, I., Koster, E. H. W., Sonuga-Barke, E. J., & de Raedt, R. (2012). The Default Mode Network and recurrent depression: A neurobiological model of cognitive risk factors. *Neuropsychology Review*, *22*(3), 229–251. https://doi.org/10.1007/S11065-012-9199-9/TABLES/1

Marks, D. M., Shah, M. J., Patkar, A. A., Masand, P. S., Park, G.-Y., & Pae, C.-U. (2009). Serotonin-Norepinephrine Reuptake Inhibitors for Pain Control: Premise and Promise. *Current Neuropharmacology*, *7*(4), 331–336. https://doi.org/10.2174/157015909790031201

Marks, W., Fournier, N. M., & Kalynchuk, L. E. (2009). Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. *Physiology & Behavior*, *98*(1–2), 67–72. https://doi.org/10.1016/J.PHYSBEH.2009.04.014

Marks, W. N., Fenton, E. Y., Guskjolen, A. J., & Kalynchuk, L. E. (2015). The effect of chronic corticosterone on fear learning and memory depends on dose and the testing protocol. *Neuroscience*, *289*, 324–333. https://doi.org/10.1016/J.NEUROSCIENCE.2015.01.011

Marshall, J. B., & Forker, A. D. (1982). Cardiovascular effects of tricyclic antidepressant drugs: Therapeutic usage, overdose, and management of complications. *American Heart Journal*, *103*(3), 401–414. https://doi.org/10.1016/0002-8703(82)90281-2

Martin, C., Tansey, K. E., Schalkwyk, L. C., & Powell, T. R. (2014). The inflammatory cytokines: molecular biomarkers for major depressive disorder? *Biomarkers in medicine*, *9*(2), 169–180. https://doi.org/10.2217/BMM.14.29

Mason, B. L., & Pariante, C. M. (2006). The effects of antidepressants on the hypothalamic-pituitary-adrenal axis. *Drug News & Perspectives*, *19*(10), 603–608. https://doi.org/10.1358/DNP.2006.19.10.1068007

Massey, P. v., Johnson, B. E., Moult, P. R., Auberson, Y. P., Brown, M. W., Molnar, E., Collingridge, G. L., & Bashir, Z. I. (2004). Differential Roles of NR2A and NR2B-Containing NMDA Receptors in Cortical Long-Term Potentiation and Long-Term Depression. *Journal of Neuroscience*, *24*(36), 7821–7828. https://doi.org/10.1523/JNEUROSCI.1697-04.2004

Mastrodonato, A., Cohensedgh, O., LaGamma, C. T., McGowan, J. C., Hunsberger, H. C., & Denny, C. A. (2020). Prophylactic (R,S)-ketamine selectively protects against inflammatory stressors. *Behavioural Brain Research*, *378*, 112238. https://doi.org/10.1016/J.BBR.2019.112238

Mastrodonato, A., Pavlova, I., Kee, N. C., Pham, V. A., Mcgowan, J. C., Mann, J. J., & Denny, C. A. (2022). Prophylactic (R,S)-Ketamine Is Effective Against Stress-Induced Behaviors in Adolescent but Not Aged Mice. *International Journal of Neuropsychopharmacology*, *25*(6), 512–523. https://doi.org/10.1093/IJNP/PYAC020

Matsuki, T., Pramatarova, A., & Howell, B. W. (2008). Reduction of Crk and CrkL expression blocks reelin-induced dendritogenesis. *Journal of Cell Science*, *121*(11), 1869–1875. https://doi.org/10.1242/JCS.027334

Matt, J., Leuthold, H., & Sommer, W. (1992). Differential Effects of Voluntary Expectancies on Reaction Times and Event-Related Potentials: Evidence for Automatic and Controlled Expectancies. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *18*(4), 810–822. https://doi.org/10.1037/0278-7393.18.4.810

Matveychuk, D., Thomas, R. K., Swainson, J., Khullar, A., MacKay, M.-A., Baker, G. B., & Dursun, S. M. (2020). Ketamine as an antidepressant: overview of its mechanisms of action and potential predictive biomarkers. *Therapeutic Advances in Psychopharmacology*, *10*, 204512532091665. https://doi.org/10.1177/2045125320916657

Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., Silva, J. A., Tekell, J. L., Martin, C. C., Lancaster, J. L., & Fox, P. T. (2013). Reciprocal limbic-cortical function and negative mood: Converging PET findings in depression and normal sadness. *Depression: The Science of Mental Health*, *6*(5), 245–253.

McCormack, J., & Korownyk, C. (2018). Effectiveness of antidepressants. *BMJ*, *360*. https://doi.org/10.1136/BMJ.K1073

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annual Review of Neuroscience*, *22*, 105-122. https://doi.org/10.1146/annurev.neuro.22.1.105

McEwen, B. S. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress*, *1*. https://doi.org/10.1177/2470547017692328

McEwen, B. S., & Morrison, J. H. (2013). The Brain on Stress: Vulnerability and Plasticity of the Prefrontal Cortex over the Life Course. *Neuron*, *79*(1), 16–29. https://doi.org/10.1016/J.NEURON.2013.06.028

McIntyre, R. S., Carvalho, I. P., Lui, L. M. W., Majeed, A., Masand, P. S., Gill, H., Rodrigues, N. B., Lipsitz, O., Coles, A. C., Lee, Y., Tamura, J. K., Iacobucci, M., Phan, L., Nasri, F., Singhal, N., Wong, E. R., Subramaniapillai, M., Mansur, R., Ho, R., … Rosenblat, J. D. (2020). The effect of intravenous, intranasal, and oral ketamine in mood disorders: A meta-analysis. *Journal of Affective Disorders*, *276*, 576–584. https://doi.org/10.1016/J.JAD.2020.06.050

McKinney, W. T., & Bunney, W. E. (1969). Animal Model of Depression: I. Review of Evidence: Implications for Research. *Archives of General Psychiatry*, *21*(2), 240–248. https://doi.org/10.1001/ARCHPSYC.1969.01740200112015

McNeill, R. V., Ziegler, G. C., Radtke, F., Nieberler, M., Lesch, K. P., & Kittel-Schneider, S. (2020). Mental health dished up—the use of iPSC models in neuropsychiatric research. *Journal of Neural Transmission*, *127*(11), 1547-1568. https://doi.org/10.1007/s00702-020-02197-9

Megías, M., Emri, Z., Freund, T. F., & Gulyás, A. I. (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, *102*(3), 527–540. https://doi.org/10.1016/S0306-4522(00)00496-6

Melas, P. A., Rogdaki, M., Lennartsson, A., Björk, K., Qi, H., Witasp, A., ... & Lavebratt, C. (2012). Antidepressant treatment is associated with epigenetic alterations in the promoter of P11 in a genetic model of depression. *International Journal of Neuropsychopharmacology*, *15*(5), 669-679.

Mendez-David, I., David, D. J., Deloménie, C., Beaulieu, J.-M., Gardier, A. M., & Hen, R. (2020). A non-linear relation between levels of adult hippocampal neurogenesis and expression of the immature neuron marker doublecortin. *BioRxiv*, 2020.05.26.115873. https://doi.org/10.1101/2020.05.26.115873

Menke, A., & Binder, E. B. (2022). Epigenetic alterations in depression and antidepressant treatment. *Dialogues in clinical neuroscience*, *16*(3), 395–404. https://doi.org/10.31887/DCNS.2014.16.3

Mertens, J., Reid, D., Lau, S., Kim, Y., & Gage, F. H. (2018). Aging in a Dish: iPSC-Derived and Directly Induced Neurons for Studying Brain Aging and Age-Related Neurodegenerative Diseases. *Annual Review of Genetics*, *52*, 271. https://doi.org/10.1146/ANNUREV-GENET-120417-031534

Mertens, J., Wang, Q. W., Kim, Y., Yu, D. X., Pham, S., Yang, B., Zheng, Y., Diffenderfer, K. E., Zhang, J., Soltani, S., Eames, T., Schafer, S. T., Boyer, L., Marchetto, M. C., Nurnberger, J. I., Calabrese, J. R., Ødegaard, K. J., McCarthy, M. J., Zandi, P. P., … Yao, J. (2015). Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature*, *527*(7576), 95–99. https://doi.org/10.1038/nature15526

Michael Deans, P. J., & Brennand, K. J. (2021). Applying stem cells and CRISPR engineering to uncover the etiology of schizophrenia. *Current Opinion in Neurobiology*, *69*, 193–201. https://doi.org/10.1016/J.CONB.2021.04.003

Michetti, C., Romano, E., Altabella, L., Caruso, A., Castelluccio, P., Bedse, G., Gaetani, S., Canese, R., Laviola, G., & Scattoni, M. L. (2014). Mapping pathological phenotypes in reelin mutant mice. *Frontiers in Pediatrics*, *2*(SEP), 95. https://doi.org/10.3389/FPED.2014.00095

Miech, R. A., & Shanahan, M. J. (2000). Socioeconomic status and depression over the life course. *Journal of Health and Social Behavior*, *41*(2), 162–176. https://doi.org/10.2307/2676303

Mill, J., & Petronis, A. (2007). Molecular studies of major depressive disorder: The epigenetic perspective. *Molecular Psychiatry*, *12*(9), 799-814. https://doi.org/10.1038/sj.mp.4001992

Miller, A. H., & Raison, C. L. (2015). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature Reviews Immunology*, *16*(1), 22–34. https://doi.org/10.1038/nri.2015.5

Miller, O. H., Moran, J. T., & Hall, B. J. (2016). Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. *Neuropharmacology*, *100*, 17–26. https://doi.org/10.1016/J.NEUROPHARM.2015.07.028

Miller, W. L., & Auchus, R. J. (2011). The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. *Endocrine Reviews*, *32*(1), 81–151. https://doi.org/10.1210/ER.2010-0013

Minichino, A., Bersani, F. S., Trabucchi, G., Albano, G., Primavera, M., Chiaie, R. D., & Biondi, M. (2014). The role of cerebellum in unipolar and bipolar depression: A review of the main neurobiological findings. *Rivista Di Psichiatria*, *49*(3), 124–131. https://doi.org/10.1708/1551.16907

Mion, G. (2017). History of anaesthesia: The ketamine story - past, present and future. *European Journal of Anaesthesiology*, *34*(9), 571–575. https://doi.org/10.1097/EJA.0000000000000638

Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby, C. R., & Kawahara, R. (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(6), 1155–1158. https://doi.org/10.1016/J.PNPBP.2006.03.036

Mitchell, C. P., Chen, Y., Kundakovic, M., Costa, E., & Grayson, D. R. (2005). Histone deacetylase inhibitors decrease reelin promoter methylation in vitro. *Journal of Neurochemistry*, *93*(2), 483–492. https://doi.org/10.1111/J.1471-4159.2005.03040.X

Mitra, R., & Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(14), 5573–5578.

Mkrtchian, A., Evans, J. W., Kraus, C., Yuan, P., Kadriu, B., Nugent, A. C., Roiser, J. P., & Zarate, C. A. (2021). Ketamine modulates fronto-striatal circuitry in depressed and healthy individuals. *Molecular Psychiatry*, *26*(7), 3292–3301. https://doi.org/10.1038/s41380-020-00878-1

Moaddel, R., Shardell, M., Khadeer, M., Lovett, J., Kadriu, B., Ravichandran, S., Morris, P. J., Yuan, P., Thomas, C. J., Gould, T. D., Ferrucci, L., & Zarate, C. A. (2018). Plasma metabolomic profiling of a ketamine and placebo crossover trial of major depressive disorder and healthy control subjects. *Psychopharmacology*, *235*(10), 3017–3030.

Moghaddam, B. (2002). Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. *Biological Psychiatry*, *51*(10), 775–787. https://doi.org/10.1016/S0006-3223(01)01362-2

Moncrieff, J., Cooper, R. E., Stockmann, T., Amendola, S., Hengartner, M. P., & Horowitz, M. A. (2022). The serotonin theory of depression: a systematic umbrella review of the evidence. *Molecular Psychiatry*, 1–14. https://doi.org/10.1038/s41380-022-01661-0

Monteggia, L. M., & Zarate, C. (2015). Antidepressant actions of ketamine: From molecular mechanisms to clinical practice. *Current Opinion in Neurobiology, 30*, 139-143. https://doi.org/10.1016/j.conb.2014.12.004

Moore, D. R. (2008). Reverse translation: Clearing a path from bedside to bench. *Nature*, *454*(7202), 274. https://doi.org/10.1038/454274a

Morgan, C. J. A., & Curran, H. V. (2012). Ketamine use: A review. *Addiction*, *107*(1), 27–38.

Morgan, C. J. A., Perry, E. B., Cho, H. S., Krystal, J. H., & D'Souza, D. C. (2006). Greater vulnerability to the amnestic effects of ketamine in males. *Psychopharmacology*, *187*(4), 405–414.

Morris, M. C., Ciesla, J. A., & Garber, J. (2010). A prospective study of stress autonomy versus stress sensitization in adolescents at varied risk for depression. *Journal of Abnormal Psychology*, *119*(2), 341–354. https://doi.org/10.1037/A0019036

Mourtzi, N., Sertedaki, A., & Charmandari, E. (2021). Glucocorticoid Signaling and Epigenetic Alterations in Stress-Related Disorders. *International Journal of Molecular Sciences*, *22*(11), 5964. https://doi.org/10.3390/IJMS22115964

Mulder, M., Jansen, P. J., Janssen, B. J. A., van de Berg, W. D. J., van der Boom, H., Havekes, L. M., de Kloet, R. E., Ramaekers, F. C. S., & Blokland, A. (2004). Low-density lipoprotein receptor-knockout mice display impaired spatial memory associated with a decreased synaptic density in the hippocampus. *Neurobiology of Disease*, *16*(1), 212–219. https://doi.org/10.1016/J.NBD.2004.01.015

Müller, H. K., Wegener, G., Liebenberg, N., Zarate, C. A., Popoli, M., & Elfving, B. (2013). Ketamine regulates the presynaptic release machinery in the hippocampus. *Journal of Psychiatric Research*, *47*(7), 892–899. https://doi.org/10.1016/J.JPSYCHIRES.2013.03.008

Muratore, C. R., Srikanth, P., Callahan, D. G., & Young-Pearse, T. L. (2014). Comparison and Optimization of hiPSC Forebrain Cortical Differentiation Protocols. *PLoS ONE*, *9*(8), e105807. https://doi.org/10.1371/JOURNAL.PONE.0105807

Murray, F., Smith, D. W., & Hutson, P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *European Journal of Pharmacology*, *583*(1), 115–127. https://doi.org/10.1016/J.EJPHAR.2008.01.014

Murrough, J. W., Perez, A. M., Pillemer, S., Stern, J., Parides, M. K., Aan Het Rot, M., Collins, K. A., Mathew, S. J., Charney, D. S., & Iosifescu, D. v. (2013). Rapid and Longer-Term Antidepressant Effects of Repeated Ketamine Infusions in Treatment-Resistant Major Depression. *Biological Psychiatry*, *74*(4), 250–256. https://doi.org/10.1016/J.BIOPSYCH.2012.06.022

Musazzi, L. (2020). Targeting metabotropic glutamate receptors for rapid-acting antidepressant drug discovery. *Expert Opinion on Drug Discovery*, *16*(2), 147–157. https://doi.org/10.1080/17460441.2020.1822814

Myers, B., & Greenwood-Van Meerveld, B. (2007). Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *292*(6). https://doi.org/10.1152/ajpgi.00080.2007

Myung, J. K., Dunah, A. W., Yu, T. W., & Sheng, M. (2005). Differential Roles of NR2A- and NR2B-Containing NMDA Receptors in Ras-ERK Signaling and AMPA Receptor Trafficking. *Neuron*, *46*(5), 745–760. https://doi.org/10.1016/J.NEURON.2005.04.031

Nabil Fikri, R. M., Norlelawati, A. T., Nour El-Huda, A. R., Hanisah, M. N., Kartini, A., Norsidah, K., & Nor Zamzila, A. (2017). Reelin (RELN) DNA methylation in the peripheral blood of schizophrenia. *Journal of Psychiatric Research*, *88*, 28–37. https://doi.org/10.1016/J.JPSYCHIRES.2016.12.020

Najjar, S., Pearlman, D. M., Devinsky, O., Najjar, A., & Zagzag, D. (2013). Neurovascular unit dysfunction with blood-brain barrier hyperpermeability contributes to major depressive disorder: a review of clinical and experimental evidence. *Journal of Neuroinflammation*, *10*(1), 1–16. https://doi.org/10.1186/1742-2094-10-142

Nakano, Y., Kohno, T., Hibi, T., Kohno, S., Baba, A., Mikoshiba, K., Nakajima, K., & Hattori, M. (2007). The extremely conserved C-terminal region of Reelin is not necessary for secretion but is required for efficient activation of downstream signaling. *Journal of Biological Chemistry*, *282*(28), 20544–20552. https://doi.org/10.1074/jbc.M702300200

Nakazawa, T., Hashimoto, R., Takuma, K., & Hashimoto, H. (2019). Modeling of psychiatric disorders using induced pluripotent stem cell-related technologies. *Journal of Pharmacological Sciences*, *140*(4), 321–324. https://doi.org/10.1016/J.JPHS.2019.06.002

Nakazawa, T., Kikuchi, M., Ishikawa, M., Yamamori, H., Nagayasu, K., Matsumoto, T., Fujimoto, M., Yasuda, Y., Fujiwara, M., Okada, S., Matsumura, K., Kasai, A., Hayata-Takano, A., Shintani, N., Numata, S., Takuma, K., Akamatsu, W., Okano, H., Nakaya, A., … Hashimoto, R. (2017). Differential gene expression profiles in neurons generated from lymphoblastoid B-cell line-derived iPS cells from monozygotic twin cases with treatment-resistant schizophrenia and discordant responses to clozapine. *Schizophrenia Research*, *181*, 75–82. https://doi.org/10.1016/J.SCHRES.2016.10.012

Nandam, L. S., Brazel, M., Zhou, M., & Jhaveri, D. J. (2020). Cortisol and Major Depressive Disorder—Translating Findings From Humans to Animal Models and Back. *Frontiers in Psychiatry*, *10*, 974. https://doi.org/10.3389/FPSYT.2019.00974/BIBTEX

Nandam, L. S., Jhaveri, D., & Bartlett, P. (2007). 5-HT7, neurogenesis and antidepressants: a promising therapeutic axis for treating depression. *Clinical and Experimental Pharmacology & Physiology*, *34*(5–6), 546–551. https://doi.org/10.1111/J.1440-1681.2007.04608.X

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of Depression. *Neuron*, *34*(1), 13–25. https://doi.org/10.1016/S0896-6273(02)00653-0

Newell-Price, J., Clark, A. J. L., & King, P. (2000). DNA Methylation and Silencing of Gene Expression. *Trends in Endocrinology & Metabolism*, *11*(4), 142–148. https://doi.org/10.1016/S1043-2760(00)00248-4

Nin, M. S., Martinez, L. A., Pibiri, F., Nelson, M., & Pinna, G. (2011). Neurosteroids Reduce Social Isolation-Induced Behavioral Deficits: A Proposed Link with Neurosteroid-Mediated Upregulation of BDNF Expression. *Frontiers in Endocrinology*, *2*(NOV). https://doi.org/10.3389/FENDO.2011.00073

Niswender, C. M., & Conn, P. J. (2010). Metabotropic Glutamate Receptors: Physiology, Pharmacology, and Disease. *Annual Review of Pharmacology and Toxicology*, *50*, 295. https://doi.org/10.1146/ANNUREV.PHARMTOX.011008.145533

Niu, S., Yabut, O., & D'Arcangelo, G. (2008). The Reelin Signaling Pathway Promotes Dendritic Spine Development in Hippocampal Neurons. *Journal of Neuroscience*, *28*(41), 10339–10348. https://doi.org/10.1523/JNEUROSCI.1917-08.2008

Noble, R. E. (2005). Depression in women. *Metabolism*, *54*(5), 49–52. https://doi.org/10.1016/J.METABOL.2005.01.014

Nogo, D., Jasrai, A. K., Kim, H., Nasri, F., Ceban, F., Lui, L. M. W., Rosenblat, J. D., Vinberg, M., Ho, R., & McIntyre, R. S. (2022). The effect of ketamine on anhedonia: improvements

in dimensions of anticipatory, consummatory, and motivation-related reward deficits. *Psychopharmacology*, *239*(7), 2011–2039.

Nosyreva, E., Szabla, K., Autry, A. E., Ryazanov, A. G., Monteggia, L. M., & Kavalali, E. T. (2013). Acute Suppression of Spontaneous Neurotransmission Drives Synaptic Potentiation. *Journal of Neuroscience*, *33*(16), 6990–7002. https://doi.org/10.1523/JNEUROSCI.4998-12.2013

Notaras, M. J., Vivian, B., Wilson, C., & van den Buuse, M. (2020). Interaction of reelin and stress on immobility in the forced swim test but not dopamine-mediated locomotor hyperactivity or prepulse inhibition disruption: Relevance to psychotic and mood disorders. *Schizophrenia Research*, *215*, 485–492. https://doi.org/10.1016/j.schres.2017.07.016

Nowak, G., Trullas, R., Layer, R. T., Skolnick, P., & Paul, I. A. (1993). Adaptive changes in the N-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid. *Journal of Pharmacology and Experimental Therapeutics*, *265*(3).

Nugent, A. C., Ballard, E. D., Gould, T. D., Park, L. T., Moaddel, R., Brutsche, N. E., & Zarate, C. A. (2018). Ketamine has distinct electrophysiological and behavioral effects in depressed and healthy subjects. *Molecular Psychiatry*, *24*(7), 1040–1052. https://doi.org/10.1038/s41380-018-0028-2

Nuvvula, S. (2016). Learned helplessness. *Contemporary Clinical Dentistry*, *7*(4), 426-427 https://doi.org/10.4103/0976-237X.194124

Ogata, N., de Souza Dantas, L. M., & Crowell-Davis, S. L. (2022). Selective Serotonin Reuptake Inhibitors. *Veterinary Psychopharmacology*, 103–128. https://doi.org/10.1002/9781119226253.ch8

Okine, T., Shepard, R., Lemanski, E., & Coutellier, L. (2020). Sex Differences in the Sustained Effects of Ketamine on Resilience to Chronic Stress. *Frontiers in Behavioral Neuroscience*, *14*, 185. https://doi.org/10.3389/FNBEH.2020.581360

Okita, K., Hong, H., Takahashi, K., & Yamanaka, S. (2010). Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nature Protocols*, *5*(3), 418–428. https://doi.org/10.1038/nprot.2009.231

Olloquequi, J., Cornejo-Córdova, E., Verdaguer, E., Soriano, F. X., Binvignat, O., Auladell, C., & Camins, A. (2018). Excitotoxicity in the pathogenesis of neurological and psychiatric disorders: Therapeutic implications. *Journal of Psychopharmacology*, *32*(3), 265–275.

Olsen, G. M., Scheel-Krüger, J., Møller, A., & Jensen, L. H. (1994). Does neuronal damage of CA1 relate to spatial memory performance of rats subjected to transient forebrain ischemia? *Acta Neurologica Scandinavica*, *89*(3), 204–209. https://doi.org/10.1111/J.1600-0404.1994.TB01662.X

Öngür, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences*, *95*(22), 13290–13295. https://doi.org/10.1073/PNAS.95.22.13290

Oo, K. Z., Aung, Y. K., Jenkins, M. A., & Win, A. K. (2016). Associations of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence: A systematic review and meta-analysis. *Australian and New Zealand Journal of Psychiatry*, *50*(9), 842–857.

Ormel, J., Hartman, C. A., & Snieder, H. (2019). The genetics of depression: successful genome-wide association studies introduce new challenges. *Translational Psychiatry*, *9*(1), 1–10. https://doi.org/10.1038/s41398-019-0450-5

O'Shea, K. S., & McInnis, M. G. (2015). Induced Pluripotent Stem Cell (iPSC) Models of Bipolar Disorder. *Neuropsychopharmacology*, *40*(1), 248. https://doi.org/10.1038/NPP.2014.221

O'Shea, K. S., & McInnis, M. G. (2016). Neurodevelopmental origins of bipolar disorder: iPSC models. *Molecular and Cellular Neuroscience*, *73*, 63–83. https://doi.org/10.1016/J.MCN.2015.11.006

Ouyang, X., Wang, Z., Luo, M., Wang, M., Liu, X., Chen, J., Feng, J. G., Jia, J., & Wang, X. (2021). Ketamine ameliorates depressive-like behaviors in mice through increasing glucose uptake regulated by the ERK/GLUT3 signaling pathway. *Scientific Reports*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-97758-7

Owens, A., Holroyd, B. R., & Mclane, P. (2020). Patient race, ethnicity, and care in the emergency department: A scoping review. *Canadian Journal of Emergency Medicine*, *22*(2), 245–253. https://doi.org/10.1017/CEM.2019.458

Padurariu, M., Ciobica, A., Mavroudis, I., Fotiou, D., & Baloyannis, S. (2012). Hippocampal neuronal loss in the CA1 and CA3 areas of Alzheimer's disease patients. *Psychiatria Danubina*, *24*(2), 152–158.

Pałucha-Poniewiera, A., Podkowa, K., & Rafało-Ulińska, A. (2021). The group II mGlu receptor antagonist LY341495 induces a rapid antidepressant-like effect and enhances the effect of ketamine in the chronic unpredictable mild stress model of depression in C57BL/6J mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *109*, 110239. https://doi.org/10.1016/J.PNPBP.2020.110239

Papakostas, G. I., Petersen, T., Denninger, J., Sonawalla, S. B., Mahal, Y., Alpert, J. E., Nierenberg, A. A., & Fava, M. (2003). Somatic symptoms in treatment-resistant depression. *Psychiatry Research*, *118*(1), 39–45. https://doi.org/10.1016/S0165-1781(03)00063-5

Papapetrou, E. P., Tomishima, M. J., Chambers, S. M., Mica, Y., Reed, E., Menon, J., Tabar, V., Mo, Q., Studer, L., & Sadelain, M. (2009). Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(31), 12759–12764.

Pariante, C. M. (2003). Depression, stress and the adrenal axis. *Journal of Neuroendocrinology*, *15*(8), 811–812. https://doi.org/10.1046/J.1365-2826.2003.01058.X

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468. https://doi.org/10.1016/J.TINS.2008.06.006

Park, T. J., & Curran, T. (2008). Crk and Crk-Like Play Essential Overlapping Roles Downstream of Disabled-1 in the Reelin Pathway. *Journal of Neuroscience*, *28*(50), 13551–13562. https://doi.org/10.1523/JNEUROSCI.4323-08.2008

Parmar, M., & Björklund, A. (2020). From Skin to Brain: A Parkinson's Disease Patient Transplanted with His Own Cells. *Cell Stem Cell*, *27*(1), 8–10. https://doi.org/10.1016/J.STEM.2020.06.008

Parsons, M. P., & Raymond, L. A. (2014). Extrasynaptic NMDA Receptor Involvement in Central Nervous System Disorders. *Neuron*, *82*(2), 279–293. https://doi.org/10.1016/J.NEURON.2014.03.030

Pashaei, Y. (2021). Drug repurposing of selective serotonin reuptake inhibitors: Could these drugs help fight COVID-19 and save lives? *Journal of Clinical Neuroscience*, *88*, 163–172. https://doi.org/10.1016/J.JOCN.2021.03.010

Paulsen, B. da S., Cardoso, S. C., Stelling, M. P., Cadilhe, D. V., & Rehen, S. K. (2014). Valproate reverts zinc and potassium imbalance in schizophrenia-derived reprogrammed cells. *Schizophrenia Research*, *154*(1–3), 30–35. https://doi.org/10.1016/J.SCHRES.2014.02.007

Pavlides, C., Nivón, L. G., & McEwen, B. S. (2002). Effects of chronic stress on hippocampal long-term potentiation. *Hippocampus*, *12*(2), 245–257. https://doi.org/10.1002/HIPO.1116

Pazini, F. L., Cunha, M. P., Rosa, J. M., Colla, A. R. S., Lieberknecht, V., Oliveira, Á., & Rodrigues, A. L. S. (2016). Creatine, Similar to Ketamine, Counteracts Depressive-Like Behavior Induced by Corticosterone via PI3K/Akt/mTOR Pathway. *Molecular Neurobiology*, *53*(10), 6818–6834. https://doi.org/10.1007/S12035-015-9580-9/FIGURES/8

Peciña, M., Karp, J. F., Mathew, S., Todtenkopf, M. S., Ehrich, E. W., & Zubieta, J. K. (2018). Endogenous opioid system dysregulation in depression: implications for new therapeutic approaches. *Molecular Psychiatry*, *24*(4), 576–587. https://doi.org/10.1038/s41380-018-0117-2

Pędzich, B. D., Rubens, S., Sekssaoui, M., Pierre, A., van Schuerbeek, A., Marin, P., Bockaert, J., Valjent, E., Bécamel, C., & de Bundel, D. (2022). Effects of a psychedelic 5-HT2A receptor agonist on anxiety-related behavior and fear processing in mice. *Neuropsychopharmacology*, *47*(7), 1304–1314. https://doi.org/10.1038/s41386-022-01324-2

Peng, B., Xu, Q., Liu, J., Guo, S., Borgland, S. L., & Liu, S. (2021). Corticosterone Attenuates Reward-Seeking Behavior and Increases Anxiety via D2 Receptor Signaling in Ventral Tegmental Area Dopamine Neurons. *Journal of Neuroscience*, *41*(7), 1566–1581. https://doi.org/10.1523/JNEUROSCI.2533-20.2020

Perez-Caballero, L., Perez, V., & Berrocoso, E. (2020). What ketamine can teach us about the opioid system in depression? *Expert Opinion on Drug Discovery, 15*(12), 1369-1372. https://doi.org/10.1080/17460441.2020.1781812

Perry, R. J., Resch, J. M., Douglass, A. M., Madara, J. C., Rabin-Court, A., Kucukdereli, H., Wu, C., Song, J. D., Lowell, B. B., & Shulman, G. I. (2019). Leptin's hunger-suppressing effects are mediated by the hypothalamic–pituitary–adrenocortical axis in rodents. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(27), 13670–13679.

Persson, J. (2010). Wherefore ketamine? *Current Opinion in Anaesthesiology*, *23*(4), 455–460. https://doi.org/10.1097/ACO.0B013E32833B49B3

Peterlik, D., J. Flor, P., & Uschold-Schmidt, N. (2015). The Emerging Role of Metabotropic Glutamate Receptors in the Pathophysiology of Chronic Stress-Related Disorders. *Current Neuropharmacology*, *14*(5), 514–539. https://doi.org/10.2174/1570159x13666150515234920

Pfisterer, U., Kirkeby, A., Torper, O., Wood, J., Nelander, J., Dufour, A., Björklund, A., Lindvall, O., Jakobsson, J., & Parmar, M. (2011). Direct conversion of human fibroblasts to dopaminergic neurons. *Proceedings of the National Academy of Sciences of the United States of America, 108*(25), 10343–10348.

Pham, K., Nacher, J., Hof, P. R., & McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *European Journal of Neuroscience*, *17*(4), 879–886. https://doi.org/10.1046/J.1460-9568.2003.02513.X

Pinna, G. (2020). Allopregnanolone, the Neuromodulator Turned Therapeutic Agent: Thank You, Next? *Frontiers in Endocrinology*, *11*, 236. https://doi.org/10.3389/FENDO.2020.00236

Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology*, *33*(1), 88-109. https://doi.org/10.1038/sj.npp.1301574

Piva, A., Caffino, L., Mottarlini, F., Pintori, N., Castillo Díaz, F., Fumagalli, F., & Chiamulera, C. (2021). Metaplastic Effects of Ketamine and MK-801 on Glutamate Receptors Expression in Rat Medial Prefrontal Cortex and Hippocampus. *Molecular Neurobiology*, *58*(7), 3443–3456. https://doi.org/10.1007/S12035-021-02352-7

Planchez, B., Surget, A., & Belzung, C. (2019). Animal models of major depression: drawbacks and challenges. *Journal of Neural Transmission, 126*(11), 1383-1408. https://doi.org/10.1007/s00702-019-02084-y

Pochwat, B., Nowak, G., & Szewczyk, B. (2019). An update on NMDA antagonists in depression. *Expert Review of Neurotherapeutics, 19*(11), 1055-1067. https://doi.org/10.1080/14737175.2019.1643237

Ponton, E., Turecki, G., Nagy, C., & Blvd, L. (2022). Sex Differences in the Behavioral, Molecular, and Structural Effects of Ketamine Treatment in Depression. *International Journal of Neuropsychopharmacology*, *25*(1), 75–84. https://doi.org/10.1093/IJNP/PYAB082

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology*, *47*(4), 379–391. https://doi.org/10.1016/0014-2999(78)90118-8

Pothula, S., Liu, R.-J., Wu, M., Sliby, A.-N., DiLeone, R., & Duman, R. (2021). GluN2B-containing NMDA Receptors on Sst-interneurons act as Initial Cellular Trigger for Antidepressant Actions of Ketamine. *The FASEB Journal*, *35*(S1). https://doi.org/10.1096/FASEBJ.2021.35.S1.02392

Price, R. B., & Duman, R. (2020). Neuroplasticity in cognitive and psychological mechanisms of depression: an integrative model. *Molecular Psychiatry, 25*(3), 530-543. https://doi.org/10.1038/s41380-019-0615-x

Prieto, M. L., & Wollmuth, L. P. (2010). Gating Modes in AMPA Receptors. *Journal of Neuroscience*, *30*(12), 4449–4459. https://doi.org/10.1523/JNEUROSCI.5613-09.2010

Pujadas, L., Gruart, A., Bosch, C., Delgado, L., Teixeira, C. M., Rossi, D., de Lecea, L., Martínez, A., Delgado-García, J. M., & Soriano, E. (2010). Reelin Regulates Postnatal Neurogenesis and Enhances Spine Hypertrophy and Long-Term Potentiation. *Journal of Neuroscience*, *30*(13), 4636–4649. https://doi.org/10.1523/JNEUROSCI.5284-09.2010

Pujadas, L., Rossi, D., Andrés, R., Teixeira, C. M., Serra-Vidal, B., Parcerisas, A., Maldonado, R., Giralt, E., Carulla, N., & Soriano, E. (2014). Reelin delays amyloid-beta fibril formation and rescues cognitive deficits in a model of Alzheimer's disease. *Nature Communications*, *5*(1), 1–11. https://doi.org/10.1038/ncomms4443

Purselle, D. C., & Nemeroff, C. B. (2002). Serotonin Transporter: A Potential Substrate in the Biology of Suicide. *Neuropsychopharmacology*, *28*(4), 613–619. https://doi.org/10.1038/sj.npp.1300092

Qi, H., Mailliet, F., Spedding, M., Rocher, C., Zhang, X., Delagrange, P., McEwen, B., Jay, T. M., & Svenningsson, P. (2009). Antidepressants reverse the attenuation of the neurotrophic MEK/MAPK cascade in frontal cortex by elevated platform stress; reversal of effects on LTP is associated with GluA1 phosphorylation. *Neuropharmacology*, *56*(1), 37–46. https://doi.org/10.1016/J.NEUROPHARM.2008.06.068

Qiu, S., Korwek, K. M., & Weeber, E. J. (2006). A fresh look at an ancient receptor family: Emerging roles for low density lipoprotein receptors in synaptic plasticity and memory

formation. *Neurobiology of Learning and Memory*, *85*(1), 16–29. https://doi.org/10.1016/J.NLM.2005.08.009

Qiu, S., Zhao, L. F., Korwek, K. M., & Weeber, E. J. (2006). Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *Journal of Neuroscience*, *26*(50), 12943–12955. https://doi.org/10.1523/JNEUROSCI.2561-06.2006

Raab, S., Klingenstein, M., Liebau, S., & Linta, L. (2014). A Comparative View on Human Somatic Cell Sources for iPSC Generation. *Stem Cells International*, *2014*. https://doi.org/10.1155/2014/768391

Rafało-Ulińska, A., Brański, P., & Pałucha-Poniewiera, A. (2022a). Combined Administration of (R)-Ketamine and the mGlu2/3 Receptor Antagonist LY341495 Induces Rapid and Sustained Effects in the CUMS Model of Depression via a TrkB/BDNF-Dependent Mechanism. *Pharmaceuticals, 15*(2), 125. https://doi.org/10.3390/PH15020125

Rafało-Ulińska, A., & Pałucha-Poniewiera, A. (2022b). The effectiveness of (R)-ketamine and its mechanism of action differ from those of (S)-ketamine in a chronic unpredictable mild stress model of depression in C57BL/6J mice. *Behavioural Brain Research*, *418*, 113633. https://doi.org/10.1016/J.BBR.2021.113633

Rahimi-Balaei, M., Jiao, X., Dalvand, A., Shabanipour, S., Chung, S. H., Amiri, S., Kong, J., & Marzban, H. (2020). Mutations in the Reelin pathway are associated with abnormal expression of microglial IgG FC receptors in the cerebellar cortex. *Molecular Biology Reports*, *47*(7), 5323–5331. https://doi.org/10.1007/S11033-020-05614-0

Rahman, M. M., Callaghan, C. K., Kerskens, C. M., Chattarji, S., & O'Mara, S. M. (2016). Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress. *Scientific Reports*, *6*(1), 1–15. https://doi.org/10.1038/srep29127

Rainer, Q., Xia, L., Guilloux, J. P., Gabriel, C., Mocaër, E., Hen, R., Enhamre, E., Gardier, A. M., & David, D. J. (2012). Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. *International Journal of Neuropsychopharmacology*, *15*(3), 321–335. https://doi.org/10.1017/S1461145711000356

Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology*, *27*(1), 24–31. https://doi.org/10.1016/J.IT.2005.11.006

Rajkowska, G., & Miguel-Hidalgo, J. J. (2008). Gliogenesis and Glial Pathology in Depression. *CNS & Neurological Disorders - Drug Targets*, *6*(3), 219–233. https://doi.org/10.2174/187152707780619326

Ramasubbu, B., James, D., Scurr, A., & Sandilands, E. A. (2022). Tricyclic Antidepressants. *BMJ Case Reports*, *2016*. https://doi.org/10.1136/BCR-2016-214685

Ramponi, C., Barnard, P. J., & Nimmo-smith, I. (2004). Recollection deficits in dysphoric mood: An effect of schematic models and executive mode? *Memory*, *12*(5), 655–670. https://doi.org/10.1080/09658210344000189

Ranaivoson, F. M., von Daake, S., & Comoletti, D. (2016). Structural insights into reelin function: Present and future. *Frontiers in Cellular Neuroscience*, *10*(MAY), 137. https://doi.org/10.3389/FNCEL.2016.00137

Räsänen, N., Tiihonen, J., Koskuvi, M., Lehtonen, Š., & Koistinaho, J. (2022). The iPSC perspective on schizophrenia. *Trends in Neurosciences*, *45*(1), 8–26. https://doi.org/10.1016/J.TINS.2021.11.002

Rawat, R., Tunc-Ozcan, E., McGuire, T. L., Peng, C. Y., & Kessler, J. A. (2022). Ketamine activates adult-born immature granule neurons to rapidly alleviate depression-like behaviors in mice. *Nature Communications*, *13*(1), 1–12. https://doi.org/10.1038/s41467-022-30386-5

Regenthal, R., Koch, H., Köhler, C., Preiss, R., & Krügel, U. (2009). Depression-like deficits in rats improved by subchronic modafinil. *Psychopharmacology*, *204*(4), 627–639. https://doi.org/10.1007/S00213-009-1493-8/FIGURES/5

Ren, Z., Pribiag, H., Jefferson, S. J., Shorey, M., Fuchs, T., Stellwagen, D., & Luscher, B. (2016). Bidirectional Homeostatic Regulation of a Depression-Related Brain State by Gamma-Aminobutyric Acidergic Deficits and Ketamine Treatment. *Biological Psychiatry*, *80*(6), 457–468. https://doi.org/10.1016/J.BIOPSYCH.2016.02.009

Rengasamy, M., Marsland, A., McClain, L., Kovats, T., Walko, T., Pan, L., & Price, R. B. (2021). Longitudinal relationships of cytokines, depression and anhedonia in depressed adolescents. *Brain, Behavior, and Immunity*, *91*, 74–80. https://doi.org/10.1016/J.BBI.2020.09.004

Reul, J. M. H. M., & de Kloet, E. R. (1985). Two Receptor Systems for Corticosterone in Rat Brain: Microdistribution and Differential Occupation. *Endocrinology*, *117*(6), 2505–2511. https://doi.org/10.1210/ENDO-117-6-2505

Rice, D. S., Sheldon, M., D'Arcangelo, G., Nakajima, K., Goldowitz, D., & Curran, T. (1998). Disabled-1 acts downstream of Reelin in a signaling pathway that controls laminar organization in the mammalian brain. *Development*, *125*(18), 3719–3729. https://doi.org/10.1242/DEV.125.18.3719

Richards, D. (2011). Prevalence and clinical course of depression: A review. *Clinical Psychology Review*, *31*(7), 1117–1125. https://doi.org/10.1016/J.CPR.2011.07.004

Richards, D. P., Jordan, I., Strain, K., & Press, Z. (2018). Patient partner compensation in research and health care: the patient perspective on why and how. *Patient Experience Journal*, *5*(3), 6–12. https://doi.org/10.35680/2372-0247.1334

Richards, D. P., Jordan, I., Strain, K., & Press, Z. (2020). Patients as partners in research: How to talk about compensation with patient partners. *Journal of Orthopaedic and Sports Physical Therapy*, *50*(8), 413–414.

Richards, E. M., Zanotti-Fregonara, P., Fujita, M., Newman, L., Farmer, C., Ballard, E. D., Machado-Vieira, R., Yuan, P., Niciu, M. J., Lyoo, C. H., Henter, I. D., Salvadore, G., Drevets, W. C., Kolb, H., Innis, R. B., & Zarate, C. A. (2018). PET radioligand binding to translocator protein (TSPO) is increased in unmedicated depressed subjects. *EJNMMI Research*, *8*(1), 1–9. https://doi.org/10.1186/S13550-018-0401-9

Rivera-Baltanas, T., Agis-Balboa, R. C., Romay-Tallon, R., Kalynchuk, L. E., Olivares, J. M., & Caruncho, H. J. (2015). Serotonin transporter clustering in blood lymphocytes predicts the outcome on anhedonia scores in naïve depressive patients treated with antidepressant medication. *Annals of General Psychiatry*, *14*(1), 1–8. https://doi.org/10.1186/s12991-015-0085-8

Rivera-Baltanas, T., Olivares, J. M., Calado-Otero, M., Kalynchuk, L. E., Martinez-Villamarin, J. R., & Caruncho, H. J. (2012). Serotonin transporter clustering in blood lymphocytes as a putative biomarker of therapeutic efficacy in major depressive disorder. *Journal of Affective Disorders*, *137*(1–3), 46–55. https://doi.org/10.1016/j.jad.2011.12.041

Rivera-Baltanas, T., Olivares, J. M., Martinez-Villamarin, J. R., Y. Fenton, E., E. Kalynchuk, L., & J. Caruncho, H. (2014). Serotonin 2A receptor clustering in peripheral lymphocytes is altered in major depression and may be a biomarker of therapeutic efficacy. *Journal of Affective Disorders*, *163*, 47–55. https://doi.org/10.1016/J.JAD.2014.03.011

Rizvi, S. J., Pizzagalli, D. A., Sproule, B. A., & Kennedy, S. H. (2016). Assessing anhedonia in depression: Potentials and pitfalls. *Neuroscience & Biobehavioral Reviews*, *65*, 21–35. https://doi.org/10.1016/J.NEUBIOREV.2016.03.004

Rock, P. L., Roiser, J. P., Riedel, W. J., & Blackwell, A. D. (2014). Cognitive impairment in depression: a systematic review and meta-analysis. *Psychological Medicine*, *44*(10), 2029–2040. https://doi.org/10.1017/S0033291713002535

Roddy, D. W., Farrell, C., Doolin, K., Roman, E., Tozzi, L., Frodl, T., O'Keane, V., & O'Hanlon, E. (2019). The Hippocampus in Depression: More Than the Sum of Its Parts? Advanced Hippocampal Substructure Segmentation in Depression. *Biological Psychiatry*, *85*(6), 487–497.

Rodriguez, M. A., Pesold, C., Liu, W. S., Kriho, V., Guidotti, A., Pappas, G. D., & Costa, E. (2000). Colocalization of integrin receptors and reelin in dendritic spine postsynaptic densities of adult nonhuman primate cortex. *Proceedings of the National Academy of Sciences*, *97*(7), 3550–3555. https://doi.org/10.1073/PNAS.97.7.3550

Rogers, J. T., Rusiana, I., Trotter, J., Zhao, L., Donaldson, E., Pak, D. T. S., Babus, L. W., Peters, M., Banko, J. L., Chavis, P., Rebeck, G. W., Hoe, H. S., & Weeber, E. J. (2011).

Reelin supplementation enhances cognitive ability, synaptic plasticity, and dendritic spine density. *Learning & Memory*, *18*(9), 558–564. https://doi.org/10.1101/LM.2153511

Rogers, J. T., Zhao, L., Trotter, J. H., Rusiana, I., Peters, M. M., Li, Q., Donaldson, E., Banko, J. L., Keenoy, K. E., Rebeck, G. W., Hoe, H. S., D'Arcangelo, G., & Weeber, E. J. (2013). Reelin supplementation recovers sensorimotor gating, synaptic plasticity and associative learning deficits in the heterozygous reeler mouse. *Journal of Psychopharmacology*, *27*(4), 386–395. https://doi.org/10.1177/0269881112463468

Rohde, P., Lewinsohn, P. M., & Seeley, J. R. (1991). Comorbidity of Unipolar Depression: II. Comorbidity With Other Mental Disorders in Adolescents and Adults. *Journal of Abnormal Psychology*, *100*(2), 214–222. https://doi.org/10.1037/0021-843X.100.2.214

Rolland, B., Jardri, R., Amad, A., Thomas, P., Cottencin, O., & Bordet, R. (2014). Pharmacology of hallucinations: Several mechanisms for one single symptom? *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/307106

Romay-Tallon, R., Kulhawy, E., Brymer, K. J., Allen, J., Rivera-Baltanas, T., Olivares, J. M., Kalynchuk, L. E., & Caruncho, H. J. (2018). Changes in membrane protein clustering in peripheral lymphocytes in an animal model of depression parallel those observed in naïve depression patients: Implications for the development of novel biomarkers of depression. *Frontiers in Pharmacology*, *9*(OCT), 1149. https://doi.org/10.3389/fphar.2018.01149

Romay-Tallon, R., Rivera-Baltanas, T., Allen, J., Olivares, J. M., Kalynchuk, L. E., & Caruncho, H. J. (2017). Comparative study of two protocols for quantitative image-analysis of serotonin transporter clustering in lymphocytes, a putative biomarker of therapeutic efficacy in major depression. *Biomarker Research*, *5*(1), 1–8. https://doi.org/10.1186/s40364-017-0107-6

Romay-Tallon, R., Rivera-Baltanas, T., Kalynchuk, L. E., & Caruncho, H. J. (2015). Differential effects of corticosterone on the colocalization of reelin and neuronal nitric oxide synthase in the adult hippocampus in wild type and heterozygous reeler mice. *Brain Research*, *1594*, 274–283. https://doi.org/10.1016/J.BRAINRES.2014.10.050

Rosburg, T., & Kreitschmann-Andermahr, I. (2016). The effects of ketamine on the mismatch negativity (MMN) in humans – A meta-analysis. *Clinical Neurophysiology*, *127*(2), 1387–1394. https://doi.org/10.1016/J.CLINPH.2015.10.062

Rosburg, T., Marinou, V., Haueisen, J., Smesny, S., & Sauer, H. (2004). Effects of Lorazepam on the Neuromagnetic Mismatch Negativity (MMNm) and Auditory Evoked Field Component N100m. *Neuropsychopharmacology*, *29*(9), 1723–1733. https://doi.org/10.1038/sj.npp.1300477

Rumbaugh, G., & Vicini, S. (1999). Distinct Synaptic and Extrasynaptic NMDA Receptors in Developing Cerebellar Granule Neurons. *Journal of Neuroscience*, *19*(24), 10603–10610. https://doi.org/10.1523/JNEUROSCI.19-24-10603.1999

Rüsch, N., Angermeyer, M. C., & Corrigan, P. W. (2005). Mental illness stigma: Concepts, consequences, and initiatives to reduce stigma. *European Psychiatry, 20*(8), 529-539. https://doi.org/10.1016/j.eurpsy.2005.04.004

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., Niederehe, G., Thase, M. E., Lavori, P. W., Lebowitz, B. D., McGrath, P. J., Rosenbaum, J. F., Sackeim, H. A., Kupfer, D. J., Luther, J., & Fava, M. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR*D report. *American Journal of Psychiatry*, *163*(11), 1905–1917.

Sacristán, J. A. (2013). Patient-centered medicine and patient-oriented research: Improving health outcomes for individual patients. *BMC Medical Informatics and Decision Making, 13*(1), 1-8. https://doi.org/10.1186/1472-6947-13-6

Sáez-Valero, J., Costell, M., Sjögren, M., Andreasen, N., Blennow, K., & Luque, J. M. (2003). Altered levels of cerebrospinal fluid reelin in frontotemporal dementia and Alzheimer's disease. *Journal of Neuroscience Research*, *72*(1), 132–136. https://doi.org/10.1002/JNR.10554

Sahay, A., & Hen, R. (2007). Adult hippocampal neurogenesis in depression. *Nature Neuroscience, 10*(9), 1110-1115. https://doi.org/10.1038/nn1969

Sahli, Z. T., Banerjee, P., & Tarazi, F. I. (2016). The Preclinical and Clinical Effects of Vilazodone for the Treatment of Major Depressive Disorder. *Expert Opinion on Drug Discovery, 11*(5), 515–523. https://doi.org/10.1517/17460441.2016.1160051

Saland, S. K., Duclot, F., & Kabbaj, M. (2017). Integrative analysis of sex differences in the rapid antidepressant effects of ketamine in preclinical models for individualized clinical outcomes. *Current Opinion in Behavioral Sciences*, *14*, 19–26. https://doi.org/10.1016/J.COBEHA.2016.11.002

Salzer, H. M., & Lurie, M. L. (1953). Anxiety and depressive states treated with isonicotinyl hydrazide (isoniazid). *Archives of Neurology and Psychiatry*, *70*(3), 317–324. https://doi.org/10.1001/archneurpsyc.1953.02320330042005

Sánchez-Lafuente, C. L., Romay-Tallon, R., Allen, J., Johnston, J. N., Kalynchuk, L. E., & Caruncho, H. J. (2022). Sex differences in basal reelin levels in the paraventricular hypothalamus and in response to chronic stress induced by repeated corticosterone in rats. *Hormones and Behavior*, *146*, 105267. https://doi.org/10.1016/J.YHBEH.2022.105267

Sanderson, D. J., & Bannerman, D. M. (2012). The role of habituation in hippocampus-dependent spatial working memory tasks: Evidence from GluA1 AMPA receptor subunit knockout mice. *Hippocampus*, *22*(5), 981–994. https://doi.org/10.1002/HIPO.20896

Sanderson, D. J., Good, M. A., Skelton, K., Sprengel, R., Seeburg, P. H., Rawlins, J. N. P., & Bannerman, D. M. (2009). Enhanced long-term and impaired short-term spatial memory in GluA1 AMPA receptor subunit knockout mice: Evidence for a dual-process memory model. *Learning & Memory*, *16*(6), 379–386. https://doi.org/10.1101/LM.1339109

Sanderson, D. J., McHugh, S. B., Good, M. A., Sprengel, R., Seeburg, P. H., Rawlins, J. N. P., & Bannerman, D. M. (2010). Spatial working memory deficits in GluA1 AMPA receptor subunit knockout mice reflect impaired short-term habituation: Evidence for Wagner's dual-process memory model. *Neuropsychologia*, *48*(8), 2303–2315.

Sansone, R. A., & Sansone, L. A. (2014). Serotonin Norepinephrine Reuptake Inhibitors: A Pharmacological Comparison. *Innovations in Clinical Neuroscience*, *11*(3–4), 37.

Santini, Z. I., Jose, P. E., York Cornwell, E., Koyanagi, A., Nielsen, L., Hinrichsen, C., Meilstrup, C., Madsen, K. R., & Koushede, V. (2020). Social disconnectedness, perceived isolation, and symptoms of depression and anxiety among older Americans (NSHAP): a longitudinal mediation analysis. *The Lancet Public Health*, *5*(1), e62–e70. https://doi.org/10.1016/S2468-2667(19)30230-0

Sapolsky, R. M. (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biological Psychiatry*, *48*(8), 755–765. https://doi.org/10.1016/S0006-3223(00)00971-9

Sapolsky, R. M. (2003). Stress and Plasticity in the Limbic System. *Neurochemical Research*, *28*(11), 1735-1742. https://doi.org/10.1023/A:1026021307833

Sarkar, A., & Kabbaj, M. (2016). Sex Differences in Effects of Ketamine on Behavior, Spine Density, and Synaptic Proteins in Socially Isolated Rats. *Biological Psychiatry*, *80*(6), 448–456. https://doi.org/10.1016/J.BIOPSYCH.2015.12.025

Sase, S., Sase, A., Sialana, F. J., Gröger, M., Bennett, K. L., Stork, O., Lubec, G., & Li, L. (2015). Individual phases of contextual fear conditioning differentially modulate dorsal and ventral hippocampal GluA1-3, GluN1-containing receptor complexes and subunits. *Hippocampus*, *25*(12), 1501–1516. https://doi.org/10.1002/HIPO.22470

Sassano-Higgins, S., Baron, D., Juarez, G., Esmaili, N., & Gold, M. (2016). A review of ketamine abuse and diversion. *Depression and Anxiety*, *33*(8), 718–727. https://doi.org/10.1002/DA.22536

Sato, Y., Kobayashi, D., Kohno, T., Kidani, Y., Prox, J., Becker-Pauly, C., & Hattori, M. (2016). Determination of cleavage site of Reelin between its sixth and seventh repeat and contribution of meprin metalloproteases to the cleavage. *The Journal of Biochemistry*, *159*(3), 305–312. https://doi.org/10.1093/JB/MVV102

Schafer, S. T., Paquola, A. C. M., Stern, S., Gosselin, D., Ku, M., Pena, M., Kuret, T. J. M., Liyanage, M., Mansour, A. A. F., Jaeger, B. N., Marchetto, M. C., Glass, C. K., Mertens, J., & Gage, F. H. (2019). Pathological priming causes developmental gene network heterochronicity in autistic subject-derived neurons. *Nature Neuroscience*, *22*(2), 243–255. https://doi.org/10.1038/s41593-018-0295-x

Schatzberg, A. F., Keller, J., Tennakoon, L., Lembke, A., Williams, G., Kraemer, F. B., Sarginson, J. E., Lazzeroni, L. C., & Murphy, G. M. (2014). HPA axis genetic variation,

cortisol and psychosis in major depression. *Molecular Psychiatry*, *19*(2), 220–227. https://doi.org/10.1038/mp.2013.129

Schiavone, S., Colaianna, M., & Curtis, L. (2015). Impact of Early Life Stress on the Pathogenesis of Mental Disorders: Relation to Brain Oxidative Stress. *Current Pharmaceutical Design*, *21*(11), 1404–1412. https://doi.org/10.2174/1381612821666150105143358

Schiffmann, S. N., Bernier, B., & Goffinet, A. M. (1997). Reelin mRNA Expression During Mouse Brain Development. *European Journal of Neuroscience*, *9*(5), 1055–1071. https://doi.org/10.1111/J.1460-9568.1997.TB01456.X

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *The American journal of psychiatry*, *122*(5), 509-522. https://doi.org/10.1176/ajp.122.5.509

Schildkraut, J. J. (1974). Biogenic amines and affective disorders. *Annual review of medicine*, *25*, 333-348. https://doi.org/10.1146/annurev.me.25.020174.002001

Schildkraut, J. J., & Kety, S. S. (1967). Biogenic Amines and Emotion. *Science*, *156*(3771), 21–30. https://doi.org/10.1126/SCIENCE.156.3771.21

Schmidt, A., Bachmann, R., Kometer, M., Csomor, P. A., Stephan, K. E., Seifritz, E., & Vollenweider, F. X. (2011). Mismatch Negativity Encoding of Prediction Errors Predicts S-ketamine-Induced Cognitive Impairments. *Neuropsychopharmacology 37*(4), 865–875. https://doi.org/10.1038/npp.2011.261

Schmidt, H. D., Shelton, R. C., & Duman, R. S. (2011a). Functional biomarkers of depression: Diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology*, *36*(12), 2375-2394. https://doi.org/10.1038/npp.2011.151

Schmidt, M. V., Wang, X. D., & Meijer, O. C. (2011b). Early life stress paradigms in rodents: Potential animal models of depression? *Psychopharmacology*, *214*(1), 131–140. https://doi.org/10.1007/S00213-010-2096-0

Schroeder, A., Buret, L., Hill, R. A., & van den Buuse, M. (2015). Gene–environment interaction of reelin and stress in cognitive behaviours in mice: Implications for schizophrenia. *Behavioural Brain Research*, *287*, 304–314.

Schubert, J. J., Veronese, M., Fryer, T. D., Manavaki, R., Kitzbichler, M. G., Nettis, M. A., Mondelli, V., Pariante, C. M., Bullmore, E. T., Wlazly, D., Dickinson, A., Foster, A., Knight, C., Leckey, C., Morgan, P., Morgan, A., O'Hagan, C., Touchard, S., Khan, S., … Turkheimer, F. E. (2021). A Modest Increase in 11C-PK11195-Positron Emission Tomography TSPO Binding in Depression Is Not Associated With Serum C-Reactive Protein or Body Mass Index. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *6*(7), 716–724. https://doi.org/10.1016/J.BPSC.2020.12.017

Schünemann, H. J., Fretheim, A., & Oxman, A. D. (2006). Improving the use of research evidence in guideline development 10. Integrating values and consumer involvement. *Health Research Policy and Systems*, *4*(1), 1-8. BioMed Central. https://doi.org/10.1186/1478-4505-4-22

Schüttler, J., Stanski, D. R., White, P. F., Trevor, A. J., Horai, Y., Verotta, D., & Sheiner, L. B. (1987). Pharmacodynamic modeling of the EEG effects of ketamine and its enantiomers in man. *Journal of Pharmacokinetics and Biopharmaceutics*, *15*(3), 241–253. https://doi.org/10.1007/BF01066320

Schwarcz, R., Bruno, J. P., Muchowski, P. J., & Wu, H. Q. (2012). Kynurenines in the mammalian brain: when physiology meets pathology. *Nature Reviews Neuroscience*, *13*(7), 465–477. https://doi.org/10.1038/nrn3257

Seki, T., Yuasa, S., Oda, M., Egashira, T., Yae, K., Kusumoto, D., Nakata, H., Tohyama, S., Hashimoto, H., Kodaira, M., Okada, Y., Seimiya, H., Fusaki, N., Hasegawa, M., & Fukuda, K. (2010). Generation of induced pluripotent stem cells from human terminally differentiated circulating t cells. *Cell Stem Cell*, *7*(1), 11-14. https://doi.org/10.1016/j.stem.2010.06.003

Seligman, M. E. P., Weiss, J. M., Weinraub, M., & Schulman, A. (1980). Coping behavior: Learned helplessness, physiological change and learned inactivity. *Behaviour Research and Therapy*, *18*(5). https://doi.org/10.1016/0005-7967(80)90011-X

Shadrina, M., Bondarenko, E. A., & Slominsky, P. A. (2018). Genetics factors in major depression disease. *Frontiers in Psychiatry*, *9*(JUL), 334. https://doi.org/10.3389/FPSYT.2018.00334/BIBTEX

Shapero, B. G., Black, S. K., Liu, R. T., Klugman, J., Bender, R. E., Abramson, L. Y., & Alloy, L. B. (2014). Stressful Life Events and Depression Symptoms: The Effect of Childhood Emotional Abuse on Stress Reactivity. *Journal of Clinical Psychology*, *70*(3), 209–223. https://doi.org/10.1002/JCLP.22011

Shavers, V. L., Fagan, P., Jones, D., Klein, W. M. P., Boyington, J., Moten, C., & Rorie, E. (2012). The state of research on racial/ethnic discrimination in the receipt of health care. *American Journal of Public Health*, *102*(5), 953–966. https://doi.org/10.2105/AJPH.2012.300773

Sheline, Y. I. (2011). Depression and the hippocampus: Cause or effect? *Biological Psychiatry*, *70*(4), 308-309. https://doi.org/10.1016/j.biopsych.2011.06.006

Sheline, Y. I., Gado, M. H., & Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. *American Journal of Psychiatry*, *160*(8), 1516–1518. https://doi.org/10.1176/APPI.AJP.160.8.1516

Sheline, Y. I., Liston, C., & McEwen, B. S. (2019). Parsing the Hippocampus in Depression: Chronic Stress, Hippocampal Volume, and Major Depressive Disorder. *Biological Psychiatry*, *85*(6), 436–438. https://doi.org/10.1016/j.biopsych.2019.01.011

Sheline, Y. I., Raichle, M. E., Snyder, A. Z., Morris, J. C., Head, D., Wang, S., & Mintun, M. A. (2010). Amyloid Plaques Disrupt Resting State Default Mode Network Connectivity in Cognitively Normal Elderly. *Biological Psychiatry*, *67*(6), 584–587. https://doi.org/10.1016/J.BIOPSYCH.2009.08.024

Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G., & Vannier, M. W. (1996). Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences*, *93*(9), 3908–3913. https://doi.org/10.1073/PNAS.93.9.3908

Shelton, R. C., Manier, D. H., & Sulser, F. (1996). cAMP-dependent protein kinase activity in major depression. *The American Journal of Psychiatry*, *153*(8), 1037–1042. https://doi.org/10.1176/AJP.153.8.1037

Shi, J., Potash, J. B., Knowles, J. A., Weissman, M. M., Coryell, W., Scheftner, W. A., Lawson, W. B., Depaulo, J. R., Gejman, P. v., Sanders, A. R., Johnson, J. K., Adams, P., Chaudhury, S., Jancic, D., Evgrafov, O., Zvinyatskovskiy, A., Ertman, N., Gladis, M., Neimanas, K., … Levinson, D. F. (2011). Genome-wide association study of recurrent early-onset major depressive disorder. *Molecular Psychiatry*, *16*(2), 193–201. https://doi.org/10.1038/mp.2009.124

Shi, S. H., Hayashi, Y., Esteban, J. A., & Malinow, R. (2001). Subunit-Specific Rules Governing AMPA Receptor Trafficking to Synapses in Hippocampal Pyramidal Neurons. *Cell*, *105*(3), 331–343. https://doi.org/10.1016/S0092-8674(01)00321-X

Shih, J. C., & Thompson, R. F. (1999). Monoamine oxidase in neuropsychiatry and behavior. *American Journal of Human Genetics*, *65*(3), 593. https://doi.org/10.1086/302562

Shinohara, K., Efthimiou, O., Ostinelli, E. G., Tomlinson, A., Geddes, J. R., Nierenberg, A. A., Ruhe, H. G., Furukawa, T. A., & Cipriani, A. (2019). Comparative efficacy and acceptability of antidepressants in the long-term treatment of major depression: protocol for a systematic review and network meta-analysis. *BMJ Open*, *9*(5), e027574. https://doi.org/10.1136/BMJOPEN-2018-027574

Shopsin, B., Friedman, E., & Gershon, S. (1976). Parachlorophenylalanine Reversal of Tranylcypromine Effects in Depressed Patients. *Archives of General Psychiatry*, *33*(7), 811–819. https://doi.org/10.1001/ARCHPSYC.1976.01770070041003

Shopsin, B., Gershon, S., Goldstein, M., Friedman, E., & Wilk, S. (1975). Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. *Psychopharmacology Communications*, *1*(2), 239–249. https://psycnet.apa.org/record/1976-22650-001

Shors, T. J. (2006). Stressful Experience and Learning Across the Lifespan. *Annual Review of Psychology*, *57*, 55. https://doi.org/10.1146/ANNUREV.PSYCH.57.102904.190205

Shors, T. J., Seib, T. B., Levine, S., & Thompson, R. F. (1989). Inescapable versus Escapable Shock Modulates Long-Term Potentiation in the Rat Hippocampus. *Science*, *244*(4901), 224–226. https://doi.org/10.1126/SCIENCE.2704997

Short, B., Fong, J., Galvez, V., Shelker, W., & Loo, C. K. (2018). Side-effects associated with ketamine use in depression: a systematic review. *The Lancet Psychiatry*, *5*(1), 65–78. https://doi.org/10.1016/S2215-0366(17)30272-9

Shumake, J., Barrett, D., & Gonzalez-Lima, F. (2005). Behavioral characteristics of rats predisposed to learned helplessness: Reduced reward sensitivity, increased novelty seeking, and persistent fear memories. *Behavioural Brain Research*, *164*(2), 222–230. https://doi.org/10.1016/J.BBR.2005.06.016

Sigtermans, M. J., van Hilten, J. J., Bauer, M. C. R., Arbous, M. S., Marinus, J., Sarton, E. Y., & Dahan, A. (2009). Ketamine produces effective and long-term pain relief in patients with Complex Regional Pain Syndrome Type 1. *Pain*, *145*(3), 304–311. https://doi.org/10.1016/j.pain.2009.06.023

Silberbauer, L. R., Spurny, B., Handschuh, P., Klöbl, M., Bednarik, P., Reiter, B., Ritter, V., Trost, P., Konadu, M. E., Windpassinger, M., Stimpfl, T., Bogner, W., Lanzenberger, R., & Spies, M. (2020). Effect of Ketamine on Limbic GABA and Glutamate: A Human In Vivo Multivoxel Magnetic Resonance Spectroscopy Study. *Frontiers in Psychiatry*, *11*, 920. https://doi.org/10.3389/FPSYT.2020.549903

Silva, A. C., Matthys, O. B., Joy, D. A., Kauss, M. A., Natarajan, V., Lai, M. H., Turaga, D., Blair, A. P., Alexanian, M., Bruneau, B. G., & McDevitt, T. C. (2021). Co-emergence of cardiac and gut tissues promotes cardiomyocyte maturation within human iPSC-derived organoids. *Cell Stem Cell*, *28*(12), 2137-2152.e6. https://doi.org/10.1016/J.STEM.2021.11.007

Sinagra, M., Verrier, D., Frankova, D., Korwek, K. M., Blahos, J., Weeber, E. J., Manzoni, O. J., & Chavis, P. (2005). Reelin, Very-Low-Density Lipoprotein Receptor, and Apolipoprotein E Receptor 2 Control Somatic NMDA Receptor Composition during Hippocampal Maturation In Vitro. *Journal of Neuroscience*, *25*(26), 6127–6136. https://doi.org/10.1523/JNEUROSCI.1757-05.2005

Skolnick, P., Layer, R. T., Popik, P., Nowak, G., Paul, I. A., & Trullas, R. (1996). Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: Implications for the pharmacotherapy of depression. *Pharmacopsychiatry*, *29*(1), 23–26. https://doi.org/10.1055/S-2007-979537

Slauson, D. O., Walker, C., Kristensen, F., Wang, Y., & de Weck, A. L. (1984). Mechanisms of serotonin-induced lymphocyte proliferation inhibition. Cellular Immunology, 84(2), 240-252.

Smith, D. F. (2013). Quest for biomarkers of treatment-resistant depression: Shifting the paradigm toward risk. *Frontiers in Psychiatry*, *4*(JUN), 57. https://doi.org/10.3389/FPSYT.2013.00057

Smith, K. E., & Pollak, S. D. (2020). Early life stress and development: potential mechanisms for adverse outcomes. *Journal of Neurodevelopmental Disorders, 12*(1), 1-15. https://doi.org/10.1186/s11689-020-09337-y

Smith-Geater, C., Hernandez, S. J., Lim, R. G., Adam, M., Wu, J., Stocksdale, J. T., Wassie, B. T., Gold, M. P., Wang, K. Q., Miramontes, R., Kopan, L., Orellana, I., Joy, S., Kemp, P. J., Allen, N. D., Fraenkel, E., & Thompson, L. M. (2020). Aberrant Development Corrected in Adult-Onset Huntington's Disease iPSC-Derived Neuronal Cultures via WNT Signaling Modulation. *Stem Cell Reports*, *14*(3), 406–419.

Soldner, F., & Jaenisch, R. (2012). iPSC disease modeling. *Science*, *338*(6111), 1155–1156.

Song, C., & Wang, H. (2011). Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(3), 760–768. https://doi.org/10.1016/J.PNPBP.2010.06.020

Soumier, A., Carter, R. M., Schoenfeld, T. J., & Cameron, H. A. (2016). New Hippocampal Neurons Mature Rapidly in Response to Ketamine But Are Not Required for Its Acute Antidepressant Effects on Neophagia in Rats. *ENeuro*, *3*(2), 66–77. https://doi.org/10.1523/ENEURO.0116-15.2016

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F. X., & Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience*, *101*(2), 483. https://doi.org/10.1016/S0306-4522(00)00465-6

Spies, M., James, G. M., Berroterán-Infante, N., Ibeschitz, H., Kranz, G. S., Unterholzner, J., Godbersen, M., Gryglewski, G., Hienert, M., Jungwirth, J., Pichler, V., Reiter, B., Silberbauer, L., Winkler, D., Mitterhauser, M., Stimpfl, T., Hacker, M., Kasper, S., & Lanzenberger, R. (2018). Assessment of Ketamine Binding of the Serotonin Transporter in Humans with Positron Emission Tomography. *International Journal of Neuropsychopharmacology*, *21*(2), 145–153.

Squire, L. R., van der Horst, A. S., McDuff, S. G. R., Frascino, J. C., Hopkins, R. O., & Mauldin, K. N. (2010). Role of the hippocampus in remembering the past and imagining the future. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(44), 19044–19048.

Stachowiak, E. K., Benson, C. A., Narla, S. T., Dimitri, A., Chuye, L. E. B., Dhiman, S., Harikrishnan, K., Elahi, S., Freedman, D., Brennand, K. J., Sarder, P., & Stachowiak, M. K. (2017). Cerebral organoids reveal early cortical maldevelopment in schizophrenia—computational anatomy and genomics, role of FGFR1. *Translational Psychiatry, 7*(11), 1–24. https://doi.org/10.1038/s41398-017-0054-x

Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., & Hochedlinger, K. (2008). Induced pluripotent stem cells generated without viral integration. *Science*, *322*(5903), 945–949.

Starkman, M. N., Schteingart, D. E., & Schork, M. A. (1986). Cushing's syndrome after treatment: Changes in cortisol and ACTH levels, and amelioration of the depressive syndrome. *Psychiatry Research*, *19*(3), 177–188. https://doi.org/10.1016/0165-1781(86)90096-X

Sterner, E. Y., & Kalynchuk, L. E. (2010). Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: Relevance to depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(5), 777-790. https://doi.org/10.1016/j.pnpbp.2010.03.005

Stetler, C., & Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: A quantitative summary of four decades of research. *Psychosomatic Medicine*, *73*(2), 114–126. https://doi.org/10.1097/PSY.0B013E31820AD12B

Stewart, R. J., Chen, B., Dowlatshahi, D., MacQueen, G. M., & Young, L. T. (2001). Abnormalities in the cAMP signaling pathway in post-mortem brain tissue from the Stanley Neuropathology Consortium. *Brain Research Bulletin*, *55*(5), 625–629. https://doi.org/10.1016/S0361-9230(01)00524-X

Stocca, G., & Vicini, S. (1998). Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. *The Journal of Physiology*, *507*(1), 13–24. https://doi.org/10.1111/J.1469-7793.1998.013BU.X

Stockmeier, C. A., Mahajan, G. J., Konick, L. C., Overholser, J. C., Jurjus, G. J., Meltzer, H. Y., Uylings, H. B. M., Friedman, L., & Rajkowska, G. (2004). Cellular changes in the postmortem hippocampus in major depression. *Biological Psychiatry*, *56*(9), 640–650. https://doi.org/10.1016/J.BIOPSYCH.2004.08.022

Strawbridge, R., Arnone, D., Danese, A., Papadopoulos, A., Herane Vives, A., & Cleare, A. J. (2015). Inflammation and clinical response to treatment in depression: A meta-analysis. *European Neuropsychopharmacology*, *25*(10), 1532–1543. https://doi.org/10.1016/J.EURONEURO.2015.06.007

Strawbridge, R., Hodsoll, J., Powell, T. R., Hotopf, M., Hatch, S. L., Breen, G., & Cleare, A. J. (2019). Inflammatory profiles of severe treatment-resistant depression. *Journal of Affective Disorders*, *246*, 42–51. https://doi.org/10.1016/J.JAD.2018.12.037

Strawbridge, R., Young, A. H., & Cleare, A. J. (2017). Biomarkers for depression: Recent insights, current challenges and future prospects. *Neuropsychiatric Disease and Treatment*, *13*, 1245–1262. https://doi.org/10.2147/NDT.S114542

Strömgren, L. S. (1977). The influence of depression on memory. *Acta Psychiatrica Scandinavica*, *56*(2), 109–128. https://doi.org/10.1111/J.1600-0447.1977.TB06670.X

Su, Y.A., Lin, J.Y., Liu, Q., Lv, X.Z., Wang, G., Wei, J., Zhu, G., Chen, Q.L., Tian, H.J., Zhang, K.R., Wang, X.Y., Zhang, N., Wang, Y., Haroon, E., Yu, X., & Si, T.M. (2020). Associations among serum markers of inflammation, life stress and suicide risk in patients

with major depressive disorder. *Journal of Psychiatric Research*, *129*, 53–60. https://doi.org/10.1016/j.jpsychires.2020.06.008

Suderman, M., McGowan, P. O., Sasaki, A., Huang, T. C. T., Hallett, M. T., Meaney, M. J., Turecki, G., & Szyf, M. (2012). Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 17266–17272.

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: Review and meta-analysis. *American Journal of Psychiatry*, *157*(10), 1552–1562.

Sumner, J. A., Griffith, J. W., & Mineka, S. (2010). Overgeneral autobiographical memory as a predictor of the course of depression: A meta-analysis. *Behaviour Research and Therapy*, *48*(7), 614–625. https://doi.org/10.1016/J.BRAT.2010.03.013

Sumner, R. L., McMillan, R., Spriggs, M. J., Campbell, D., Malpas, G., Maxwell, E., Deng, C., Hay, J., Ponton, R., Kirk, I. J., Sundram, F., & Muthukumaraswamy, S. D. (2020a). Ketamine Enhances Visual Sensory Evoked Potential Long-term Potentiation in Patients With Major Depressive Disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *5*(1), 45–55. https://doi.org/10.1016/J.BPSC.2019.07.002

Sumner, R. L., McMillan, R., Spriggs, M. J., Campbell, D., Malpas, G., Maxwell, E., Deng, C., Hay, J., Ponton, R., Sundram, F., & Muthukumaraswamy, S. D. (2020b). Ketamine improves short-term plasticity in depression by enhancing sensitivity to prediction errors. *European Neuropsychopharmacology*, *38*, 73–85. https://doi.org/10.1016/J.EURONEURO.2020.07.009

Sun, L., Verkaik-Schakel, R. N., Biber, K., Plösch, T., & Serchov, T. (2021). Antidepressant treatment is associated with epigenetic alterations of Homer1 promoter in a mouse model of chronic depression. *Journal of Affective Disorders*, *279*, 501-509.

Svanborg, P., & Åsberg, M. (2001). A comparison between the Beck Depression Inventory (BDI) and the self-rating version of the Montgomery Åsberg Depression Rating Scale (MADRS). *Journal of Affective Disorders*, *64*(2–3), 203–216. https://doi.org/10.1016/S0165-0327(00)00242-1

Szczepankiewicz, A., Leszczyńska-Rodziewicz, A., Pawlak, J., Rajewska-Rager, A., Wilkosc, M., Zaremba, D., Dmitrzak-Weglarz, M., Skibinska, M., & Hauser, J. (2013). Epistatic interaction between CRHR1 and AVPR1b variants as a predictor of major depressive disorder. *Psychiatric Genetics*, *23*(6), 239–246.

Tackenberg, C., Grinschgl, S., Trutzel, A., Santuccione, A. C., Frey, M. C., Konietzko, U., Grimm, J., Brandt, R., & Nitsch, R. M. (2013). NMDA receptor subunit composition determines beta-amyloid-induced neurodegeneration and synaptic loss. *Cell Death and Disease*, *4*(4), e608–e608. https://doi.org/10.1038/cddis.2013.129

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell*, *131*(5), 861–872. https://doi.org/10.1016/J.CELL.2007.11.019

Takei, S., Morinobu, S., Yamamoto, S., Fuchikami, M., Matsumoto, T., & Yamawaki, S. (2011). Enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in an animal model of posttraumatic stress disorder. *Journal of Psychiatric Research*, *45*(4), 460–468. https://doi.org/10.1016/J.JPSYCHIRES.2010.08.009

Tan, H.K., Delon Toh, C.X., Ma, D., Yang, B., Ming Liu, T., Lu, J., Wong, C.W., Tan, T.K., Li, H., Syn, C., Tan, E.L., Lim, B., Lim, Y.P., Cook, S. A., Loh, Y.H., Tan, H., Delon Toh, C., Wong, C., Tan, T., … Loh, Y. (2014). Human Finger-Prick Induced Pluripotent Stem Cells Facilitate the Development of Stem Cell Banking. *Stem Cells Translational Medicine*, *3*(5), 586–598. https://doi.org/10.5966/SCTM.2013-0195

Tan, S., Wang, Y., Chen, K., Long, Z., & Zou, J. (2017). Ketamine Alleviates Depressive-Like Behaviors via Down-Regulating Inflammatory Cytokines Induced by Chronic Restraint Stress in Mice. *Biological and Pharmaceutical Bulletin*, *40*(8), 1260–1267. https://doi.org/10.1248/BPB.B17-00131

Tartt, A. N., Mariani, M. B., Hen, R., Mann, J. J., & Boldrini, M. (2022). Dysregulation of adult hippocampal neuroplasticity in major depression: pathogenesis and therapeutic implications. *Molecular Psychiatry*, *27*(6), 2689-2699. https://doi.org/10.1038/s41380-022-01520-y

Teixeira, C. M., Martín, E. D., Sahún, I., Masachs, N., Pujadas, L., Corvelo, A., Bosch, C., Rossi, D., Martinez, A., Maldonado, R., Dierssen, M., & Soriano, E. (2011). Overexpression of Reelin Prevents the Manifestation of Behavioral Phenotypes Related to Schizophrenia and Bipolar Disorder. *Neuropsychopharmacology*, *36*(12), 2395–2405. https://doi.org/10.1038/npp.2011.153

Telese, F., Ma, Q., Perez, P. M., Notani, D., Oh, S., Li, W., Comoletti, D., Ohgi, K. A., Taylor, H., & Rosenfeld, M. G. (2015). LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation. *Neuron*, *86*(3), 696–710. https://doi.org/10.1016/J.NEURON.2015.03.033

Thacker, J. S., Yeung, D. H., Staines, W. R., & Mielke, J. G. (2016). Total protein or high-abundance protein: Which offers the best loading control for Western blotting? *Analytical Biochemistry*, *496*, 76–78.

Thase, M. E., Entsuah, A. R., & Rudolph, R. L. (2001). Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *British Journal of Psychiatry*, *178*(MARCH.), 234–241. https://doi.org/10.1192/bjp.178.3.234

Thome, J., Henn, F. A., & Duman, R. S. (2002). Cyclic AMP response element-binding protein and depression. *Expert Review of Neurotherapeutics*, *2*(3), 347-354. https://doi.org/10.1586/14737175.2.3.347

Thompson, S. M., Kallarackal, A. J., Kvarta, M. D., van Dyke, A. M., LeGates, T. A., & Cai, X. (2015). An excitatory synapse hypothesis of depression. *Trends in Neurosciences*, *38*(5), 279–294. https://doi.org/10.1016/J.TINS.2015.03.003

Thornicroft, G., Rose, D., & Kassam, A. (2007). Discrimination in health care against people with mental illness. *International Review of Psychiatry*, *19*(2), 113-122. https://doi.org/10.1080/09540260701278937

Tian, A., Muffat, J., Li, Y., Rehbach, K., Fernando, M. B., Kristen, X., & Brennand, J. (2020). Integrating CRISPR Engineering and hiPSC-Derived 2D Disease Modeling Systems. *Journal of Neuroscience*, *40*(6), 1176–1185. https://doi.org/10.1523/JNEUROSCI.0518-19.2019

Tiger, M., Veldman, E. R., Ekman, C. J., Halldin, C., Svenningsson, P., & Lundberg, J. (2020). A randomized placebo-controlled PET study of ketamine´s effect on serotonin1B receptor binding in patients with SSRI-resistant depression. *Translational Psychiatry*, *10*(1), 1–8. https://doi.org/10.1038/s41398-020-0844-4

Tipton, K. F., Boyce, S., O'Sullivan, J., Davey, G. P., & Healy, J. (2012). Monoamine Oxidases: Certainties and Uncertainties. *Current Medicinal Chemistry*, *11*(15), 1965–1982. https://doi.org/10.2174/0929867043364810

Tissir, F., & Goffinet, A. M. (2003). Reelin and brain development. *Nature Reviews Neuroscience 2003 4:6*, *4*(6), 496–505. https://doi.org/10.1038/nrn1113

Tkacs, N. C., & Thompson, H. J. (2006). From bedside to bench and back again: Research issues in animal models of human disease. *Biological Research for Nursing*, *8*(1), 78–88. https://doi.org/10.1177/1099800406289717

Tolentino, J. C., & Schmidt, S. L. (2018). DSM-5 criteria and depression severity: Implications for clinical practice. *Frontiers in Psychiatry*, *9*(OCT), 450. https://doi.org/10.3389/fpsyt.2018.00450

Toral-Rios, D., Pichardo-Rojas, P. S., Alonso-Vanegas, M., & Campos-Peña, V. (2020). GSK3β and Tau Protein in Alzheimer's Disease and Epilepsy. *Frontiers in Cellular Neuroscience*, *14*, 19. https://doi.org/10.3389/FNCEL.2020.00019

Toth, E., Gersner, R., Wilf-Yarkoni, A., Raizel, H., Dar, D. E., Richter-Levin, G., Levit, O., & Zangen, A. (2008). Age-dependent effects of chronic stress on brain plasticity and depressive behavior. *Journal of Neurochemistry*, *107*(2), 522–532. https://doi.org/10.1111/J.1471-4159.2008.05642.X

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate Receptor Ion Channels: Structure, Regulation, and Function. *Pharmacological Reviews*, *62*(3), 405–496. https://doi.org/10.1124/PR.109.002451

Treadway, M. T., & Zald, D. H. (2011). Reconsidering anhedonia in depression: Lessons from translational neuroscience. *Neuroscience & Biobehavioral Reviews*, *35*(3), 537–555. https://doi.org/10.1016/J.NEUBIOREV.2010.06.006

Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R. E., Richardson, J. A., & Herz, J. (1999). Reeler/Disabled-like Disruption of Neuronal Migration in Knockout Mice Lacking the VLDL Receptor and ApoE Receptor 2. *Cell*, *97*(6), 689–701. https://doi.org/10.1016/S0092-8674(00)80782-5

Tsankova, N., Renthal, W., Kumar, A., & Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience*, *8*(5), 355-367. Nature Publishing Group. https://doi.org/10.1038/nrn2132

Tueting, P., Doueiri, M. S., Guidotti, A., Davis, J. M., & Costa, E. (2006). Reelin down-regulation in mice and psychosis endophenotypes. *Neuroscience & Biobehavioral Reviews*, *30*(8), 1065–1077. https://doi.org/10.1016/J.NEUBIOREV.2006.04.001

Turkheimer, F. E., Althubaity, N., Schubert, J., Nettis, M. A., Cousins, O., Dima, D., Mondelli, V., Bullmore, E. T., Pariante, C., & Veronese, M. (2021). Increased serum peripheral C-reactive protein is associated with reduced brain barriers permeability of TSPO radioligands in healthy volunteers and depressed patients: implications for inflammation and depression. *Brain, Behavior, and Immunity*, *91*, 487–497. https://doi.org/10.1016/J.BBI.2020.10.025

Underwood, M. D., Kassir, S. A., Bakalian, M. J., Galfalvy, H., Dwork, A. J., Mann, J. J., & Arango, V. (2018). Serotonin receptors and suicide, major depression, alcohol use disorder and reported early life adversity. *Translational Psychiatry*, *8*(1), 1–15. https://doi.org/10.1038/s41398-018-0309-1

Universities of Canada (2019). Equity, diversity, and inclusion at Canadian Universities. Available from: https://www.univcan.ca/wp-content/uploads/2019/11/Equity-diversity-and-inclusion-at-Canadian-universities-report-on-the-2019-national-survey-Nov-2019-1.pdf.

University of Victoria. (2018). Employment Equity Survey. Available from: https://www.uvic.ca/equity/employment-equity/equitysurvey/index.php#snapshot.

Vadodaria, K. C., Ji, Y., Skime, M., Paquola, A. C., Nelson, T., Hall-Flavin, D., Heard, K. J., Fredlender, C., Deng, Y., Elkins, J., Dani, K., Le, A. T., Marchetto, M. C., Weinshilboum, R., & Gage, F. H. (2019a). Altered serotonergic circuitry in SSRI-resistant major depressive disorder patient-derived neurons. *Molecular Psychiatry*, *24*(6), 808–818. https://doi.org/10.1038/s41380-019-0377-5

Vadodaria, K. C., Ji, Y., Skime, M., Paquola, A., Nelson, T., Hall-Flavin, D., Fredlender, C., Heard, K. J., Deng, Y., Le, A. T., Dave, S., Fung, L., Li, X., Marchetto, M. C., Weinshilboum, R., & Gage, F. H. (2019b). Serotonin-induced hyperactivity in SSRI-resistant major depressive disorder patient-derived neurons. *Molecular Psychiatry*, *24*(6), 795–807. https://doi.org/10.1038/s41380-019-0363-y

Valbuena, S., & Lerma, J. (2021). Kainate receptors, homeostatic gatekeepers of synaptic plasticity. *Neuroscience*, 456, 17-26.

Varela, M. J., Lage, S., Caruncho, H. J., Cadavid, M. I., Loza, M. I., & Brea, J. (2015). Reelin influences the expression and function of dopamine D2 and serotonin 5-HT2A receptors: A comparative study. *Neuroscience*, *290*, 165–174. https://doi.org/10.1016/J.NEUROSCIENCE.2015.01.031

Veldic, M., Caruncho, H. J., Liu, W. S., Davis, J., Satta, R., Grayson, D. R., Guidotti, A., & Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proceedings of the National Academy of Sciences*, *101*(1), 348–353. https://doi.org/10.1073/PNAS.2637013100

Ventruti, A., Kazdoba, T. M., Niu, S., & D'Arcangelo, G. (2011). Reelin deficiency causes specific defects in the molecular composition of the synapses in the adult brain. *Neuroscience*, *189*, 32–42. https://doi.org/10.1016/j.neuroscience.2011.05.050

Verhagen, M., van der Meij, A., van Deurzen, P. M., Janzing, J., Arias-Vásquez, A., Buitelaar, J., & Franke, B. (2010). Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: Effects of gender and ethnicity. *Molecular Psychiatry*, *15*(3), 260–271. https://doi.org/10.1038/mp.2008.109

Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: A meta-analysis of MRI studies. *American Journal of Psychiatry*, *161*(11), 1957-1966. https://doi.org/10.1176/appi.ajp.161.11.1957

Vílchez-Acosta, A., Manso, Y., Cárdenas, A., Elias-Tersa, A., Martínez-Losa, M., Pascual, M., ... & Soriano, E. (2022). Specific contribution of Reelin expressed by Cajal–Retzius cells or GABAergic interneurons to cortical lamination. *Proceedings of the National Academy of Sciences*, 119(37), e2120079119.

Villoutreix, B., Creemers, J., Léger, Y., Siegfried, G., Decroly, E., Evrard, S., & Khatib, A.M. (2020). Targeting furin activity through in silico and in vitro drug repurposing strategy for SARS-CoV-2 spike glycoprotein cleavage repression. *Res. Sq.*, 1–16. https://doi.org/10.21203/rs.3.rs-25856/v1

Wang, D. S., Penna, A., & Orser, B. A. (2017). Ketamine Increases the Function of γ-Aminobutyric Acid Type A Receptors in Hippocampal and Cortical Neurons. *Anesthesiology*, *126*(4), 666–677. https://doi.org/10.1097/ALN.0000000000001483

Wang, K., Wang, H., Lou, W., Ma, L., Li, Y., Zhang, N., Wang, C., Li, F., Awais, M., Cao, S., She, R., Fu, Z. F., & Cui, M. (2018). IP-10 promotes blood-brain barrier damage by inducing tumor necrosis factor alpha production in Japanese encephalitis. *Frontiers in Immunology*, *9*(MAY), 1148. https://doi.org/10.3389/FIMMU.2018.01148

Wang, M., Zhang, L., & Gage, F. H. (2020). Modeling neuropsychiatric disorders using human induced pluripotent stem cells. *Protein and Cell*, *11*(1), 45–59. https://doi.org/10.1007/S13238-019-0638-8

Wang, N., Yu, H. Y., Shen, X. F., Gao, Z. Q., Yang, C., Yang, J. J., & Zhang, G. F. (2015). The rapid antidepressant effect of ketamine in rats is associated with down-regulation of pro-inflammatory cytokines in the hippocampus. *Upsala Journal of Medical Sciences*, *120*(4), 241–248. https://doi.org/10.3109/03009734.2015.1060281

Wang, W., Liu, L., Yang, X., Gao, H., Tang, Q. K., Yin, L. Y., Yin, X. Y., Hao, J. R., Geng, D. Q., & Gao, C. (2019). Ketamine improved depressive-like behaviors via hippocampal glucocorticoid receptor in chronic stress induced- susceptible mice. *Behavioural Brain Research*, *364*, 75–84.

Warden, D., Rush, A. J., Trivedi, M. H., Fava, M., & Wisniewski, S. R. (2007). The STAR*D project results: A comprehensive review of findings. *Current Psychiatry Reports*, *9*(6), 449–459. https://doi.org/10.1007/S11920-007-0061-3

Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y. H., Li, H., Lau, F., Ebina, W., Mandal, P. K., Smith, Z. D., Meissner, A., Daley, G. Q., Brack, A. S., Collins, J. J., Cowan, C., Schlaeger, T. M., & Rossi, D. J. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, *7*(5), 618–630. https://doi.org/10.1016/j.stem.2010.08.012

Wasser, C. R., & Herz, J. (2017). Reelin: Neurodevelopmental Architect and Homeostatic Regulator of Excitatory Synapses. *Journal of Biological Chemistry*, *292*(4), 1330–1338. https://doi.org/10.1074/JBC.R116.766782

Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Förster, E., David Sweatt, J., & Herz, J. (2002). Reelin and apoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *Journal of Biological Chemistry*, *277*(42), 39944–39952. https://doi.org/10.1074/jbc.M205147200

Weingartner, H., Cohen, R. M., Murphy, D. L., Martello, J., & Gerdt, C. (1981). Cognitive Processes in Depression. *Archives of General Psychiatry*, *38*(1), 42–47. https://doi.org/10.1001/ARCHPSYC.1981.01780260044004

Weintraub, A., Singaravelu, J., & Bhatnagar, S. (2010). Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity. *Brain Research*, *1343*, 83–92. https://doi.org/10.1016/J.BRAINRES.2010.04.068

Weissman, M. M., Kidd, K. K., & Prusoff, B. A. (1982). Variability in Rates of Affective Disorders in Relatives of Depressed and Normal Probands. *Archives of General Psychiatry*, *39*(12), 1397–1403. https://doi.org/10.1001/ARCHPSYC.1982.04290120033006

West, E. D., & Dally, P. J. (1959). Effects of iproniazid in depressive syndromes. *British Medical Journal*, *1*(5136), 1491–1494. https://doi.org/10.1136/bmj.1.5136.1491

Whishaw, I. Q., Rod, M. R., & Roland Auer, N. (1994). Behavioral deficits revealed by multiple tests in rats with ischemie damage limited to half of the CA1 sector of the hippocampus. *Brain Research Bulletin*, *34*(3), 283–289. https://doi.org/10.1016/0361-9230(94)90065-5

Wichers, M. C., Koek, G. H., Robaeys, G., Verkerk, R., Scharpé, S., & Maes, M. (2004). IDO and interferon-α-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Molecular Psychiatry*, *10*(6), 538–544. https://doi.org/10.1038/sj.mp.4001600

Williams, N. R., Heifets, B. D., Blasey, C., Sudheimer, K., Pannu, J., Pankow, H., Hawkins, J., Birnbaum, J., Lyons, D. M., Rodriguez, C. I., & Schatzberg, A. F. (2018). Attenuation of antidepressant effects of ketamine by opioid receptor antagonism. *American Journal of Psychiatry*, *175*(12), 1205–1215.

Williamson, C. (2008). The patient movement as an emancipation movement. *Health Expectations*, *11*(2), 102–112. https://doi.org/10.1111/J.1369-7625.2007.00475.X

Willner, P. (1990). Animal models of depression: An overview. *Pharmacology & Therapeutics*, *45*(3), 425–455. https://doi.org/10.1016/0163-7258(90)90076-E

Willner, P., & Belzung, C. (2015). Treatment-resistant depression: are animal models of depression fit for purpose? *Psychopharmacology*, *232*(19), 3473–3495. https://doi.org/10.1007/S00213-015-4034-7

Wixted, J. T., & Squire, L. R. (2010). The role of the human hippocampus in familiarity-based and recollection-based recognition memory. *Behavioural Brain Research*, *215*(2), 197–208. https://doi.org/10.1016/J.BBR.2010.04.020

Woltjen, K., Hämäläinen, R., Kibschull, M., Mileikovsky, M., & Nagy, A. (2011). Transgene-Free Production of Pluripotent Stem Cells Using piggyBac Transposons. *Methods in Molecular Biology*, *767*, 87–103.

Wong, E. Y. H., & Herbert, J. (2006). Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience*, *137*(1), 83–92. https://doi.org/10.1016/J.NEUROSCIENCE.2005.08.073

Wong, M. L., & Licinio, J. (2001). Research and treatment approaches to depression. *Nature Reviews Neuroscience*, *2*(5), 343–351. https://doi.org/10.1038/35072566

Wong, W. K., Ou, X. M., Chen, K., & Shih, J. C. (2002). Activation of human monoamine oxidase B gene expression by a protein kinase C MAPK signal transduction pathway involves c-Jun and Egr-1. *Journal of Biological Chemistry*, *277*(25), 22222–22230. https://doi.org/10.1074/jbc.M202844200

World Health Organization. (2019). Suicide in the world: Global Health Estimates. *World Health Organization,Geneva*, 32.

Wu, H. H., & Wang, S. (2010). Strain differences in the chronic mild stress animal model of depression. *Behavioural Brain Research*, *213*(1), 94–102. https://doi.org/10.1016/J.BBR.2010.04.041

Wu, M., Minkowicz, S., Dumrongprechachan, V., Hamilton, P., & Kozorovitskiy, Y. (2021a). Ketamine Rapidly Enhances Glutamate-Evoked Dendritic Spinogenesis in Medial

Prefrontal Cortex Through Dopaminergic Mechanisms. *Biological Psychiatry*, *89*(11), 1096–1105. https://doi.org/10.1016/J.BIOPSYCH.2020.12.022

Wu, M., Minkowicz, S., Dumrongprechachan, V., Hamilton, P., Xiao, L., & Kozorovitskiy, Y. (2021b). Attenuated dopamine signaling after aversive learning is restored by ketamine to rescue escape actions. *ELife*, *10*. https://doi.org/10.7554/ELIFE.64041

Wulf, H. A., Browne, C. A., Zarate, C. A., & Lucki, I. (2022). Mediation of the behavioral effects of ketamine and (2R,6R)-hydroxynorketamine in mice by kappa opioid receptors. *Psychopharmacology*, *239*(7), 2309–2316. https://doi.org/10.1007/S00213-022-06118-4

Wurst, C., Schiele, M. A., Stonawski, S., Weiß, C., Nitschke, F., Hommers, L., Domschke, K., Herrmann, M. J., Pauli, P., Deckert, J., & Menke, A. (2021). Impaired fear learning and extinction, but not generalization, in anxious and non-anxious depression. *Journal of Psychiatric Research*, *135*, 294–301. https://doi.org/10.1016/J.JPSYCHIRES.2021.01.034

Xiao, Z., Liu, W., Gao, K., Wan, Q., Yang, C., Wang, H., Wang, X., Wang, G., & Liu, Z. (2011). Interaction between CRHR1 and BDNF genes increases the risk of recurrent major depressive disorder in Chinese population. *PLoS ONE*, *6*(12), e28733. https://doi.org/10.1371/journal.pone.0028733

Xie, N., Zhou, Y., Sun, Q., & Tang, B. (2018). Novel epigenetic techniques provided by the CRISPR/Cas9 System. *Stem Cells International*, *2018*. https://doi.org/10.1155/2018/7834175

Xu, J., Kurup, P., Zhang, Y., Goebel-Goody, S. M., Wu, P. H., Hawasli, A. H., Baum, M. L., Bibb, J. A., & Lombroso, P. J. (2009). Extrasynaptic NMDA Receptors Couple Preferentially to Excitotoxicity via Calpain-Mediated Cleavage of STEP. *Journal of Neuroscience*, *29*(29), 9330–9343. https://doi.org/10.1523/JNEUROSCI.2212-09.2009

Yamada, J., & Jinno, S. (2019). Potential link between antidepressant-like effects of ketamine and promotion of adult neurogenesis in the ventral hippocampus of mice. *Neuropharmacology*, *158*, 107710. https://doi.org/10.1016/J.NEUROPHARM.2019.107710

Yamanaka, H., Yokoyama, C., Mizuma, H., Kurai, S., Finnema, S. J., Halldin, C., Doi, H., & Onoe, H. (2014). A possible mechanism of the nucleus accumbens and ventral pallidum 5-HT1B receptors underlying the antidepressant action of ketamine: A PET study with macaques. *Translational Psychiatry*, *4*(1), e342–e342. https://doi.org/10.1038/tp.2013.112

Yang, C., Ren, Q., Qu, Y., Zhang, J. C., Ma, M., Dong, C., & Hashimoto, K. (2018). Mechanistic Target of Rapamycin–Independent Antidepressant Effects of (R)-Ketamine in a Social Defeat Stress Model. *Biological Psychiatry*, *83*(1), 18–28. https://doi.org/10.1016/J.BIOPSYCH.2017.05.016

Yang, C., Wardenaar, K. J., Bosker, F. J., Li, J., & Schoevers, R. A. (2019). Inflammatory markers and treatment outcome in treatment resistant depression: A systematic review. *Journal of Affective Disorders*, *257*, 640–649. https://doi.org/10.1016/J.JAD.2019.07.045

Yao, N., Skiteva, O., Zhang, X., Svenningsson, P., & Chergui, K. (2017). Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit. *Molecular Psychiatry*, *23*(10), 2066–2077. https://doi.org/10.1038/mp.2017.239

Yao, W., Cao, Q., Luo, S., He, L., Yang, C., Chen, J., Qi, Q., Hashimoto, K., & Zhang, J. chun. (2022). Microglial ERK-NRBP1-CREB-BDNF signaling in sustained antidepressant actions of (R)-ketamine. *Molecular Psychiatry*, *27*(3), 1618–1629. https://doi.org/10.1038/s41380-021-01377-7

Yasui, N., Nogi, T., & Takagi, J. (2010). Structural Basis for Specific Recognition of Reelin by Its Receptors. *Structure*, *18*(3), 320–331. https://doi.org/10.1016/J.STR.2010.01.010

Yau, S. Y., Li, A., Tong, J. bin, Bostrom, C., Christie, B. R., Lee, T. M. C., & So, K. F. (2016). Chronic corticosterone administration reduces dendritic complexity in mature, but not young granule cells in the rat dentate gyrus. *Restorative Neurology and Neuroscience*, *34*(5), 849–857. https://doi.org/10.3233/RNN-160662

Yirmiya, R., & Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain, Behavior, and Immunity*, *25*(2), 181–213. https://doi.org/10.1016/J.BBI.2010.10.015

You, Z., Luo, C., Zhang, W., Chen, Y., He, J., Zhao, Q., Zuo, R., & Wu, Y. (2011). Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. *Behavioural Brain Research*, *225*(1), 135–141. https://doi.org/10.1016/J.BBR.2011.07.006

Youdim, M. B. H., Banerjee, D. K., Kelner, K., Offutt, L., & Pollard, H. B. (1989). Steroid regulation of monoamine oxidase activity in the adrenal medulla. *The FASEB Journal*, *3*(6), 1753–1759. https://doi.org/10.1096/FASEBJ.3.6.2495232

Youdim, M. B. H., Edmondson, D., & Tipton, K. F. (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nature Reviews Neuroscience*, *7*(4), 295-309. https://doi.org/10.1038/nrn1883

Youdim, M. B. H., & Weinstock, M. (2004). Therapeutic Applications of Selective and Non-Selective Inhibitors of Monoamine Oxidase A and B that do not Cause Significant Tyramine Potentiation. *NeuroToxicology*, *25*(1–2), 243–250. https://doi.org/10.1016/S0161-813X(03)00103-7

Young, J. J., Bruno, D., & Pomara, N. (2014). A review of the relationship between proinflammatory cytokines and major depressive disorder. *Journal of Affective Disorders*, *169*, 15–20. https://doi.org/10.1016/J.JAD.2014.07.032

Zacharko, R. M., Bowers, W. J., Kokkinidis, L., & Anisman, H. (1983). Region-specific reductions of intracranial self-stimulation after uncontrollable stress: Possible effects on reward processes. *Behavioural Brain Research*, *9*(2), 129–141. https://doi.org/10.1016/0166-4328(83)90123-7

Zakzanis, K., Leach, L., Kaplan, E. K. (1998). On the nature and pattern of neurocognitive function in major depressive disorder. *Neuropsychiatry, Neurobiology, and Behavioral Neurology*.

Zanos, P., & Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Molecular Psychiatry*, *23*(4), 801–811. https://doi.org/10.1038/mp.2017.255

Zanos, P., Highland, J. N., Stewart, B. W., Georgiou, P., Jenne, C. E., Lovett, J., Morris, P. J., Thomas, C. J., Moaddel, R., Zarate, C. A., & Gould, T. D. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor dependent antidepressant actions. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(13), 6441–6450.

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., Alkondon, M., Yuan, P., Pribut, H. J., Singh, N. S., Dossou, K. S. S., Fang, Y., Huang, X. P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481–486.

Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E. F. R., Albuquerque, E. X., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2018a). Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms. *Pharmacological Reviews*, *70*(3), 621–660. https://doi.org/10.1124/pr.117.015198

Zanos, P., Thompson, S. M., Duman, R. S., Zarate, C. A., & Gould, T. D. (2018b). Convergent Mechanisms Underlying Rapid Antidepressant Action. *CNS Drugs*, *32*(3), 197-227. https://doi.org/10.1007/s40263-018-0492-x

Zarate, C. A., Brutsche, N. E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C. A., Liberty, V., & Luckenbaugh, D. A. (2012). Replication of Ketamine's Antidepressant Efficacy in Bipolar Depression: A Randomized Controlled Add-On Trial. *Biological Psychiatry*, *71*(11), 939–946.

Zarate, C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S., & Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*, *63*(8), 856–864. https://doi.org/10.1001/archpsyc.63.8.856

Zhang, F., Hillhouse, T. M., Anderson, P. M., Koppenhaver, P. O., Kegen, T. N., Manicka, S. G., Lane, J. T., Pottanat, E., van Fossen, M., Rice, R., & Porter, J. H. (2021). Opioid receptor system contributes to the acute and sustained antidepressant-like effects, but not the hyperactivity motor effects of ketamine in mice. *Pharmacology Biochemistry and Behavior*, *208*, 173228. https://doi.org/10.1016/J.PBB.2021.173228

Zhang, H., Zhao, Y., & Wang, Z. (2015). Chronic corticosterone exposure reduces hippocampal astrocyte structural plasticity and induces hippocampal atrophy in mice. *Neuroscience Letters*, *592*, 76–81. https://doi.org/10.1016/J.NEULET.2015.03.006

Zhang, J. C., Li, S. X., & Hashimoto, K. (2014). R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology Biochemistry and Behavior*, *116*, 137–141. https://doi.org/10.1016/J.PBB.2013.11.033

Zhang, J., Ma, L., Wan, X., Shan, J., Qu, Y., & Hashimoto, K. (2021a). (R)-Ketamine attenuates LPS-induced endotoxin-derived delirium through inhibition of neuroinflammation. *Psychopharmacology*, *238*(10), 2743–2753. https://doi.org/10.1007/S00213-021-05889-6

Zhang, J., Yue, Y., Thapa, A., Fang, J., Zhao, S., Shi, W., Yang, Z., Li, Y., & Yuan, Y. (2019). Baseline serum C-reactive protein levels may predict antidepressant treatment responses in patients with major depressive disorder. *Journal of Affective Disorders*, *250*, 432–438. https://doi.org/10.1016/J.JAD.2019.03.001

Zhang, K., Xu, T., Yuan, Z., Wei, Z., Yamaki, V. N., Huang, M., Huganir, R. L., & Cai, X. (2016). Essential roles of AMPA receptor GluA1 phosphorylation and presynaptic HCN channels in fast-Acting antidepressant responses of ketamine. *Science Signaling*, *9*(458).

Zhang, M. (2010). The Prevalence and Impact of Depression. *The Journal of Clinical Psychiatry*, *71*(3), 26446. https://doi.org/10.4088/JCP.8001TX17C

Zhang, Y., Lu, C., Zhang, J., Hu, L., Song, H., Li, J., & Kang, L. (2013). Gender differences in abusers of amphetamine-type stimulants and ketamine in southwestern China. *Addictive Behaviors*, *38*(1), 1424–1430. https://doi.org/10.1016/J.ADDBEH.2012.06.024

Zhao, J., Goldberg, J., Bremner, J. D., & Vaccarino, V. (2013). Association Between Promoter Methylation of Serotonin Transporter Gene and Depressive Symptoms: A Monozygotic Twin Study. *Psychosomatic Medicine*, *75*(6), 523–529. https://doi.org/10.1097/PSY.0B013E3182924CF4

Zhao, X., Cao, F., Liu, Q., Li, X., Xu, G., Liu, G., Zhang, Y., Yang, X., Yi, S., Xu, F., Fan, K., & Ma, J. (2019). Behavioral, inflammatory and neurochemical disturbances in LPS and UCMS-induced mouse models of depression. *Behavioural Brain Research*, *364*, 494–502. https://doi.org/10.1016/J.BBR.2017.05.064

Zhao, X., Venkata, S. L. V., Moaddel, R., Luckenbaugh, D. A., Brutsche, N. E., Ibrahim, L., Zarate, C. A., Mager, D. E., & Wainer, I. W. (2012). Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. *British Journal of Clinical Pharmacology*, *74*(2), 304–314. https://doi.org/10.1111/J.1365-2125.2012.04198.X

Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., & Du, L. (2008). A mouse model of depression induced by repeated corticosterone injections. *European Journal of Pharmacology*, *581*(1–2), 113–120. https://doi.org/10.1016/j.ejphar.2007.12.005

Zhou, W., Wang, N., Yang, C., Li, X. M., Zhou, Z. Q., & Yang, J. J. (2014). Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. *European Psychiatry*, *29*(7), 419–423. https://doi.org/10.1016/J.EURPSY.2013.10.005

Zhou, Y., Zheng, W., Liu, W., Wang, C., Zhan, Y., Li, H., Chen, L., Li, M., & Ning, Y. (2018). Antidepressant effect of repeated ketamine administration on kynurenine pathway metabolites in patients with unipolar and bipolar depression. *Brain, Behavior, and Immunity*, *74*, 205–212. https://doi.org/10.1016/J.BBI.2018.09.007

Zhu, Q. S., Chen, K., & Shih, J. C. (1994). Bidirectional promoter of human monoamine oxidase A (MAO A) controlled by transcription factor Sp1. *Journal of Neuroscience*, *14*(12), 7393–7403. https://doi.org/10.1523/JNEUROSCI.14-12-07393.1994

Zisook, S., John Rush, A., Albala, A., Alpert, J., Balasubramani, G. K., Fava, M., Husain, M., Sackeim, H., Trivedi, M., & Wisniewski, S. (2004). Factors that differentiate early vs. later onset of major depression disorder. *Psychiatry Research*, *129*(2), 127–140. https://doi.org/10.1016/J.PSYCHRES.2004.07.004

Zörner, B., Wolfer, D. P., Brandis, D., Kretz, O., Zacher, C., Madani, R., Grunwald, I., Lipp, H. P., Klein, R., Henn, F. A., & Gass, P. (2003). Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than "depressive". *Biological Psychiatry*, *54*(10), 972–982. https://doi.org/10.1016/S0006-3223(03)00418-9

Zunszain, P. A., Hepgul, N., & Pariante, C. M. (2013). Inflammation and Depression. *Current Topics in Behavioral Neurosciences*, *14*, 135–151. https://doi.org/10.1007/7854_2012_211/FIGURES/1

Appendix A (Immunocytochemical analysis of iPSC-derived neurons treated with reelin and (2R,6R)-HNK)

As described in Chapter 6, immunocytochemical (ICC) analyses of various proteins were undertaken to ascertain region-specific (soma, neurite, whole cell) protein expression. In addition, ICC was used to confirm Western blot findings, which ascertained a drug-dependent increase in protein expression of Synapsin I, GluA1, TrkB, PSD-95 after one hour of exposure, but decreased indicators of protein expression after 24 hours.

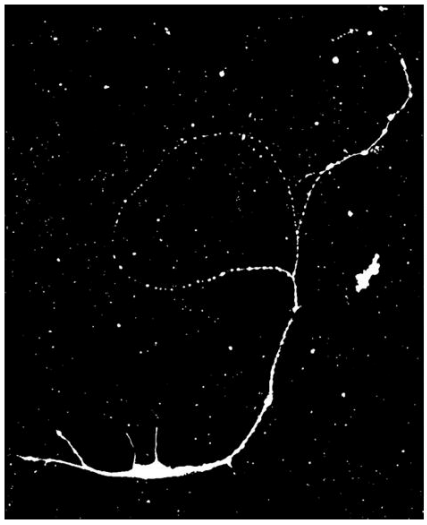
As there is significant debate regarding proper characterization of fluorescent markers, I conducted a preliminary analysis through a modified punctal analysis adapted from the membrane protein clustering protocol (Romay-Tallon et al., 2017). While this provided a proxy indicator of protein expression, further research is necessary to determine the best methodological approach to ICC analyses. This work is presented as a preliminary overview, and results should be interpreted with caution.

Protocol and analysis

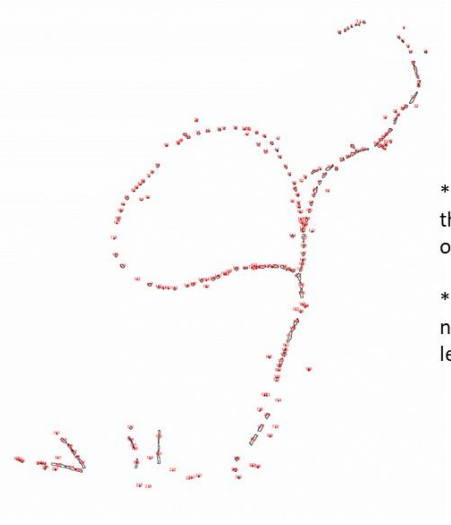
After fixation, cells for ICC analyses were permeabilized by incubating in 0.5% Triton X-100 in PBS for 5 minutes. To reduce background fluorescence, cells were blocked in 5% BSA for 1 hour, then incubated overnight in primary antibodies. Cells were then double-labeled with Synapsin I and NR2B, BDNF and TrkB, and GluA1 and PSD-95 (antibodies used were parallel to those mentioned above). Appropriate fluorochrome-conjugated secondaries were for 1 hour, then coverslips were placed onto slides for fluorescent imaging. Fluorescent images were taken using a confocal microscope at 60x magnification.

For analyses, 5 neurons from each coverslip were traced manually, then analyzed for region-specific puncta (soma, neurites, whole cell) using an Otsu correction method to first threshold the image in Fiji to determine changes in protein expression. For an example of punctal analysis, please see Figure 1. Measures of punctal count (to determine amount of protein expression hot spots), average punctal area (to estimate amount of protein expression), and percent punctal area (to ascertain what percent of traced cell was labeled). As discussed in Chapter 6, Western blotting data demonstrated no significant differences across all measures in the HC cell lines, so these were not analyzed with ICC.

Traced Neuron Example:



Punctal Analysis:



*Otsu corrections used for image thresholding, then a modified version of particle analysis (3D)

*Normalized Count is Count/Length as neurons measured will be varying lengths

Punctal Analysis Output:

ID	Count	Average Size	Length	Normalized Count
834-24-B6-005-01	216	2.183	969	0.22

Figure 1. Punctal analysis example on ICC stains. Individual neurons were traced (n = 5/sample), then assessed through a modified particle analysis after Otsu correction. Count was normalized to length of neuron, and divided into three sections (soma, neurite, and whole cell) for further analysis.

Results

All statistics can be viewed in Table 1. Of note, TrkB puncta count was significantly increased at 1 hour and decreased at 24 hours in neurites at the highest concentration of reelin and (2R,6R)-HNK. Reelin also significantly changed NR2B puncta count, cluster size, and area in a parallel manner. Unfortunately, GluA1 and PSD-95 were not able to be quantified at 24 hours, and were left out of the analysis.

Whole Cell

Significance Against Vehicle Control (Whole Cell)		Whole Cell Cluster Size				Whole Cell Puncta Count				Whole Cell % Punctal Area			
		Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM
BDNF	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	p = .029*	NS	p = .016*	NS	NS	NS	NS
TrkB	1 hour	NS	NS	NS	NS	NS	NS	p < 0.001***	p = 0.015*	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	NS	NS	p = 0.021*	NS	NS	NS	NS
PSD-95	1 hour	NS	p = 0.009**	NS	NS	NS	NS	NS	p = 0.002**	NS	p = 0.001**	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
GluA1	1 hour	NS	NS	NS	NS	p = 0.031*	p = 0.002**	NS	NS	NS	NS	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
Synapsin I	1 hour	NS	p = 0.024*	NS	p = 0.027*	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NR2B	1 hour	NS	NS	p < 0.001***	NS	NS	NS	p = 0.002**	NS	p = 0.038*	NS	p < 0.001***	NS
	24 hours	NS	NS	NS	NS	NS	NS	p = 0.008**	NS	NS	NS	p < 0.001***	NS

Neurites

Significance Against Vehicle Control (Neurites)		Neurite Cluster Size				Neurite Puncta Count				Neurite Punctal % Area			
		Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM
BDNF	1 hour	NS	NS	NS	NS	NS	NS	p = .002**	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	NS	NS	p = .028*	NS	NS	NS	NS
TrkB	1 hour	NS	NS	NS	NS	NS	NS	p < 0.001***	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	p = 0.044*	NS	NS	NS	p = 0.006**	NS	NS	NS	NS
PSD-95	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	p = 0.004**	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
GluA1	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
Synapsin I	1 hour	NS	p = 0.04*	NS	p = 0.016*	NS	NS	NS	NS	NS	NS	NS	p = 0.005**
	24 hours	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	p = 0.047*	p = 0.016*
NR2B	1 hour	NS	NS	p = 0.024*	NS	NS	NS	p = 0.005**	NS	NS	NS	p < 0.001***	NS
	24 hours	NS	NS	p = 0.043*	NS	NS	NS	p < 0.001***	p = 0.007**	NS	NS	p < 0.001***	p = 0.008**

Soma

Significance Against Vehicle Control (Soma)		Soma Cluster Size				Soma Puncta Count				Soma Punctal % Area			
		Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM	Reelin 5nM	Reelin 10nM	Reelin 50nM	HNK 1uM
BDNF	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TrkB	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	p = 0.013*	NS	NS	NS	p = 0.005**	NS	NS
PSD-95	1 hour	NS	NS	NS	NS	NS	NS	p = 0.035*	p = 0.045*	NS	p = 0.001**	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
GluA1	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED	NED
Synapsin I	1 hour	NS	NS	NS	NS	NS	p = 0.044*	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	p = 0.038*	NS	NS	NS	NS	NS	NS	NS	NS	NS
NR2B	1 hour	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	24 hours	NS	NS	NS	NS	NS	NS	p = 0.01**	p = 0.025*	NS	NS	NS	NS

Table 1. Significant differences in cluster size, puncta count, and % punctal area of various proteins from iPSC-derived neurons. Whole cell, neurites, and soma were considered in analyses. Significant changes from vehicle-administered treatment-resistant depression cell lines are highlighted. NS = Not significant, NED = No Existing Data

Immunocytochemical analyses revealed significant differences in protein expression across treatment groups in whole cell, soma, and neurite regions. In parallel to Western blotting data, the highest concentration of Reelin (50 nM) and (2R,6R)-HNK were most likely to upregulate (at 1 hour) or downregulate (at 24 hours) protein expression. Interestingly, significant differences were found mainly in NR2B expression, which did not parallel Western blot results.

However, a major limitation to this method is that there are no consistent protocols for proper quantification of protein expression in ICC imaging. In addition, challenges such as uneven cell populations complicated interpretation of the results. Further development of punctal analysis should attempt to further validate this method of study in addition to co-localization focused analyses. Measurements that focus on histology, such as dendrite count and length should also be considered in future research.

Appendix B (Comparison of inflammatory markers between diagnosis, treatment, and timepoint)

With mounting evidence that immune dysregulation plays a role in the pathogenesis and treatment of major depressive disorder (MDD), there is a growing body of research on measurement of peripheral pro- and anti-inflammatory cytokines in MDD. While the majority of research has linked pro-inflammatory cytokines with the severity and progression of MDD, there have been mixed results about the role they play (reviewed in (Young et al., 2014)). The mixed literature on the role of cytokines in depression could suggest that inflammatory tone indicates a certain subset of depression (such as treatment resistant depression (TRD)) patients, or a potential indicator of treatment responsiveness (Chen et al., 2018; Li et al., 2017).

Ketamine, an N-methyl-d-aspartate receptor antagonist has been a breakthrough treatment for MDD, with fast-acting effects that reduce depressive symptomology within 24 hours. Though researchers primarily attribute ketamine's therapeutic effect to upregulated neuroplasticity induced via glutamatergic modulation (Duman, Shinohara, et al., 2019b), there is growing evidence that ketamine may also regulate acute inflammatory reactions and restore immune homeostasis. At anesthetic doses in healthy patients, ketamine significantly reduces pro-inflammatory cytokine expression without affecting anti-inflammatory production levels (Chen et al., 2018; Kawasaki et al., 1999). In animal models, ketamine has dose-dependently blocked inflammatory responses associated with an antidepressant-like effect (do Vale et al., 2016; Tan et al., 2017). However, there is a gap in research regarding the effects of ketamine on pro- and anti-inflammatory cytokines and the immune response in MDD (particularly TRD) patients. This study will analyze the baseline characteristics of cytokine expression between healthy controls and TRD patients, as well as the impact of ketamine on these cytokine profiles. This also includes other measures of inflammation such as TNF-α and other proteins demonstrated to be impacted in depression such as Substance P and reelin expression.

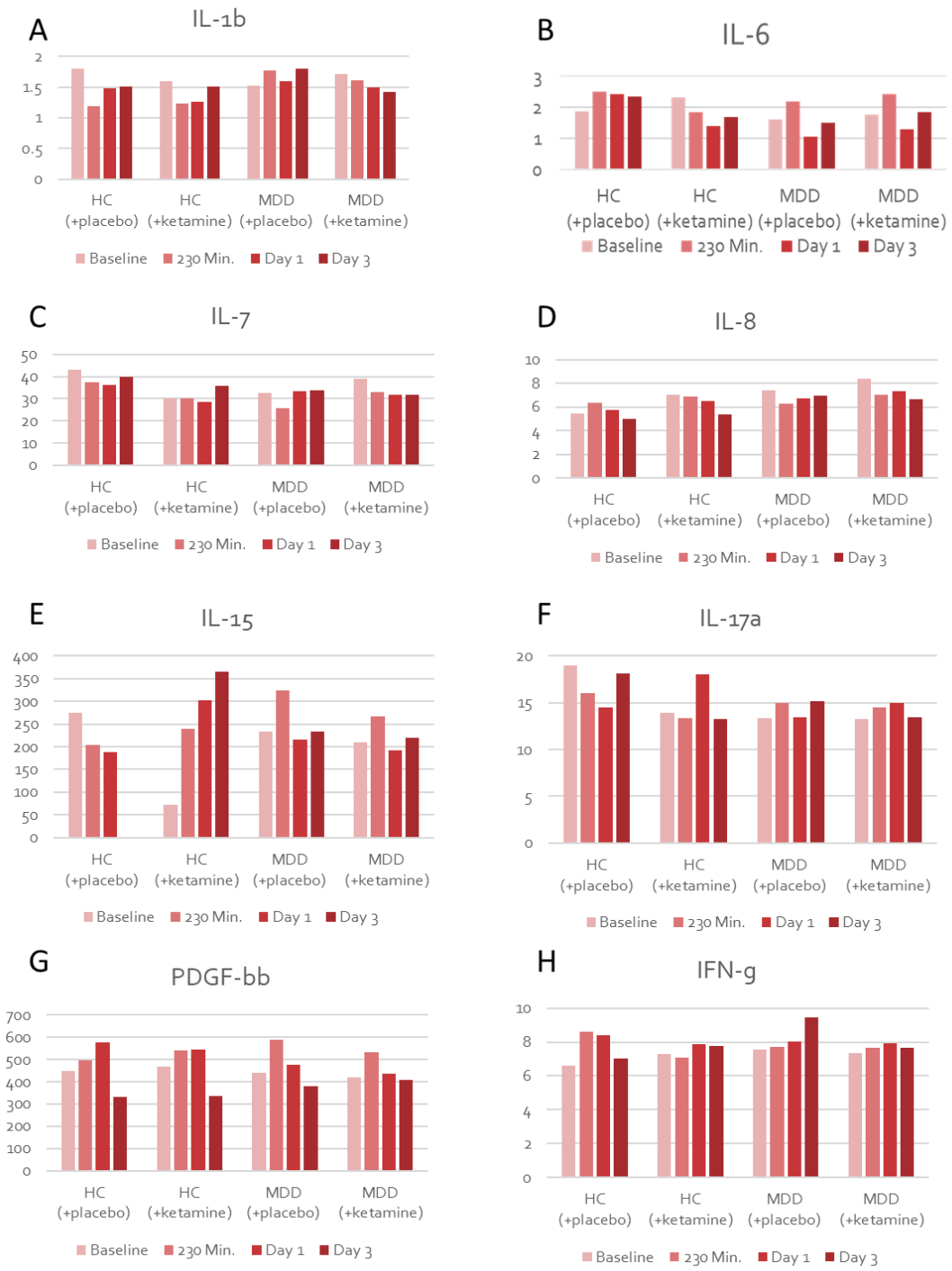
For the purpose of this study, four major hypotheses were tested: *(1)* ketamine will affect immune markers in a disparate manner to placebo, *(2)* baseline immune markers will affect clinical response to ketamine (as measured by MADRS), *(3)* there will be differences in treatment response across diagnostic groups, and *(4)* there will be baseline differences between TRD participants and healthy controls.

Methodology

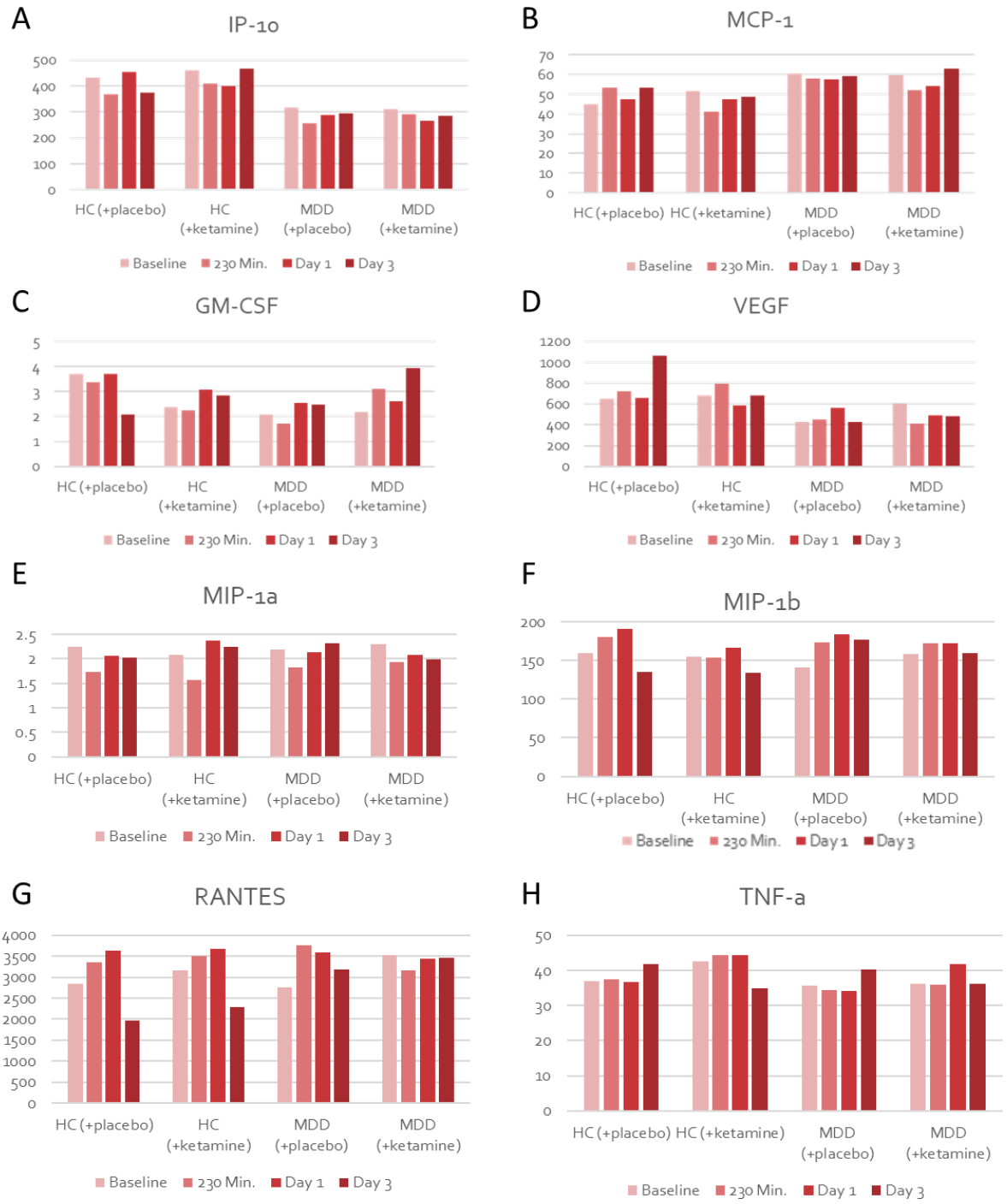
In accordance with NIH ethics, whole blood was collected from participants with treatment-resistant depression (n = 39) or healthy controls (n = 25) enrolled in a double-blind placebo crossover design study with a single ketamine infusion (0.5 mg/kg). Blood was collected at four timepoints: 1 hour before infusion, 230 min after infusion, 1 day after infusion, and 3 days after infusion. The Bio-Plex Pro Human Cytokine 27-plex assay kit (BioRad, Hercules, CA) was used to evaluate inflammatory markers in plasma. Inflammation measures included were fibroblast growth factor (FGF)-basic, Eotaxin, Granulocyte colony-stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), Interleukin (IL)-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17A, interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor (PDGF)-BB, C-C motif chemokine 5 (CCL5), regulated upon activation normal T cell expressed and presumably secreted (RANTES), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF). In addition, ELISA kits were used to assay levels of Substance P (#ab133029, abcam, Cambridge, UK) and reelin expression (#LS-F7023, LifeSpan Biosciences, Seattle, WA). Linear mixed model analyses were used to assess each hypothesis.

Results

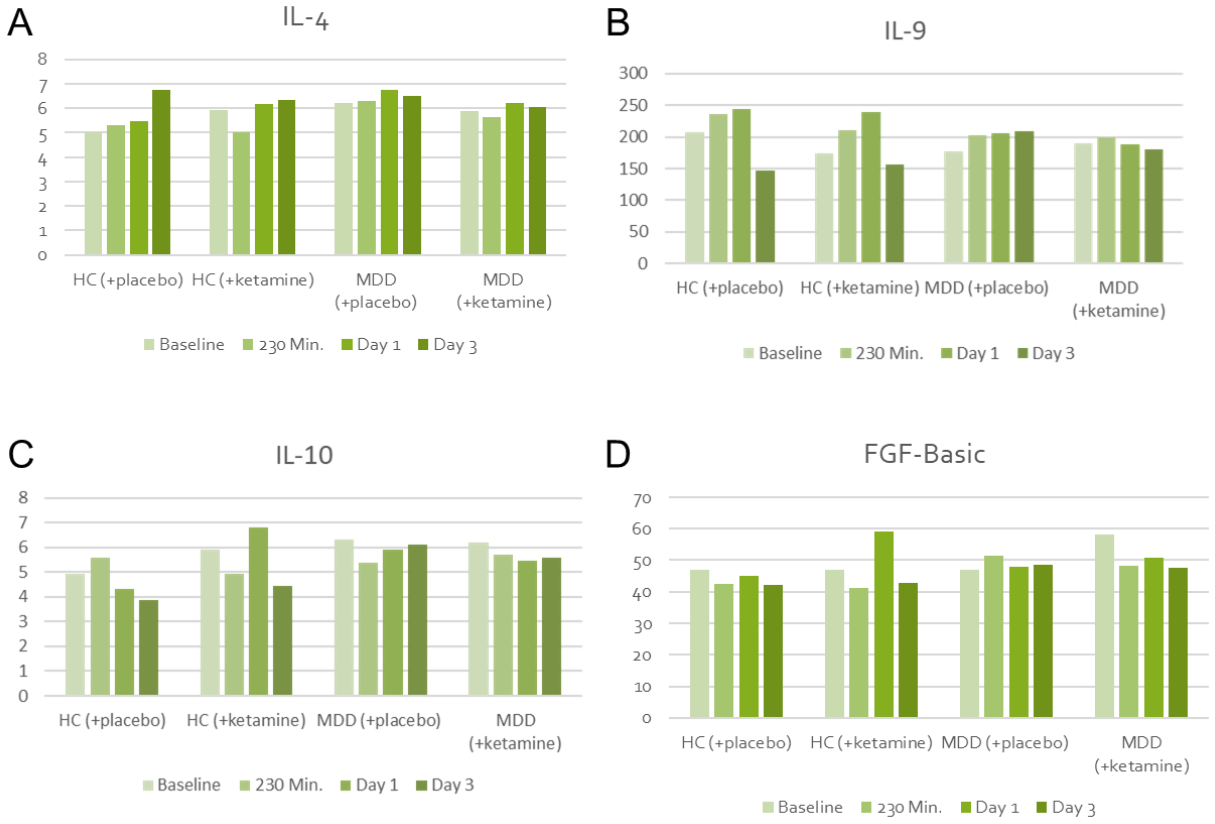
No significant differences were found across any of the four hypotheses for all inflammatory markers. Figures 1 – 5.



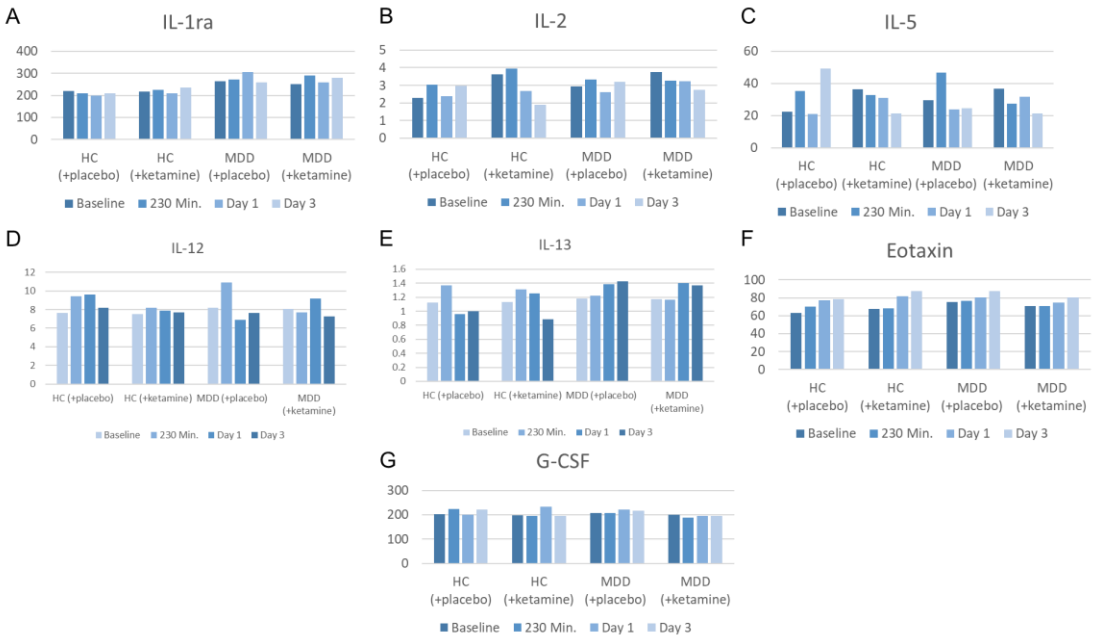
Appendix B. Figure 1. Effect of diagnosis, treatment, and timepoint, on markers with pro-inflammatory effects (Part I). (A – H) No significant differences were found across diagnosis, treatment group, or time for Interleukin (IL)-1β, IL-6, IL-7, IL-8, IL-15, IL-17a, platelet-derived growth factor (PDGF)-bb, or interferon gamma (IFN-γ). All data is expressed as average pg/ml found in plasma.



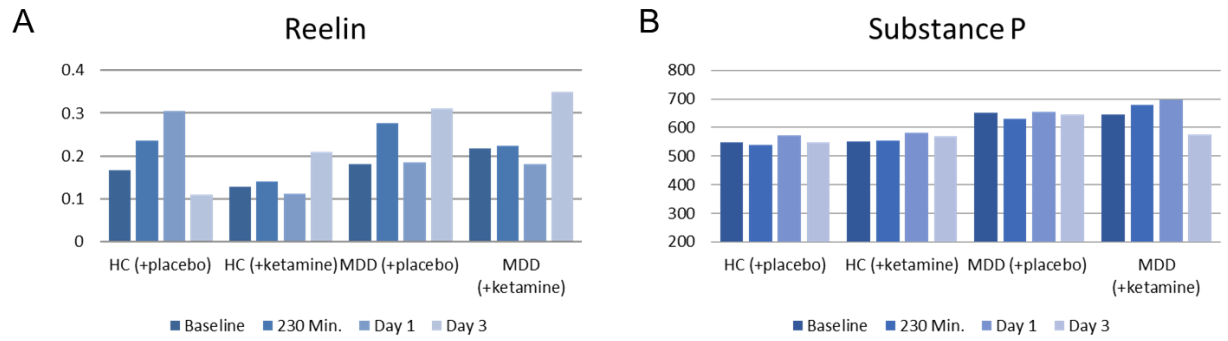
Appendix B. Figure 2. Effect of diagnosis, treatment, and timepoint, on markers with pro-inflammatory effects (Part I). (A – H) No significant differences were found across diagnosis, treatment group, or time for interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, Granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated upon activation normal T cell expressed and presumably secreted (RANTES), tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF). All data is expressed as average pg/ml found in plasma.



Appendix B. Figure 3. Effect of diagnosis, treatment, and timepoint, on markers with anti-inflammatory effects. (A-D) No significant differences were found across diagnosis, treatment group, or time for Interleukin (IL)-4, IL-9, IL-10, or fibroblast growth factor (FGF)-basic. All data is expressed as average pg/ml found in plasma.



Appendix B. Figure 4. Effect of diagnosis, treatment, and timepoint, on markers with mixed effects on inflammation. (A-G) No significant differences were found across diagnosis, treatment group, or time for Interleukin (IL-1ra), IL-2, IL-5, IL-12, IL-13, Eotaxin, or Granulocyte colony-stimulating factor (G-CSF). All data is expressed as average pg/ml found in plasma. All data is expressed as average pg/ml found in plasma.



Appendix B. Figure 5. Effect of diagnosis, treatment, and timepoint, on plasma reelin and Substance P expression. (A-B) No significant differences were found for either reelin or substance P expression across any comparison groups. All data is expressed as average ng/ml (reelin) or pg/ml (Substance P) found in plasma.

While the four major hypotheses tested in this study failed to capture any significant differences, it should be imperative to determine whether there were sex- or age-specific effects that could be impacting results. Future research should also assess the prophylactic effects of ketamine, which has shown significant promise in animal models (Camargo et al., 2021; Mastrodonato et al., 2020, 2022; Zhang et al., 2021).

Appendix C (comparisons between inflammatory markers in CSF and plasma)

Despite the ubiquity of depression, peripheral biomarkers of treatment responsiveness and diagnostics have been scarce (Gururajan et al., 2016; Schmidt et al., 2011; Strawbridge et al., 2017). Animal models and various *in vitro* assays have implicated inflammation in depression, however there are mixed results when measured in human participants, as demonstrated by Appendix B. The lack of reliable biomarkers may be due, in part, to disconnect between central and peripheral levels of response and inflammation.

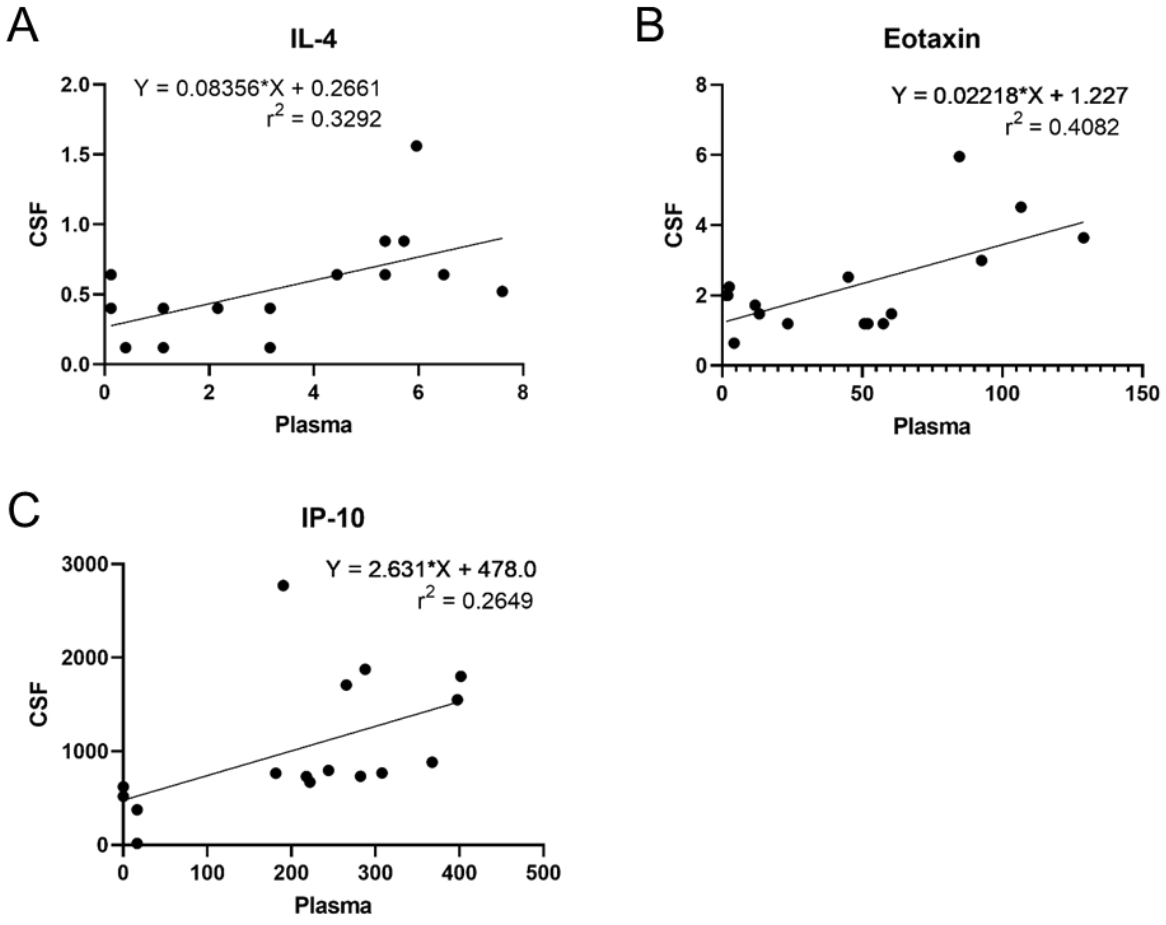
Generally, most research focuses either on peripheral or central markers, rarely combining or correlating these measures (Enache et al., 2019). However, knowledge on which peripheral markers are most reflective of central inflammatory processes could help inform future biomarker research and clinical studies, particularly since a blood draw is significantly less invasive than cerebrospinal fluid (CSF) collection.

Methodology

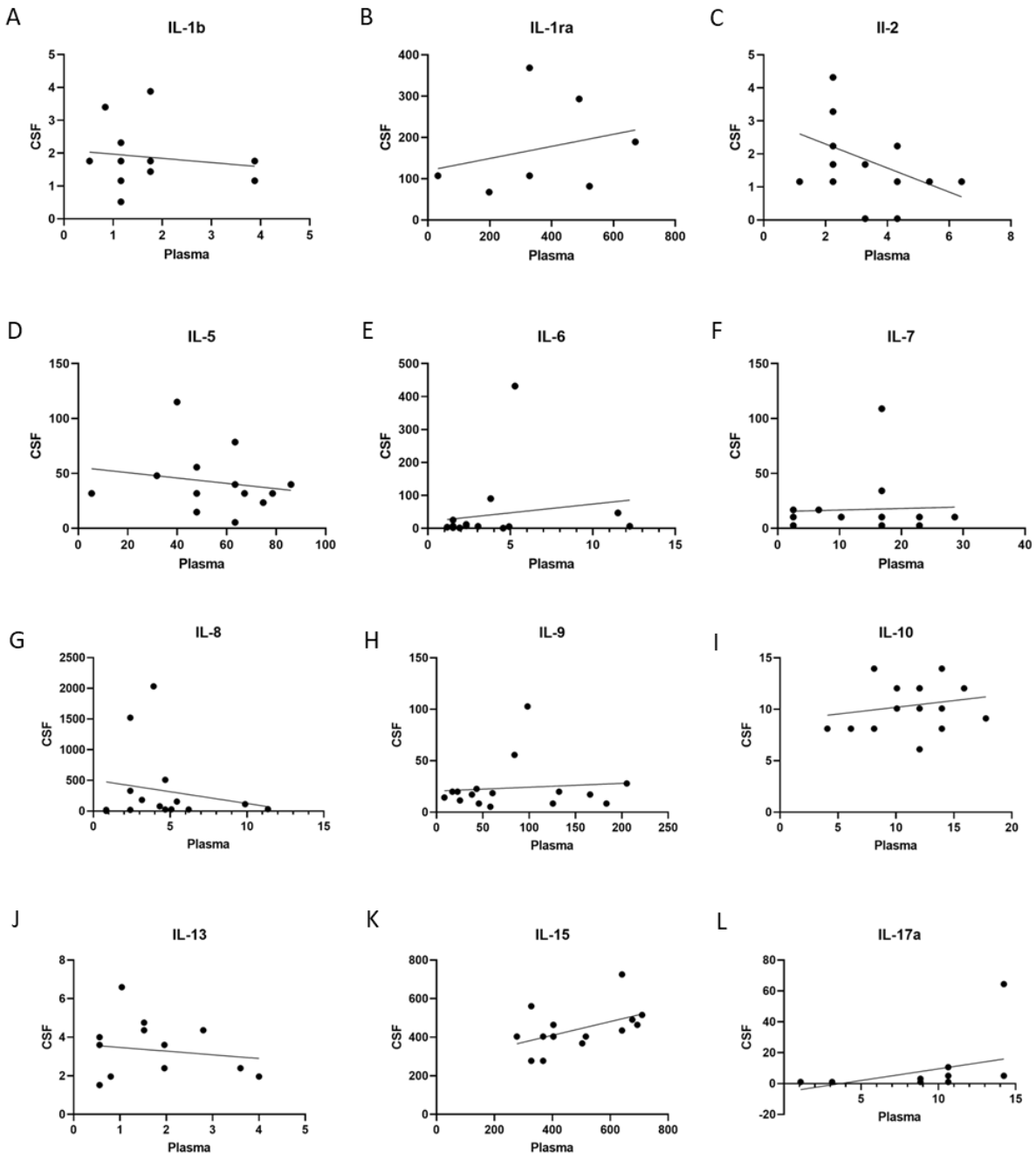
In this study, healthy control adults (n=16) who were enrolled in a double-blind, randomized, placebo-controlled design study of a single ketamine infusion (0.5 mg/kg) and had samples of both CSF and plasma collected at baseline, allowing the unique opportunity to measure if central levels of various pro- and anti-inflammatory markers were correlated with peripheral plasma measures. The Bio-Plex Pro Human Cytokine 27-plex assay kit (BioRad, Hercules, CA) was used to evaluate inflammatory markers in plasma and CSF in parallel to the markers measured above.

Results

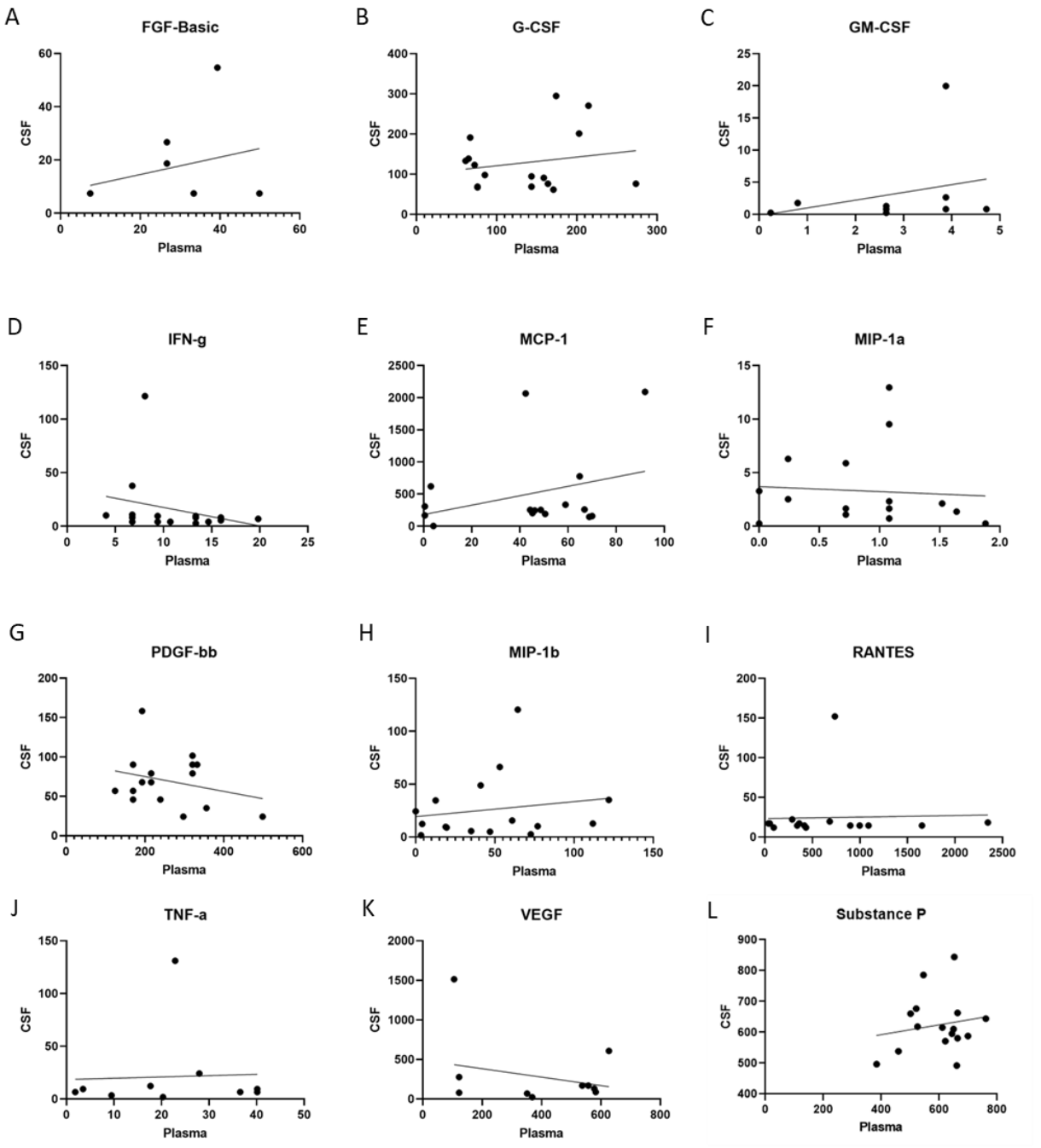
Significant correlations were found between plasma and CSF for levels of IL-4 (r^2 = 0.3292, F(1,13) = 6.379, p = 0.0253), Eotaxin (r^2 = 0.4082, F(1,14) = 9.658, p = 0.0077), and IP-10 (r^2 = 0.2649, F(1,14) = 5.046, p = 0.0413) (Figure 1). All other inflammatory markers had no significant correlations between central and peripheral expression levels (Figure 2-3).



Appendix C. Figure 1. Significant correlations between inflammatory markers in CSF and plasma. (A-C) Significant correlations were found between CSF and plasma expression levels for Interleukin (IL)-4, interferon gamma-induced protein (IP)-10, and Eotaxin. All data is expressed as pg/ml found in plasma or CSF.

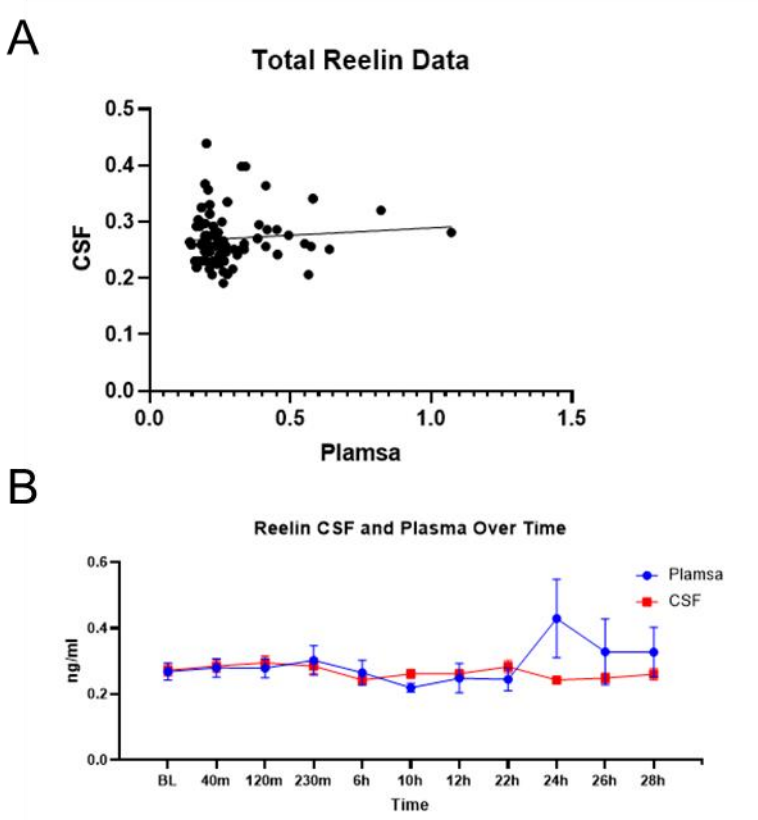


Appendix C. Figure 2. Non-significant correlations between inflammatory markers in CSF and plasma (part I). (A-L) No significant correlations were found in levels of various pro- and anti-inflammatory markers between CSF and plasma of participants for Interleukin (IL)-1b, IL-1ra, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-15, or IL-17a. All data is expressed as average pg/ml found in plasma or CSF.



Appendix C. Figure 3. Non-significant correlations between inflammatory markers in CSF and plasma (part II). (A-L) No significant correlations were found in levels of various pro- and anti-inflammatory markers between CSF and plasma of participants for fibroblast growth factor (FGF)-basic, Eotaxin, Granulocyte colony-stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor (PDGF)-BB, regulated upon activation normal T cell expressed and presumably secreted (RANTES), tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), and Substance P. All data is expressed as average pg/ml found in plasma or CSF.

Another area of great interest was the central and peripheral expression of reelin after ketamine administration. For this, we correlated plasma and CSF reelin expression levels over all timepoints, but also looked at the differences in expression over time after ketamine administration. No significant correlation was found in CSF and plasma levels or any significant differences between samples over time (Figure 4).



Appendix C. Figure 4. Correlation between reelin expression in CSF and plasma. (A) Correlations between reelin expression in cerebrospinal fluid (CSF) and plasma for all subjects and timepoints. (B) Average reelin expression in CSF and plasma across timepoints. All data is expressed as average ng/ml found in plasma or CSF.

There is still debate about the ability of certain interleukins to cross the blood-brain barrier (BBB), and it appears to be highly variable (Banks et al., 1995). However, the central markers shown in this study to have correlations with plasma Certain markers that demonstrated strong correlations in this study are known to cross or affect the BBB. Eotaxin, for example, has significant rapid bidirectional transport across the BBB (Erickson et al., 2014). In addition, IP-10 has been shown

to damage the BBB through an upregulation of TNF-α signaling (K. Wang et al., 2018). Lastly, blocking the action of IL-4 has been shown to protect BBB integrity, suggesting that IL-4 signaling is able to weaken the BBB as well (Bok et al., 2018).

Evidently, the majority of peripheral inflammation measurements do not reliably reflect central inflammation. This does not discount the utility of peripheral measurements, which can still be an invaluable tool for determining changes related to depression or treatment-responsiveness. However, inferences regarding neurochemical changes should be made sparingly. For increased translatability in depression research, animal models should report peripheral measures in conjunction with central markers. Future research should also determine if correlations are strengthened or worsened in participants with a diagnosis of depression, where it is hypothesized that the blood-brain barrier may be weaker (Dudek et al., 2020; Najjar et al., 2013).