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Putative Mechanisms Underlying the Antidepressant Actions of Ketamine: A Review and Study Proposal

A Thesis Presented

by

Tristan Reece

To the Keck Science Department

of

Claremont McKenna, Scripps, and Pitzer Colleges

In Partial Fulfillment of

The Degree of Bachelor of Arts

Senior Thesis in Neuroscience

April 25th, 2022

Abstract

Major Depressive Disorder (MDD) is a highly debilitating and common psychiatric disorder that affects over 250 million people globally; it is among the most financially and emotionally burdensome illnesses in the world. Currently approved antidepressants are suboptimal in their efficacy and latency of therapeutic action. In contrast, single administrations of sub-anesthetic ketamine have been shown to rapidly alleviate depressive symptoms within hours, even in treatment-resistant patients. Ketamine is believed to exert these effects by increasing glutamate-mediated neurotransmission and promoting rapid neurotrophic factor release, restoring the integrity of neural circuits that are compromised in depression. However, uncertainty surrounding its specific antidepressant mechanism of action has stalled distribution of this promising drug. Here, a chronology of antidepressant treatment advances that preceded the ketamine discovery is detailed, and current hypotheses for ketamine's antidepressant mechanism are critically reviewed to identify the limits of our understanding. Then, a study addressing a poorly characterized aspect of this mechanism is proposed. The study aims to assess whether isolated ketamine or its active antidepressant metabolite, (2*R*,6*R*)-hydroxynorketamine, differ in their ability to ameliorate stress-induced depressive phenotypes within the hippocampus of mice, compared to when they are present in combination. This study will provide insight for ongoing drug discovery efforts. An improved understanding of how ketamine exerts its effects within the brain will help foster future therapeutic innovation, which may lighten the ever-increasing burden of MDD on society.

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CHAPTER 1

Major Depressive Disorder and its Treatment Through History

Depression is Not Simple

Major Depressive Disorder (MDD), also known simply as depression, is among the most burdensome of all illnesses, accounting for significant disability, morbidity, and mortality worldwide (WHO 2020). The often chronic and relapsing course of this disorder leads to marked reductions in overall quality of life. Still, pinpointing exactly what depression *is* can be deceptively challenging, for depressive states, including periods of prolonged sadness and bereavement, are an unavoidable and perfectly ‘normal’ part of the human experience. Indeed, a life completely free of depression is idealistic. Transitory bouts of depressed mood, especially those caused by some precipitating external event, are not necessarily indicative of any mental illness and usually self-resolve without intervention (Proudman et al. 2021). True, *clinical* depression denotes a much more debilitating form thereof, one that badly impairs motivation and causes relationships and life-goals to deteriorate. MDD thus goes far beyond the normal human experience of sadness. The symptoms of this disorder do not remit when the external cause of these emotions dissipate, and they are often disproportionate to their cause. In fact, classic severe states of depression may not have any discernible external cause (Kessler & Bromet 2013). Though historically believed to be purely ‘mental’ or even spiritual in nature, it has become recognized in modernity that genetic predispositions and physical alterations to one’s neurobiology are deeply intertwined with the onset of MDD (Ebert & Bar 2010).

Researchers studying MDD are challenged by the complex nature of the disease, which is frequently comorbid with other chronic and acute conditions (Belmaker & Agam 2008). There is

immense variability in disease presentation and symptom profile, which leads to strong heterogeneity in the effects of treatments (Boku et al. 2017). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the primary symptoms of MDD include: persistent sadness, loss of pleasure in usual activities (anhedonia), apathy and avolition, feelings of guilt or worthlessness, fatigue, concentration and memory problems, rapid change in appetite or weight, insomnia or hypersomnia, loss of sex drive, psychomotor retardation, and suicidal ideation (APA 2013). The combination of symptoms that is experienced differs between patients, or even between depressive episodes within the same patient, though anhedonia is nearly always present. Furthermore, the duration and severity of symptoms during a depressive episode are highly idiosyncratic and may last anywhere from two weeks (the minimum required for formal MDD diagnosis) to months or even years. The impact of these symptoms can be very intrusive and far-reaching in a patient's life. They significantly impair activities of daily living, cognitive function, interpersonal relationships, employment status and productivity.

Prevalence

In addition to its severity, MDD is also distressingly common. The World Health Organization labeled depression as a leading cause of disability in the world, with well over 270 million people having received at least one formal diagnosis; countless more cases remain undiagnosed (WHO 2020; Liu et al. 2020). Depression straddles all genders, ethnicities, races, and walks of life. However, the distribution of MDD diagnoses is not homogenous. Depression disproportionately affects women, with the ratio of female to male diagnoses being roughly 2:1; this relationship has been observed in numerous countries worldwide (Kessler & Bromet 2013). The most common age demographic to receive a diagnosis is 18-25, though MDD can occur at

any age (NIMH 2021). In the United States, individuals with the lowest incomes are twice as likely to be depressed as those with the highest incomes (Kessler & Bromet 2013, Bailey et al. 2019). Interestingly, though, low socioeconomic status only appears to be a significant risk factor for MDD within highly developed countries, for rates of depression are higher in industrialized countries than in the developing world. However, even within developed countries, rates of MDD are inconsistent, being significantly lower in certain nations (e.g. Japan at 7%) than in others (e.g. France at 21%; Bailey et al 2019).

Much of the disparity in these numbers can be attributed to sociocultural factors rather than neurobiological ones. The likelihood of a person seeking clinical help for depression is swayed by values inculcated into that person through their family, peers, community, society, and government (Shim et al. 2012). For example, female rates of MDD may appear higher than rates in males because men are demonstrably more likely to avoid seeking help and leave their condition untreated (Nolan-Hoeksema 2001; Cunningham et al. 2021). Similarly, poorer socioeconomic groups generally receive inferior education about mental health issues and are presented with fewer treatment opportunities, often leading to a more negative prognosis (Bailey et al 2019).

Regardless of demographic differences, overall rates of depression also appear to be increasing. The sharpest increases have been seen in young people aged 18-34 (Proudman et al 2021). An obvious contributor is the COVID-19 pandemic, which has caused a drastic spiking in the diagnosis of mood disorders. Recent data from the Centers for Disease Control and Prevention show that the US prevalence of MDD has increased substantially from 7% pre-pandemic to 27% during the pandemic's first year, and MDD with anxiety disorders has increased from 11 to 38% (CDC 2021). However, the pandemic merely exacerbated a trend that

already existed, for depression rates were seeing spikes before Sars-CoV-2 emerged (CDC 2019). Another contributing factor could be the increased usage of the internet and social media in recent years, which has been correlated with damaged self-esteem and reduced self-satisfaction in children and adolescents, potentially explaining the spiking of MDD in these age groups (Keles et al. 2020; Cunningham et al. 2021). The current pandemic and internet usage are only two highly visible examples-- sociocultural factors influencing depression are complex and far too varied to exhaustively describe.

The public health implications of depression are alarming. MDD has been associated with increased risk and severity of numerous medical conditions, lowered productivity, and an elevated risk of early death. A lack of effective treatment can lead to negative coping strategies like substance abuse, self-harm and self-mutilation, and at worst, attempted or completed suicide (Riggs & Gould 2021). The emotional burden of this disease severely affects patients and reverberates outwardly into their loved ones and peers. Furthermore, MDD is extremely costly. Even pre-pandemic, the National Survey on Drug Use and Health (NSDUH) estimated that the incremental economic burden of adults with MDD sat at roughly 326.2 billion dollars per year (Greenberg et al 2021). This total was derived from the collation of direct costs (e.g. medical bills, drug prescriptions), suicide-related costs (e.g. hospital bills, funerals), and workplace costs (e.g. loss of job productivity). Individuals with severe MDD cost employers approximately double in health care expenses, are more likely to file for disability or be unemployed, and missed approximately 13.7 more work hours per month compared to healthy individuals (Birnbaum et al., 2010).

Altogether, the burdens of MDD are both severe and highly prevalent. The treatment of this disorder is thus one of the most urgent clinical priorities that we face today. Unfortunately,

the growth of our treatment methods over time has been slow, mainly due to the fact that our understanding of depressive pathophysiology is incomplete and does not provide the strong empirical foundation upon which tangible treatment advances critically rely. An overemphasis on incomplete mechanistic hypotheses of antidepressant action has caused progress to lag in recent decades (Riggs & Gould 2021). While a number of demonstrable risk factors have been identified, the etiology of depression remains elusive, and there is a glaring lack of consensus regarding its neurological and psychosocial underpinnings. MDD's etiology is obfuscated by the heterogeneity of its clinical presentation, disease course, and response to treatment (Mikulska et al. 2021). To better grasp how future antidepressant therapies can address the shortcomings of those currently administered, it is important to first lay out how our understanding of MDD has evolved through time.

Etiological History

Though depression has been acknowledged in art, literature, and philosophy since antiquity, early explanations for it were typically mystical rather than physiological. Mental illness was thought to be supernatural in origin, caused by evil spirits, demonic possession, punishment for sins, or similar non-scientific or pseudoscientific phenomena. 'Treatment' for such ailments consisted of exorcisms and other equivalent spiritual catharses, performed by priests rather than physicians (Kendler 2020). Medicinally, a primitive solution was found in trepanation, a surgical procedure where burr holes were drilled in the skull, perhaps as a means of allowing some evil to be released (Faria 2013). Though the practice of trepanation was discontinued in developed nations (except for niche surgical applications), the idea that mental

illness was essentially a spiritual or moral problem appears to have dominated for thousands of years.

Things began to change in the late 19th century, facilitated by the advent of psychiatry and the disease entity model, which led to the discovery of similarities across patients in onset, manifestations, and course of illness (Kane & Correll 2011). It was German psychiatrist Emil Kraepelin who first coined the term ‘depressive states’ to describe various forms of melancholia (Ebert & Bar 2010). He separated depression into two categories: *manic depression* (today’s bipolar disorder concept) and *dementia praecox* (today’s schizophrenia concept). The essences of his ideas live on in psychiatric discourse today. Unfortunately, though, neurotransmitter-based models of these concepts were still a half-century away.

Until then, a main form of care for depressed and suicidal patients was *institutionalization*, or the sequestering of mentally ill people into closed system psychiatric prison-hospitals that were removed from society. Patients’ freedoms were stripped and replaced with monotonous routinization, often in overcrowded and undersupplied facilities with aloof staff (Chow & Priebe 2013). The electroconvulsive therapies, insulin shock therapies, and frontal leukotomies/lobotomies popularized at that time reflect our cluelessness about where exactly mental illness manifested within the brain. Some patients actually responded quite well to institutional care (which is why psychiatric hospitals remain in use today), but this was overshadowed by failures on the larger scale. Thankfully, this era was brief. Deinstitutionalization occurred after WW2 due to infeasible burdens on the welfare state, the burgeoning civil rights movement, and, most importantly, milestone developments in antidepressant drugs (Chow & Priebe 2013).

The Genesis of the Monoamine Hypothesis

In the late 1940's, the indole alkaloid reserpine, isolated from *Rauvolfia serpentina* as a treatment for hypertension, was noticed to cause a strong blunting of affect in many patients. It essentially precipitated depressive symptoms in previously healthy people (Muller et al. 1955). We soon learned that this is due to reserpine's ability to deplete catecholamine and monoamine transmitters from sympathetic nerve endings (Hillhouse & Porter 2015). This is accomplished by its binding to vesicular uptake transporters VMAT1 and VMAT2, which package amines like norepinephrine (NE), dopamine (DA), and serotonin (5-HT) into synaptic vesicles. Their blockage serves to decrease the size of releasable neurotransmitter pools, which consequently decreases the amplitude of signal communication across synapses (Miyamoto et al. 2012). The result is a global tranquilizing effect on brain and body. Interestingly, reserpine's side effects were highly resemblant of depressive symptomology. This led to the prolific realization that depression itself might be caused by the depletion of these aminergic transmitters (Kirshner 1962; Shore et al. 1957; Shore et al. 1955).

Right around this same time, in 1952, the tuberculosis drug isoniazid was serendipitously found to have mood elevating properties (Ramachandrai et al. 2011). Its derivative, iproniazid, was then shown to be capable of improving a variety of MDD symptoms, as well as 'reversing' many of the depressive effects of reserpine (Pletscher 1991). The mechanism responsible for this was the inhibition of monoamine oxidase, which slowed the breakdown of monoamine transmitters, thereby increasing serotonin, norepinephrine, and dopamine transmission in the synapse (Lopez-Munos et al. 2007; Mafioletti et al. 2020). Contemporaneously, Swiss psychiatrist Roland Kuhn discovered the antidepressant properties of compound G22355 while

searching for derivatives of chlorpromazine that could deliver similar antipsychotic effects (Kuhn 1957; Brown & Rosdolsky 2015). This tricyclic drug, which would later be called imipramine, seemed to attenuate many depressive symptoms by blocking the SERT and NET transporters, again increasing the duration of monoamine action within synapses (Brown & Rosdolsky 2015). Combined with the concurrent discoveries about depression through reserpine, depression itself became perceived as a disorder of monoaminergic transmission. The subsequent distribution of iproniazid and imipramine as the first officially marketed antidepressants saw promising results, at least in juxtaposition to other treatments available at the time (Mafioletti et al. 2020). By this point, there was compelling evidence that deficiencies in levels of 5-HT, NE, and DA within diffuse brain regions were the underlying cause of MDD, and that rescuing these deficits through antidepressant drugs were sufficient to ‘fix’ the maladaptive behavioral changes caused by the deficiencies (Boku et al. 2018). Thusly, the focus of psychiatrists began to shift from a psychodynamic to a biological basis for depression (Pletscher 1991).

Monoamine Antidepressants and Their Shortcomings

Over the next few decades, the monoamine hypothesis would quickly become cemented as the most classically accepted theory regarding the pathoetiology of MDD, with particular emphasis placed on 5-HT (Boku et al. 2018). Diffuse modulatory serotonergic pathways originate in the Raphe nucleus and influence diverse functions in both brain and body. 5-HT transmission is heavily interactive on conscious perceptions of pain, eating, sleeping, and mood, all of which are known to be disturbed in MDD (Mafioletti et al. 2020; Hirschfield 2000). A deficiency of 5-HT could therefore explain many of depression’s symptoms. It could also explain why depression has such a diverse constellation of symptoms (5-HT influence is pervasive). In

fact, we have evidence of this, for inhibition of the 5-HT biosynthetic precursor tryptophan hydroxylase by PCPA causes rats to elicit depressive symptoms (Ellison & Bresler 1974). Furthermore, reduced cerebrospinal concentrations of the proprietary NE and 5-HT metabolites (6-fluoronorepinephrine and 5-hydroxyindoleacetic acid) have been found in depressed patients, especially in suicide victims (Barton 2008; Cowen 2001).

NE's role in depression is less clear, though a correlation is now known to exist between antidepressant drug efficacy and stronger downregulation of NE β -receptors (meaning NE transmission has increased in the antidepressant's presence; Esler et al. 1982). Since NE transmission helps modulate arousal, attention, memory, reward, and motivation, deficiencies of NE could explain some elements of depressive pathology (Moret & Briley 2011; Delgado & Moreno 2000). Likewise, reductions in DA transmission could at least partially explain why anhedonia and avolition are characteristic of MDD (Tye et al. 2013, Belujon & Grace 2017). The ability of MAOIs to block DA breakdown may therefore contribute to their antidepressant properties (Tekes et al. 1988). Taken together, it is no surprise that monoamine transmission has been so strongly implicated in MDD's pathology, and why nearly all antidepressant drugs focus their action on them.

Several classes of antidepressants have been widely distributed to date, all of which primarily aim to increase monoamine transmission. After the relative success of iproniazid, many other MAOIs were developed (e.g. phenelzine; Lopez-Munos et al. 2007). To reiterate, MAOIs inhibit monoamine oxidase, slowing breakdown of monoamines, thereby increasing 5-HT, NE, & DA transmission in the synapse. Since MAOIs primarily act on MAO_A, they inevitably also bind to MAO_A in the liver, leading to hepatotoxic tyramine buildup and significant dietary restrictions (Hillhouse & Porter 2015; Ramachandrai et al. 2011). In addition, more tricyclic

antidepressants (e.g. desipramine) were developed in the likeness of imipramine. TCAs attempted to subvert the liver-binding problem of MAOI's by instead inhibiting CNS-specific uptake transporters for 5-HT and NE, again leading to extended synaptic action of monoamines (Lopez-Munos et al. 2007). Though TCAs successfully reduced hepatotoxicity, they did it by substituting one problem for several others. TCAs collaterally block muscarinic acetylcholine receptors, as well as adrenergic α -1 receptors and histamine H1 receptors (Otte et al. 2016). TCAs thus had an even lower margin of safety than MAOIs in addition to diverse side effects and extensive drug-drug interactions (Ramachandrai et al. 2011).

Attempts to address these shortcomings in later decades gave rise to a second generation of antidepressants that, while not more effective per se, have narrower side effect profiles and a wider margin of safety (Butler & Meegan 2008). Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) and Selective Serotonin Reuptake Inhibitors (SSRIs) are the pinnacle of these (e.g. duloxetine and fluoxetine, respectively; Connelly & Thase 2012). SNRIs work in a manner reminiscent of TCAs but with lower affinity for receptors outside their targets, improving their tolerability (Hillhouse & Porter 2015; Ramachandrai et al. 2011). The SSRIs strayed from 5-HT/NE dual action, selectively blocking the SERT presynaptic transporter, increasing synaptic concentrations of 5-HT only (Thase 2008). Even newer antidepressants within the second generation include Serotonin Antagonists and Reuptake Inhibitors (SARIs) such as trazodone, Noradrenergic and Specific Serotonergic Antidepressants (NaSSA) such as mirtazapine, catecholamine releasers such as bupropion, and triple reuptake inhibitors like venlafaxine (Chang & Fava 2010; Connelly & Thase 2010).

Though it is undeniable that the safety, tolerability, and side effect profiles of antidepressant drugs have improved significantly in the last fifty years, a glaring issue remains: the efficacy of monoamine antidepressants is *not* increasing (e.g. Thase 2008; Jacobsen et al. 2012). Only 60-70% of MDD patients experience clinically significant symptom relief with currently available antidepressants, and this includes patients that may require up to four different treatment steps after failing to respond to first-line medications (Connelly & Thase 2010; Rush et al. 2006). In fact, only one-third of patients appear to respond fully to current pharmacotherapies; large clinical trials have shown that half of patients fail to experience at least a 50% reduction in depressive symptoms, even after 12-14 weeks of treatment (e.g. Jakobsen et al. 2017). The relapse of symptoms is also common if the drug's administration is altered or abandoned—sustained remission of depressive symptoms in the long term is rare (Chang & Fava 2010; Abdallah et al. 2016).

Furthermore, patients that do experience remission of depressive symptoms from antidepressants usually take weeks or even months to do so (Jakobsen et al. 2017; Katz et al. 2004). This latency of onset is well-documented and represents an immense obstacle for the success of currently administered antidepressants. Dropout rates are typically high during clinical trials as patients become frustrated or disheartened by the delays (Rush et al. 2006). The latency can also be dangerous for MDD patients with imminent risk of self-harm or suicide (Belujon & Grace 2017). The high latency and inconsistency is made worse by the fact that even the most successful antidepressants still have side effect profiles harmful enough to render them intolerable to a significant fraction of patients (Jakobsen et al. 2017; Crawford et al. 2014). SSRIs like fluoxetine cause various side effects (e.g. nausea, insomnia, sexual dysfunction, restlessness, dizziness) as a consequence of altering 5-HT transmission (Jacobsen et al. 2012;

Belujon & Grace 2017). This contributes to clinical dropout rates; many patients cannot justify a life-altering constellation of side effects just to experience only a modest or partial reduction in their depressive symptoms (Crawford et al. 2014; Hirschfield 2000).

Overall, there is a paucity of convincing evidence for the monoamine hypothesis, which relies on the assumption that monoamines have a primary, causative role in the etiology of MDD (Heninger et al. 1996). Predictions that arise from this hypothesis are that (a) a reduction in monoamines increases depression susceptibility; (b) the extent of that reduction is proportional to the severity of depressive symptoms; (c) antidepressant response is contingent upon, and mirrors the time course of, monoamine restoration; and (d) symptom relapse is due to the recurrence of a monoamine deficit (Hirschfield 2000; Jacobsen et al. 2012; Riggs & Gould 2021). The evidence in support of these predictions has become exhausted and, as a result, it is generally accepted that the monoamine hypothesis cannot fully explain depression symptomatology, nor can it foster the development of treatments that will be universally effective (Heninger et al. 1996; Hirschfeld 2000; Belmaker & Agam 2008).

Statement of the Problem

Depression therapeutics were basically nonexistent when the antidepressant effects of iproniazid were discovered half a century ago. Unfortunately, the individual and societal impacts of depression have since been managed using medications with suboptimal efficacy, high latency of therapeutic action, and side effects significant enough to cause high rates of treatment discontinuation (Riggs & Gould 2021). This situation is, in part, a direct result of depression being poorly understood at the level of its etiology, pathophysiology, and clinical manifestation.

The lack of understanding has prevented us from discovering an ‘ideal’ antidepressant that attenuates MDD symptoms in a supermajority of patients with the smallest side effect profile necessary to achieve that effect (Heninger et al. 1996). Though many depressed patients display aberrant monoaminergic signaling, a sizable minority do not; they consequently fail to experience significant remission of symptoms when these transmitters are targeted by antidepressant drugs (Belmaker & Agam 2008; Heninger 1996). Thus, while monoamines are certainly a core aspect of depressive pathology, they do not describe the full picture, and treatments that focus on them are inadequate *en bloc* (Hirschfield 2000). Until the lesser known, monoaminergic-independent aspects of MDD’s pathology are better understood, our progress in treating the disorder will continue to stagnate. All the while, MDD continues to become more financially and emotionally burdensome on society with each passing year (Greenberg et al. 2021, Birnbaum et al. 2010). There is a pressing need to develop new, faster acting, more universally effective pharmacological approaches to treat depression, particularly for patients who are unresponsive to traditional antidepressants. Thankfully, modern research has revealed several monoamine-independent aspects of MDD pathology that provide footholds for novel treatment methods.

CHAPTER 1 REFERENCES

- Abdallah CG, Adams TG, Kelmendi B, Esterlis I, Sanacora G, Krystal JH. (2016). Ketamine's Mechanism of Action: A Path to Rapid-Acting Antidepressants. *Depression and anxiety*. **33**:689-97.
- Bailey RK, Mokonogho J, Kumar A. (2019). Racial and Ethnic Differences in Depression: Current Perspectives. *Neuropsychiatry Dis Treat*. **16**: 603-609
- Barton DA. (2008). Elevated Brain Serotonin Turnover in Patients With Depression: Effect of Genotype and Therapy. *Arch Gen Psychiatry*. 65(1):38-46.
doi:10.1001/archgenpsychiatry.2007.11
- Belmaker RH, Agam G. (2008). Major Depressive Disorder. *N Engl J Med*. **358**(1): 55-68
- Belujon P, Grace AA. (2017). Dopamine System Dysregulation in Major Depressive Disorders. *The international journal of neuropsychopharmacology*. 20(12): 1036–1046.
doi:10.1093/ijnp/pyx056
- Birnbaum HG, Kessler RC, Kelley D, Ben-Hamadi R, Joish VN, Greenberg PE. (2010). Employer burden of mild, moderate, and severe major depressive disorder: mental health services utilization and costs, and work performance. *Depression & Anxiety*. **27**:78–89.
- Boku S, Nakagawa S, Toda H, Hishimoto A. (2018). Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry and clinical neurosciences*. **72**:3-12.
- Brown WA, Rosdolsky M. (2015). The clinical discovery of imipramine. *Am J Psychiatry*. 172(5): 426-429. doi:10.1176/appi.ajp.2015.141013
- Butler SG, Meegan MJ. (2008). Recent developments in the design of antidepressant therapies: Targeting the serotonin transporter. *Curr Med Chem*. **15**:1737–61.
- Centers for Disease Control and Prevention. (2020). *Early release of selected mental health estimates based on data from the January–June 2019 National Health Interview Survey*. Atlanta: Centers for Disease Control and Prevention.
- Centers for Disease Control and Prevention. (2021). *Anxiety and Depression*. (2021). Atlanta: Centers for Disease Control and Prevention.
- Chang T, Fava M. (2010). The future of psychopharmacology of depression. *J Clin Psychiatry*. 71(8):971-975. doi: 10.4088/JCP.10m06223blu.
- Chow WS, Priebe S. (2013). Understanding psychiatric institutionalization: a conceptual review. *BMC Psychiatry*. 169(13)

- Connolly KR, Thase ME. (2012). Emerging drugs for major depressive disorder. *Expert Opin Emerg Drugs*. 17(1):105-26. doi: 10.1517/14728214.2012.660146.
- Cowen PJ. (2001). Neuroendocrine markers of depression and antidepressant drug action. *Antidepressants*. 95-107. doi:10.1007/978-3-0348-8344-3_7
- Crawford AA, Lewis S, Nutt D, et al. 2014. Adverse effects from antidepressant treatment: randomised controlled trial of 601 depressed individuals. *Psychopharmacology*. **231**:2921-31.
- Cunningham S, Hudson C, Harkness K. (2021). Social Media and Depression Symptoms: A Meta-Analysis. *Research on Child and Adolescent Psychopathology*. **49**: 241-253
- Delgado PL, Moreno FA. (2000). Role of norepinephrine in depression. *J Clin Psychiatry*. PMID: 10703757
- Ebert A, Bar KJ. (2010). Emil Kraepelin: A pioneer of scientific understanding of psychiatry and psychopharmacology. *Indian J Psychiatry*. **52**(2): 191-191
- Depressive Disorders. (2013). Diagnostic and Statistical Manual of Mental Disorders: American Psychiatric Association.
- Ellison GD, Bresler DE. (1974). Tests of emotional behavior in rats following depletion of norepinephrine, of serotonin, or of both. *Psychopharmacologia*. 34(4), 275–288. doi: 10.1007/BF00422552
- Esler M, Turbott J, Schwarz R. (1982). The Peripheral Kinetics of Norepinephrine in Depressive Illness. *Arch Gen Psychiatry*. 39(3): 295-300. doi:10.1001/archpsyc.1982.04290030035006
- Faria MA. (2013). Violence, mental illness, and the brain – A brief history of psychosurgery: Part 1 – From trephination to lobotomy. *Surg Neurol Int*. **4**: 49
- Greenberg P, Fournier AA, Sisitsky T, et al. (2021). The economic burden of adults with major depressive disorder in the United States (2010 and 2018). *Pharmacoeconomics*.
- Hillhouse TM, Porter JH. (2015). A brief history of the development of antidepressant drugs: From monoamines to glutamate. *Exp Clin Psychopharmacol*. 23(1): 1-21
- Heninger GR, Delgado PL, Charney DS. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry*. 29:2–11
- Hirschfeld, RM. (2000). History and evolution of the monoamine hypothesis of depression. *J Clin Psychiatry*. 6: 4-6. PMID: 10775017
- Jacobsen JP, Medvedev IO, Caron MG. (2012). The 5-HT deficiency theory of depression: perspectives from a naturalistic 5-HT deficiency model, the tryptophan hydroxylase 2Arg439His knockin mouse. *Philos. Trans. R. Soc. B* 367:2444–59

- Jakobsen JC, Katakam KK, Schou A, Hellmuth SG, Stallknecht SE, et al. (2017). Selective serotonin reuptake inhibitors versus placebo in patients with major depressive disorder. A systematic review with meta-analysis and Trial Sequential Analysis. *BMC Psychiatry* 17:58
- Kane JM, Correll CU. (2011). Past and present progress in the pharmacological treatment of schizophrenia. *J Clin Psychiatry*. 71(9): 1115-1124
- Keles B, McCrae N, Grealish A. (2020). A systematic review: the influence of social media on depression, anxiety and psychological distress in adolescents. *International Journal of Adolescence & Youth*. 25(1)
- Kendler KS. (2020). The Origin of Our Modern Concept of Depression—The History of Melancholia From 1780-1880. *JAMA Psychiatry*. 77(8): 863-883
- Kessler RC, Bromet EJ. (2013). The epidemiology of depression across cultures. *Annual review of Public Health*. 34:119-38.
- Kirshner N. (1962). Uptake of Catecholamines by a Particulate Fraction of the Adrenal Medulla. *Journal of Biological Chemistry*. 237(7): 2311–2317
- Kuhn R. (1957). Über die Behandlung depressiver Zustände mit einem Iminodibenzyl-derivat (G22355). *Schweiz Med Wochenschr*. 87:1135–1140
- Liu Q, He H, Yang J, Feng X, Zhao F, Lyu J. (2020). Changes in the global burden of depression from 1990 to 2017: findings from the Global Burden of Disease Study. *J Psychiatr Res*. 126:134–40.
- Lopez-Munos F, Alamo C, Juckel G, Assion HJ. (2007). Half a century of antidepressants: On clinical introduction of monoamine oxidase inhibitors, tricyclics and tetracyclics. Part 1: Monoamine oxidase inhibitors. *J Clin Pharmacol*. 27:555–9.
- Maffioletti E, Minelli A, Tardito D, Gennarelli M. (2020). Blues in the Brain and Beyond: Molecular Bases of Major Depressive Disorder and Relative Pharmacological and Non-Pharmacological Treatments. *Genes (Basel)*. 11(9): 1089
- Mikulska J, Juszczak G, Gawronska-Grzywacz M, Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sciences*.
- Miyamoto S, Miyake N, Jarskog LF, Fleischhacker, WW, Lieberman, JA. (2012). Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Molecular Psychiatry*. 17(12): 1206–27.
- Moret C, Briley M. (2011). The importance of norepinephrine in depression. *Neuropsychiatry Dis Treat*. 7: 9-13. doi: 10.2147/NDT.S19619

Muller JC, Pryor WW, Gibbons JE, Orgain, ES. (1955). Depression and anxiety occurring during rauwolfia therapy. *Journal of the American Medical Association*. **159**:836–839

National Institute of Mental Health. (2022). *Major Depression*.

Nolan-Hoeksema S. (2001). Gender Differences in Depression. *Current Directions in Psychological Science*. **10**(5), 173–176.

Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, Schatzberg AF. (2016). Major depressive disorder. *Nat Rev Dis Primers*. 2:160-65. doi: 10.1038/nrdp.2016.65

Pletscher A. (1991). The Discovery of antidepressants: A winding path. *Experientia*. **47**:4–8.

Proudman D, Greenberg P, Nellesen D. (2021). The Growing Burden of Major Depressive Disorders (MDD): Implications for Researchers and Policy Makers. *Pharmacoeconomics*. **39**(6): 619-625

Ramachandrai CT, Subramanyam N, Kral Jurgen B, Baker, G, Yeragani, VK. (2011). Antidepressants: From MAOI's to SSRI's and More. *Indian J Psychiatry*. **53**(2): 180-182
Riggs LM, Gould TD. (2021). Ketamine and the Future of Rapid-Acting Antidepressants. *Annual Review of Clinical Psychology*. **15**(7)

Rush AJ, Trivedi MH, Wisniewski SR, et al. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *The American journal of psychiatry*. **163**:1905-17.

Shim RS, Ye J, Baltrus P, Fry-Johnson Y, Daniels E, Rust G. (2012). Racial/ethnic disparities, social support, and depression: examining a social determinant of mental health. *Ethn Dis*. **22**(1):15–20.

Shore PA, Pletscher A, Tomich EG, Carlsson A, Kuntzman R, Brodie BB. (1957). Role of brain serotonin in reserpine action. *Annals of the New York Academy of Sciences*. 66:609–617. doi:10.1111/j.1749-6632.1957.tb40751.x.

Shore PA, Silver SL, Brodie BB. (1955). Interaction of reserpine, serotonin, and lysergic acid diethylamide in brain. *Science*. 122:284–285. doi:10.2307/1751305

Substance Abuse and Mental Health Services Administration. (2020). *National Survey of Drug Use and Health (NSDUH) Releases*. U.S. Department of Health & Human Services

Thase ME. (2008). Are SNRIs more effective than SSRIs? A Review of the current state of the controversy. *Psychopharmacol Bull*. **41**:58–85.

Tye KM, Mirzabekov JJ, Warden MR, Ferenczi EA, Tsai HC, Finkelstein J, Kim SY, Adhikari A, Thompson KR, Andalman AS, Gunaydin LA, Witten IB, Deisseroth K. (2013). Dopamine

neurons modulate neural encoding and expression of depression-related behaviour. *Nature*. **493**:537–541.

Tye KM, et al. (2013). Dopamine alters expression of depression-related behaviour. *Nature*. 493: 537-541. doi:10.1038/nature11740

Uher R, Payne JL, Pavlova B, Perlis RH. (2014). Major depressive disorder in DSM-5: implications for clinical practice and research of changes from DSM-IV. *Depression and anxiety* **31**:459-71.

World Health Organization (WHO). (2020). *WHO Depression Fact Sheet*.

CHAPTER 2

Glutamate and Chronic Stress: A Contemporary MDD Framework

Chronic Stress on Depression

Research in recent decades has demonstrated that MDD emerges from a complex interaction of genetic and environmental factors (Jafee & Price 2007; Uher 2014). Among the most notable of these environmental factors is exposure to chronic stress, which may induce maladaptive structural changes in the brain that underlie depressive pathology. The chronic stress model for depression is well documented (e.g. Han and Nestler 2017; McEwen & Akil 2020; Tornese et al. 2019). It posits that the etiopathogenesis of MDD can be explained through alterations in stress hormone patterns, particularly a hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA axis is built into the body as a system for managing stress and maintaining homeostasis. Upon perceiving a stress-inducing stimulus, the hypothalamus uses corticotropin releasing hormone to stimulate adrenocorticotrophic hormone release out of the anterior pituitary, which in turn stimulates the adrenal cortex to release glucocorticoids, such as cortisol, into the blood (Mikulska et al. 2021). Glucocorticoids induce various physiological changes (e.g. augmenting the amount of glucose available to muscles) to temporarily enhance fight-or-flight responses to life-threatening stimuli (Jacobson 2005). These changes improve the probability of survival. Glucocorticoids then negatively feedback to the brain by inhibiting hypothalamic and hippocampal neurons, terminating their own release once the stressor has subsided (Joseph & Whirledge 2017).

The HPA axis evolved to briefly enhance performance in acute and urgent situations (e.g. running from a predator), and has been conserved across many species due to its benefits (Sapolsky 1994). Unfortunately, though, the stresses of modern life differ greatly from those faced by ancestral humans; the HPA axis was not designed to accommodate the long-term emotional, occupational, and existential stressors faced by people today (Sapolsky 1994). Internalized, abstract stressors activate the HPA axis all the same, but differ from acute environmental stressors in the sense that they may persist relentlessly. If stress persists, HPA signaling may become dysfunctional in individuals genetically vulnerable to the adverse effects of chronic stress (Tornese et al. 2019). Downregulation of the brain's negative feedback mechanisms cause glucocorticoid levels to remain protracted in the blood (rather than self-attenuating), where they are free to exert unsavory effects on the CNS (Joseph & Whirledge 2017). Long term, these include hippocampal cell atrophy and dendritic spine reduction, immunodeficiency, forebrain desynchronization, impaired hippocampal and frontal neurogenesis, and neurotransmitter deficiencies (Mikulska et al. 2021; Tornese et al. 2019; Andres et al. 2013). Given these effects, it is no mystery why chronic stress has become recognized as an undercarriage of depressive pathology: nearly all systems compromised by chronic stress are also compromised in MDD. In fact, we know that MDD correlates with HPA axis dysfunction because depressed patients lack normal cortisol suppression when other glucocorticoids such as dexamethasone are administered (Dogra 2021).

Summed together, chronic stress results in numerous maladaptive structural changes within the brain that may underlie depressive pathology. Deficits of monoaminergic signaling are among these changes and have received the most attention because they contribute the most outwardly visible affective symptoms of MDD (Riggs & Gould 2021). The recent promulgation

of this fact has changed our understanding of how depression develops. While it is very much clear that monoamines are involved in the behavioral and affective domains of depression, they primarily serve a neuromodulatory role, impinging on other physiological processes that are more etiologically relevant in the onset of depression, rather than themselves being the cause (Heninger et al. 1996, Hirschfeld 2000, Lee & Han 2019). There are adverse effects of chronic stress (i.e. reduced synaptic plasticity) whose contributions to depressive symptomology are less obvious, and have received little to no attention until recent years (Mikulska et al. 2021; Tornese et al. 2019; Andres et al. 2013). Shifting the focus of antidepressant drug action towards other systems compromised by chronic stress may be essential for addressing the shortcomings of current treatment methods. Thankfully, several drugs that accomplish this have already been identified, some of which show great promise (Murrough et al. 2017). The most emblematic of these is none other than ketamine.

Ketamine as a Novel Antidepressant

Ketamine is a dissociative anesthetic derived from phencyclidine. It was first synthesized to reduce the severity of postanesthetic emergence delirium while simultaneously minimizing length of anesthetic action (Domino et al. 1965). Though not by design, ketamine dose-dependently enhances sympathetic processes that modify cardiovascular function, potentially causing hypertension and tachycardia (Zanos et al. 2018a). Early on, it was believed that ketamine exerts these sympathomimetic effects by inhibiting reuptake of NE in a manner similar to TCAs (Liebe et al. 2017, Miletich et al. 1973). While it was later discovered that ketamine has little to no affinity for monoamine transporters (Can et al. 2016), this initial hypothesis led Sofia & Harakal (1975) to test whether these secondary sympathomimetic effects confer ketamine with

any preclinical antidepressant properties. They found that orally administered ketamine appeared moderately capable of reversing reserpine-induced hypothermia in rats, but that such actions were relatively unimpressive compared to those of imipramine (Sofia & Harakal 1975). So, despite preliminary evidence that subanesthetic doses of ketamine could facilitate psychotherapy and alleviate depressive symptoms, projects investigating its antidepressant potential were shelved for the time being (Khorramzadeh & Lotfy 1973).

The failure of researchers to recognize ketamine's potential fifty years ago is understandable given how different the proposed mechanisms for MDD were at that time, all of which assumed that an increase in monoamine release was necessary and sufficient for antidepressant action (Domino 2010). Antidepressant efficacy was thus defined as the extent to which a compound could reverse the physiological effects of monoamine depletion. Since ketamine was found to possess poor affinity for monoamine transporters, its candidacy for antidepressant use was rescinded (Can et al. 2016). It would be some years before ketamine's use as an antidepressant would be revisited. Until then, it slowly gained notoriety amongst laymen, law enforcement, and policymakers as a recreational 'club drug' due to its euphoric effects, ability to modulate sensory perception, and psychotomimetic properties at high doses (Zanos et al. 2018a). This reputation as a drug of abuse earned ketamine (as well as its salts and isomers) illegality status as a Schedule III controlled substance (U.S. Department of Justice 1999), which appears to have overshadowed many anecdotal hints that subanesthetic doses of ketamine can attenuate symptoms of depression with efficacy possibly exceeding that of SSRIs (Domino 2010).

Luckily, ketamine's antidepressant effects were eventually re-examined after in a double-blind, placebo-controlled, clinical trial conducted by Berman et al. (2000). They revealed conclusively that ketamine alleviated depressive symptoms in a modest sample of seven patients. When a subanesthetic dose of ketamine (0.5 mg/kg) was intravenously infused over a forty-minute period, depression rating scores reduced significantly within 4 hours—a rapid effect that lasted for up to 3 days post-administration (Berman et al. 2000). They also demonstrated that the psychotomimetic effects of ketamine, which occur shortly after infusion, diminish fully by 4 hours post-administration, and are thus temporally separable from the drug's antidepressant properties (Berman et al. 2000; Krystal et al. 2019). Not long after, Zarate et al. (2006a) tested ketamine's applicability for treatment-resistant MDD patients who, on average, had failed six prior antidepressant trials. Despite the severity and tenacity of their depressive symptoms, ketamine restored well-being and behavioral functionality in roughly 70% of treatment-resistant patients for 7 days, while also significantly outperforming other anesthetic controls under blinded conditions (Zarate et al. 2006a; Murrough et al. 2013). Many more subsequent studies would corroborate these promising claims (for review, see Kryst et al. 2020). Soon, the sustained and rapid antidepressant effects of ketamine would become undeniable, sparking great interest in recent years about ketamine's mechanism of action-- a topic that is still actively debated today.

Ketamine on Glutamatergic Transmission

Roughly 20 years after ketamine's synthesis, it was discovered that it acts as a noncompetitive *N*-Methyl-*D*-Aspartate receptor (NMDAR) antagonist (Lodge et al. 1983; Anis et al. 1983). NMDARs, along with 3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) and kainate receptors, are the proprietary ionotropic receptors for glutamate.

Glutamate is the principal excitatory mediator in the CNS, both in terms of abundance and importance—its signaling is crucial for information processing, learning and memory, and neuronal plasticity (Murrough et al. 2017). The discovery of ketamine’s binding target turned the attention of many researchers away from monoamines toward the glutamate system. A graphical overview of glutamatergic signaling is shown in Figure 1.

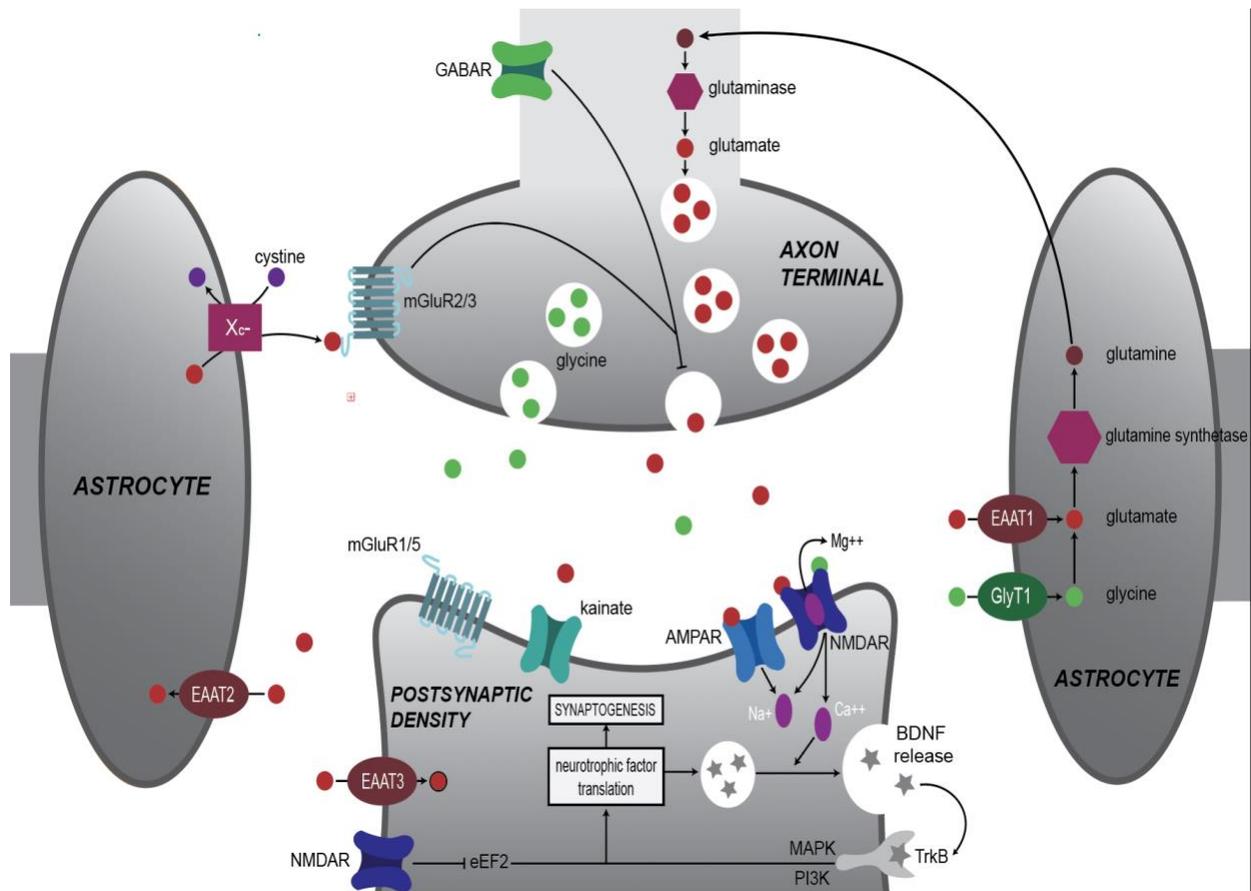


Figure 1. Overview of glutamatergic transmission. Glutamate is synthesized from glutamine by glutaminase. It is packaged into presynaptic vesicles and released alongside other compounds such as glycine. Once released, glutamate binds to post-synaptic AMPARs, NMDARs, kainite receptors, and mGluR1/5. NMDAR-induced calcium influx stimulates neurotrophin synthesis and release. Released BDNF binds to TrkB, consequently activating synaptogenic signaling pathways such as MAPK and PI3K, leading to upregulated translation of proteins associated with cell survival and neuronal growth. Glutamate is recycled with the help of glia (astrocytes) that uptake glutamate, reconvert it to glutamine, and transport it back to the presynaptic terminal for re-conversion to glutamate. Glutamate release is terminated through action of mGluR2/3

autoreceptors, which work in tandem with glial X_c- antiporters to regulate glutamate concentrations in the synapse (Murrough et al. 2017; Duman et al. 2016; Duman & Monteggia 2006; Banasr et al. 2010; Sanacora et al. 2008; Papouin & Oliet 2014; Hardingham & Bading 2010)

NMDARs are ligand-gated, ion channel receptors which exist as heterotetramers. Seven different receptor subunits have been identified: GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A and GluN3B (Vyklicky et al. 2014). NMDAR activation requires concurrent binding of L-glutamate and glycine/D-serine at the GluN2 and GluN1 subunits, respectively (Zanos & Gould 2018). NMDARs differ from other ionotropic glutamate receptors in that their calcium permeability is ten-fold higher than that of other cations, and four-fold higher than that of calcium-permeable AMPARs (Traynelis et al. 2010). Furthermore, NMDARs are more sensitive to glutamate binding than AMPARs ($EC_{50} = 0.5\text{--}3$ versus $3\text{--}560 \mu\text{M}$, respectively) and take longer to desensitize after being activated ($\tau = 59\text{--}2,000$ versus $1\text{--}10$ ms, respectively; Traynelis et al. 2010). These biochemical properties make the NMDAR a vital transducer of intracellular Ca^{2+} , which acts as a second messenger to modulate the efficacy of synaptic transmission, as well as many other cellular processes (Sanacora et al. 2008). While prolonged elevations of intracellular calcium are known to be excitotoxic, extracellular Mg^{2+} counteracts calcium-dependent excitotoxicity by occluding the NMDA channel pore at negative membrane potentials (Riggs & Gould 2021). The blockage of the channel by magnesium confers NMDARs with a high voltage-dependence for activation; AMPARs must be successively activated to sufficiently depolarize the membrane before NMDARs can themselves activate and pass further ionic current (Sanacora et al. 2008). In this way, NMDARs are known to function as ‘coincidence detectors’-- they amplify excitatory transmission for converging inputs (Murrough et al. 2017).

Ketamine is a use-dependent open channel blocker that competes with magnesium to bind deep within the channel pore, where it remains bound when the receptor transitions to a closed conformation (Riggs & Gould 2021; Glasgow et al. 2018). The result is an antagonistic blockage of the NMDA channel without opportunity for voltage-dependent removal. Ketamine also interacts with opioid and cholinergic receptors, although the contribution of these NMDAR-independent actions to ketamine's overall mechanism remains unclear (Murrrough et al. 2017). Furthermore, while the biophysical action of ketamine on NMDA is relatively well-characterized, its downstream effects, and the way that ketamine binding results in altered mood states, is still very much disagreed upon. A variety of putative models have been proposed and will be shortly discussed herein. Despite any differences in their means, however, nearly all of them reach the same end: enhanced synaptic plasticity.

Synaptic Plasticity and MDD

Glutamate and its receptors have been highlighted in the molecular mechanisms of synaptic plasticity that underlie learning and memory. Models such as Long-Term Potentiation are very well characterized (e.g. Cole et al 1989; Sanacora et al. 2008; Murrrough et al. 2017). NMDAR signaling promotes cell survival and neurotrophic functions or, conversely, can activate cell death pathways, depending on the timing of receptor activation, the location of the receptor, and the cellular and extracellular environment at the time of activation (Andres et al. 2013; Hardingham et al. 2002; Stanika et al. 2009; Papouin & Oliet 2014). Moderate levels of NMDAR activation stimulate neuroprotective signaling pathways, such as the RAS–mitogen-activated protein kinase (RAS–MAPK) pathway and the cAMP-responsive element-binding protein (CREB) pathway, both of which mediate induction of genes pertaining to cell survival

(Murrrough et al. 2017). In particular, CREB promotes the expression of brain-derived neurotrophic factor (BDNF), which plays an essential role in neuroprotective, neuroplastic, and neurotrophic processes that are demonstrably altered in stress and mood disorders (Krishnan & Nestler 2008; Krishnan & Monteggia 2006; Tornese et al. 2019). By contrast, abnormally elevated or inappropriate NMDAR signaling leads to deleterious effects on neurons (Hardingham et al. 2010).

The realization that ketamine's antidepressant properties are glutamatergic, rather than monoaminergic, marked a pivotal turning point in our understanding of what depression is (Riggs & Gould 2021). Dysfunctions of the glutamate system (i.e. those caused by chronic stress) have thus become integral in modern explanations for the etiopathogenesis of MDD (Duman et al. 2016; Musazzi et al. 2013; Sanacora et al. 2012). Neuroimaging studies on depressed brains consistently report volume and connectivity abnormalities in corticolimbic areas such as the hippocampus (HPC), prefrontal cortex (PFC), and amygdala, where glutamatergic synapses dominate (Autry & Monteggia 2012). Furthermore, various pre-clinical studies with stress-based animal models of MDD have confirmed that dendritic atrophy/remodeling occurs in the same brain regions altered in humans, suggesting that stress-induced maladaptive changes are very consequential in the chain of cellular events underlying the onset of depressive symptoms (e.g. Autry and Monteggia 2012; Krishnan and Nestler 2008; McEwen 2017; Sanacora et al. 2012).

By altering glutamate release and uptake, chronic stress damages the cortex and hippocampus through several known pathways: (a) diminishing synapse density and diameter; (b) reducing AMPAR and NMDAR availability; (c) deteriorating and/or destabilizing dendritic

spines; (d) reducing dendritic arborization (for review, see Popoli et al. 2011). Several of these changes may be explained through a decline in extracellular glutamate clearance by glial cells (Murrough et al. 2017). Glia are vital for glutaminergic regulation, and may therefore factor into the cellular basis of depression (Sanacora & Banasr 2013). In health, astrocytes clear glutamate from the synapse using excitatory amino acid transporters (EAATs); metabolize glutamate to glutamine; release the NMDAR co-agonist d-serine; release trophic factors; and express group I and group II metabotropic mGluR receptors (Ongur et al. 1998; Banasr et al. 2010). These glial actions are demonstrably interrupted during MDD. Studies have suggested that elevated glial death occurs in the cortices of MDD patients (Sanacora & Banasr 2013), and that chronic stress may therefore lead to depression by impairing cortical astrocytes (Banasr et. al 2010; Mikulska et al. 2021).

The stress-induced death of glia responsible for glutamate/glutamine cycling may result in elevated extrasynaptic glutamate concentrations (Krystal et al. 2013). A somewhat counterintuitive consequence of this increase may actually be the downstream suppression of glutamatergic signaling through activation of metabotropic glutamate receptor 2 (mGluR2) autoreceptors. (Zanos et al. 2018). The result is a deficit of glutamate transmission in brain areas responsible for mood regulation, pleasure and reward, concentration and memory, and various physiological processes. Not coincidentally, all of these adverse outcomes are symptoms of MDD. In addition to this, chronic stress appears to activate extrasynaptic NR2B-containing NMDARs, contributing to synaptic loss and further cell death through the triggering of apoptotic pathways (Murrough et al. 2017). Furthermore, synapse loss from NR2B may compound with damage caused by calpain-2: a calcium-dependent protease known to rapidly atrophy dendritic

spines when recruited by the co-action of stress hormones and NMDARs (Wang et al. 2020; Andres et al. 2013).

Summed together, the net effects of chronic stress are multitudinous. Among them are disruptions in cellular signaling, glial death, destabilized dendritic spine dynamics, and diminished cellular resilience within brain circuits that are crucial for mood regulation (for review, see Kempton et al. 2011). These microstructural and molecular changes are believed to underlie several gross abnormalities that have been found in the brains of MDD patients, such as atypical glutamate signaling, altered functional connectivity within corticolimbic networks, and reduced overall brain volume (Manji et al. 2003; Kempton et al. 2011; Andres et al. 2013; Duman et al. 2016). Altogether, a consensus has been reached that maladaptive structural changes induced by chronic stress are sufficient to explain many aspects of depressive symptomology (for review, see Mikulska et al. 2021).

Where the Consensus Ends

A great deal of progress on MDD has been made during the 21st century. The monoamine hypothesis has evolved into more contemporary models emphasizing chronic stress. With these new models have come targets for new putative antidepressants, such as ketamine, that appear capable of addressing many shortcomings of those previously administered. The discovery of ketamine's potential has been hailed by some as the most valuable psychiatric discovery in sixty years (e.g. Duman 2018). However, at this point the ceiling of our knowledge is scraped.

The (*S*)-Ketamine stereoisomer (Spravato) was approved by the FDA in 2019 and is currently used as a second-line treatment (under highly controlled conditions) for patients with

imminent suicide risk (Riggs & Gould 2021). Still, ketamine's other salts and enantiomers have not received any widespread distribution, even though its impressive antidepressant properties have been recognized for two decades. Why is this? The reasons are multifaceted. For one, ketamine's reputation precedes it in discussions with law and policymakers; the recreational stigma it acquired before its clinical potential was recognized has not gone away, making the legitimization of its use divisive (Corrigan & Pickering 2019). Ketamine also has well-documented abuse potential (Caddy et al. 2015). Furthermore, it has several short-term side effects (e.g. elevated blood pressure, elevated heart rate, dizziness, dissociation, altered sensory processing), as well as long-term adverse effects that are not well documented, both of which beget skepticism toward ketamine's mainstream viability (Riggs & Gould 2021; Caddy et al. 2015). Though it is improbable that these drawbacks will subvert its usefulness (the antidepressant potential of ketamine is immense and urgently needed), the drug's imperfections have made private companies tentative to offer funding for further research and FDA applications.

Arguably the biggest obstacle for ketamine distribution, however, is the fact that its antidepressant mechanism remains elusive. Ketamine's antidepressant properties are nearly doubtless, but no consensus has been reached about *how* exactly ketamine goes about instigating these changes. Many models have been suggested yet none have been able to fully lift the 'black box' and settle the debate. Until there is more clarity surrounding ketamine's antidepressant mechanism, the drug will fail to gain the momentum it needs to modernize antidepressant therapies at a large scale. For this reason, a review of postulated models for ketamine's action will now be detailed. Afterwards, a study will be proposed that may help fill gaps in our

understanding of ketamine's mechanism, further illuminating whether ketamine is truly viable as a mainstream antidepressant.

CHAPTER 2 REFERENCES

- Andres AL, Regev L, Phi L, Seese RR, Chen Y, Gall CM, Baram TZ. (2013). NMDA receptor activation and calpain contribute to disruption of dendritic spines by the stress neuropeptide CRH. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 33(43): 16945–16960. doi: 10.1523/JNEUROSCI.1445-13.2013
- Anis NA, Berry SC, Burton NR, Lodge D. (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br. J. Pharmacol.* 79:565–75
- Autry AE, Monteggia LM. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol Rev.* 64(2): 238-58. doi:10.1124/pr.111.005108.
- Banasr M, *et al.* (2010). Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry*. **15**: 501–511
- Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, *et al.* (2000). Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry*. 47:351–54
- Caddy C, Amit BH, McCloud TL, Rendell JM, Furukawa TA, McShane R, Hawton K, Cipriani A. (2015). Ketamine and other glutamate receptor modulators for depression in adults. *Cochrane Database Syst Rev*. doi:10.1002/14651858.CD011612.pub2.
- Can A, Zanos P, Moaddel R, Kang HJ, Dossou KS, *et al.* (2016). Effects of ketamine and ketamine metabolites on evoked striatal dopamine release, dopamine receptors, and monoamine transporters. *J. Pharmacol. Exp. Ther.* 359:159–70
- Cole AJ, Saffen DW, Baraban, JM, Worley, PF. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature*. **340**: 474–476
- Corrigan A, Pickering G. (2019). Ketamine and depression: a narrative review. *Drug Des Devel Ther.* 13: 3051-3067. doi:10.2147/DDDT.S221437
- Diversion Control Division. (1999). *[DEA-183F], 21 CFR Part 1308, Schedules of Controlled Substances: Placement of Ketamine into Schedule III*. U.S. Department of Justice: Drug Enforcement Administration.
- Dogra P, Vijayashankar NP. (2021). Dexamethasone Suppression Test. *StatPearls*.
- Domino EF, Chodoff P, Corssen G. (1965). Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. *Clin. Pharmacol. Ther.* 6: 279–91

- Duman RS. (2018). The Dazzling Promise of Ketamine. *Cerebrum: The Dana forum on brain science*. PMID: 30746033
- Duman RS, Aghajanian GK, Sanacora G, Krystal JH. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat. Med.* **22**: 238–249.
- Duman RS, Monteggia LM. (2006). A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry.* **59**: 1116–1127.
- Glasgow NG, Wilcox MR, Johnson JW. (2018). Effects of Mg²⁺ on recovery of NMDA receptors from inhibition by memantine and ketamine reveal properties of a second site. *Neuropharmacology.* 137:344–58
- Han MH, Nestler EJ. (2017). *Neural Substrates of Depression and Resilience*. *Neurotherapeutics.* 14(3): 677-686. doi: 10.1007/s13311-017-0527-x.
- Hardingham GE, Bading H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* **11**: 682–696
- Hardingham GE, Fukunaga Y, Bading H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* **5**: 405–414
- Heninger GR, Delgado PL, Charney DS. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry.* 29:2–11
- Hirschfield, RM. (2000). History and evolution of the monoamine hypothesis of depression. *J Clin Psychiatry.* 6: 4-6. PMID: 10775017
- Jaffee SR, Price TS. (2007). Gene-environment correlations: a review of the evidence and implications for prevention of mental illness. *Mol Psychiatry.* 12(5): 432-42. doi: 10.1038/sj.mp.4001950.
- Jacobson L. (2005). Hypothalamic–Pituitary–Adrenocortical Axis Regulation. *Endocrinol. Metab. Clin. N. Am.* 34: 271–292
- Joseph DN, Whirledge S. (2017). Stress and the HPA Axis: Balancing Homeostasis and Fertility. *Int. J. Mol. Sci.*
- Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. (2015). Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting- state functional connectivity. *JAMA Psychiatry.* **72**: 603–611
- Kempton MJ, *et al.* (2011). Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder. *Arch. Gen. Psychiatry.* **68**: 675–690

- Khorramzadeh E, Lotfy AO. (1973). The use of ketamine in psychiatry. *Psychosomatics*. 14: 344–46
- Krishnan V, Nestler EJ. (2008). The molecular neurobiology of depression. *Nature*. **455**: 894–902
- Kryst J, Kawalec P, Mitoraj AM, Pilc A, Lason W, Brzostek T. (2020). Efficacy of single and repeated administration of ketamine in unipolar and bipolar depression: a meta-analysis of randomized clinical trials. *Pharmacol. Rep.* 72:543–62
- Krystal JH, Abdallah CG, Sanacora G, Charney DS, Duman RS. (2019). Ketamine: a paradigm shift for depression research and treatment. *Neuron*. 101:774–78
- Krystal JH, Sanacora G and Duman RS. (2013). Rapidacting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry*. 73: 1133–1141.
- Lee EH, Han PL. (2019). Reciprocal interactions across and within multiple levels of monoamine and cortico-limbic systems in stress-induced depression: a systematic review. *Neurosci. Biobehav. Rev.* 101:13–31
- Liebe T, Li S, Lord A, Colic L, Krause AL, et al. (2017). Factors influencing the cardiovascular response to subanesthetic ketamine: a randomized, placebo-controlled trial. *Int. J. Neuropsychopharmacol.* 20:909–18
- Lodge D, Anis N, Berry S, Burton N. (1983). Arylcyclohexylamines selectively reduce excitation of mammalian neurons by aspartate-like amino acids. In *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*, ed. JM Kamenka, EF Domino, P Geneste, pp. 595–616. Ann Arbor, MI: NPP Books
- Manji HK, et al. (2003). Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol. Psychiatry*. **53**: 707–742
- McEwen BS. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress (Thousand Oaks)*. doi:10.1177/2470547017692328.
- McEwen BS, Akil H. (2020). Revisiting the stress concept: implications for affective disorders. *Journal of Neuroscience*. 40(1): 12-21. doi: 10.1523/JNEUROSCI.0733-19.2019
- Mikulska J, Juszczyk G, Gawronska-Grzywacz M, Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sciences*.
- Miletich DJ, Ivankovic AD, Albrecht RF, Zahed B, Ilahi AA. (1973). The effect of ketamine on catecholamine metabolism in the isolated perfused rat heart. *Anesthesiology*. 39:271–77

- Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry*. 170:1134–42
- Murrough JW, Abdallah CG, Mathew SJ. (2017). Targeting glutamate signalling in depression: progress and prospects. *Nat Rev Drug Discov*. 16(7):472-486. doi: 10.1038/nrd.2017.16.
- Musazzi L, Treccani G, Mallei A, Popoli M. (2013). The action of antidepressants on the glutamate system: regulation of glutamate release and glutamate receptors. *Biol Psychiatry*. 73(12): 1180-8. doi:10.1016/j.biopsych.2012.11.009.
- Niciu MJ, Ionescu DF, Richards EM, Zarate CA. (2014). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm (Vienna)*. 121(8):907-924. doi:10.1007/s00702-013-1130-x
- Ongur D, Drevets WC, Price JL. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc. Natl Acad. Sci. USA*. **95**: 13290–13295
- Papouin T, Oliet SH. (2014). Organization, control and function of extrasynaptic NMDA receptors. *Phil. Trans. R. Soc. B*. **369**, 20130601
- Popoli M, Yan Z, McEwen BS, Sanacora G. (2011). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat. Rev. Neurosci*. **13**: 22–37
- Riggs LM, Gould TD. (2021). Ketamine and the Future of Rapid-Acting Antidepressants. *Annual Review of Clinical Psychology*. **15**(7)
- Sanacora G, Banasr M. (2013). From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry*. **73**: 1172–1179
- Sanacora G, Treccani G, Popoli M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*. 62(1): 63-77. doi:10.1016/j.neuropharm.2011.07.036.
- Sanacora G, Zarate CA, Krystal JH, Manji HK. (2008). Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Discov*. 7(5):426-437. doi:10.1038/nrd2462
- Sapolsky, R. (1994). *Why Zebras Don't Get Ulcers: A Guide to Stress, Stress Related Diseases, and Coping*. W.H. Freeman.
- Sofia RD, Harakal JJ. (1975). Evaluation of ketamine HCl for anti-depressant activity. *Arch. Int. Pharmacodyn. Ther*. 214: 68–74

Stanika RI, *et al.* (2009). Coupling diverse routes of calcium entry to mitochondrial dysfunction and glutamate excitotoxicity. *Proc. Natl Acad. Sci. USA.* **106**: 9854–9859

Tornese P, Sala N, Bonini D, Bonifacino T, La Via L, Milanese M, Treccani G, Seguíni M, Ieraci A, Mingardi J, Nyengaard JR, Calza S, Bonanno G, Wegener G, Barbon A, Popoli M, Musazzi L. (2019). Chronic mild stress induces anhedonic behavior and changes in glutamate release, BDNF trafficking and dendrite morphology only in stress vulnerable rats. The rapid restorative action of ketamine. *Neurobiol Stress.* 10:100-160. doi: 10.1016/j.ynstr.2019.100160.

Traynelis SF, Wollmuth LP, McBain CJ, *et al.* (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev.* 62(3):405-496. doi:10.1124/pr.109.002451

Uher R. (2014). Gene-environment interactions in severe mental illness. *Front Psychiatry.* 5:48. doi: 10.3389/fpsy.2014.00048.

Wang Y, Liu Y, Bi X, Baudry M. (2020). Calpain-1 and Calpain-2 in the Brain: New Evidence for a Critical Role of Calpain-2 in Neuronal Death. *Cells.* 9(12):2698. doi:10.3390/cells9122698

Vyklicky V, Korinek M, Smejkalova T, Balik A, Krausova B, Kaniakova M, *et al.* (2014). Structure, function, and pharmacology of NMDA receptor channels. *Physiol Res.* 63(Suppl 1):S191–203.

Zanos P, Gould TD. (2018). Mechanisms of ketamine action as an antidepressant. *Mol Psychiatry.* 23(4):801-811. doi:10.1038/mp.2017.255

Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, *et al.* (2018a). Ketamine and ketamine metabolite pharmacology: insights into therapeutic mechanisms. *Pharmacol. Rev.* 70: 621–60

CHAPTER 3

Putative Mechanisms for Ketamine's Antidepressant Action

Ketamine Has Multiple Downstream Targets

Part of the difficulty in determining ketamine's mechanism of action is that no singular pathway appears to be entirely responsible for its antidepressant properties (Zanos & Gould 2018). Current literature has identified multiple downstream targets for ketamine's antidepressant action, all of which are empirically supported. These pre-clinically demonstrated mechanisms of ketamine are not mutually exclusive and may act in concert to effect behavioral changes. Furthermore, the antidepressant and anesthetic properties may be functionally separable and occur via different mechanisms (Riggs & Gould 2021).

The antidepressant effects of ketamine probably emerge from a cascade of downstream events independent of ketamine's NMDAR antagonism. We can infer this because other NMDAR antagonists (e.g. memantine, lanicemine, nitrous oxide) fail to exert consistent antidepressant effects in clinical trials (Newport et al. 2015). Furthermore, after Kishimoto et al. (2016) performed a meta-analysis of single-infusion non-ketamine NMDAR antagonists, such as traxoprodil and rapastinel (GLYX-13), they found much smaller effect sizes in reduction of depression symptoms compared to ketamine. It is therefore likely that ketamine's mechanism involves downstream targets, especially considering that it is metabolized within hours, yet it demonstrates antidepressant effects lasting days to weeks (Matveychuk et al. 2020).

The NMDA Disinhibition Hypothesis

The antagonism of NMDARs should, intuitively, reduce excitatory transmission. However, a compelling body of evidence suggests that ketamine remedies glutamatergic MDD symptoms by *increasing* glutamate signaling. This hypothesis stemmed from Moghaddam et al. (1997), who showed that ketamine evoked transient, dose-dependent increases in the concentration of extrasynaptic glutamate within the medial PFC. Ergo, a single administration of ketamine led to net increases in cortical excitation that balanced out the glutamatergic deficits caused by chronic stress (Duman et al. 2016; Musazzi et al. 2013; Sanacora et al. 2012). Moghaddam et al. explained their counterintuitive results by suggesting that ketamine preferentially attenuates inhibitory tonal activity that impinges on many excitatory (pyramidal) neurons within the cortex. This occurs because fast-spiking inhibitory interneurons are normally active, which places cortical pyramidal neurons in a state of functional quiescence (Duman 2014; Riggs & Gould 2021). In support of this, Zorumski et al. (2016) noted that ketamine has a greater affinity for NMDA receptors on γ -aminobutyric acid (GABA) interneurons, which suppress the activity of downstream glutamatergic neurons. Low doses of ketamine may preferentially antagonize NMDARs on these GABA interneurons over other cells, blocking their inhibitory action and disinhibiting glutamatergic signaling (Niciu et al. 2014; Li et al. 2010; Matveychuk et al. 2020).

Consistent with these findings, Homayoun & Moghaddam (2007) showed that the NMDAR antagonist MK-801 reduces firing in fast-spiking inhibitory interneurons, leading to surges in cortical pyramidal neuron activity. A similar phenomenon was observed by Widman & McMahon (2018) in ex vivo hippocampal slice preparations, suggesting that ketamine's disinhibition may extend beyond the cortex to subcortical regions involved in mood regulation.

Overall, there is evidence that disinhibition of glutamate release by ketamine could rapidly restore the integrity of synaptic connections compromised in depression.

NMDAR Disinhibition is AMPAR/BDNF/mTORC1-Dependent

The disinhibition hypothesis predicts that the activational balance in the cortex and hippocampus is acutely shifted toward excitatory AMPAR-dependent transmission under ketamine's effect, which could underlie its antidepressant efficacy. Indeed, ketamine may require AMPAR activity, for its antidepressant effects were abolished in subjects pretreated with AMPAR antagonists, as shown in Maeng et al. 2008. Activation of AMPARs increases intracellular calcium, potentiating the release of brain derived neurotrophic factor (BDNF) and other neurotrophins known to augment synaptic plasticity and neurogenesis (Jourdi et al. 2009). A great deal of emphasis has been placed on BDNF in recent years; many studies have purported that it is crucial for ketamine to exert its effects (e.g. Lepack et al. 2016; Li et al. 2010; Liu et al. 2012). For example, subjects with the Val66Met single-nucleotide polymorphism in the BDNF gene exhibit impairments in BDNF release and mRNA trafficking, nullifying the antidepressant properties of ketamine when it is administered to them (Liu et al. 2012). The necessity of BDNF for ketamine to act has been verified in both rodents and humans (Laje et al. 2012; Chen et al. 2019).

BDNF enhances plasticity by binding to tropomyosin receptor kinase B (TrkB), which recruits intracellular signaling molecules that execute structural changes through several known pathways: mitogen-activated protein kinase (MAPK), phospholipase C γ (PLC γ), and phosphatidylinositol 3-kinase (PI3K; Yoshii et al. 2010; Niciu et al. 2014; Murrough et al. 2017). A well-characterized example of these molecules is the mammalian target of rapamycin complex

1 (mTORC1), a component of the PI3K pathway that performs diverse regulatory functions, playing a role in cell proliferation and the healthy formation of neural circuits (Sun et al. 2016; Saxton & Sabatini 2017; Costa-Mattioli et al. 2009). Ketamine appears to rapidly increase mTORC1 signaling by promoting cell growth through action on multiple targets, as demonstrated by Li et al. (2010). A downstream effector of mTORC1, called the eukaryotic translation initiation factor 4E-binding protein 2 is reported to evoke a lasting increase in the efficacy of synaptic transmission (Deyama & Duman 2020; Duman et al. 2021; Aguilar-Valles et al. 2020). Another target of mTORC1 is p70S6K, whose action has also been implicated in neuroplasticity (Sun et al. 2016).

The roles of BDNF and mTOR expression in ketamine's action are further corroborated by studies from Yang et al. (2012, 2013), who found that BDNF and mTORC1 expression was upregulated in the rat hippocampus and PFC after ketamine administration. Interestingly, they also found that pre-treatment with the analgesic tramadol enhanced the antidepressant effects of ketamine in a forced-swim test through the potentiation of mTOR (Yang et al. 2012). The upregulation of BDNF and mTOR may be AMPAR-mediated, for when Zhou et al. (2014) pre-treated rats with an AMPAR antagonist it decreased levels of BDNF and mTOR and increased forced-swim test immobility time. Other studies have also reported that ketamine's antidepressant potential is abolished when animals are pre-treated with the mTORC1 inhibitor rapamycin (e.g. Li et al. 2011).

Despite this evidence, the role of mTORC1 in ketamine's antidepressant action may not be as clear-cut as previously surmised (Matveychuk et al. 2020). A study by Autry et al. (2011) found that ketamine administration did *not* affect mTOR phosphorylation in hippocampal or

cortical tissue, and that rapamycin failed to abrogate ketamine's antidepressant effects. Furthermore, a randomized controlled trial conducted by Abdallah et al. (2018) found that pre-treatment with rapamycin actually tripled ketamine's response rate at 14 days after treatment in human patients. The authors speculated that rapamycin may have augmented ketamine's effects through an mTOR-independent action, such as by suppressing neuroinflammation (Abdallah et al. 2018).

In sum, ketamine's preferential blockade of GABAergic interneurons can disinhibit pyramidal neuron signaling, exerting antidepressant effects by promoting synaptogenesis in an AMPAR/BDNF/mTORC1-dependent manner (Lepack et al. 2016; Duman et al. 2021; Li et al. 2010; Liu et al. 2012). Ketamine's ability to affect mood for many days after it is metabolized may be due to sustained adaptations in the number or function of AMPARs, a common mechanism by which synaptic plasticity is known to manifest (e.g. Huganir & Nicoll 2013). In support of this, ketamine increases the expression of AMPARs containing the GluA1 subunit 24 hours after administration (Adaikkan et al. 2018). This suggests that ketamine's glutamatergic disinhibition initiates synaptogenic processes that sustainedly increase AMPAR expression, which might underlie ketamine's ability to restore the synaptic deficits evoked by chronic stress (Yamada & Jinno 2019; Zanos et al. 2016; Li et al. 2011). Indeed, longitudinal observations of cortical dendritic spines by Moda-Sava et al. (2019) have revealed that reduced synaptic integrity is tied to the emergence of depressive phenotypes in mice. Ketamine might rescue these deficits through targeted spine remodeling at destabilized synapses (Moda-Sava et al. 2019; Riggs & Gould 2021; Tornese et al. 2019). Though the exact mechanism is still unclear, AMPA, mTOR, and especially BDNF all appear to play a role in ketamine's action.

Ketamine Upregulates BDNF Independent of Glutamate Disinhibition

While the fact that ketamine upregulates BDNF is mostly agreed upon, some studies suggest it may go about doing this through a mechanism that does not involve glutamate disinhibition (Riggs & Gould 2021). For example, Autry et al. (2011) suggested that ketamine enhances BDNF synthesis by preventing ‘spontaneous’ NMDAR activation. This refers to the fact that some glutamate is released stochastically from the presynaptic terminal without action potentials, which prevents the Ca^{2+} /calmodulin-dependent eukaryotic elongation factor 2 kinase (CaMKII) from inhibiting eEF2 activity, suppressing BDNF synthesis under resting conditions (Autry et al. 2011; Nosyreva et al. 2013). Ketamine’s acute blockage of NMDARs at rest reduces eEF2 phosphorylation, thereby increasing rapid translation of target mRNA transcripts, including AMPAR subunits (Kavalali & Monteggia 2020). This model distinguishes itself from the disinhibition hypothesis by predicting that ketamine enhances plasticity through blockage of pyramidal NMDARs, *not* by triggering activity-dependent BDNF release (Autry et al. 2011; Riggs & Gould 2021). Interestingly, though, when Yang et al. (2013) studied the response of real MDD patients to ketamine, they observed surges in both mTOR and eEF2 phosphorylation. While the increase in mTOR corroborates animal studies, the increase in eEF2 phosphorylation contradicts the reductions seen in rodent models by Autry et al. (2011).

The uncertainty of the previous model has led to an alternative hypothesis offered by Miller et al. (2014), who suggest that ketamine selectively inhibits GluN2B-containing NMDARs that are preferentially activated by ambient glutamate at sites outside of the synapse. However, there is evidence that ketamine does not functionally inhibit NMDARs with subunit-level specificity (Dravid et al. 2007; Li et al. 2010; Maeng et al. 2008), and although GluN2B-

specific antagonists exert preclinical antidepressant-like actions, they fail to display strong antidepressant effects in clinical studies (for review, see Gould et al. 2019).

The NMDA Inhibition Hypothesis

While glutamatergic hypoactivity has been implicated in depressive pathology for certain brain areas (e.g. dentate gyrus, which could explain the concentration and memory problems characteristic of MDD), glutamate signaling is known to be *hyperactive* in other brain areas (e.g. the amygdala and lateral habenula (LHb), which could explain the increased agitation and anxiety states characteristic of MDD; Yang et al. 2010; Yang et al. 2018). In support of this, four positron emission tomographic studies by Drevets et al. (1992) repeatedly confirmed that amygdala activity is significantly increased in MDD patients; a finding corroborated by fMRI neuroimaging studies performed by Matthews et al. (2008) and Fales et al. (2008). Furthermore, elevated serum and plasma glutamate levels have been documented in many depressed patients, with the extent of glutamate abnormalities correlating with ratings of anxiety (Altamura et al. 1995; Kim et al. 1982). These findings continue to implicate abnormal glutamate signaling in MDD, but whether glutamate is hyperactive or hypoactive appears to vary between patients, or even between brain areas within the same patient (Niciu et al. 2014; Sanacora et al. 2012).

Evidence of glutamatergic hyperactivity has fostered an alternative hypothesis that ketamine exerts its antidepressant effects by decreasing excitation in brain regions whose activity promotes depressive phenotypes, rather than increasing the activity of euthymic-related regions (Riggs & Gould 2021). Indeed, Yang et al. (2018) reported that ketamine reduces NMDAR-mediated burst firing in the LHb, where excess activity is associated with behavioral despair and anhedonia. In contrast with the NMDA disinhibition hypothesis, studies supporting NMDA inhibition propose a model in which monoaminergic circuits are rapidly disinhibited by

ketamine's blocking of LHb activity (Yang et al. 2010; Yang et al. 2018; Riggs & Gould 2021).

The NMDA inhibition hypothesis is intuitive considering ketamine's role as an NMDAR antagonist. However, whether ketamine is truly inhibitory, disinhibitory, or some combination of the two remains disputed.

There is also evidence that the endogenous opioid system (EOS) is in some way involved in ketamine's ability to inhibit hyperactivity within the LHb (e.g. Klein et al. 2020; Williams et al. 2018; Heifits et al. 2021). For example, Klein et al. (2020) found that pretreatment with the μ -opioid receptor antagonist naltrexone abolished the ability of ketamine to reduce depressive LHb phenotypes in rodents. However, activation of opioid receptors is not sufficient to mimic ketamine-like effects, nor does ketamine mimic the hedonic effects of opioid agonists like buprenorphine or methadone (Klein et al. 2020). This indicates that the opioid system does not mediate the actions of ketamine, but rather plays some permissive role whose intricacies are poorly understood. Their findings echo results in humans; studies by Williams et al. (2018, 2019) also showed that naltrexone blocked the antidepressant effect of ketamine. The fact that ketamine requires both NMDAR and opioid receptor signaling suggests that an interaction between these systems may not only exist within the midbrain, but may also be necessary for ketamine's antidepressant action (Heifits et al. 2021; Williams et al. 2019).

Ketamine Metabolites Have Antidepressant Properties

As stated previously, ketamine is rapidly metabolized within several hours after administration. Despite this, its antidepressant effects extend long after it is broken down, persisting for days or even weeks. The metabolism of ketamine is performed extensively in the liver via CYP2B6- and CYP3A4-mediated N-demethylation to norketamine (Zanos et al. 2018). Norketamine then undergoes further catabolism to several hydroxynorketamine (HNK)

stereoisomers and dehydronorketamine. Of these possible metabolites, (2*R*,6*R*)-HNK is the major metabolite found in the plasma and brain of mice (Zanos et al. 2016), as well as the plasma of humans (Zarate et al. 2012).

It has been speculated that (2*R*,6*R*)-HNK is active and may exert its own antidepressant effects. This idea arose from pre-clinical observations by Zanos et al. (2016) showing that when ketamine was chemically deuterated at the C6 position to prevent it from correctly metabolizing into (2*R*,6*R*)-HNK, its antidepressant effects were abolished. This suggests that the metabolism of ketamine into (2*R*,6*R*)-HNK could be necessary for its antidepressant action (Riggs & Gould 2021). Additionally, direct administration of both the (2*S*,6*S*)- or (2*R*,6*R*)-HNK enantiomers are sufficient on their own to exert dose-dependent antidepressant actions loosely resembling of ketamine (Zanos et al. 2016; Pham et al. 2018).

To complicate things, this occurs even though (2*R*,6*R*)-HNK has no measurable binding to, or functional effects on, the NMDAR at antidepressant dosages (Lumsden et al 2019; Fukumoto et al. 2019; Zanos et al. 2016; Zanos et al. 2019a). However, (2*R*,6*R*)-HNK still seems to somehow promote glutamatergic transmission (Pham et al. 2018, Riggs et al. 2020, Zanos et al. 2016) and trigger an acute increase in BDNF release, which --as with ketamine itself-- is required for it to exert its antidepressant effects (Fukumoto et al. 2019; Riggs & Gould 2021). The way this is accomplished is unclear, though AMPARs have been strongly implicated (e.g. Zanos et al. 2016). A somewhat controversial hypothesis built off these observations is that NMDAR inhibition is not essential for the rapid antidepressant properties of ketamine. Rather, they might emerge from a pathway contingent on its metabolism to (2*R*,6*R*)-HNK (Zanos & Gould 2018). While it is possible that ketamine and its metabolites work synergistically, there are plausible claims that NMDAR inhibition accounts for only the anesthetic properties of

ketamine, with its antidepressant effects arising from some NMDA-independent mechanism that has yet to be illuminated (Riggs & Gould 2021). Interestingly, (2*R*,6*R*)-HNK does not possess the dissociative, euphoric, or psychotomimetic properties of its parent drug (Fukumoto et al. 2019). This lends further credibility to the idea that the anesthetic and antidepressant actions of ketamine are mechanistically separable, with NMDAR antagonism primarily underlying the former. The lack of abuse potential, combined with more tolerable side effects, has caused (2*R*,6*R*)-HNK to be lauded by some as an antidepressant with equal, if not greater, potential than ketamine (Pham et al. 2018).

The role of (2*R*,6*R*)-HNK in ketamine's overall mechanism is further obfuscated by continued disagreement about the source of its antidepressant efficacy. A meta-analysis by Fukumoto and Duman (2021) details that (2*R*,6*R*)-HNK did not exert antidepressant-like effects in chronic social defeat stress and lipopolysaccharide-induced models of depression, although ketamine itself elicited robust antidepressive actions in those same animal models. Additionally, a recent study by Hare et al. (2020) reported that (2*R*,6*R*)-HNK (even at strong 10 μ M concentrations) did not bind orthosterically to, or have functional activity on, AMPARs. If the amelioration of depressive states by (2*R*,6*R*)-HNK does involve AMPARs, their role appears to be indirect (Hare et al. 2020). A contemporaneous study by Zanos et al. (2019) found evidence that (2*R*,6*R*)-HNK might increase glutamate concentrations by inhibiting mGluR2 presynaptic autoreceptors, rather than postsynaptic AMPARs or NMDARs (Zanos et al. 2019). If this is the case, the presynaptic disinhibition of glutamate release by (2*R*,6*R*)-HNK could explain why the metabolite is also antidepressive, despite being substantially different in action from its parent drug. Collectively, (2*R*,6*R*)-HNK has shown promising antidepressant effects

in rodents, though recent research hints that the action of ketamine metabolites may be less similar to ketamine than originally surmised (Fukumoto & Duman 2021).

Addressing the Unknown

The fact that ketamine's exact antidepressant mechanism has eluded scientists for twenty years is self-evident of its complexity. Multiple downstream targets from ketamine's initial NMDAR blockade have been identified, all of which are empirically validated and seem partially sufficient to explain the drug's effects. However, no singular pathway appears wholly responsible. The proposed hypotheses discussed herein include: direct NMDAR inhibition and consequent activity reduction in brain regions that promote depressive phenotypes, inhibition of GABAergic interneuron NMDARs resulting in pyramidal neuron disinhibition, and the metabolism of ketamine into the mGluR2 inhibitor (2*R,6R*)-HNK (Figure 2). These putative ketamine mechanisms are not mutually exclusive and may complement each other, culminating in the unique antidepressant effects of the drug. Indeed, a net result of all these processes is a sustained potentiation of excitatory synapses in brain circuits involved in the maintenance of mood and stress-reactivity (Zanos & Gould 2018). This potentiation may be sufficient to rescue the stress-induced synaptic deficits that underlie depressive pathology.

There is a consensus from most pre-clinical research that AMPAR activity is required for the antidepressant actions of ketamine. Increased glutamate release, either by interneuron-mediated disinhibition or by (2*R,6R*)-HNK autoreceptor inhibition, activates pyramidal AMPARs, which potentiates downstream neuroplasticity-related signaling pathways, including those regulated by BDNF and mTORC1, to promote synaptic plasticity (Riggs & Gould 2021). Alternatively, eEF2 inactivation as a result of NMDAR inhibition at rest may promote

production of BDNF, causing AMPARs to be upregulated. Further research is needed to better elucidate the technicalities of these pathways, as well as how they might work in tandem to exert ketamine's antidepressant actions. To this end, a hypothetical experiment will now be proposed to help the scientific community gain further insight into ketamine's mechanism. Its aim is to assess whether isolated ketamine or (2*R*,6*R*)-HNK differ in their ability to ameliorate stress-induced depressive cellular phenotypes within the hippocampus of mice, compared to when they are present in combination.

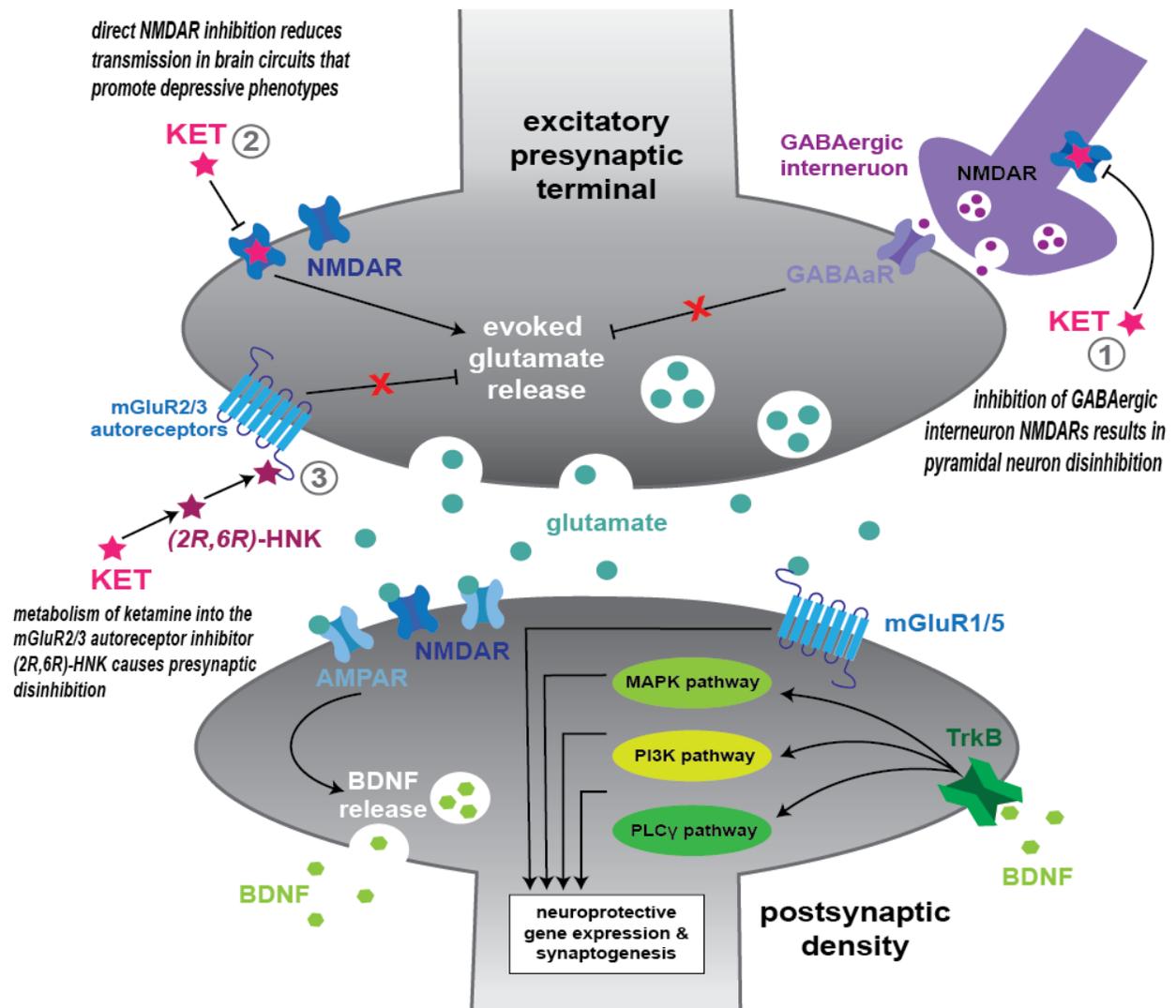


Figure 2. Three thoroughly supported hypotheses for ketamine’s antidepressant mechanism of action. These hypotheses are not mutually exclusive of one another and may occur simultaneously.

CHAPTER 3 REFERENCES:

Abdallah CG, Averill LA, Gueorguieva R, et al. (2018). Rapamycin, an immunosuppressant and mTORC1 inhibitor, triples the antidepressant response rate of ketamine at 2 weeks following treatment: a double-blind, placebo-controlled, cross-over, randomized clinical trial. *bioRxiv*. 500959.

Adaikkan C, Taha E, Barrera I, David O, Rosenblum K. (2018). Calcium/calmodulin-dependent protein kinase II and eukaryotic elongation factor 2 kinase pathways mediate the antidepressant action of ketamine. *Biol. Psychiatry* 84:65–75

Aguilar-Valles A, De Gregorio D, Matta-Camacho E, Eslamizade MJ, Khlaifia A, et al. (2020). Antidepressant actions of ketamine engage cell-specific translation via eIF4E. *Nature*. doi:10.1038/s41586-020-03047-0

Autry AE, Adachi M, Nosyreva E, et al. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*. 475: 91–95.

Altamura C, Maes M, Dai J, et al. Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. (1995). *Eur Neuropsychopharmacol*. 5(Suppl. 1): 71–75.

Chen MH, Lin WC, Wu HJ, et al. (2019). Antisuicidal effect, BDNF Val66Met polymorphism, and low-dose ketamine infusion: reanalysis of adjunctive ketamine study of Taiwanese patients with treatment-resistant depression (AKSTPTRD). *J Affect Disord*. 251: 162–169.

Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron*. 61(1):10-26. doi: 10.1016/j.neuron.2008.10.055.

Deyama S, Duman RS. (2020). Neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine. *Pharmacol. Biochem. Behav*. 188:172837

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME. (1992) A functional anatomical study of unipolar depression. *J Neurosci*. 12(9):3628-41. doi: 10.1523/JNEUROSCI.12-09-03628.1992.

Duman RS, Deyama S, Fogaça MV. (2021). Role of BDNF in the pathophysiology and treatment of depression: activity-dependent effects distinguish rapid-acting antidepressants. *Eur. J. Neurosci*. 53:1

Duman RS. (2014). Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. *Dialogues Clin. Neurosci*. 16:11–27

Fales CL, Barch DM, Rundle MM, Mintun MA, Snyder AZ, Cohen JD, Mathews J, Sheline YI. (2008). Altered emotional interference processing in affective and cognitive-control brain

circuitry in major depression. *Biol Psychiatry*. 63(4):377-84. doi: 10.1016/j.biopsych.2007.06.012.

Fukumoto K, Fogaça MV, Liu RJ, Duman C, Kato T, et al. (2019). Activity-dependent brain-derived neurotrophic factor signaling is required for the antidepressant actions of (2R,6R)-hydroxynorketamine. *PNAS* 116(1):297–302

Gould TD, Zarate CA Jr., Thompson SM. (2019). Molecular pharmacology and neurobiology of rapid-acting antidepressants. *Annu. Rev. Pharmacol. Toxicol.* 59:213–36

Hare BD, Pothula S, DiLeone RJ, Duman RS. (2020). Ketamine increases vmPFC activity: effects of (R)- and (S)-stereoisomers and (2R,6R)-hydroxynorketamine metabolite. *Neuropharmacology*. 166:107947

Heifets, B.D., Bentzley, B.S., Williams, N. et al. (2021). Unraveling the opioid actions of S-ketamine and R-ketamine: comment on Bonaventura et al. *Mol Psychiatry*. 6104–6106. <https://doi.org/10.1038/s41380-021-01167-1>

Homayoun H, Moghaddam B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J. Neurosci.* 27:11496–500

Huganir RL, Nicoll RA. (2013). AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80:704–17

Jourdi H, Hsu YT, Zhou M, Qin Q, Bi X, Baudry M. (2009). Positive AMPA receptor modulation rapidly stimulates BDNF release and increases dendritic mRNA translation. *J. Neurosci.* 29:8688–97

Kavalali ET, Monteggia LM. (2020). Targeting homeostatic synaptic plasticity for treatment of mood disorders. *Neuron* 106:715–26

Kishimoto T, Chawla JM, Hagi K, et al. (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. *Psychol Med.* 46: 1459–1472.

Kim JS, Schmid-Burgk W, Claus D, et al. (1982). Increased serum glutamate in depressed patients. *Arch Psychiatr Nervenkr.* 232: 299–304.

Klein ME, Shandra J, Sheriff S, Malinow R. (2020). Opioid System is Necessary but not Sufficient for Antidepressive Actions of Ketamine in Rodents. *Biological Sciences*. 117:5 (2656-2662)

Laje G, Lally N, Mathews D, et al. (2012). Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. *Biol Psychiatry*. 72: e27–e28.

Lepack AE, Bang E, Lee B, Dwyer JM, Duman RS. (2016). Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures. *Neuropharmacology* 111:242–5

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

Li N, Lee B, Liu R-J, et al. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 329: 959–964.

Liu RJ, Duman C, Kato T, Hare B, Lopresto D, et al. (2017). GLYX-13 produces rapid antidepressant responses with key synaptic and behavioral effects distinct from ketamine. *Neuropsychopharmacology*

Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK. (2012). Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol. Psychiatry* 71:996–1005

Lumsden EW, Troppoli TA, Myers SJ, Zanos P, Aracava Y, et al. (2019). Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function. *PNAS* 116:5160–69

Maeng S, Zarate CA Jr., Du J, Schloesser RJ, McCammon J, et al. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry* 63:349–52

Matthews SC, Strigo IA, Simmons AN, Yang TT, Paulus MP. (2008). Decreased functional coupling of the amygdala and supragenual cingulate is related to increased depression in unmedicated individuals with current major depressive disorder. *J Affect Disord*. 111(1):13-20. doi: 10.1016/j.jad.2008.05.022

Matveychuk D, Thomas RK, Swainson J, et al. (2020). Ketamine as an antidepressant: overview of its mechanisms of action and potential predictive biomarkers. *Therapeutic Advances in Psychopharmacology*. doi:10.1177/2045125320916657

Moda-Sava RN, Murdock MH, Parekh PK, Fetcho RN, Huang BS, et al. (2019). Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science* 364(6436):eaat8078

Moghaddam B, Adams B, Verma A, Daly D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J. Neurosci*. 17:2921–27

- Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry*. 170:1134–42
- Newport DJ, Carpenter LL, McDonald WM, et al. (2015). Ketamine and other NMDA antagonists: early clinical trials and possible mechanisms in depression. *Am J Psychiatry*. 172: 950–966.
- Niciu MJ, Ionescu DF, Richards EM, Zarate CA. (2014). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm (Vienna)*. 121(8):907-924. doi:10.1007/s00702-013-1130-x
- Nosyreva E, Szabla K, Autry AE, Ryazanov AG, Monteggia LM, Kavalali ET. (2013). Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *J Neurosci*. 33(16):6990-7002. doi: 10.1523/JNEUROSCI.4998-12.2013.
- Pham TH, Defaix C, Xu X, Deng SX, Fabresse N, Alvarez JC, Landry DW, Brachman RA, Denny CA, Gardier AM. (2018). Common Neurotransmission Recruited in (R,S)-Ketamine and (2R,6R)-Hydroxynorketamine-Induced Sustained Antidepressant-like Effects. *Biol Psychiatry*. 84(1):e3-e6. doi: 10.1016/j.biopsych.2017.10.020.
- Riggs LM, Aracava Y, Zanos P, Fischell J, Albuquerque EX, et al. (2020). (2R,6R)-hydroxynorketamine rapidly potentiates hippocampal glutamatergic transmission through a synapse-specific presynaptic mechanism. *Neuropsychopharmacology*. 45:426–36
- Riggs LM, Gould TD. (2021). Ketamine and the Future of Rapid-Acting Antidepressants. *Annual Review of Clinical Psychology*. 15(7)
- Sanacora G, Treccani G, Popoli M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*. 62(1): 63-77. doi:10.1016/j.neuropharm.2011.07.036.
- Saxton RA and Sabatini DM. (2017). mTOR signaling in growth, metabolism, and disease. *Cell*. 169: 361–371.
- Sun J, Liu Y, Tran J, O'Neal P, Baudry M, Bi X. (2016). mTORC1-S6K1 inhibition or mTORC2 activation improves hippocampal synaptic plasticity and learning in Angelman syndrome mice. *Cell Mol Life Sci*. 73(22):4303-4314. doi:10.1007/s00018-016-2269-z
- Tornese P, Sala N, Bonini D, Bonifacino T, La Via L, Milanese M, Treccani G, Seguíni M, Ieraci A, Mingardi J, Nyengaard JR, Calza S, Bonanno G, Wegener G, Barbon A, Popoli M, Musazzi L. (2019). Chronic mild stress induces anhedonic behavior and changes in glutamate release, BDNF trafficking and dendrite morphology only in stress vulnerable rats. The rapid restorative action of ketamine. *Neurobiol Stress*. 10:100-160. doi: 10.1016/j.ynstr.2019.100160.

- Widman AJ, McMahon LL. (2018). Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. *PNAS* 115:E3007–16
- Williams NR, Heifets BD, Blasey C, et al. (2018). Attenuation of Antidepressant Effects of Ketamine by Opioid Receptor Antagonism. *Am J Psychiatry*. 175(12):1205-1215. doi:10.1176/appi.ajp.2018.1802013
- Williams NR, Heifets BD, Bentzley BS, Blasey C, Sudheimer KD, Hawkins J, et al. (2019). Attenuation of antidepressant and antisuicidal effects of ketamine by opioid receptor antagonism. *Mol Psychiatry*. 24:1779–86.
- 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
- Yang Y, Cui Y, Sang K, Dong Y, Ni Z, et al. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature*. 554:317–22
- Yang C, Hu YM, Zhou ZQ, et al. (2013). Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. *Ups J Med Sci*. 118: 3–8.
- Yang C, Li WY, Yu HY, et al. (2012). Tramadol pretreatment enhances ketamine-induced antidepressant effects and increases mammalian target of rapamycin in rat hippocampus and prefrontal cortex. *J Biomed Biotechnol*. 2012: 175619.
- Yang TT, Simmons AN, Matthews SC, et al. (2010). Adolescents with major depression demonstrate increased amygdala activation. *J Am Acad Child Adolesc Psychiatry*. 49(1):42-51. doi:10.1097/00004583-201001000-00008
- Yoshii A, Constantine-Paton M. (2010). Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol*. 70(5):304-322. doi:10.1002/dneu.20765
- Zanos P, Gould TD. (2018). Mechanisms of ketamine action as an antidepressant. *Mol Psychiatry*. 2018;23(4):801-811. doi:10.1038/mp.2017.255
- Zanos P, Highland J, Stewart BW, Gould T. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. *Pharmacology*. 116 (13): 6441-6450. doi: 10.1073/pnas.1819540116
- Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*. 533(7604):481–486.

Zanos P, Highland JN, Liu X, Troppoli TA, Georgiou P, et al. (2019a). (*R*)-Ketamine exerts antidepressant actions partly via conversion to (*2R,6R*)-hydroxynorketamine, while causing adverse effects at sub-anaesthetic doses. *Br. J. Pharmacol.* 176:2573–92

Zarate CA, Jr, Brutsche N, Laje G, Luckenbaugh DA, Venkata SL, Ramamoorthy A, et al. (2012). Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. *Biol Psychiatry.* 72(4):331–338.

Zhou W, Wang N, Yang C, et al. (2014). Ketamine induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. *Eur Psychiatry.* 29: 419–423.

Zorumski CF, Izumi Y and Mennerick. (2016). S. Ketamine: NMDA receptors and beyond. *J Neurosci.* 36: 11158–11164.

CHAPTER 4

Determining the Relative Contributions of (*R,S*)-6,6-dideuteroketamine and (*2R,6R*)-hydroxynorketamine to Ketamine's Antidepressant Mechanism

Purpose of the Study

It has been roughly twenty years since ketamine's potential as a rapid-acting antidepressant was properly recognized. During that time, the ability of ketamine to rescue various plasticity-related deficits present in the depressed brain has become well documented (Tornese et al. 2019; Murrough et al. 2017; Zanos & Gould 2018). Similarly, the active ketamine metabolite (*2R,6R*)-HNK has become known to have its own antidepressant properties, the likes of which may match or even exceed its parent drug according to some researchers (e.g. Pham et al. 2018; Zanos et al. 2016). Both ketamine and (*2R,6R*)-HNK act primarily on glutamate transmission-- their antidepressant actions occur in a manner that is fundamentally different from currently prescribed monoaminergic antidepressants. Despite this, the specific mechanism underlying ketamine and (*2R,6R*)-HNK action is not identical (Zanos et al. 2019a).

To reiterate, ketamine is a NMDAR antagonist that is believed to remedy depressive symptoms mainly by increasing activity within brain regions that are known to be hypoactive in depression (e.g. hippocampus). It likely does so by inhibiting GABAergic interneuron NMDARs, leading to disinhibited excitatory transmission that promotes neuroprotective and neuroplastic signaling pathways (Niciu et al. 2014; Li et al. 2010; Matveychuk et al. 2020). This occurs in an AMPAR/BDNF/mTORC1-dependent manner (Yang et al. 2012; Yang et al. 2013). Meanwhile, (*2R,6R*)-HNK also appears to upregulate glutamatergic signaling despite having no direct action on NMDARs or AMPARs. Rather, it may antagonize presynaptic mGluR2/3

autoreceptors, disinhibiting the tonic blockade of presynaptic glutamate release, thereby enhancing glutamatergic concentrations in the synapse (Zanos et al. 2019). This results in an indirect activation of AMPAR/BDNF/mTORC1-dependent plasticity pathways that overlap with those affected by ketamine (Zanos et al. 2016; Zanos et al. 2019a).

Further evidence has suggested that the metabolism of ketamine to (2R,6R)-HNK is functionally intertwined with ketamine's antidepressant efficacy (Lumsden et al 2019; Fukumoto et al. 2019; Li et al. 2010), and that conversion to (2R,6R)-HNK may even be required for any meaningful antidepressant actions to occur (Zanos & Gould 2018). Since both ketamine and its metabolite activate similar signaling pathways (albeit through different means), it is not agreed upon whether the observed effects of ketamine administration are coming from ketamine itself or from its metabolite. The totality of ketamine's action *in vivo* likely involves both ketamine-dependent and (2R,6R)-HNK-dependent components that occur in parallel, though the proportional contributions of each chemical to the overall antidepressant outcome are difficult to parse out. To date, there has been very little research done attempting to do so (Zanos et al. 2016). For this reason, the aim of this study is to assess whether ketamine or (2R,6R)-HNK in isolation differ in their ability to ameliorate the stress-induced synaptic deficits associated with MDD compared to when they are present in combination.

The study will begin by employing a CMS model on mice, followed by a characterization of depression-like behavioral changes. All experimentation and analysis will be performed in a manner blind to treatment assignments. The animals will then be randomly partitioned into one of several possible drug treatments (Figure 3). Mice will be euthanized 6 hours after receiving the drug treatments. Hippocampi will be extracted from each group, and several molecular assays will be conducted to elucidate the effects of the antidepressant drug treatments on the

synaptic changes induced by chronic stress. Analysis of all treatment groups will be accomplished using one-way ANOVA tests, followed by Tukey's post hoc multiple-comparisons test. This test will denote any significant differences across treatment groups. Data will be expressed as mean \pm standard error of the mean, and analysis will be carried out using GraphPad Prism6 (GraphPad Software, La Jolla, CA, USA) or R version 3.5.1 (R Foundation). In sum, this collection of procedures will further illuminate the antidepressant actions of ketamine and (2R,6R)-HNK in isolation, which may yield distinct results compared to when they act in tandem. Given the difference in their mechanisms of action, it should be interesting to see whether ketamine and (2R,6R)-HNK differ in their performance on the same behavioral and molecular tests of antidepressant efficacy.

Materials and Methods

Subjects

Experiments will be performed in full accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and reported in accordance to *ARRIVE* guidelines. Live mice will be used because intact neural circuits are necessary to assess the *in vivo* neurobiological mechanisms underlying antidepressant efficacy.

A sample of 125 male Swiss-Webster mice aged 8-10 weeks old (Charles River Laboratories, San Diego, CA) will be group housed in standard shoebox-style cages for 1 week prior to the start of the experiment. Mice will be housed at 20–22 °C, with a 12 h light/dark cycle (light on at 0700h) and food/water provided *ad libitum*, except when required for CMS. All animals will be stress- and drug-naïve at the start of testing. Mice will be randomly assigned to treatment groups in all experiments.

Chronic Mild Stress Paradigm

To induce depressive phenotypes in rodents, the Chronic Mild Stress (CMS) model of depression-like anhedonic behavior will be used. CMS is an empirically validated and common procedure for mimicking clinical depression in animals (Hill et al. 2012). Exposure to CMS is tied to altered performance on various behavioral assays and cognitive tests, both of which indicate that MDD-like anhedonia and memory impairments have occurred (Tang et al. 2015; Zhang et al. 2014). At a molecular level, the behavioral changes induced by CMS have been explained through a sequential chain of events in the cortex and hippocampus involving: (a) reductions in glutamatergic transmission; (b) decreased BDNF transcription and trafficking; (c) reductions in BDNF/TrkB signaling pathway activation; (d) decreased translation of glutamate receptors and dendritic spine proteins; (e) destabilized spine dynamics, dendritic atrophy, and increased cell death (Tornese et al. 2019; Sanacora et al. 2012; Popoli et al. 2011; Mikulska et al. 2021).

The CMS paradigm for this experiment will consist of a 4-week rotation of mild stressors administered daily. These will include restraint in a 50 ml tube for 6 hours, cage tilted for 45° for 24 hours, cage shaking at high speed (200 rpm) for 40 minutes, water deprivation for 24 hours, food deprivation for 24 hours, soiled bedding (200 ml) for 20 hours and overnight illumination (12 hours) (Strekalova et al. 2011). Mice will receive a stress daily with the order of the stressors changing weekly, except that overnight illumination will be administered twice a week, 3–4 days apart (Tang et al. 2015). Stress-naïve mice in the vehicle-only group will be housed under normal conditions.

Drug Treatments

Figure 1 illustrates the drug treatment group design to be used. 6,6-dideuteroketamine ((R,S)-d₂-KET) will be synthesized by deuterating the C6 position on ketamine, which prevents metabolism to (2R,6R)-HNK while retaining the same NMDAR binding affinity, ensuring that most of the observed antidepressant effects are attributable to ketamine alone, rather than (2R,6R)-HNK (Zanos et al. 2016; Gant 2014). Meanwhile, direct administration of (2R,6R)-HNK in the absence of ketamine will ensure the opposite. Administration of (R)-ketamine will allow ketamine to metabolize to (2R,6R)-HNK at its normal rate, producing the combinatory effects of both drugs that occurs *in vivo*. 0.9% saline administration will be used as the vehicle. Similarly, a control group of stress-naïve mice will act as a performance baseline for behavioral and molecular tests.

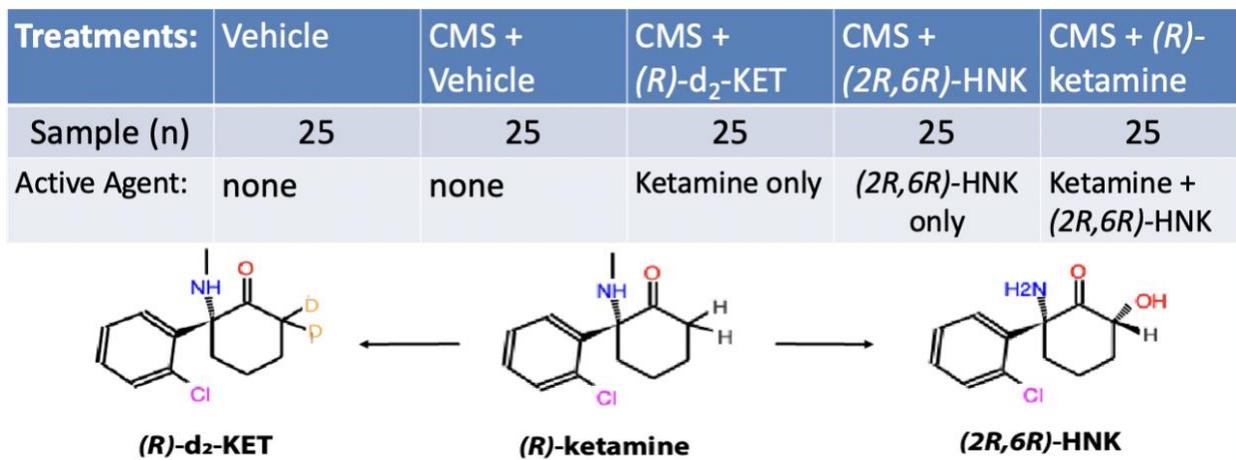


Figure 3. Experimental antidepressant drug and stress treatment groups. Illustrations of (R)-ketamine and its modified chemical derivatives are included (BioModel)

As per Zanos et al. (2019), (R)-ketamine hydrochloride (#K2753) will be purchased from Sigma-Aldrich (MO, USA). 6,6-dideuteroketamine hydrochloride ((R,S)-d₂-KET) and (2R,6R)-

HNK hydrochloride will be synthesized and characterized internally at the National Center for Advancing Translational Sciences and at SRI International (Menlo Park, CA, USA). Absolute and relative stereochemistry for (2R,6R)-HNK will be confirmed by small molecule x-ray crystallography (Morris et al. 2017). All drugs will be dissolved in 0.9% saline and administered via intraperitoneal injection in a volume of 7.5 ml/kg of body mass (Zanos et al. 2019). The concentrations of (R)-ketamine, (R,S)-d₂-ketamine, and HNK metabolites in plasma and brain tissue will be determined by achiral liquid chromatography-tandem mass spectrometry following the previously described methods of Moaddel et al. (2015).

Forced Swim Test (FST)

The FST is a valuable rodent behavioral test used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering depressive-like states. Following the methods reported by Can et al. (2012), mice will be subjected to a 6-min swim session in transparent Plexiglass cylinders (30 cm height × 20 cm diameter) filled with 15 cm of water (23 ± 1°C). The FST will be performed under normal light conditions. Sessions will be recorded using a digital video-camera. Immobility time, defined as passive floating with no additional activity other than that necessary to keep the animal's head above the water, was scored for the last 4 min of the 6-min test by a trained observer.

Typically, depressed rodents exhibit longer immobility times (e.g. Can et al. 2012; Tang et al. 2015). Changes in performance as a result of the drug treatments will be calculated by normalization to the performance observed in the saline-treated control groups. Based on relevant literature, it can be surmised that immobility times will increase in chronically stressed mice compared to stress-naïve mice, and that (R)-ketamine will restore behavioral normalcy (e.g.

Fitzgerald et al. 2019; Can et al. 2012; Tornese et al. 2019). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

Novelty-Suppressed Feeding Test (NSFT)

The NSFT is a rodent assay that models anxiety-like behaviors-- it was designed to assess the anxiolytic and/or antidepressant effects of chronic antidepressant treatment (Bodnoff et al. 1988). Following the procedure of Tang et al. (2015), the testing apparatus will consist of a plastic box measuring 50 × 50 × 20 cm. At 18 hours prior to behavioral testing, all food will be removed from the rodent's home cage to induce hunger. At the time of testing, a weighed single pellet of normal food chow for mice will be placed on a white paper platform positioned in the center of the plastic box. Each mouse will be placed in a corner of the box and allowed to explore for up to 10 minutes. Sessions will be recorded using a digital video-camera. The trial will be considered 'over' when the mouse chews a part of the chow. The amount of food consumed in the home cage will be calculated as the weight of chow consumed in 10 minutes. The time elapsed before mouse started to consume the food pellet was recorded as the latency.

Typically, anxious or depressed rodents will display longer latencies and consume less food overall (Bodnoff 1988). Changes in performance as a result of the drug treatments will be calculated by normalization to the performance observed in the saline-treated control groups. Based on relevant literature, it can be surmised that latency times will increase in chronically stressed mice compared to stress-naïve mice, and that (R)-ketamine will restore behavioral normalcy (e.g. Bodnoff et al. 1988; Tang et al. 2015). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

Sucrose Preference Test (SPT)

The SPT is useful for modeling anhedonia in rodents (Liu et al. 2018). The SPT that will be conducted blends the procedures of Liu et al. (2018) and Opal et al. (2014). Mice will be briefly transferred to individual housing and exposed to a sucrose solution (1% in tap water) for 72 hours, followed by 18 hours of water deprivation and a 2 hour exposure to two identical bottles, one filled with 1% sucrose solution and the other with tap water. The volumes of sucrose solution and water will be measured for the 2-hour exposure. Sucrose preference will be defined as the ratio of the volume of sucrose consumed versus total volume (sucrose + water) consumed during the 2-hour test, normalized to each individual animals' body weight.

Typically, rodents experiencing anhedonia will show a diminished preference for sucrose compared to water, while healthy rodents will almost universally prefer sucrose (e.g. Liu et al. 2018). Changes in performance as a result of the drug treatments will be calculated by normalization to the performance observed in the saline-treated control groups. Based on relevant literature, it can be surmised that immobility times will increase in chronically stressed mice compared to stress-naïve mice, and that (R)-ketamine will restore behavioral normalcy (e.g. Kim & Johnston 2020; Tornese et al. 2019; Tang et al. 2015). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

In Situ Hybridization

The ISH assay is intended to measure the degree of BDNF mRNA trafficking into hippocampal dendrites as a consequence of glutamatergic signaling. It will act as a representative measure of

neurotrophin signaling. ISH will be performed using methods adapted from Colliva and Tongiorgi (2021), as well as Colliva et al. (2018). Probe synthesis will follow the protocol designed by Chiaruttini et al. (2009).

Hippocampal cells from 9 animals per treatment group ($n = 9$) will be fixed for 15 minutes at room temperature in 4% PFA in PBS, washed in PBS 0.1% Tween20 (Sigma), and permeabilized in absolute ethanol for 15 min at -20°C . After rehydration with increasing concentration of PBST (50% ethanol/50% PBST, 30% ethanol/70% PBST, and finally PBST), cells will be hybridized with approximately 50–100 ng/coverlip of antisense or sense probes for a GFP or BDNF coding sequence. Before probe hybridization, coverslips will be equilibrated at 55°C in hybridization buffer containing 20 mM Tris-HCl pH 7.5 (Sigma), 300 mM NaCl (Sigma), 1 mM EDTA (Sigma), 0.5 mg/ml polyadenylic acid (Sigma), 0.5 mg/ml salmon sperm (Labtek Eurobio), $1\times$ Denhardt's solution, 100 mM Dithiothreitol (DTT, Sigma) and 50% deionised formamide (Sigma). After 1 h, this hybridization buffer will be removed and replaced with a hybridization mix (hybridization buffer + 10% dextran sulfate, Sigma) containing probes. Coverslips will be incubated in this mixture overnight at 55°C . Then, the hybridization mix will be removed, and the coverslips washed with $2\times$ Sodium Saline Citrate (Sigma) buffer (150 mM NaCl, 15 mM $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$) containing 0.1% Tween20 (SSCT $2\times$) and 50% formamide at 55°C twice, then with SSCT $2\times$ at 55°C once and finally in SSCT $0.1\times$ at 60°C twice. Coverslips will then be further incubated with PBST 5% fetal bovine serum (FBS; Euroclone) for 1 hour at room temperature to minimize nonspecific binding of primary antibodies, then incubated with anti-digoxigenin alkaline phosphatase-conjugated antibody (Roche) in PBST 5% FBS for 2 hour. After washing with PBST to remove the excess of unbound antibody, hybridized probes will be detected by developing an *in situ* signal with 70 mg/ml 4-nitroblue tetrazolium (NBT, Labtek

Eurobio) and 50 mg/ml 5-bromo-4-chloro-3-indolyl-phosphate (BCIP, Sigma) in a buffer solution containing 100 mM Tris-HCL pH 9.5, 50 mM MgCl₂, 100 mM NaCl and 1 mM Levamisole (Sigma). Image development will be carried out in a darkroom. The reaction will be stopped by removing the developing buffer and replacing it with a stop solution containing 10 mM Tris-HCl pH 8.0 and 1 mM EDTA pH 8.0. Coverslips were washed with PBS and deionized water and then mounted with Mowiol (Sigma).

Changes in the levels of BDNF mRNA trafficking as a result of the drug treatments will be calculated by normalization to the levels observed in the saline treated control groups. Based on relevant literature, it can be surmised that BDNF trafficking will be reduced in chronically stressed mice compared to stress-naïve mice, and that (R)-ketamine will successfully rescue these deficits (e.g. Tornese et al. 2019). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

Western Blot Analysis

The Western blot assay is intended to quantify the levels of phosphor-mTOR within the hippocampus. This will act as a representative measure for the activation of neuroprotective signaling pathways by BDNF/TrkB. The procedure used will follow the designs of Zanos et al. (2016), with minor modifications.

Synaptosomes will be purified from 8 mouse hippocampi per treatment group (n = 8) through dissection and homogenization in Syn-PER Reagent (ThermoFisher Scientific, Waltham, MA, USA; Cat # 87793) with a 1X protease and phosphatase inhibitor cocktail (ThermoFisher Scientific, Waltham, MA, USA; Cat # 78440). The homogenate will be centrifuged for 10 min at 1,200 × g at 4 °C. The supernatant will be centrifuged at 15,000 × g for

20 min at 4 °C. After centrifugation, the pellet (containing the synaptosomal fraction) will be re-suspended and sonicated in N-PER Neuronal Protein Extraction Reagent (ThermoFisher Scientific, Waltham, MA, USA; Cat # 87792). Protein concentration will be determined via a BCA protein assay kit (ThermoFisher Scientific, Waltham, MA, USA; Cat # 23227). Equal amount of proteins (10-40 µg as optimal for each antibody) for each sample will be loaded into NuPage 4-12% Bis-Tris gel for electrophoresis; the gel transfer will be performed with the TransBlot Turbo Transfer System (Bio-Rad, Hercules, CA, USA). Nitrocellulose membranes with transferred proteins will be blocked with 5% milk in TBST (TBS + 0.1% Tween-20) for 1 hour and kept with primary antibodies overnight at 4 °C. The primary antibody for phospho-mTOR (at Ser2448; Cell Signaling Technology, Danvers, MA, USA; Cat # 2971) and total mTOR (Cell Signaling Technology, Danvers, MA, USA; Cat # 2983) will then be applied to the sample.

The next day, blots will be washed three times in TBST and incubated with horseradish peroxidase conjugated anti-mouse or anti-rabbit secondary antibody (1:5000 to 1:10000) for 1 hour. After final three washes with TBST, bands will be detected using enhanced chemiluminescence (ECL) via a Syngene Imaging System (G:Box ChemiXX9). After imaging, the blots will be incubated in the stripping buffer (ThermoFisher Scientific, Waltham, MA, USA; Cat # 46430) for 10-15 min at room temperature followed by three washes in TBST. The stripped blots will be incubated in blocking solution for 1 hour and incubated with the primary antibody directed against total levels of the respective protein or GAPDH for loading control. Densitometric analysis of phospho- and total immunoreactive bands for each protein will be conducted using Syngene's GenTools software.

The values for the phosphorylated form of mTOR will be normalized to phosphorylation-independent levels of the same protein. Phosphorylation-independent levels of mTOR will be normalized to GAPDH. Changes in the ratio of mTOR to phospho-mTOR as a result of the drug treatments will be calculated by normalization to the ratios observed in saline-treated control groups. Based on relevant literature, it can be surmised that mTOR phosphorylation will be reduced in chronically stressed mice compared to stress-naïve mice, and that (R)-ketamine will successfully rescue these deficits (e.g. Tornese et al. 2019; Zanos et al. 2016). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

Golgi Staining and Sholl Analysis

The Golgi staining and Sholl analysis assays are intended to visualize dendritic morphology and dendritic arborization. All techniques that will be used follow the methods previously described by Nava et al. (2017) and Tornese et al. (2019).

Immediately after sacrifice, hippocampi from 8 animals per treatment group (n = 8) will be processed for Golgi staining using the Rapid Golgi Stain Kit (FD NeuroTechnologies, Inc., Columbia, MD, USA) (Nava et al., 2017). Hemispheres will be coronally sliced (200 µm) on a VT1200S vibratome (Leica, Wetzlar, Germany). CA1 and CA3 areas of HPC will be identified on a BX50 light microscope (Olympus, Tokyo, Japan) using newCAST software (Visiopharm, Hørsholm, Denmark). Z-stacks (1 µm per Z-step size) of 5 CA1 or CA3 pyramidal neurons with untruncated branches will be acquired using a × 60 oil objective. Collapsed Z-stacks will be imported into Bitplane Imaris software (version 7.7.1, Andor Technology Ltd, Belfast, Northern

Ireland), and dendrites will be reconstructed using the FilamentTracer function. Dendrite length and branching, as well as Sholl analysis will be assessed.

Changes in dendritic length and arborization as a result of the drug treatments will be calculated by normalization to the results observed in saline-treated control groups. Based on relevant literature, it can be surmised that dendritic branching will diminish, and that dendrites themselves will deteriorate, in chronically stressed mice compared to stress-naïve mice. (R)-ketamine should successfully rescue these morphological changes (e.g. Tornese et al. 2019; Tang et al. 2015; Suarez-Santiago et al. 2020). However, it is not easy to predict how the results will change when (R,S)-d₂-ketamine and (2R,6R)-HNK are administered in isolation.

CHAPTER 4 REFERENCES

- Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology*. 95: 298–302
- Bonifacino T, Musazzi L, Milanese M, Seguíni M, Marte A, Gallia E, Cattaneo L, Onofri F, Popoli M, Bonanno G. (2016). Altered mechanisms underlying the abnormal glutamate release in amyotrophic lateral sclerosis at a pre-symptomatic stage of the disease. *Neurobiol Dis*. 95:122-33. doi:10.1016/j.nbd.2016.07.011.
- Chen J, Peng LH, Luo J, Liu L, Lv F, Li P, Ao L, Hao XC, Min S. (2015). Effects of low-dose ketamine combined with propofol on phosphorylation of AMPA receptor GluR1 subunit and GABAA receptor in hippocampus of stressed rats receiving electroconvulsive shock. *J ECT*. 31(1):50-6. doi: 10.1097/YCT.000000000000148.
- Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, et al. (2009). Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. *Proc Natl Acad Sci*. 106(38):16481–6.
- Colliva, A, Tongiorgi, E. (2021). Distinct role of 5'UTR sequences in dendritic trafficking of BDNF mRNA: additional mechanisms for the BDNF splice variants spatial code. *Mol Brain*. 14, 10. <https://doi.org/10.1186/s13041-020-00680-8>
- Colliva A, Maynard KR, Martinowich K, Tongiorgi E. (2018). Detecting single and multiple BDNF transcripts by in situ hybridization in neuronal cultures and brain sections. In: Duarte CB, Tongiorgi E, editors. Brain-derived neurotrophic factor (BDNF), (*Neuromethods; vol. 143*). New York: Springer. p. 27–53.
- Collo G, Cavalleri L, Chiamulera C, Merlo Pich E. (2019). Ketamine increases the expression of GluR1 and GluR2 α -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subunits in human dopaminergic neurons differentiated from induced pluripotent stem cells. *Neuroreport*. 30(3):207-212. doi: 10.1097/WNR.0000000000001185
- Fitzgerald PJ, Yen JY, Watson BO. (2019). Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test. *PLoS One*. 14(4):e0215554. doi: 10.1371/journal.pone.0215554.
- Fukumoto K, Fogaça MV, Liu RJ, Duman C, Kato T, et al. (2019). Activity-dependent brain-derived neurotrophic factor signaling is required for the antidepressant actions of (2R,6R)-hydroxynorketamine. *PNAS* 116(1):297–300
- Gant TG. (2014) Using deuterium in drug discovery: leaving the label in the drug. *J Med Chem*. 57(9):3595-611. doi: 10.1021/jm4007998.

Hill MN, Hellemans KG, Verma P, Gorzalka BB, Weinberg J. (2012). Neurobiology of chronic mild stress: parallels to major depression. *NeurosciBiobehav R* 36, 2085–2117

Kim CS, Johnston D. (2020). Antidepressant Effects of (S)-Ketamine through a Reduction of Hyperpolarization-Activated Current I_h . *iScience*. 23(6):101239. doi:10.1016/j.isci.2020.101239

Langhammer CG, Previtiera ML, Sweet ES, Sran SS, Chen M, Firestein BL. (2010). Automated Sholl analysis of digitized neuronal morphology at multiple scales: Whole cell Sholl analysis versus Sholl analysis of arbor subregions. *Cytometry A*. 77(12):1160-1168. doi:10.1002/cyto.a.20954

Li N, Lee B, Liu R-J, et al. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. 329: 959–964.

Liu MY, Yin CY., Zhu LJ. et al. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc*. 13:1686–1698. <https://doi.org/10.1038/s41596-018-0011-z>

Lumsden EW, Troppoli TA, Myers SJ, Zanos P, Aracava Y, et al. (2019). Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function. *PNAS* 116:5160–69

Lynch G, Rex CS, Gall CM. (2007). LTP consolidation: substrates, explanatory power, and functional significance. *Neuropharmacology*. 52:12–23.

Matveychuk D, Thomas RK, Swainson J, et al. (2020). Ketamine as an antidepressant: overview of its mechanisms of action and potential predictive biomarkers. *Therapeutic Advances in Psychopharmacology*. doi:10.1177/2045125320916657

Mikulska J, Juszczak G, Gawronska-Grzywacz M, Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sciences*.

Moaddel R, Sanghvi M, Dossou KS, Ramamoorthy A, Green C, Bupp J, Swezey R, O'Loughlin K, Wainer IW. (2015). The distribution and clearance of (2S,6S)-hydroxynorketamine, an active ketamine metabolite, in Wistar rats. *Pharmacol Res Perspect*. 3(4):e00157. doi: 10.1002/prp2.157

Monge-Acuña AA, Fornaguera-Trías J. (2009). A high performance liquid chromatography method with electrochemical detection of gamma-aminobutyric acid, glutamate and glutamine in rat brain homogenates. *J Neurosci Methods*. 183(2):176-81. doi: 10.1016/j.jneumeth.2009.06.042.

Morris PJ, et al. (2017). Synthesis and N-Methyl-d-aspartate (NMDA) Receptor Activity of Ketamine Metabolites. *Org Lett*. 19(17):4572-4575.

Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry*. 170:1134–42

Murrough JW, Abdallah CG, Mathew SJ. (2017). Targeting glutamate signalling in depression: progress and prospects. *Nat Rev Drug Discov*. 16(7):472-486. doi:10.1038/nrd.2017.16.

Nava N, Treccani G, Alabsi A, Kaastrup Mueller H, Elfving B, Popoli M, Wegener G, Nyengaard JR. Temporal Dynamics of Acute Stress-Induced Dendritic Remodeling in Medial Prefrontal Cortex and the Protective Effect of Desipramine. *Cereb Cortex*. 2017 Jan 1;27(1):694-705. doi:10.1093/cercor/bhv254.

Nava N, Treccani G, Alabsi A, Kaastrup Mueller H, Elfving B, Popoli M, Wegener G, Nyengaard J.R. (2017). Temporal dynamics of acute stress-induced dendritic remodeling in medial prefrontal cortex and the protective effect of desipramine. *Cerebr. Cortex*. 27:694–705. doi: 10.1093/cercor/bhv254.

Niciu MJ, Ionescu DF, Richards EM, Zarate CA. (2014). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm (Vienna)*. 121(8):907-924. doi:10.1007/s00702-013-1130-x

Noam Y, Zha Q, Phan L, Wu RL, Chetkovich DM, Wadman WJ, Baram TZ. (2010). Trafficking and surface expression of hyperpolarization-activated cyclic nucleotide-gated channels in hippocampal neurons. *J Biol Chem*. 285:14724 –14736.

Opal MD, et al. (2014). Serotonin 2C receptor antagonists induce fast-onset antidepressant effects. *Mol Psychiatry*. 19:1106–14

Pham TH, Defaix C, Xu X, Deng SX, Fabresse N, Alvarez JC, Landry DW, Brachman RA, Denny CA, Gardier AM. (2018). Common Neurotransmission Recruited in (R,S)-Ketamine and (2R,6R)-Hydroxynorketamine-Induced Sustained Antidepressant-like Effects. *Biol Psychiatry*. 84(1):e3-e6. doi: 10.1016/j.biopsych.2017.10.020.

Popoli M, Yan Z, McEwen BS, Sanacora G. (2011). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat. Rev. Neurosci*. **13**: 22–37

Sanacora G, Treccani G, Popoli M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*. 62(1): 63-77. doi:10.1016/j.neuropharm.2011.07.036.

Suárez-Santiago JE, Orozco-Suárez S, Vega-García A, Bautista-Orozco LÁ, Picazo O. (2020). Repeated ketamine administration induces recognition memory impairment together with morphological changes in neurons from ventromedial prefrontal cortex, dorsal striatum, and hippocampus. *Behav Pharmacol*. 31(7):633-640. doi: 10.1097/FBP.0000000000000571.

Strekalova T, Couch Y, Kholod N, Boyks M, Malin D, Leprince P, Steinbusch HM. (2011). Update in the methodology of the chronic stress paradigm: internal control matters. *Behav Brain Funct.* 27;7:9. doi: 10.1186/1744-9081-7-9.

Tang, J. *et al* (2015). Involvement of normalized NMDA receptor and mTOR-related signaling in rapid antidepressant effects of Yueju and ketamine on chronically stressed mice. *Sci. Rep.* 5, 13573; doi: 10.1038/srep13573

Tornese P, Sala N, Bonini D, Bonifacino T, La Via L, Milanese M, Treccani G, Seguni M, Ieraci A, Mingardi J, Nyengaard JR, Calza S, Bonanno G, Wegener G, Barbon A, Popoli M, Musazzi L. (2019). Chronic mild stress induces anhedonic behavior and changes in glutamate release, BDNF trafficking and dendrite morphology only in stress vulnerable rats. The rapid restorative action of ketamine. *Neurobiol Stress.* 10:100-160. doi: 10.1016/j.ynstr.2019.100160.

Treccani G, Musazzi L, Perego C., Milanese M, Nava N, Bonifacino T, Lamanna J, Malgaroli A., Drago F, Racagni G, Nyengaard JR, Wegener G, Bonanno G, Popoli M. (2014). Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Mol. Psychiatry.* 19:433–443. doi: 10.1038/mp.2014.5.

Willner P. (2016). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiol Stress.* 6:78-93. doi: 10.1016/j.ynstr.2016.08.002.

Yang C, Hu YM, Zhou ZQ, et al. (2013). Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. *Ups J Med Sci.* 118: 3–8.

Yang C, Li WY, Yu HY, et al. (2012). Tramadol pretreatment enhances ketamine-induced antidepressant effects and increases mammalian target of rapamycin in rat hippocampus and prefrontal cortex. *J Biomed Biotechnol.* 2012: 175619.

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature.* 533(7604):481–486.

Zanos P, Gould TD. (2018). Mechanisms of ketamine action as an antidepressant. *Mol Psychiatry.* 2018;23(4):801-811. doi:10.1038/mp.2017.255

Zanos P, Highland J, Stewart BW, Gould T. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. *Pharmacology.* 116 (13): 6441-6450. doi: 10.1073/pnas.1819540116

Zanos P, Highland JN, Liu X, Troppoli TA, Georgiou P, et al. (2019a). (R)-Ketamine exerts antidepressant actions partly via conversion to (2R,6R)-hydroxynorketamine, while causing adverse effects at sub-anaesthetic doses. *Br. J. Pharmacol.* 176:2573–92

Zhang JC, Li SX, Hashimoto KR. (2014). (-)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacol Biochem Behav.* 116, 137–141.

CHAPTER 5

Summary, Concluding Remarks, and Future Directions for Research

Summary

Chapter 1 lays out why Major Depressive Disorder (MDD) is an important topic of study, as well as how our understanding of the disorder has evolved through time. Depression is a highly debilitating and common mental illness that straddles all genders, ages, nationalities, and walks of life. Those affected are burdened by a constellation of symptoms centering around uncontrollably negative mood states, accompanied by marked anhedonia, sleep and appetite disturbances, social dysfunctionality, and suicidal ideation. MDD develops from a complex interplay of genetic predispositions and environmental risk factors that vary strongly between patients-- this makes prevention of the disorder difficult. In addition to its emotional impacts, the financial impacts of depression are immense and only continue to grow with each passing year. The treatment of MDD is therefore among the most urgent clinical issues faced by humankind today.

Depression therapeutics were basically nonexistent when the antidepressant properties of iproniazid were serendipitously discovered in the 1950s. The ensuing realization that the affective symptoms of MDD can be managed by restoring deficits of monoamine signaling led further drug development efforts to focus almost exclusively on this mechanism. Since then, it has become obvious that monoaminergic drugs are suboptimal in their efficacy and latency of therapeutic action. Roughly 30% of patients experience little to no benefit, and those who do benefit must tolerate side effect profiles severe enough to cause high rates of treatment

discontinuation (Riggs & Gould 2021). Despite these drawbacks, monoaminergic antidepressants remain the only MDD therapeutics in mainstream distribution today. The SSRIs and SNRIs prescribed *en masse* are well-suited to the functions they perform. Yet they are still not enough. This, of course, suggests that the drugs are not to blame, but rather that our understanding of depression's etiology, pathophysiology, and clinical manifestation is incomplete (Duman & Monteggia 2006). In recent years, widespread acceptance of the monoamine hypothesis's inadequacy has galvanized the search for new ways with which the pathology can be described. Thankfully, great strides have been made that have shifted our understanding of what depression is.

Chapter 2 describes how modern explanations of MDD characterize it as a multifaceted disorder precipitated by chronic stress; the monoamine reductions emphasized previously are but one pathological component of a bigger picture. Hyperactive HPA axis signaling leads to dysfunctional neurotransmission, particularly in regard to glutamate, which acts as the primary excitatory mediator within brain circuits that are crucial for mood regulation (Murrough et al. 2017). Glutamatergic signaling is also the basis for nearly all mechanisms of synaptic plasticity. The glutamate reductions induced by chronic stress lead to a concatenation of adverse cellular changes including: (a) decreased BDNF transcription and trafficking; (b) decreased recruitment of BDNF/TrkB and downstream effectors like mTORC1; (c) decreased translation of glutamate receptors and dendritic spine proteins; (e) reduced glutamate receptor availability, dendritic atrophy, destabilized spine dynamics, increased neuronal and glial death; (f) neurotransmitter deficiencies and impaired synaptogenesis (Duman et al. 2016; Musazzi et al. 2013; Niciu et al. 2014; Sanacora et al. 2013; Tornese et al. 2019). Behaviorally, these maladaptive structural changes may underlie the affective and cognitive symptoms of MDD.

Thankfully, the glutamatergic model for depression provides ample molecular targets for novel treatment methods. The ‘poster child’ of drugs that have emerged to act on this system is ketamine. Through non-competitive NMDAR inhibition at select synapses, ketamine has consistently demonstrated robust, sustained, and rapid antidepressant effects on a supermajority of MDD patients, even those who are considered resistant to traditional antidepressants. The implications of this are immense. Ketamine’s ability to rapidly ameliorate suicidal ideation and anhedonia contrasts starkly with the delayed onset of currently approved antidepressants (Matveychuk et al. 2020). This is particularly important for severely depressed patients, where a lag in the onset of antidepressant action has been associated with increased risk for suicidal behavior (Duman et al. 2016).

However, even though ketamine’s rapid antidepressant properties have been acknowledged within the scientific community for several decades, ketamine has yet to receive any widespread distribution or large-scale funding efforts. To date, intranasal (*S*)-ketamine is the only one of these compounds to receive FDA approval for treatment-resistant MDD with concomitant administration of at least one traditional antidepressant (Zheng et al. 2020). The skepticism surrounding ketamine’s viability as a mainstream antidepressant stems from its well-documented abuse potential, as well as its dissociative, anesthetic, and psychomimetic properties at doses beyond those used for the treatment of MDD. Furthermore, a glaring lack of consensus surrounding ketamine’s mechanism of action has stymied efforts to upscale its distribution. This situation is unlikely to change until there is more clarity about how ketamine achieves its rapid antidepressant effects.

The goal of Chapter 3 was to assess various putative mechanisms for ketamine’s antidepressant action and identify the limits of our understanding. The most empirically

validated hypotheses in the current literature include: (a) direct NMDAR inhibition and consequent activity reduction in brain regions that promote depressive phenotypes; (b) inhibition of GABAergic interneuron NMDARs resulting in pyramidal neuron disinhibition; (c) metabolism of ketamine into the mGluR2 inhibitor (2R,6R)-HNK. These putative ketamine mechanisms are not mutually exclusive and may act synergistically, culminating in the unique antidepressant properties of the drug (Riggs & Gould 2021). The net result of all these processes is a sustained potentiation of excitatory synapses in brain circuits involved in the maintenance of mood and stress-reactivity (Zanos & Gould 2018), which may be sufficient to rescue the stress-induced synaptic deficits that underlie depressive pathology (e.g. Tornese et al. 2019).

Based on the mechanisms laid out in Chapter 3, the goal of Chapter 4 was to propose a study that will further investigate a poorly characterized aspect of ketamine's action. The study described herein aims to parse out the relative contributions of ketamine and (2R,6R)-HNK in ketamine's overall mechanism of action, for ketamine and (2R,6R)-HNK work in parallel after ketamine is administered *in vivo*. To do this, the CMS model will be used to induce depressive phenotypes in mouse models, after which the mice will be treated with either: (a) unaltered (R)-ketamine that readily metabolizes into (2R,6R)-HNK; (b) 6,6-dideuteroketamine that is unable to metabolize to (2R,6R)-HNK; (c) (2R,6R)-HNK in the absence of ketamine; (d) a saline vehicle to act as a control. To analyze the antidepressant efficacy of each drug, CMS-induced anhedonia, despair, and anxiety-like behaviors will be measured using a sucrose preference test, forced swim test, and novelty-suppressed feeding test, respectively. Behavioral analysis will be reinforced by molecular evidence measuring BDNF mRNA trafficking, mTOR phosphorylation, and dendritic arborization in hippocampal dendrites. This collection of procedures will further illuminate the antidepressant effects of both ketamine and (2R,6R)-HNK in isolation, which may

differ compared to when they act in tandem. Since (2R,6R)-HNK does not possess dissociative properties and has limited abuse potential, it may have higher potential for widespread distribution if it can be demonstrated to perform comparably to (R)-ketamine.

Limitations

If the mechanism underlying ketamine's antidepressant properties was simple it would have already been ascertained decades ago. Ketamine has multiple empirically verified targets that exist downstream of its NDMAR inhibition, some of which are themselves inhibitory, while others are disinhibitory (e.g. Yang et al. 2018; Yamada & Jinno 2019; Murrough et al. 2017). Combined with the observed heterogeneity in results between MDD patients, the action of ketamine within the brain is undoubtedly complex. However, slow progress may also be attributable to the fact that our overall understanding of MDD's pathophysiology is still incomplete, and the methods available for broadening that understanding are limited (Duman & Monteggia 2006). In most research contexts, genetic, pharmacological, environmental, and circuit-level manipulations are used to induce behavioral states in model organisms that are believed to reflect depressive phenotypes in humans (e.g. Can et al. 2012; Fitzgerald et al. 2019; Tang et al. 2015; Liu et al. 2018). Our predictions about whether a compound has antidepressant potential depend almost entirely on the robustness of these behavioral outputs (Riggs & Gould 2021). Unfortunately, though, the sheer complexity of the human mental illnesses that we use preclinical approaches to make inferences about may not be so easily reducible (Zanos & Gould 2018). Given that basic scientific insights have largely failed to produce novel compounds that are universally successful in late-stage clinical trials, it is worth considering whether animal

models and preclinical depression-related assays lack the translational power to properly test the veracity of mechanistic hypotheses (Duman et al. 2016; Riggs & Gould 2021).

To this end, mechanisms identified preclinically should be subjected to rigorous tests of their underlying assumptions before studies on humans can legitimately be used to verify the clinical relevance of those hypotheses in depressed patients. Since the success of clinical trials fundamentally relies on the precision of preclinical studies, further improvements of preclinical assays are needed to ensure that research findings will hold water in clinical settings. An example of this in the context of ketamine is the role of mTORC1, which is considered by many to be an essential component in the synaptogenic pathway that confers ketamine with its unique antidepressant properties (e.g. Duman et al. 2019; Zanos et al. 2016; Dwyer et al. 2012; Harraz et al. 2016). This conclusion is drawn partly from preclinical observations that the mTOR inhibitor rapamycin blocks the antidepressant effects of ketamine when infused directly into the rat mPFC (Li et al. 2010). However, contrary to what preclinical studies have predicted, Abdallah et al. (2020) recently reported that peripheral administration of rapamycin prolongs the antidepressant effects of ketamine in depressed patients. Autry et al. (2011) also reported that systemic rapamycin administration failed to block the antidepressant effects of ketamine. While it is interesting to wonder whether these results are due to a poorly characterized interaction between ketamine and immune function in depressed patients (rapamycin is used as an immunosuppressant), discrepancies such as these highlight the inherent challenge of translating scientific discoveries from the lab into the clinic.

Future Research Directions

Without question, the discovery that ketamine possesses robust, rapid, and sustained antidepressant properties is one of the greatest advances in psychiatry of the last fifty years. Ketamine has paved the way for significant theoretical, scientific, and clinical progress regarding the treatment of MDD, which has been long overdue (Duman 2018; Krystal et al. 2019). Central to ketamine's antidepressant mechanism of action is the finding that it fundamentally exerts its antidepressant effects by enhancing excitatory glutamatergic transmission at select synapses, which initiates synaptogenic processes that restore the integrity of neural circuits compromised by stress-induced depression (Murrough et al. 2017; Riggs & Gould 2021; Krystal et al. 2019). However, as stated previously, the utility of ketamine for psychiatric indications is limited by its dose-dependent dissociative and psychotomimetic properties, which confer it with noteworthy abuse potential (Krystal et al. 2013). A better understanding of the demographic, clinical, and neurobiological predictors of drug abuse will foster a safer, more targeted usage of ketamine in the future. Furthermore, while ketamine is already known to possess a relatively modest side effect profile in the short term (Duman 2018), more longitudinal research is needed to ascertain long-term risks associated with ketamine use; these may affect its viability as a mainstream antidepressant.

It remains unclear why other NMDAR antagonists like MK-801 lack antidepressant properties when juxtaposed with ketamine, which calls the role of NMDAR inhibition into question (Gould et al. 2019; Newport et al. 2015; Lener et al. 2017). Future research should aim to solidify the cellular and synaptic processes that are crucial to ketamine's antidepressant actions. These identified processes must be translationally robust and empirically sound, as a higher burden of proof will be needed for ketamine to overcome the obstacles that have thus far prevented it from receiving widespread distribution (Riggs & Gould 2021). There are several

preclinically validated targets beyond NMDAR inhibition that provide hope for the development of novel rapid-acting antidepressant drugs (Mikulska et al. 2021). As discussed previously, the active ketamine metabolite (2R,6R)-HNK is reported to possess NMDAR- and AMPAR-independent antidepressant properties without the euphoric and dissociative properties of ketamine (Zanos et al. 2016; Zanos et al. 2019a). Future research should place a heavier emphasis on this compound. If (2R,6R)-HNK can demonstrate antidepressant actions that are comparable to ketamine, its superior pharmacological and toxicological profile could potentially render its parent drug obsolete (Zanos et al. 2019).

Following the promising findings with ketamine, a plethora of preclinical and clinical studies have assessed alternative, putative rapid-acting antidepressant medications that attempt to remedy synaptic deficits induced by chronic stress. These include, but are not limited to: (a) subunit-specific NMDAR antagonists (e.g. MK-0657); (b) muscarinic acetylcholine receptor (mAChR) antagonists (e.g. scopolamine); (c) group II metabotropic glutamate receptor (mGluR2/3) antagonists beyond (2R,6R)-HNK (e.g. LY341495); (d) NMDAR glycine binding modulators (e.g. GLYX-13); (e) positive and negative allosteric modulators of GABA_A receptors (brexanolone); (f) serotonin 2C (5-HT_{2C}) receptor antagonists (e.g. promazine); (g) excitatory amino acid transporter 2 (EAAT2) enhancers (riluzole); (h) low trapping NMDA channel blockers (e.g. lanicemine) (Sanacora et al. 2013; Sanacora & Banasr 2012; Riggs & Gould 2021; Fasipe et al. 2020; Krystal et al. 2013; Ibrahim et al. 2012; Matveychuk et al. 2020). While none of these compounds appear to have usurped ketamine in terms of antidepressant potential, some have shown efficacy in several animal assays predictive of rapid-acting antidepressant actions. Still, immense volumes of further research are needed to determine if any of these compounds can achieve similar antidepressant effects to ketamine without dissociative properties and abuse

potential. Hopefully, an improved understanding of depression pathophysiology will help to support the development of more precise, mechanistically accurate treatments for MDD in the decades ahead.

Conclusion

The improvement of treatment options for MDD is of extremely high clinical and societal priority. Glutamatergic drugs like ketamine have spearheaded a new generation of antidepressants that act beyond monoamines to ameliorate maladaptive cellular changes induced by chronic stress. These novel antidepressants show great promise and appear capable of addressing many shortcomings of those currently administered. However, widespread distribution of ketamine is impossible until a consensus is reached surrounding its antidepressant mechanism of action. The goal of this paper was to lay out putative mechanisms within the literature and propose a study that will help advance our understanding. A great deal of future research is needed to hone our knowledge of ketamine's behavior within the brain and body. In time, the prescription of ketamine, and/or drugs that act in a similar manner to it may replace SSRIs as the most common pharmacological strategies for MDD.

Evidently, though, we are still far from finding any sort of universally effective treatment for depression. Despite great advances in recent years, MDD remains among the most severe psychiatric disorders, associated with a chronic relapsing course and marked functional impairment in the majority of patients. It is an unrelenting illness that saps away the pleasure from even the most gratifying parts of life, leading to profound misery. There is more imperative now than ever for the scientific community to uncover newer and better treatment strategies, so

that our progeny may one day look back at major depressive disorder as a harrowing bygone of past generations.

CHAPTER 5 REFERENCES

- Abdallah CG, Averill LA, Gueorguieva R, et al. (2018). Rapamycin, an immunosuppressant and mTORC1 inhibitor, triples the antidepressant response rate of ketamine at 2 weeks following treatment: a double-blind, placebo-controlled, cross-over, randomized clinical trial. *bioRxiv*. 500959.
- Autry AE, Adachi M, Nosyreva E, et al. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*. 475: 91–95.
- Duman RS. (2018). The Dazzling Promise of Ketamine. *Cerebrum: The Dana forum on brain science*. PMID: 30746033
- Duman RS, Aghajanian GK, Sanacora G, Krystal JH. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat. Med.* **22**: 238–249.
- Duman RS, Monteggia LM. (2006). A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry*. **59**: 1116–1127.
- Fasipe OJ, Agede OA, Enikuomihin AC (2020). Announcing the novel class of GABA-A receptor selective positive allosteric modulator antidepressants. *Future Sci OA*. 2020;7(2):FSO654. doi:10.2144/fsoa-2020-0108
- Fitzgerald PJ, Yen JY, Watson BO. (2019). Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test. *PLoS One*. 14(4):e0215554. doi: 10.1371/journal.pone.0215554.
- Harraz MM, Tyagi R, Cortés P, Snyder SH. (2016). Antidepressant action of ketamine via mTOR is mediated by inhibition of nitrenergic Rheb degradation. *Mol Psychiatry*. 21(3):313-9. doi:10.1038/mp.2015.211.
- Jason M. Dwyer, Ashley E. Lepack, Ronald S. Duman. (2012). mTOR activation is required for the antidepressant effects of mGluR_{2/3} blockade, *International Journal of Neuropsychopharmacology*. 15(4): 429–434,
- Ibrahim L, Diaz Granados N, Jolkovsky L, et al. (2012). A Randomized, placebo-controlled, crossover pilot trial of the oral selective NR2B antagonist MK-0657 in patients with treatment-resistant major depressive disorder. *J Clin Psychopharmacol*. 32(4):551-557. doi:10.1097/JCP.0b013e31825d70d6
- Krystal JH, Abdallah CG, Sanacora G, Charney DS, Duman RS. (2019). Ketamine: a paradigm shift for depression research and treatment. *Neuron*. 101:774–78
- Krystal JH, Sanacora G and Duman RS. (2013). Rapidacting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry*. 73: 1133–1141.

Liu MY, Yin CY., Zhu LJ. *et al.* (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc.* 13:1686–1698. <https://doi.org/10.1038/s41596-018-0011-z>

Matveychuk D, Thomas RK, Swainson J, et al. (2020). Ketamine as an antidepressant: overview of its mechanisms of action and potential predictive biomarkers. *Therapeutic Advances in Psychopharmacology*. doi:10.1177/2045125320916657

Murrough JW, Abdallah CG, Mathew SJ. (2017). Targeting glutamate signalling in depression: progress and prospects. *Nat Rev Drug Discov.* 16(7):472-486. doi: 10.1038/nrd.2017.16.

Musazzi L, Treccani G, Mallei A, Popoli M. (2013). The action of antidepressants on the glutamate system: regulation of glutamate release and glutamate receptors. *Biol Psychiatry.* 73(12): 1180-8. doi:10.1016/j.biopsych.2012.11.009.

Niciu MJ, Ionescu DF, Richards EM, Zarate CA. (2014). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *J Neural Transm (Vienna).* 121(8):907-924. doi:10.1007/s00702-013-1130-x

Sanacora G, Banasr M. (2013). From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry.* **73**: 1172–1179

Sanacora G, Treccani G, Popoli M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology.* 62(1): 63-77. doi:10.1016/j.neuropharm.2011.07.036.

Tornese P, Sala N, Bonini D, Bonifacino T, La Via L, Milanese M, Treccani G, Seguíni M, Ieraci A, Mingardi J, Nyengaard JR, Calza S, Bonanno G, Wegener G, Barbon A, Popoli M, Musazzi L. (2019). Chronic mild stress induces anhedonic behavior and changes in glutamate release, BDNF trafficking and dendrite morphology only in stress vulnerable rats. The rapid restorative action of ketamine. *Neurobiol Stress.* 10:100-160. doi: 10.1016/j.ynstr.2019.100160.

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

Yang Y, Cui Y, Sang K, Dong Y, Ni Z, et al. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature.* 554:317–22

Zanos P, Highland J, Stewart BW, Gould T. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. *Pharmacology.* 116 (13): 6441-6450. doi: 10.1073/pnas.1819540116

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*. 533(7604):481–486.

Zanos P, Highland JN, Liu X, Troppoli TA, Georgiou P, et al. (2019a). (*R*)-Ketamine exerts antidepressant actions partly via conversion to (*2R,6R*)-hydroxynorketamine, while causing adverse effects at sub-anaesthetic doses. *Br. J. Pharmacol.* 176:2573–92