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Ketamine and the Future of Rapid-Acting Antidepressants

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Abstract

The therapeutic onset of traditional antidepressants is delayed by several weeks and many depressed patients fail to respond to treatment altogether. In contrast, subanesthetic ketamine can rapidly alleviate symptoms of depression within hours of a single administration, even in patients who are considered treatment-resistant. Ketamine is thought to exert these effects by restoring the integrity of neural circuits that are compromised in depression. This hypothesis stems in part from preclinical observations that ketamine can strengthen synaptic connections by increasing glutamate-mediated neurotransmission and promoting rapid neurotrophic factor release. An improved understanding of how ketamine, and other novel rapid-acting antidepressants, give rise to these processes will help foster future therapeutic innovation. Here, we review the history of antidepressant treatment advances that preceded the ketamine discovery, critically examine mechanistic hypotheses for how ketamine may exert its antidepressant effects, and discuss the impact this knowledge has had on ongoing drug discovery efforts.

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INTRODUCTION

Major depressive disorder is the leading cause of disability in the world and is characterized by an amalgam of symptoms that can severely undermine overall quality of life (Vos et al. 2017, WHO 2017). Depressed individuals are not only burdened by near-constant sadness, but also suffer from a diminished sense of self-worth, extreme fatigue, difficulty concentrating, disruptions to sleep, and a reduced ability to experience pleasure (i.e., anhedonia). Antidepressants, which historically were thought to exert their effects by modulating monoamines (e.g., serotonin, norepinephrine, dopamine) in the central nervous system, include monoamine oxidase inhibitors, tricyclic and tetracyclic compounds, and selective monoamine reuptake inhibitors. While these antidepressants can be effective (Cipriani et al. 2018), several weeks of continuous treatment is typically required for them to exert a clinically significant therapeutic effect (Sinyor et al. 2010). Additionally, many patients are prone to symptom relapse (Fornaro et al. 2019, Sim et al. 2016) or may fail to respond to antidepressant treatment altogether (Gaynes et al. 2020, Trevino et al. 2014). Antidepressants also have undesirable side effects (Jakobsen et al. 2017) that contribute to treatment discontinuation in those who do respond (Demyttenaere et al. 2001). Unfortunately, a lack of effective treatment can lead to negative coping strategies, like substance abuse, deliberate self-harm, and at worst, attempted or completed suicide. For these reasons, novel antidepressant treatment development is one of the most urgent clinical priorities that we face today.

While it is important to improve treatment options for depression, our understanding of depression pathophysiology is incomplete, and does not provide the strong empirical foundation upon which tangible treatment advances critically rely. This issue is due in part to an overreliance on incomplete mechanistic hypotheses of antidepressant action, which represents a major theoretical barrier to therapeutic innovation. Indeed, only a handful of new antidepressants have been approved by the US Food and Drug Administration (FDA) in the last several decades, and all but one, (*S*)-ketamine (esketamine; Spravato®), act on the monoaminergic system (Protti et al.

2020). Some of the recent impetus to investigate novel antidepressant mechanisms of action came from the discovery that subanesthetic (*R*,*S*)-ketamine (hereafter referred to as ketamine) has rapid antidepressant effects in patients with treatment-resistant major depression (Berman et al. 2000, Zarate et al. 2006a). Ketamine is thus the first pharmacotherapy proven to relieve depression within hours of a single administration, and, no less, in patients who are unresponsive to existing forms of treatment. The antidepressant efficacy of ketamine not only led to the FDA's eventual approval of the (*S*)-ketamine stereoisomer for treatment-resistant major depression (Zheng et al. 2020) but also prompted extensive investigation into the mechanisms that could account for its unique antidepressant effects. Early work focused on ketamine's anesthetic mechanism of action as an *N*-methyl-D-aspartate glutamate receptor (NMDAR) antagonist, whereas more recent studies have examined its NMDAR-inhibition-independent actions on synaptic transmission and behavior. Collectively, these studies have provided insight into the cellular processes that appear to be necessary for ketamine to exert its antidepressant effects. However, a better understanding of rapid antidepressant mechanisms of action is still needed to develop novel treatments with improved tolerability, as ketamine has dissociative properties and abuse potential that limit its use for psychiatric indications.

While the ketamine discovery provides hope for patients suffering from treatment-resistant major depression, it is also accompanied by many unknowns that require further investigation. For instance, why are some, but not all, treatment-resistant patients responsive to ketamine, and does it have similar efficacy in patients who do respond to traditional antidepressants? Why do symptoms tend to reemerge within a few weeks of treatment, even in those who respond optimally to a single administration of ketamine? Are there unique side effects associated with long-term ketamine treatment in patients who require repeated infusions to prevent symptom relapse? Can the antidepressant effects of ketamine be extended by other antidepressant interventions, or is it possible that ketamine can enhance the efficacy of existing treatments? And probably the most actively discussed: What is the mechanism that accounts for the antidepressant actions of ketamine, and is this functionally separable from those underlying its side effects? To date, there has been a significant effort to prove the legitimacy of the NMDAR-inhibition hypothesis in the antidepressant actions of ketamine, but it is also important to consider whether theoretical advancement is stifled by the lack of rigorous attempts to falsify it. Indeed, an equivalent burden of proof was needed before it was fully appreciated that depression is not simply due to an imbalance of monoamines; it was the persistence of this belief, however, that led monoamine-based treatments to dominate the drug market for several decades. Moving forward, it is incumbent upon us to investigate rapid antidepressant mechanisms of action in more depth, and with the precision and scrutiny needed to readily make tangible improvements in depression treatment. We discuss this by providing an overview of the antidepressant treatment advances that preceded the ketamine discovery, and then present current mechanistic hypotheses for how ketamine may be exerting its antidepressant effects. We also discuss the impact this knowledge has had on current drug discovery efforts and provide recommendations for translating mechanistic insights into clinical application.

HISTORICAL PERSPECTIVE ON ANTIDEPRESSANT TREATMENT ADVANCES

First-Generation Antidepressants

The first medication generally considered to have significant antidepressant efficacy was the hydrazine compound iproniazid, which is an isopropyl derivative of the antituberculosis agent isoniazid. In tuberculosis patients, iproniazid led to a marked improvement in appetite, emotional

well-being, and quality of sleep (Salzer & Lurie 1953, Smith 1953). Subsequent studies showed that iproniazid reduced symptoms of depression in nontuberculosis patients, which contributed to its use as an antidepressant (Ayd 1957, Crane 1957, Loomer et al. 1957). More than 600,000 depressed patients were estimated to have been prescribed iproniazid during its first year in psychiatric use (Maxwell & Eckhardt 1990), attesting to how pervasive the need for depression pharmacotherapy was at that time.

The enhanced antidepressant efficacy of iproniazid relative to isoniazid provided some initial insight into its possible antidepressant mechanism of action. Iproniazid is chemically distinguished from isoniazid by its isopropyl moiety, which enhances its potency to inhibit monoamine oxidase (MAO) (Smith et al. 1963, Zeller & Barsky 1952, Zeller et al. 1955). MAO is an enzyme that catalyzes the oxidative deamination of amine-containing molecules. Among its substrates are the monoamines serotonin, norepinephrine, and dopamine, which act as prominent neuromodulators in the central nervous system. Consistent with its actions as an MAO inhibitor (MAOI), iproniazid administration was found to acutely enhance the concentration of endogenous brain serotonin in mice, rats, and rabbits, whereas peripheral serotonin levels remained unchanged (Udenfriend et al. 1957). Additionally, iproniazid treatment led to greater enhancements in serotonin than in norepinephrine, specifically in brainstem nuclei from which serotonergic and noradrenergic projections originate (Shore & Brodie 1958). This finding suggested that serotonin might be implicated in iproniazid's antidepressant actions. Indeed, the antihypertensive agent reserpine was known to have prodepressive properties (Freis 1954), and was thought to exert its effects, in part, by reducing serotonin release (Sheppard & Zimmerman 1960, Shore et al. 1957) by blocking its transport into synaptic vesicles (Stitzel 1976). While at that time serotonin had only recently been discovered in the central nervous system (Twarog & Page 1953, Whitaker-Azmitia 1999), these data provided circumstantial evidence that depression may be caused by an imbalance of serotonin and, possibly, other brain-localized monoamines (Brodie & Shore 1957, Woolley & Shaw 1954). Although the use of iproniazid was eventually discontinued because of its hepatotoxicity, these findings formed the basis of future drug development efforts (López-Muñoz et al. 2007), which led to the synthesis of additional, more selective MAOIs that were later used as antidepressants (**Figure 1**). Importantly, these discoveries also revealed depression to be a neuropsychiatric disease that is partly physiological in its origin.

The role of monoamines in the antidepressant actions of MAOIs gained additional traction when the antidepressant properties of monoamine reuptake inhibitors were discovered. As with iproniazid, this discovery arose through clinical happenstance: The tricyclic monoamine reuptake inhibitor imipramine was being investigated as a potential neuroleptic, but instead was found to improve depressive symptoms (Kuhn 1958, Lehmann et al. 1958). This discovery led to the development of other imipramine-like tricyclic antidepressants (TCAs) (Fangmann et al. 2008), which, together with MAOIs, represent the first generation of antidepressants (**Figure 1**). One of the major distinctions between these two drug classes is that MAOIs enhance the concentration of monoamines by blocking their degradation, whereas TCAs prevent them from being transported back into the presynaptic terminal once they have been released. Thus, while MAOIs increase the availability of monoamines, TCAs prolong the actions of monoamines during ongoing activity by blocking their reuptake from the synaptic cleft. While some TCAs, such as amitriptyline and imipramine, impose a greater inhibition of serotonin reuptake (Carlsson et al. 1969, Lidbrink et al. 1971), others, like nortriptyline and desipramine, exert more potent effects on the reuptake of norepinephrine (Bunney & Davis 1965, Carlsson 1970). These observations contributed to the monoamine hypothesis of depression (Bunney & Davis 1965, Schildkraut 1965), which predicts a causative relationship between decreased monoaminergic signaling and depression susceptibility (for a detailed discussion, see Hirschfeld 2000).

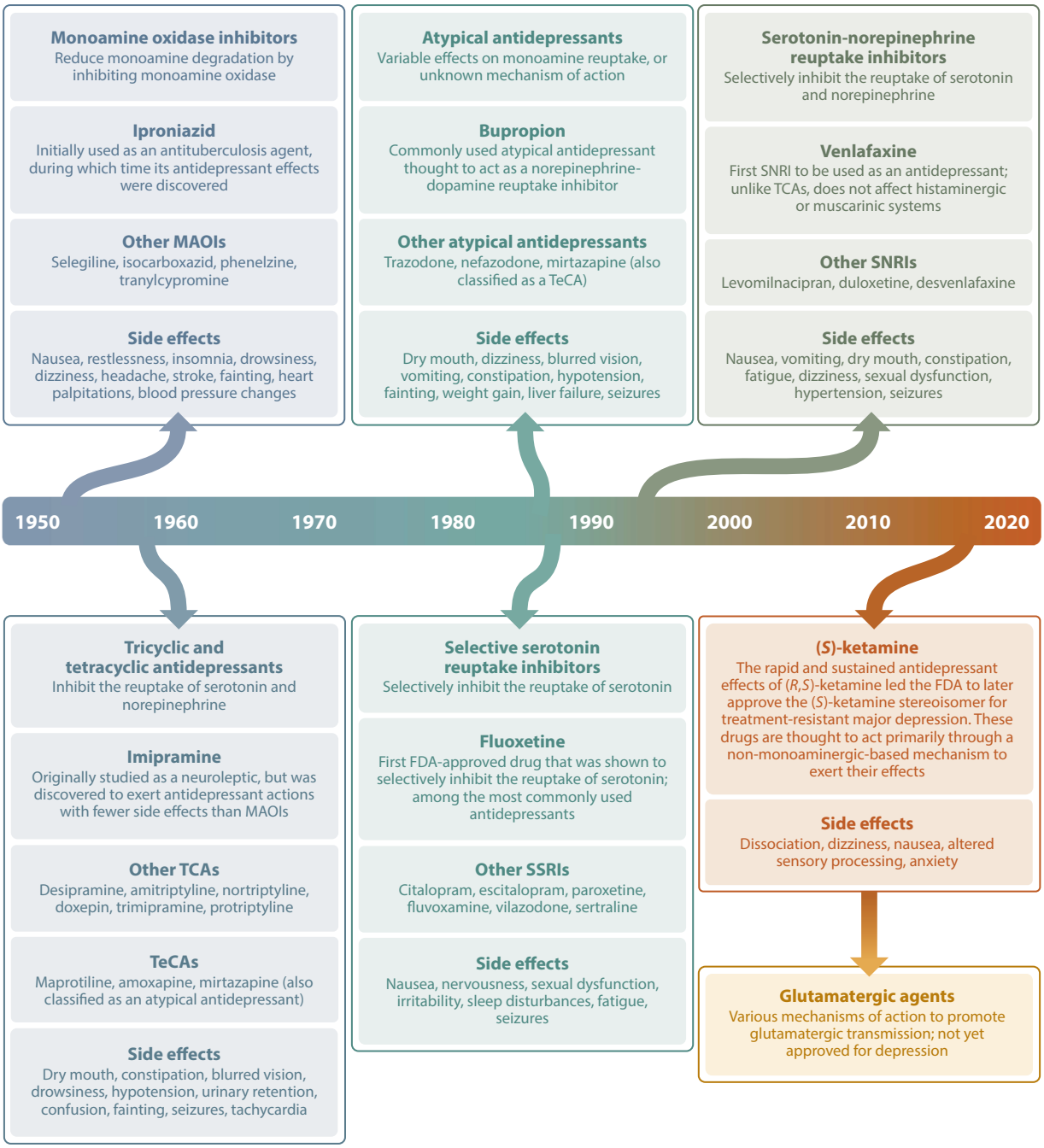

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Figure 1 (*Figure appears on preceding page*)

Antidepressant treatment advances receiving FDA approval for major depression. Iproniazid, a monoamine oxidase inhibitor, was initially used in the treatment of tuberculosis. It was soon discovered that iproniazid possesses antidepressant properties, which were attributed to its actions as an MAOI. Iproniazid is often credited for having brought pharmacotherapeutic strategies to the forefront of depression treatment. Later, it was discovered that the chlorpromazine derivative, imipramine, also has antidepressant properties. Imipramine is a TCA that is thought to exert its effects by reducing monoamine reuptake. Thus, while MAOIs reduce monoamine degradation, TCAs help maintain them in the synapse after having been released. These observations led to the hypothesis that monoamines have a direct and causal role in the etiology of depression, and formed the basis of future drug design. While additional MAOIs and TCAs entered the drug market, their use was associated with adverse effects that warranted more specific therapeutic approaches. Upon investigating the specificity of other chlorpromazine derivatives, it was discovered that fluoxetine (then, LY110140) acts as an SSRI, and thus it became the first SSRI to be implemented as an antidepressant. The improved side effect profile of SSRIs made them a favored alternative to the first generation of antidepressants (*blue*) and spurred the use and development of other monoamine-selective agents that comprised the second generation of antidepressants (*teal* and *green*) that include SNRIs. However, while these advances represent a major turning point in the treatment of depression, monoaminergic-based treatments require several weeks of continuous administration to take effect, whereas many patients fail to respond altogether. The possibility for a rapid-acting therapeutic option came at the turn of the century when it was discovered that (*R*,*S*)-ketamine rapidly alleviates symptoms of depression within hours of a single subanesthetic dose administration (Berman et al. 2000). It was later shown that the rapid antidepressant effects of ketamine extend to patients who do not respond to traditional monoaminergic-based pharmacotherapies (Zarate et al. 2006a). This finding led the FDA to later approve the (*S*)-ketamine stereoisomer for treatment-resistant major depression in 2019 (*orange*). Drug discovery efforts thus shifted from monoaminergic-based mechanisms of action to those that modulate glutamatergic transmission (*gold*). While the success of ketamine as an antidepressant spurred active investigation into glutamatergic-based mechanisms of action, other NMDAR antagonists and glutamatergic-based compounds have not yet shown the rapid and sustained antidepressant properties of ketamine (Newport et al. 2015). Abbreviations: FDA, US Food and Drug Administration; MAOI, monoamine oxidase inhibitor; NMDAR, *N*-methyl-D-aspartate glutamate receptor; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; TeCA, tetracyclic antidepressant.

Second-Generation Antidepressants

The advent of first-generation antidepressants was a significant advance for the treatment of depression and helped prompt investigation into its underlying pathophysiology. However, MAOIs have life-threatening side effects, such as hypertension, nephrotoxicity, hepatic necrosis, and tyramine-precipitated intracranial hemorrhage. TCAs also have an unfavorable (if less severe) side effect profile (e.g., tachycardia, dizziness, hypotension, blurred vision, memory impairment, drowsiness) due to their tendency to also inhibit muscarinic and histaminergic receptors (Otte et al. 2016). Unfortunately, both classes of drugs have been used as a means of committing suicide, which contributes to their overall risk profile. It was therefore necessary to identify novel compounds that could exert antidepressant effects with improved biological specificity and safety. Since imipramine had been previously derived from the diphenhydramine congener chlorpromazine, diphenhydramine analogs were synthesized to identify derivatives that had the therapeutic efficacy of imipramine but that lacked its undesirable side effects (Molloy et al. 1994). Indeed, it was known that serotonin and norepinephrine were present in distinct and separable neuronal populations with distinct projections, and thus it was hypothesized that more precise pharmacological approaches could be used to target a single monoamine system. This led to subsequent investigation into the selectivity with which diphenhydramine-derived compounds could inhibit monoamine reuptake (Wong et al. 2005). Initial studies focused on serotonin because of its then purported role in depression (Schildkraut 1965, Weil-Malherbe & Szara 1971), and because there was at least some evidence implicating enhanced serotonin in the antidepressant actions of MAOIs (Coppen et al. 1963, Shore & Brodie 1958) and TCAs (Carlsson 1970, Carlsson et al. 1968). It was discovered that one of these compounds, LY110140, acted as a selective serotonin reuptake inhibitor (SSRI) (Wong et al. 1974), which subsequently became the first SSRI to be approved as an antidepressant, named fluoxetine (Prozac®) (Wong et al. 2005).

SSRIs are distinguished from TCAs mainly by their higher potency and selectivity to inhibit serotonin reuptake. The antidepressant efficacy of SSRIs was taken as further support for the unique contribution of serotonin in the actions of antidepressant compounds. However, while some first-generation antidepressants showed modest specificity to enhance serotonin, pharmacological observations seemed to collectively suggest that norepinephrine (and to a lesser extent, dopamine) could still have a prominent role in the pathophysiology or heterogeneity of depression (Bunney & Davis 1965, Maas 1978, Schildkraut 1965). Given that many patients failed to respond to SSRIs, it was hypothesized that some patients may belong to a biochemical subgroup that responds more favorably to compounds that preferentially block the reuptake of norepinephrine (Nolen et al. 1988, Nyström & Hällström 1987). While crossover studies, in which both drug classes are sequentially administered within each patient, failed to show convincing support for such specificity in drug response (Nolen et al. 1988, Nyström & Hällström 1987), this idea contributed in part to the development of compounds that differentially target the serotonin, norepinephrine, and dopamine reuptake transporters. These include selective norepinephrine-, serotonin-norepinephrine-, and dopamine-norepinephrine reuptake inhibitors, as well as serotonin partial agonist/reuptake inhibitors, which, together with SSRIs, represent the second generation of antidepressants (**Figure 1**). Today, these drugs are the first line of treatment for depression and can be prescribed in combination when patients do not fully remit with monotherapy.

Limitations of Existing Pharmacotherapies

While second-generation antidepressants have less off-target effects than do first-generation antidepressants, they still have adverse properties that affect their tolerability (e.g., nausea, irritability, insomnia, sexual dysfunction) (Jakobsen et al. 2017, Papakostas 2008). Furthermore, monoamine antidepressants as a whole typically require several weeks of continuous treatment to exert their full therapeutic effect (Sinyor et al. 2010). Even so, many patients either fail to respond (Gaynes et al. 2020, Trevino et al. 2014) or experience symptom relapse (Fornaro et al. 2019, Sim et al. 2016), which suggests that traditional antidepressants are inadequate en bloc. There is also a lack of convincing evidence for the monoamine hypothesis, which is based on the assumption that monoamines have a direct and causal role in the etiology of depression itself. Predictions that extend from this hypothesis are that (*a*) a reduction in monoamines will increase depression susceptibility; (*b*) the extent of that reduction is associated with the severity of depression; (*c*) antidepressant response is contingent upon, and mirrors the time course of, monoamine restoration; and (*d*) symptom relapse is due to the reemergence of a deficit in monoamine levels. There has been a lack of convincing evidence in support of these predictions, 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 widely effective (Hirschfeld 2000).

While it is clear that monoaminergic synaptic transmission is involved in the affective, behavioral, and cognitive domains of depression (Delgado 2000, Jacobsen et al. 2012, Schildkraut 1965), monoamines primarily serve a neuromodulatory role, and thus likely impinge on other physiological processes that are of more direct etiological significance to the onset and maintenance of depression (Heninger et al. 1996, Hirschfeld 2000, Lee & Han 2019). Even during an era focused on monoamines, it seemed probable that—while traditional antidepressants do increase monoamines—their therapeutic efficacy may be due to other molecular and/or cellular adaptations that emerge over time, and are revealed after chronic treatment (Hyman & Nestler 1996). Indeed, changes in neural connectivity were becoming increasingly implicated in depression, so it was feasible to consider that antidepressants were capable of producing structural change over several weeks of continuous treatment (Duman 1998, Manji et al. 2001). The mechanisms underlying

this process could form the basis of rational drug design (Duman et al. 1997), aimed at strengthening synapses in a lasting and therapeutically beneficial way (Gould et al. 2019, Thompson et al. 2015). Despite these early propositions and the limitations of existing treatments, virtually all antidepressants that were subsequently approved by the FDA act primarily on the monoaminergic system (**Figure 1**); the assumption that antidepressants exert their effects by restoring deficits in monoamine signaling led drug discovery and development efforts to focus almost exclusively on this mechanism of action. Ultimately, this narrow focus contributed to the lack of new treatment modalities since the initial discovery of iproniazid's antidepressant properties in the 1950s. It should be recognized, however, that the advent of traditional antidepressants is responsible for many clinical and theoretical advances that have been essential, such as recognizing depression as a biological disease, triggering active investigation into its pathophysiology, and providing different treatment options for individuals suffering from depression.

RAPID ANTIDEPRESSANT MECHANISMS OF ACTION

Ketamine as a Prototype Rapid-Acting Antidepressant

Ketamine is a phencyclidine-derived dissociative anesthetic that was synthesized in an effort to minimize the length of anesthetic action and to reduce the severity of postanesthetic emergence delirium (Domino 2010, Domino et al. 1965). Ketamine also augments sympathetic processes that alter cardiovascular function (although to a lesser extent than phencyclidine), which can lead to tachycardia and hypertension (Zanos et al. 2018a). It was initially thought that ketamine exerts these sympathomimetic effects by inhibiting norepinephrine reuptake (Liebe et al. 2017, Miletich et al. 1973). While it was later shown that ketamine lacks affinity for monoamine transporters (Can et al. 2016), these initial hypotheses led Sofia & Harakal (1975) to test whether these secondary sympathomimetic effects confer ketamine with preclinical antidepressant-like actions. At the time, preclinical antidepressant screening procedures were based on existing mechanistic hypotheses that an increase in monoamine release is necessary and sufficient for antidepressant action. Antidepressant efficacy was thus established to the extent that a compound could reverse the physiological effects of monoamine depletion (i.e., ptosis, hypothermia) or exacerbate the physiological effects of an increase in monoamines (i.e., tremor, toxicity). While orally administered ketamine appeared to exert these antidepressant-like actions in mice and rats, such effects were relatively modest when compared to the TCA imipramine (Sofia & Harakal 1975). Despite preliminary evidence that subanesthetic ketamine could facilitate psychotherapy and relieve symptoms of psychiatric disease (Khorramzadeh & Lotfy 1973), its clinical antidepressant potential was not further investigated at that time.

While the antidepressant effects of ketamine awaited discovery, its dose-dependent psychotomimetic properties gained it attention as a pharmacological strategy to understand the pathophysiology of psychosis (Krystal et al. 1994). Subanesthetic ketamine also has euphoric properties and influences sensory perception—features that promote its recreational use and abuse liability (Zanos et al. 2018a). These properties appear to have initially overshadowed anecdotal hints that subanesthetic ketamine could attenuate symptoms of depression, and possibly with greater efficacy than existing antidepressants (Domino 2010). The clinical antidepressant effects of ketamine were first reported in a double-blind, placebo-controlled, randomized clinical trial by Berman et al. (2000), which revealed that ketamine could rapidly alleviate symptoms of depression. When a subanesthetic dose of ketamine (0.5 mg/kg) was infused intravenously over a period of 40 min, depression rating scores were reduced within 4 h, and this effect lasted for up to 3 days postadministration (Berman et al. 2000). Consistent with previous reports (Krystal

et al. 1994), the authors demonstrated that the psychotomimetic properties of subanesthetic ketamine, which primarily occur during the time of the infusion (Berman et al. 2000, Krystal et al. 2019), are absent by 4 h posttreatment, and are thus temporally separable from the drug's antidepressant effects. Zarate et al. (2006a) later examined the antidepressant actions of ketamine in treatment-resistant depressed patients, who, on average, had previously failed six antidepressant trials. Despite the length and severity of their depression, ketamine led to a rapid reduction of symptoms in the majority of patients, which lasted for up to 1 week postadministration (Zarate et al. 2006a). Importantly, the antidepressant effect of ketamine in treatment-resistant depressed patients maintains a significant separation from placebo when an active anesthetic control is used under blinded conditions (Murrough et al. 2013). The rapid and sustained antidepressant effects of ketamine have now been reported in a number of clinical studies (Kryst et al. 2020), which have prompted specialized treatment clinics to open in private and academic sectors (Wilkinson et al. 2017).

Ketamine Is an *N*-methyl-D-aspartate Receptor Antagonist

Two decades after it was initially synthesized, it was discovered that ketamine acts as a noncompetitive NMDAR antagonist (Anis et al. 1983, Lodge et al. 1983). NMDARs belong to the ionotropic class of glutamate receptors (i.e., those that allow ions to flow across the cellular membrane upon glutamate binding) that specialize in fast synaptic transmission, which also includes α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and kainate receptors. NMDARs are unique 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). NMDARs are more sensitive than AMPARs to glutamate binding ($EC_{50} = 0.5–3$ versus 3–560 μM, respectively) and are also slower to desensitize (τ = 59–2,000 versus 1–10 ms, respectively) (Traynelis et al. 2010). These biophysical properties make NMDARs an essential transducer of intracellular calcium, which acts as a second messenger to modulate the efficacy of synaptic transmission and many other cellular processes. While prolonged elevations of intracellular calcium can be excitotoxic, extracellular magnesium helps to oppose calcium-dependent excitotoxicity by occluding the channel pore at negative membrane potentials (i.e., NMDARs are not permeable to magnesium as they are to sodium, potassium, and calcium). The presence of magnesium within the channel confers NMDARs with a voltage-dependence of activation, such that a depolarization threshold needs to be reached before NMDARs will become activated by glutamate binding. That is, the interior of the cell must be sufficiently positive from other ongoing ionic fluxes for NMDARs to themselves pass ionic current when glutamate is bound; this is due to electrostatic repulsion of magnesium from within the channel. Ketamine is a use-dependent open channel blocker that competes with magnesium binding deep within the channel pore, where it remains bound when the receptor transitions to a closed conformation (Glasgow et al. 2018, MacDonald et al. 1987). Its rapid off-rate upon channel reopening is thought to confer it with a shorter duration of anesthetic action than its structural analog, phencyclidine (Domino 2010, Domino et al. 1965).

While ketamine is believed to exert its anesthetic effects by blocking NMDAR-mediated synaptic transmission (Zanos et al. 2018a), some early preclinical data suggested that NMDAR antagonism could have antidepressant potential (Trullas & Skolnick 1990). In contrast to the early monoamine-based screening procedures described above (Sofia & Harakal 1975), increases in escape-directed behavior in forced swim test (Porsolt et al. 1977) and tail suspension test (Steru et al. 1985) were later considered a more accurate predictor of antidepressant action. With this approach, NMDAR antagonists were found to dose-dependently reduce behavioral

despair, and to a degree that was comparable to that of traditional antidepressants with known clinical efficacy (Trullas & Skolnick 1990). Similar results were later observed following ketamine administration (e.g., Chaturvedi et al. 1999, Maeng et al. 2008, Mantovani et al. 2003), which provided circumstantial support for the role of NMDAR inhibition in its clinical antidepressant actions (Berman et al. 2000, Zarate et al. 2006a).

Ketamine Enhances Glutamatergic Transmission and Promotes Synaptogenesis

There has been a significant effort to understand how blocking NMDAR transmission could account for the unique antidepressant properties of ketamine. Some initial insight came from a preclinical study by Moghaddam et al. (1997), which revealed that a single administration of ketamine evokes a transient dose-dependent increase in the concentration of extrasynaptic glutamate in the medial prefrontal cortex in vivo. This study showed that subanesthetic ketamine leads to a net increase in cortical excitation—a finding in contrast to what may have been predicted of a compound that blocks NMDAR-mediated excitatory synaptic current. The authors proposed that lower doses of ketamine may promote glutamatergic transmission by preferentially attenuating the inhibitory tone that typically impinges on the excitatory (pyramidal) neurons in this region. This occurs because inhibitory interneurons are normally (tonically) active, which places excitatory pyramidal neurons in a state of functional quiescence. Since ketamine is a blocker of open/active NMDARs, it is predicted that ketamine would more readily inhibit tonically active inhibitory interneurons. Consistent with this prediction, Homayoun & Moghaddam (2007) showed that the NMDAR antagonist dizocilpine (MK-801) reduces the firing rate of putative fast-spiking inhibitory interneurons, which disinhibits cortical pyramidal neuron activity. A similar disinhibition phenomenon has been observed with ketamine in ex vivo hippocampal slice preparations (Widman & McMahon 2018), suggesting that these effects may extend to other brain regions that are involved in mood regulation. Overall, this work contributed to the hypothesis that an acute disinhibition of glutamate release could restore the integrity of synaptic connections that are compromised in depression (Duman 2014, Li et al. 2010). That is, ketamine may exert its antidepressant actions by strengthening the efficacy of synaptic transmission, which resembles a plasticity-related process that is made possible by the brain's intrinsic ability to constantly undergo change at the level of individual synapses. Alternatively, it is possible that ketamine exerts its effects by decreasing excitation in regions whose activity promotes depressive-like phenotypes, as opposed to increasing the activity of euthymic-related regions. Indeed, ketamine has been reported to block NMDAR-mediated burst firing in the lateral habenula, where excess activity has been associated with behavioral despair and anhedonia (Yang et al. 2018). In contrast to the glutamatergic disinhibition hypothesis, these studies support a model in which monoaminergic circuits are rapidly disinhibited by ketamine's inhibition of lateral habenula activity (Yang et al. 2018).

The disinhibition hypothesis predicts that the activational balance in these regions is acutely shifted toward excitatory, and in particular AMPAR, transmission, which could underlie its antidepressant efficacy. In support of this hypothesis, the preclinical antidepressant-like actions of ketamine require AMPAR activity as they are blocked by pretreatment with an AMPAR antagonist (Maeng et al. 2008). Thus, ketamine's effects may rely on cellular processes that are at, or downstream of, the AMPAR itself. There are a number of possibilities for what these processes could be, as the AMPAR is a highly abundant unit of fast synaptic transmission that serves many diverse physiological roles throughout the brain. Unlike NMDARs, however, AMPARs faithfully pass excitatory current upon glutamate binding as they do not have a voltage-dependence of activation. As a result, AMPARs are capable of gating the voltage-dependent processes that dictate the extent of calcium influx. This is relevant to the AMPAR-dependent antidepressant actions of ketamine because calcium acts as a second messenger to regulate synaptic transmission,

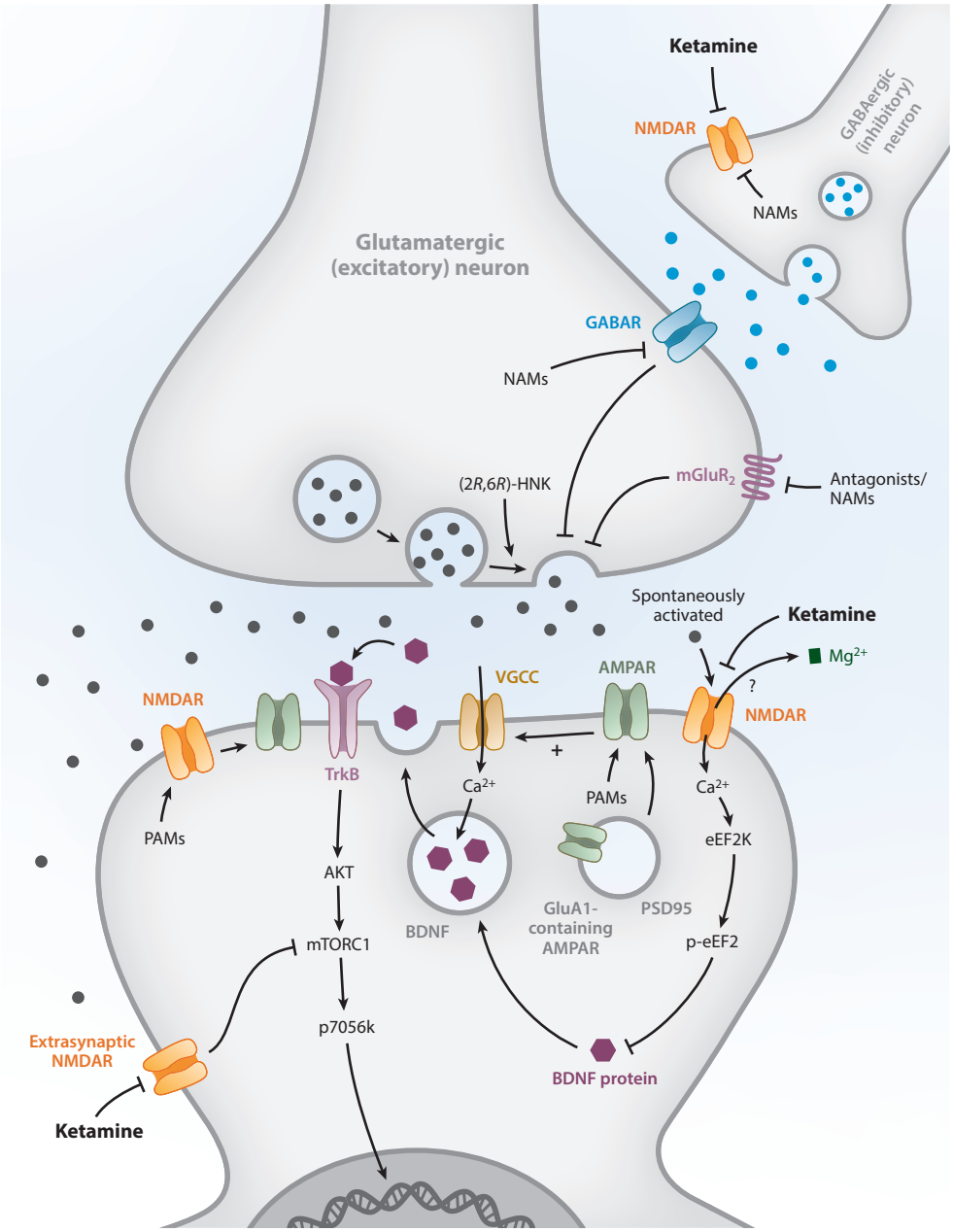
membrane excitability, gene expression, and synaptogenesis (Kawamoto et al. 2012, Redmond & Ghosh 2005). Indeed, the AMPAR-mediated activity-dependent rise in intracellular calcium triggers the release of brain-derived neurotrophic factor (BDNF) (Jourdi et al. 2009, Zhang & Lipton 1999), which is required for ketamine to exert its antidepressant-like effects (Lepack et al. 2015, 2016; Li et al. 2010; Liu et al. 2012). BDNF is a soluble protein that promotes neuronal survival and can contribute to the formation of new synaptic connections (i.e., synaptogenesis). BDNF accomplishes this by binding to its receptor, tropomyosin receptor kinase B (TrkB), which recruits the intracellular signaling molecules that are needed to execute structural changes within the cell. For instance, BDNF-TrkB activity can orchestrate the formation of mechanistic target of rapamycin complex 1 (mTORC1), which is a specialized protein complex that regulates protein synthesis and cell proliferation (Jourdi et al. 2009, Zhang & Lipton 1999). Interestingly, ketamine exerts its antidepressant-like effects by initiating synaptogenesis in an AMPAR/BDNF/mTORC1-dependent manner (Lepack et al. 2015, 2016; Li et al. 2010; Liu et al. 2012), which involves recruitment of the mTORC1 effector, eukaryotic translation initiation factor 4E–binding protein 2 (4E-BP2) (Aguilar-Valles et al. 2020), and is thought to evoke a lasting increase in the efficacy of synaptic transmission (Deyama & Duman 2020, Duman et al. 2021). The sustained actions of ketamine also appear to require AMPAR activity, as its preclinical antidepressant-like effects are blocked by AMPAR inhibition immediately before testing, 24 h after treatment (Koike & Chaki 2014, Zanos et al. 2016). Thus, the long-lasting antidepressant effects of ketamine may be due to sustained adaptations in the number or function of AMPARs—a common mechanism by which synaptic plasticity has been shown to manifest (Huganir & Nicoll 2013). Indeed, ketamine increases the expression of AMPARs containing the GluA1 subunit 24 h after systemic administration (Adaikkan et al. 2018, Li et al. 2010, Yamada & Jinno 2019, Zanos et al. 2016) but not 1 h after treatment (Li et al. 2010, Zanos et al. 2016). These findings suggest that the acute AMPAR-activity-dependent actions of ketamine on BDNF signaling initiate synaptogenic processes that involve sustained increases in AMPAR expression (**Figure 2**). This process may underlie the ability of ketamine to restore synaptic deficits following chronic stress exposure (Li et al. 2011)—for instance, by promoting dendritic outgrowth in regions like the medial prefrontal cortex (Li et al. 2010). Consistent with this hypothesis, longitudinal observations of cortical spine formation in vivo have revealed that the emergence of depressive-like phenotypes in mice is associated with reduced synaptic integrity, and that ketamine reverses these deficits in part through targeted dendritic spine remodeling at those synapses (Moda-Sava et al. 2019).

While glutamatergic disinhibition could explain the acute antidepressant actions of ketamine, an alternative explanation is that ketamine exerts its effects through a BDNF-dependent process that is independent of disinhibition (**Figure 2**). One such hypothesis has been proposed by Autry et al. (2011), who suggested that ketamine promotes BDNF synthesis by blocking NMDAR activation by spontaneously released glutamate—that is, glutamate released stochastically from the presynaptic terminal and not synchronously evoked by an increase in presynaptic activity. Spontaneously released glutamate prevents the eukaryotic elongation factor 2 (eEF2) kinase–dependent inhibition of eEF2 activity, which normally suppresses BDNF synthesis under resting conditions (Kavalali & Monteggia 2020). This marks an important distinction from the disinhibition hypothesis in predicting that ketamine exerts its effects by blocking synaptic NMDARs on pyramidal neurons and not by triggering activity-dependent BDNF release or mTORC1 signaling (Autry et al. 2011). An additional alternative hypothesis has been proposed by Miller et al. (2014), who suggested that ketamine selectively inhibits GluN2B-containing NMDARs that are preferentially activated by ambient glutamate at extrasynaptic sites (i.e., outside of the synapse). However, there is evidence that ketamine does not functionally inhibit NMDARs with subunit specificity (Dravid et al. 2007), and although GluN2B-specific antagonists exert preclinical antidepressant-like

actions (Li et al. 2010, Maeng et al. 2008), they have failed to show antidepressant effects in clinical studies (Gould et al. 2019).

The Conflicting Role of *N*-methyl-D-aspartate Receptor Inhibition in the Antidepressant Actions of Ketamine

A major assumption of the hypotheses described above is that NMDAR blockade is essential to the antidepressant actions of ketamine. However, in the same year in which it was discovered that



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Putative rapid antidepressant mechanisms of action. Ketamine is an NMDAR antagonist that acts as an anesthetic at higher doses than those effective for depression (Zanos et al. 2018a). At subanesthetic doses, ketamine possesses acute dissociative and psychotomimetic effects, which are followed by its rapid antidepressant properties (occurring within hours) that are sustained for several days to weeks. A few hypotheses have been set forth to explain how ketamine exerts its antidepressant actions. Subanesthetic doses of ketamine lead to an acute increase in extracellular glutamate, which is proposed to shift the activational balance toward increased glutamatergic versus inhibitory mediated synaptic transmission. It is proposed that this increase in glutamate release is due to ketamine's preferential blockade of NMDARs localized to inhibitory interneurons that typically decrease activity of excitatory transmission (Duman 2014). Such disinhibition would result in an increase in AMPAR-mediated synaptic transmission, leading to the activity-dependent release of BDNF. BDNF-TrkB-dependent recruitment of mTORC1 subsequently increases the synthesis of synaptic proteins that can enhance the efficacy of synaptic transmission. For instance, this process may underlie the synaptogenic properties of ketamine, which involve an upregulation of GluA1-containing AMPARs in the PSD. Alternatively, ketamine has been proposed to block synaptic NMDARs that respond preferentially to spontaneously released glutamate at rest (Kavalali & Monteggia 2020), though magnesium typically occludes the NMDAR channel pore at negative membrane potentials. This blockade is proposed to remove the eukaryotic elongation factor 2–mediated inhibition of BDNF synthesis, a process that is independent of mTORC1 activity. Ketamine may also exert its effects by selectively inhibiting extrasynaptic NMDARs whose activity is associated with excitotoxicity through mTORC1 inhibition of protein synthesis (Miller et al. 2014). Lastly, ketamine is rapidly metabolized to a number of molecules, including hydroxynorketamines, which have been implicated in ketamine's rapid antidepressant mechanism of action (Highland et al. 2021). Ketamine metabolites, such as (2*R*,6*R*)-HNK, promote glutamate release independent of NMDAR blockade or glutamatergic network disinhibition (Riggs et al. 2020), possibly through a mechanism downstream of $mGluR_2$ activity (Zanos et al. 2019b). Other compounds that manifest ketamine-like preclinical antidepressant-relevant actions are proposed to exert similar synaptic effects, but through mechanisms that are distinct from NMDAR inhibition. NMDAR-PAMs promote NMDAR activity upon glutamate binding, which induces the activity-dependent release of BDNF. GABAR-NAMs decrease tonic inhibition of glutamatergic neurons, while $mGluR_2$ antagonists attenuate $mGluR_2$-dependent inhibition of glutamate vesicle release. Abbreviations: AKT, protein kinase B; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF, brain-derived neurotrophic factor; eEF2K, eukaryotic elongation factor 2 kinase; GABAR, γ-aminobutyric acid receptor; HNK, hydroxynorketamine; $mGluR_2$, metabotropic glutamate receptor 2; mTORC1, mechanistic target of rapamycin complex 1; NAM, negative allosteric modulator; NMDAR, *N*-methyl-D-aspartate glutamate receptor; PAM, positive allosteric modulator; p-eEF2, phosphorylated eukaryotic elongation factor 2; PSD, postsynaptic density; TrkB, tropomyosin receptor kinase B; VGCC, voltage-gated calcium channel.

ketamine relieves treatment-resistant major depression (Zarate et al. 2006a), it also was shown that the NMDAR inhibitor memantine lacks clinical antidepressant effects, even after 7 weeks of continuous treatment (Zarate et al. 2006b). Furthermore, a meta-analysis of six double-blind, randomized, placebo-controlled trials revealed that memantine is not superior to placebo in antidepressant efficacy (Kishi et al. 2017). While memantine functionally inhibits the NMDAR comparably to ketamine, it is possible that modest differences in its trapping behavior [71% versus 86%, respectively (Mealing et al. 1999)] or pharmacokinetic properties could account for its lack of antidepressant effects. However, even high-affinity NMDAR channel blockers do not show the same preclinical antidepressant-like actions of ketamine (e.g., Autry et al. 2011, Maeng et al. 2008, Zanos et al. 2016), nor has any NMDAR antagonist that has been tested clinically (Gould et al. 2019). It is thought that other NMDAR antagonists lack antidepressant effects because they inhibit the NMDAR in a manner that is adequately distinct from that of ketamine (Duman et al. 2019), though this remains untested.

An alternative explanation is that NMDAR inhibition accounts for the anesthetic properties of ketamine but not its antidepressant effects (Zanos et al. 2018a). This possibility was raised by preclinical observations with the use of ketamine metabolites, including (2*R*,6*R*)-hydroxynorketamine

(HNK). Specifically, preventing the metabolism of ketamine to (2*R*,6*R*)-HNK blocks its antidepressant-like effects, whereas direct administration of (2*R*,6*R*)-HNK exerts antidepressant-like actions that are similar to those of ketamine. This is despite the fact that (2*R*,6*R*)-HNK has no measurable binding to, or functional effects on, the NMDAR at antidepressant-relevant concentrations (Fukumoto et al. 2019; Lumsden et al. 2019; Zanos et al. 2016, 2019a). Interestingly, (2*R*,6*R*)-HNK promotes glutamatergic synaptic transmission (Pham et al. 2018, Riggs et al. 2020, Zanos et al. 2016) and triggers an acute increase in BDNF release, which, as with ketamine, is required for it to exert its antidepressant-like effects (Fukumoto et al. 2019). A controversial view that has stemmed from these observations is that the antidepressant actions of ketamine depend, in part, on its metabolism to (2*R*,6*R*)-HNK. While it is possible that ketamine and its metabolites work synergistically, these findings suggest that NMDAR inhibition is not essential to the rapid antidepressant actions of ketamine.

ADVANCING ANTIDEPRESSANT TREATMENT DEVELOPMENT

Theoretical Advances and Treatment Developments Emerging from the Ketamine Discovery

While there remains debate regarding the role of NMDAR inhibition in the antidepressant actions of ketamine, there is compelling evidence that an AMPAR-dependent increase in BDNF-TrkB signaling is necessary for its antidepressant effects, independent of whether they are initiated by NMDAR blockade. Providing further support for this hypothesis, ketamine promotes dendritic arborization (i.e., structural growth at neuronal sites that receive synaptic input) of patient-derived induced pluripotent stem cell neurons, which is blocked by AMPAR-, BDNF-, and mTORC1 inhibition (Cavalleri et al. 2018, Collo et al. 2018). Interestingly, BDNF signaling has also been implicated in the antidepressant efficacy of monoaminergic-based treatments (Duman et al. 1997), which suggests that BDNF-dependent signaling could be a general antidepressant mechanism regardless of the initial pharmacological site of action. Consistent with the role of BDNF signaling, the delay in therapeutic onset of traditional antidepressants mirrors the slow time course along which adaptations in BDNF expression occur upon chronic administration (Deyama & Duman 2020, Duman et al. 2021). In a detailed review, Alt et al. (2006) proposed that by modulating AMPAR activity, one could potentially overcome the limitations of traditional antidepressants by rapidly triggering BDNF signaling—a process that could give rise to the "holy grail" of treatment, as they aptly described. It would now appear that ketamine is one such treatment, and indeed, ketamine has been shown to initiate synaptogenic processes through a rapid AMPAR-dependent increase in BDNF release (**Figure 2**). While the rapid increase in BDNF release is proposed to be related to the rapid antidepressant actions of ketamine, additional studies are needed to understand whether this process also accounts for the increased response rate among treatment-resistant depressed patients who respond favorably only to ketamine.

Additional mechanistic validity for the role of BDNF-TrkB signaling in general antidepressant actions comes from preclinical discoveries with compounds that do not inhibit the NMDAR to exert their antidepressant-like effects. These include NMDAR glycine site agonists and antagonists, NMDAR positive allosteric modulators (PAMs), γ-aminobutyric acid receptor negative allosteric modulators, metabotropic glutamate receptor 2 antagonists, muscarinic acetylcholine receptor antagonists, and ketamine metabolites, including (2*R*,6*R*)-HNK (**Figure 2**). Similar to ketamine, these compounds are proposed to exert their effects by promoting AMPAR-dependent BDNF-TrkB activity (Duman et al. 2019, Zanos et al. 2018b). For example, the NMDAR-PAM GLYX-13 (rapastinel) has shown rapid antidepressant-like preclinical effects in the absence of psychotomimetic properties (Donello et al. 2019, Moskal et al. 2014). Additionally, GLYX-13

administration leads to a BDNF-mediated increase in mTORC1 signaling (Kato et al. 2018, Liu et al. 2017) and promotes the efficacy of synaptic transmission (Zhang et al. 2008), similar to that induced by ketamine (Burgdorf et al. 2013). Consistent with these findings, a phase II clinical trial showed that GLYX-13 has rapid dose-dependent antidepressant effects in treatment-resistant depressed patients (Preskorn et al. 2015). Importantly, given that GLYX-13 acts as a PAM to directly potentiate NMDAR-mediated currents, it lacks the psychotomimetic and dissociative properties of ketamine that are associated with NMDAR inhibition (Preskorn et al. 2015). Overall, the literature suggests that antidepressants exert their effects through an increase in the efficacy of excitatory synaptic transmission, and that the onset of those effects depends in part on the time course along which adaptations in BDNF occur.

An important extension of this work is to translate preclinical discoveries and insights to advance clinical trials in humans. The drug discovery process involves preclinical identification and testing of new molecules that either target a cellular process that is implicated in the disease or that mimics the proposed mechanism of action of existing treatments. When such molecules (with favorable toxicology profiles) are identified, clinical trials are then conducted to determine their safety and efficacy in humans. As discussed above, it is also possible for an existing drug to be used off-label for a novel indication for which it has shown therapeutic efficacy. This was initially the case for iproniazid and imipramine: The off-label use of these drugs for the treatment of depression led to the development of novel MAOIs and TCAs that went on to dominate the drug market for several years. The proposed mechanism of these antidepressants contributed to rational drug design, which led to the development of SSRIs and other second-generation antidepressants that now represent the first line of treatment for depression. The discovery of ketamine's antidepressant effects has led to its off-label use for depression in specialized treatment clinics (Wilkinson et al. 2017) and has increased an interest in the use and development of NMDAR antagonists and other selective glutamatergic modulators for the treatment of depression. Investigational drugs that have reached clinical trials include nonsubunit-selective NMDAR antagonists, NR2B-selective NMDAR antagonists, NMDAR glycine site agonists, metabotropic glutamate receptor modulators, the NMDAR-PAM GLYX-13 (discussed above), and the ketamine enantiomers, (*S*)-ketamine and (*R*)-ketamine. To date, intranasal (*S*)-ketamine is the only one of these compounds to receive FDA approval for treatment-resistant major depression with concomitant administration of at least one traditional antidepressant (Zheng et al. 2020). Thus far, other NMDAR antagonists appear to lack the antidepressant efficacy of ketamine (Gould et al. 2019, Lener et al. 2017, Newport et al. 2015)—an observation that provides additional evidence that racemic ketamine and (*S*)-ketamine may not exert their antidepressant effects solely through NMDAR inhibition.

Considerations for Translating Basic Scientific Findings to Improve Clinical Care

While the ketamine discovery has led to significant clinical and theoretical advances, the field has not developed a novel rapid-acting antidepressant that has succeeded in late-stage clinical efficacy trials based on knowledge regarding ketamine's antidepressant mechanism of action. The conflicting evidence for the role of NMDAR inhibition in the antidepressant actions of ketamine only further complicates the situation; the preclinical data discussed herein suggest that NMDAR inhibition is not necessary for ketamine to exert its antidepressant effects, nor is it sufficient, as other NMDAR antagonists lack ketamine's clinical antidepressant properties. But it has become increasingly recognized that the AMPAR-dependent activation of BDNF-TrkB signaling may be the functional point of relevance (**Figure 2**) with regard to restoring the integrity of neural circuits in the depressed brain. With this in mind, it is critical to better understand how to safely initiate

these synaptic processes as a means of treatment, but without also inducing ketamine's euphoric, dissociative, or psychotomimetic effects.

To this end, mechanisms identified preclinically should be subjected to rigorous tests of their underlying assumptions, whereas proof-of-principle human studies should be used to readily verify the clinical relevance of those emergent hypotheses in depressed patients. But the success of clinical trials intrinsically relies on the actualization and precision of preclinical studies, and there is at least some evidence to suggest that improvements can be made in this area. As one example, many consider mTORC1 an essential component in the synaptogenic pathway that confers ketamine with its unique antidepressant properties (Duman et al. 2019, Zanos et al. 2018b). This conclusion is drawn in part from preclinical observations that rapamycin, an mTOR inhibitor, blocks the antidepressant-like effects of ketamine when infused directly into the rat medial prefrontal cortex (Li et al. 2010). As rapamycin is already in clinical use as an immunosuppressant, it serves as an available tool to address the role of mTORC1 in the clinical antidepressant actions of ketamine. However, Abdallah et al. (2020) recently reported that peripheral administration of rapamycin modestly extends the antidepressant effects of ketamine in depressed patients, contrary to what preclinical studies predicted. The method and route of administration may account for this discrepancy, as systemic administration of rapamycin fails to block the preclinical antidepressant-like effects of ketamine (Autry et al. 2011). While it is interesting to consider whether these results are due to a unique interaction between ketamine and immune function in depressed patients, discrepancies such as these serve to highlight the inherent challenge of translating basic scientific discoveries into the clinic.

These challenges are due in part to the complexity of the human physiological processes that we use preclinical approaches to make inferences about. Put simply, it is difficult to develop treatments based on a limited understanding of depression pathophysiology (and rapid antidepressant mechanisms of action, for that matter). Often, genetic, pharmacological, environmental, and circuit-level manipulations are used to induce behavioral states in model organisms that are thought to reflect depressive-like phenotypes. Our predictions about whether a compound or intervention will have antidepressant potential depend almost entirely on the robustness of these behavioral outputs. Additionally, behavioral tests that are sensitive to the actions of existing antidepressants are commonly used as an indicator of a clinically relevant antidepressant-like response. But while traditional antidepressants enhance rodents' escape-directed behavior (e.g., forced swimming), these assays are also sensitive to compounds that do not exert clinical antidepressant actions (thus, a negative result may be useful in ruling out compounds that do not have antidepressant efficacy, but the presence of a positive result does not fully/unequivocally predict antidepressant potential). Given that basic scientific insights have largely failed to produce novel compounds that are successful in late-stage clinical trials, it is worth considering whether preclinical depression-related assays lack the translational power to test the veracity of mechanistic hypotheses. While this is a multifaceted issue, it may be due in part to a tendency of the field to model new drug candidates after the therapeutic actions of existing monoaminergic-based antidepressants that are not widely effective themselves. However, recent efforts to develop compounds based on the proposed mechanism of action of ketamine (**Figure 2**) have surprisingly encountered similar issues when tested in the clinic. For instance, while GLYX-13 showed antidepressant potential in preclinical studies and early clinical trials, it failed to improve depressive symptoms compared to placebo in recent phase III studies (Kato & Duman 2020). Instances like these can shed doubt on the therapeutic potential of compounds under preclinical investigation and can minimize the perceived relevance of preclinical procedures in developing robust treatments for complex psychiatric conditions. This is a valid concern because preclinical approaches are inherently limited by the current understanding of how depression is thought to arise and manifest. Since the etiology and

pathophysiology of depression are not well understood, behavioral assays may be useful when addressing scientific questions that are relevant to underlying neural processes, but not necessarily for their perceived congruence with depression symptomatology in humans. Even so, using behavioral assays to investigate aberrant neural processes in depression will require better description of the underlying neurobiological substrates.

Ultimately, the antidepressant drug discovery process will benefit from a willingness to test major predictions of mechanistic hypotheses in the pursuit of truth, as opposed to confirming scientific beliefs with observations that are based on assumptions that have not been independently verified (for a detailed and engaging discussion of the scientific hypothesis and its utility, see Alger 2019). This approach can help thwart the tendency for epiphenomena to be overinterpreted as having causal influence on experimental end points and can improve the theoretical foundation upon which novel treatments are designed. This is of equal benefit to reverse- and forward-translational approaches, in which novel compounds are developed from existing antidepressant mechanisms of action versus a pathophysiological target, respectively. The field also stands to benefit from increasing interactions among basic scientists and clinicians. These can lead to improved preclinical disease models that are a more valid predictor of clinically relevant end points and can encourage the use of patient-derived, high-throughput preparations in preclinical studies. Additionally, clinicians can use translational methodologies to investigate potential biomarkers of antidepressant action. For instance, putative rapid-acting antidepressants have been shown to increase synchronization of neural activity in preclinical studies, which can be detected with cortical electrophysiological recording approaches as an increase in network oscillations in the gamma frequency range (Gould et al. 2019). The measurement of cortical electrophysiological recordings may serve as an effective biomarker for the preclinical actions of putative rapid-acting antidepressants (Fitzgerald & Watson 2019), as increases in gamma power have been consistently reported in patients receiving subanesthetic ketamine administration (Gilbert & Zarate 2020). While using electrophysiological approaches as an indicator of antidepressant action is not without limitation (McMillan & Muthukumaraswamy 2020), these studies suggest that these measures have significant translational relevance, and may provide an avenue for preclinical mechanistic discoveries to be tested clinically in depressed patients.

CONCLUSIONS

Depression therapeutics were essentially nonexistent when the antidepressant efficacy of iproniazid was discovered in the 1950s. Unfortunately, the individual and societal impact of depression has since been managed with medications that are suboptimal in their efficacy and latency of therapeutic action, and whose significant side effects lead to high rates of treatment discontinuation. This situation is in part a direct result of depression being poorly understood in terms of its etiology, pathophysiology, and clinical manifestation. While great strides have undoubtedly been made, an improved understanding of depression pathophysiology will help to support the development of more precise, mechanistically accurate treatments in the decades ahead.

Without question, the robust rapid and sustained antidepressant effects of ketamine have initiated significant theoretical, scientific, and clinical advancements with regard to depression treatment, which have been long overdue. Likely central to ketamine's antidepressant mechanism of action is the preclinical finding that it fundamentally exerts its antidepressant effects by enhancing excitatory (glutamatergic) transmission at select synapses, which initiates a synaptogenic process that restores the integrity of neural circuits that are affected in depression. However, the utility of ketamine for psychiatric indications is limited by its dissociative properties and abuse potential. A better understanding of demographic, clinical, and neurobiological predictors of dissociation

and abuse will allow a safer, more targeted use of ketamine and future rapid-acting antidepressants. Additionally, it is unclear why NMDAR antagonists lack antidepressant properties relative to ketamine, and thus the role of NMDAR inhibition has been called into question (Gould et al. 2019, Lener et al. 2017, Newport et al. 2015). Future studies should aim to better understand the cellular and synaptic processes that are critical to the rapid antidepressant actions of ketamine and that are both translationally robust and empirically sound. Rigorous attempts need to be made to either verify or falsify key mechanistic hypotheses, as opposed to providing circumstantial or epiphenomenal evidence in support of them; a higher burden of proof will be needed if the next major advance is going to be a true improvement on ketamine.

SUMMARY POINTS

1. Depression is a highly debilitating condition that is difficult to treat with existing pharmacotherapies.
2. The antidepressant efficacy of traditional antidepressants was discovered by chance, and their monoamine-based mechanism of action formed the basis of drug development for more than half a century.
3. Antidepressant mechanisms of action, as well as the requisite delayed time course of their onset, were reconsidered following the discovery that subanesthetic ketamine has rapid, robust, and sustained antidepressant effects.
4. It has been hypothesized that ketamine exerts its rapid antidepressant effects by inhibiting the *N*-methyl-D-aspartate glutamate receptor (NMDAR), but there is evidence that ketamine and its metabolites act through NMDAR-independent mechanisms to yield their antidepressant-relevant effects.
5. There is convincing evidence that ketamine engages plasticity-related mechanisms to restore neuronal integrity in areas of the brain that are thought to be compromised in depression. This process involves an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-dependent increase in brain-derived neurotrophic factor (BDNF) release.
6. While the discovery of ketamine's antidepressant effects has led to significant clinical and theoretical advances, its acute antidepressant mechanism of action is still debated, and the development of novel ketamine-like antidepressant drug treatments has been slow to progress.
7. An improved understanding of depression pathophysiology and rapid antidepressant mechanisms of action is needed in order to develop more effective treatments in the future.

DISCLOSURE STATEMENT

L.M.R. reports no conflicts of interest. T.D.G. is a coauthor on patents and patent applications related to the pharmacology and use of (2*R*,6*R*)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation, and posttraumatic stress disorder. He has assigned patent rights to the University of Maryland, Baltimore, but will share a percentage of any royalties that may be received. T.D.G. has received research funding from Allergan and Roche Pharmaceuticals and has served as a consultant for FSV7, LLC, during the preceding 3 years.

AUTHOR CONTRIBUTIONS

L.M.R. conceptualized and wrote the manuscript; T.D.G. edited the manuscript for critical intellectual content.

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