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# Neurobiology of rapid acting antidepressants: convergent effects on GluA1-synaptic function

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## Abstract

Efforts to develop efficacious antidepressant agents with novel mechanisms have largely unsuccessful since the 1950's until the discovery of ketamine, an NMDA receptor antagonist that produces rapid and sustained antidepressant actions even in treatment resistant patients. This finding has ushered a new era for the development of novel rapid acting antidepressants that act at the NMDA receptor complex, but without dissociative and psychotomimetic side effects of ketamine. Here we review the current state of rapid acting antidepressant drug development, including NMDA channel blockers, glycine site agents, and allosteric modulators, as well as ketamine stereoisomers and metabolites. In addition, we focus on the neurobiological mechanisms underlying the actions of these diverse agents and discuss evidence of convergent mechanisms including increased brain derived neurotrophic factor signaling, increased synthesis of synaptic proteins, and most notably increased GluR1 and increased synaptic connectivity in the medial prefrontal cortex. These convergent mechanisms provide insight for potential additional novel targets for drug development (e.g., agents that increase synaptic protein synthesis and plasticity). Importantly, the convergent effects on synapse formation and plasticity also reverse the well-documented neuronal and synaptic deficits associated with stress and depression, and thereby target the underlying pathophysiology of major depressive disorder.

## Introduction

Major depressive disorder effects approximately 17 percent of the population exacting enormous personal and economic burden and is on pace to be the leading cause of disability worldwide by 2020 [1–3]. Currently available medications, notably monoamine reuptake blockers are modestly effective, but require weeks to months of treatment and for many patients multiple prescriptions and/or drug combinations, and still these agents are ineffective in approximately one third of patients who are considered treatment resistant [4]. These limitations of time lag and efficacy are extremely serious for a patient population that is at increased risk of suicide [5].



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Conflict of Interest

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The development of pharmacological interventions that produce rapid and efficacious actions has been the holy grail of antidepressant therapeutics since the discovery of the monoaminergic agents in the 1950's, and in recent decades was largely considered out of the realm of possibility. That is until the discovery that a single dose of ketamine produces rapid and sustained antidepressant actions in depressed patients [6]. Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist developed as a dissociative anesthetic, which at low doses (0.5 mg/kg, i.v. slow infusion) produces mild dissociative and psychotomimetic effects, leading to its use in clinical research studies. Based on early studies implicating the NMDA receptor in the actions of antidepressant treatments, Krystal and colleagues tested the effects of ketamine in depressed patients and found that a single low dose produced a rapid antidepressant response within hours that lasted for 3 days [6, 7]. Zarate and colleagues replicated and extended this finding, reporting significant antidepressant actions 2 hours after a single low dose of ketamine that lasted for 7 days [8]. In addition, ketamine has proven effective for reducing bipolar depression and suicidal ideation [9, 10]. The rapid and sustained antidepressant actions of ketamine have now been replicated in multiple studies [11–13], even in treatment resistant patients [8, 10]. The discovery of the rapid actions of ketamine by a completely different mechanism than typical antidepressants represents the most significant advance in the treatment of depression since the discovery of monoaminergic agents over 60 years ago.

Although ground breaking, ketamine has serious dissociative and psychotomimetic side effects, as well as abuse potential, limiting its wide spread use [14]. Nevertheless, a nasal preparation of (S)-ketamine has proven effective in phase 3 clinical trials and is expected to receive approval from the FDA in 2019. In addition, the discovery of the rapid actions of ketamine has paved the way for a new era of drug development focused on agents that influence the glutamatergic-NMDA system. This includes other noncompetitive open channel blockers like ketamine, but also agents that act at other NMDA receptor sites, as well as allosteric modulators [13, 15–17]. In addition, there have been reports that ketamine metabolites and enantiomers have antidepressant actions in rodent models with fewer side effects [18, 19].

These findings highlight the potential for the development of novel rapid acting antidepressants, instilling renewed interest by pharmaceutical companies. However, a major question is what neurobiological mechanisms underlie the rapid and sustained antidepressant actions of an NMDA receptor antagonist? Characterization of the molecular, cellular, and circuit level actions of ketamine and other rapid acting glutamatergic agents, will provide key insights for new drug development and is a key focus of the current review. Where appropriate we also compare the effects of ketamine with typical monoaminergic agents. Moreover, these studies have the potential to shed light on the neurobiology of depression, particularly as it relates to synaptic deficits that are targeted by novel glutamatergic agents.

## Neurobiology underlying the antidepressant actions of glutamate-NMDA receptor modulating agents

Insight on the fast actions of ketamine vs. typical antidepressants may come from the difference between rapid neurotransmitter effects of glutamate vs. the modulatory effects of

the monoamine neurotransmitter systems. This could account in part for the delayed response of the monoaminergic agents, which requires time for gradual adaptations of postsynaptic signaling and gene expression [16, 20]. In contrast, ketamine acts on glutamate, the major excitatory neurotransmitter in the brain which could account for the rapid and robust therapeutic response even in treatment resistant patients. Here we discuss the actions of ketamine as a NMDA receptor channel blocker and the rapid paradoxical increase in glutamate that leads to rapid and sustained synaptogenic and behavioral responses.

**Ketamine blocks the NMDA receptor channel: differences with other channel blockers—**Ketamine is an antagonist of the NMDA receptor, an ionotropic multimeric complex that gates Ca2+, leading to stimulation of intracellular signaling pathways that underlie synaptic plasticity involved in cellular and behavioral models of learning and memory (Figure 1) [21, 22]. The NMDA receptor is a heteromeric complex made up of 4 subunits, with obligatory GluN1 combined with different GluN2 subunits (GluN2A, B, C, and D) [23, 24]. This can include di- and triheteromeric receptors (i.e., 2 GluN1 and either 2 of the same or 2 different GluN2 subunits). Since many forebrain neurons express both GluN2A and GluN2B, along with GluN1 subunits, they have the potential to express triheteromeric receptors, which could result in complex effects on glutamate binding, channel conductance, deactivation, and Ca2+ permeability [23, 24]. There are two other classes of glutamate ionotropic receptors, AMPA and kainate, that gate Na+ and mediate fast excitatory transmission [23]. Importantly, the function of the NMDA receptor is tightly linked to the AMPA receptor: glutamate-AMPA stimulated depolarization is required for opening the NMDA channel, allowing removal of Mg2+ that blocks the channel pore; this is required for ketamine entry and subsequent blockade of the NMDA channel (Figure 1) [23, 24].

There are several NMDA receptor channel blocking agents that have been used in clinical trials with varying degrees of efficacy. However, these agents have not been uniformly efficacious. Once such agent is memantine, an NMDA channel blocker approved for cognitive enhancement for Alzheimer's disease patients [25]. Clinical trials with memantine in depressed patients have been negative, suggesting that memantine has channel blocking properties that differ from ketamine[26]. Another agent, AZD6765 (lanicemine) was initially reported to produce rapid antidepressant actions in depressed patients but subsequent studies failed to support this conclusion [27–29].

These negative findings have led to several alternate theories for the actions of ketamine, including evidence that a metabolite, (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) acts at an unknown, non-NMDA site to mediate an antidepressant response[18], and/or that actions at other targets play an important role in the antidepressant actions of ketamine [30]. However, a closer look reveals that NMDA receptor channel antagonists have different channel blocking properties that could be related to their antidepressant efficacy.

While all of these agents block the NMDA receptor channel in an activity dependent manner, the binding kinetics within the channel and effects on binding of glutamate differ. For example, ketamine is a high affinity channel blocker that acts as a dissociative anesthetic at high doses [24, 28, 31]. In addition, because of ketamine's binding characteristics, it is trapped inside the channel pore as it closes, allowing glutamate to dissociate from the

agonist binding site; ketamine is therefore classified as a high trapping blocker, estimated at 86 percent. In contrast, memantine is a lower affinity channel blocker with faster on/off kinetics and is classified as a partial trapping blocker at 71 percent [31]. These characteristics may contribute to a broader therapeutic window for memantine with respect to the psychotomimetic effects observed with ketamine. AZD6765 is another uncompetitive channel blocker, with even lower (54 percent) partial trapping blocking characteristics [31]. The reduced trapping and blocking efficacy of memantine and AZ6765 could account for the lower efficacy of these agents in clinical trials. In contrast, findings with MK801, a higher affinity, blocking, and near complete trapping agent, demonstrate a rapid, [32] but not sustained response in rodent models [18, 33, 34], indicating that more compete blockade and trapping results in adaptive responses that counter the sustained antidepressant actions observed with ketamine.

Additional studies of these and other open channel blocking agents are required to fully elucidate the optimal trapping and blocking properties required for antidepressant actions. However, these finding raise the possibility of a “Goldilocks effect”: not too much or too little, just the right amount of blocking/trapping of the NMDA complex is necessary to produce the rapid and sustained antidepressant actions of ketamine. It is also possible that the diverse ligands act differently depending on the composition of the NMDA receptor (e.g., di- or triheteromeric receptors). Further studies are required to test this hypothesis.

**Ketamine increases extracellular glutamate and synapse number, and reverses synaptic deficits caused by chronic stress—**While the initial actions of ketamine at the NMDA receptor complex are well established, the mechanism by which NMDA receptor blockade causes a rapid and sustained antidepressant response are unclear. An early study reported that ketamine produces a rapid, but transient increase of extracellular glutamate levels in the mPFC (Figure 2) [35]. A requirement for excitatory glutamate transmission has been further demonstrated by evidence that pretreatment with an AMPA receptor antagonist completely blocks the antidepressant behavioral actions of ketamine in rodents [33, 34, 36].

The glutamate burst and requirement for AMPA activation are puzzling effects for an NMDA receptor blocker, but stimulation of burst firing of excitatory neurons is known to cause synaptic plasticity in cellular models of learning and memory [21, 22]. This raises the possibility that ketamine, through a burst of glutamate, produces rapid synaptic actions that underlie the antidepressant behavioral responses. The possibility that synaptogenic effects play a role in the antidepressant actions of ketamine is also supported by evidence that stress and depression are associated with decreased synapse number and atrophy of cortical and limbic brain regions [37–40].

Studies directly testing this hypothesis in rodent models show that a single dose of ketamine produces a rapid increase in levels of synaptic proteins, including GluA1, PSD95, and synapsin 1 in the mPFC[36, 41]. This is consistent with other reports that ketamine increases levels of synaptic proteins [18, 33]. This effect was observed as early as 2 hours after ketamine dosing, consistent with the onset of the therapeutic actions of ketamine [6, 8]. Increased levels of synaptic proteins, particularly GluA1, a major subunit of the AMPA receptor, indicates

that ketamine increases synapse formation, which was examined by electrophysiological and morphological studies. The results show that ketamine increases the number and function of spine synapses on layer V pyramidal neurons in the mPFC (Figure 2) [36, 41, 42]. This includes increased 5-HT and hypocretin induced EPSCs, increased spine density, and increased number of large diameter mushroom spines that have high levels of synaptic efficacy.

These findings suggest that ketamine could reverse the synaptic deficits caused by stress and depression [38, 39]. To directly test this possibility, we utilized a chronic unpredictable stress (CUS) model, which is considered one of the more valid rodent models of depression [41]. Exposure to CUS for 3–4 weeks causes anhedonic behavior, a core symptom of depression. Typical monoaminergic antidepressant agents reverse this anhedonic behavior but only after chronic administration (three weeks), making CUS a rigorous preclinical model for testing rapid acting agents. Moreover, CUS exposure causes a reduction in synapse number in the mPFC and hippocampus [38, 43, 44]. We found that a single dose of ketamine caused a rapid reversal of anhedonia resulting from CUS exposure, and also rapidly reversed the synaptic deficits caused by CUS [41].

While it is difficult to test this hypothesis in depressed patients, the results of these rodent studies suggest that ketamine reverses the atrophy and synaptic loss reported in depressed patients and thereby targets the underlying neurobiology of depression. Brain imaging studies are required to directly test this hypothesis in human depressed patients. This could include studies to determine if ketamine reverses the volumetric changes observed in PFC and hippocampus of depressed patients. Alternatively, a new synaptic PET ligand, which binds to the synaptic vesicle protein 2A (SV2A), could be used to assess synaptic density in vivo, including in human studies [45]. This ligand binds to presynaptic elements but can be used to assess the influence of ketamine on synaptic processes to further test the synaptic actions of ketamine in patients.

## Cellular signaling pathways underlying the synaptic and behavioral actions of ketamine

The neurobiology of synaptic plasticity and synapse formation is one of the most highly studied and fundamental brain functions. It represents the ability of neuronal circuits to respond to and store stimuli from multiple inputs and make appropriate adaptive responses to the same or similar future stimuli [46]. Conditions that cause loss of plasticity can lead to cognitive deficits, habit related disorders, drug abuse, and major depression [47, 48]. One of the signaling pathways that has been linked with protein synthesis dependent synaptic plasticity is the mechanistic target of rapamycin complex 1 (mTORC1) [48, 49]. The mTORC1 complex is located in synaptic terminals as well as cell bodies and regulates the synthesis of synaptic proteins in response to a variety of stimuli, including neuronal activity, neurotrophic factor signaling, amino acid levels, and energy demand [49]. We have reported that ketamine rapidly increases mTORC1 signaling in the mPFC, within 30 to 60 minutes after dosing (Figure 2) [36]; this includes increased levels of the phosphorylated and activated forms of mTOR and p70 ribosomal S6 kinase (S6K). This effect has been replicated by multiple laboratories [50–55]. Further studies show that infusion of a selective inhibitor of mTORC1, rapamycin, into the mPFC completely blocks the synaptic and antidepressant behavioral actions of ketamine, including in animals exposed to CUS [36, 41].

A role for protein synthesis signaling in the actions of ketamine is also supported by studies of a related pathway, eukaryotic elongation factor 2 (eEF2) kinase, that is regulated by NMDA receptors [56]. In the absence of neuronal depolarization, spontaneous neurotransmission leads to low levels of NMDA activity that result in a phenomenon referred to as long-term depression; this occurs in part via stimulation of eEF2 kinase signaling which inhibits synaptic protein synthesis. Monteggia and collaborators have reported that ketamine blockade of NMDA receptors at rest leads to suppression of eEF2 kinase activity, and subsequent increased synaptic protein synthesis [34]. They also found that pretreatment with a protein synthesis inhibitor, actinomycin D, blocked the actions of ketamine and that a selective eEF2 kinase inhibitor reproduces the actions of ketamine.

One perplexing consideration for the role of NMDA-eEF2 kinase signaling is that this model proposes that the effects of ketamine occur at rest, in the absence of neuronal activity. However, it is well established that ketamine causes a rapid increase in extracellular glutamate that stimulates neuronal activity, supported by evidence that ketamine increases the number of cFos positive neurons in the mPFC [57]. In addition, the behavioral actions of ketamine are activity dependent (i.e., blocked by an AMPA receptor antagonist) [33, 34, 36]. In any case, these studies support a role for eEF2 kinase and downstream proteins such as brain derived neurotrophic factor (BDNF).

**Role of BDNF in the actions of ketamine—**Ketamine is also reported to rapidly stimulate the synthesis of BDNF in the hippocampus and PFC, and the antidepressant behavioral actions of ketamine are blocked in BDNF deletion mutant mice [34]. BDNF is a major neurotrophic factor in brain that plays a critical role during development as well as the in the function of neurons in adult brain [58, 59]. Expression of BDNF is decreased by chronic stress exposure in rodent models and is reduced in depressed postmortem hippocampus and PFC [20, 60–63]. BDNF plays an important role in synaptic plasticity [58, 64]; indicating that BDNF alterations could be involved in the synaptic deficits caused by stress and conversely the antidepressant action of ketamine. We have directly tested this hypothesis in mice with a knockin of the BDNF Val66Met allele, a human polymorphism that blocks the processing and activity dependent release of BDNF [65, 66]. We found that the ability of ketamine to increase the number and function of synapses, as well as antidepressant behavioral actions are blocked in BDNF Val66Met mice [42].

Neurons of the BDNF Val66Met mice are able to synthesize BDNF, but the processing and activity dependent release of BDNF is restricted, suggesting that the actions of ketamine require BDNF release [65]. We tested this possibility by mPFC infusion of a function blocking antibody that binds and neutralizes extracellular BDNF. BDNF antibody infusion completely blocked the antidepressant actions of ketamine in the forced swim and novelty suppressed feeding tests (FST and NSFT) [67]. In addition, studies in cultured primary cortical neurons demonstrate that ketamine stimulates BDNF release, which is blocked by AMPA receptor inhibition [67, 68]. The ability of ketamine to stimulate the release of BDNF, not just the synthesis is a critical distinguishing action, as previous studies demonstrate that chronic, but not acute administration of typical monoaminergic antidepressants increase the synthesis, but not the release of BDNF [16, 69]. Together these studies indicate that ketamine stimulates

activity-dependent release of BDNF, and that this leads to increased synaptic connectivity that underlies the rapid and sustained antidepressant actions of ketamine (Figure 2).

These findings indicate the functional polymorphisms, such as the BDNF Val66Met polymorphism, could impact the response to ketamine. One study found that the BDNF Met allele, found in approximately 25% of Caucasions, is associated with a significant reduction in the response to ketamine [70]. Most of these patients carried a single allele and still showed a partial response to ketamine. However, a recent study conducted in China where a much higher percentage of the population carry the BDNF Met allele reported little or no effect on the response to ketamine [71]. The reason for this discrepancy is unclear but may suggest that different populations express polymorphisms that oppose the Met allele and allow for a complete ketamine response. Further studies are needed to examine the influence of the BDNF Met allele on the response to ketamine in different populations and to identify additional polymorphisms that influence the response in both a negative and positive manner.

## Initial cellular trigger for ketamine: direct vs. indirect hypothesis

Stimulation of BDNF release, mTORC1 signaling, and synapse formation are key downstream cellular responses required for the antidepressant actions of ketamine. But an important question is what is the initial cellular trigger? There are two major theories to explain the initial target of ketamine [16, 72, 73]. The first, related to the rapid paradoxical burst of glutamate [35], states that ketamine initially blocks NMDA receptors on GABA inhibitory neurons leading to “indirect” disinhibition of glutamate transmission. The second is that ketamine acts directly at NMDA receptors located on excitatory neurons, referred to as the “direct” hypothesis [16, 72, 73].

Evidence in support of the disinhibition hypothesis includes in vivo studies demonstrating that ketamine initially blocks the firing of GABA interneurons, which is followed by increased activity of excitatory neurons [74]. The open channel blocking activity of ketamine also fits with the disinhibition hypothesis as GABA interneurons are more active due to tonic firing compared to excitatory neurons, which increases the probability that NMDA receptors on GABA neurons are in an open channel conformation required for ketamine to block the channel [23, 24]. There is also recent electrophysiology evidence in hippocampal slices demonstrating that a low concentration of ketamine completely blocks basal levels of inhibitory postsynaptic currents (IPSCs) but has no effect on EPSCs [75]. A very recent study reports a similar blockade of inhibitory activity using a novel synaptic imaging approach [76].

To directly test this hypothesis, we are conducting studies to selectively manipulate NMDA receptors in a neuron specific manner using floxed shRNA and cell specific Cre recombinase mice. For these studies we have focused on the GluN2B subunit based on clinical and preclinical studies reporting that selective GluN2B antagonists produce rapid antidepressant responses [33, 36, 77]. Preliminary studies indicate that knockdown of GluN2B in GABA but not glutamate neurons in the mPFC blocks the antidepressant behavioral actions of ketamine [78]. We have used a similar approach to test the direct vs. indirect hypothesis in the actions of another rapid antidepressant scopolamine, that supports this hypothesis. Scopolamine, a nonselective acetylcholine muscarinic (ACh-M) antagonist, also produces rapid

antidepressant actions in depressed patients [79, 80], although a recent study reports that scopolamine was not effective in treatment resistant depressed patients [81]. Preclinical studies demonstrate that scopolamine also increases mTORC1 signaling and synapse formation in the mPFC [82]. Knockdown of ACh-M1 on GABA but not glutamate neurons blocks the antidepressant actions of scopolamine [83]. Further studies show that knockdown of ACh-M1 on somatostatin, but not parvalbumin subtype GABA interneurons blocks the effects of scopolamine [83]. These studies suggest that ketamine might also function via blockade of NMDA receptors on somatostatin but not parvalbumin neurons, which would be consistent with a previous negative report [84]. Studies are ongoing to further test the disinhibition hypothesis and to determine the exact cell types that mediate the actions of ketamine.

Studies supporting the direct hypothesis include the work by Monteggia and colleagues and their model that blockade of NMDA-eEF2 kinase signaling on excitatory neurons mediates the actions of ketamine [34]. However, as discussed there appears to be some discrepancy since this model requires ketamine to act at NMDA receptors at rest, which contradicts the well-established evidence of a glutamate burst. There is evidence for this hypothesis from a study of mice with global deletion of GluN2B on excitatory neurons, demonstrating occlusion of the antidepressant behavioral actions of ketamine [51]. However, these mice display a hyperlocomotive phenotype that makes it impossible to interpret the results [85]. Further studies are needed of cell and region specific or inducible deletion of NMDA receptors to further test the direct vs. indirect hypothesis.

## Ketamine stereoisomers, metabolites and other NMDA receptor modulating agents

The discovery of ketamine's antidepressant actions has led to the search for additional agents that act at the NMDA receptor complex that could produce ketamine like rapid actions but without the side effects.

**Ketamine stereoisomers and metabolites—**Ketamine metabolites, as well as it's R and S stereoisomers, have varying degrees of NMDA receptor channel blocking activity as well as antidepressant behavioral actions. Importantly, these metabolites and stereoisomers also have varying degrees of dissociative and psychotomimetic side effects. (S)-ketamine has approximately 4-fold greater affinity for the NMDA receptor complex compared with (R)-ketamine; both have antidepressant actions but (R)-ketamine has fewer side effects in rodent models (i.e., prepulse inhibition, conditioned place preference, and locomotor sensitization) [86]. The antidepressant actions of (S)- but not (R)-ketamine are blocked by rapamycin [87]. The major metabolites norketamine and hydroxynorketamine, as well as their stereoisomers also have different levels of NMDA receptor affinity, blocking activity, behavioral actions, and side effect profiles [13, 19]. A recent study demonstrates that (S)-norketamine produces greater antidepressant actions than (R)-norketamine and has fewer side effects [54]. The actions of (S)-norketamine are blocked by inhibition of the BDNF-TrkB receptor or by mTORC1 blockade, but not by pretreatment with an AMPA receptor antagonist [54].

Studies of the (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) metabolite have also been very interesting. Gould and colleagues noted that female mice are more sensitive to the antidepressant actions of ketamine and found that females metabolize ketamine to (2R,6R)-

HNK to a greater rate [18]. They went on to show that (2R,6R)-HNK is produced at relatively high levels and that if the metabolism of ketamine to (2R,6R)-HNK is blocked the antidepressant actions of ketamine are blocked [18]. They found that the antidepressant actions of (2R,6R)-HNK are greater than (2S,6S)-HNK in different rodent models, without the side effect profile of ketamine. Surprisingly, (2R,6R)-HNK has extremely low activity at the NMDA receptor complex in ligand binding assays and electrophysiology studies, indicating that this metabolite acts at a different, as yet unidentified site. (2R,6R)-HNK is reported to increase extracellular levels of glutamate [88] and the antidepressant actions of (2R,6R)-HNK are blocked by pretreatment with an AMPA receptor antagonist [18]. (2R,6R)-HNK also increases eEF2 kinase activity [18]; we have also reported that (2R,6R)-HNK increases levels of mTORC1 signaling and that the antidepressant actions of (2R,6R)-HNK are blocked by rapamycin infusion [89]. It should be noted that another group did not observe antidepressant actions of (2R,6R)-HNK in a chronic social defeat test (different mouse strain) [90] and in a rat learned helplessness model [91], and that a high concentration of (2R,6R)-HNK (50 μM) blocks NMDA receptors [92].

These findings indicate that (2R,6R)-HNK, as well as other ketamine metabolites could be efficacious antidepressants with fewer side effects than ketamine, and clinical studies have been initiated to test the safety and efficacy of these agents in humans.

**Allosteric modulators of the NMDA-GluN2B subunit—**GluN2B negative allosteric modulators were initially developed in the 1990's for the treatment of stroke, but due to side effects and efficacy problems these programs were discontinued. However, preclinical as well as clinical studies have resurrected interest in this class of agents for the treatment of depression. Rodent studies of a prototypical GluN2B negative allosteric modulator, RO 25–6981, reported antidepressant responses in a number of different behavioral models, including CUS [33, 36, 41].

Clinical studies have also been promising, although reports have been mixed. An early clinical trial reported that a single dose of CP101,606 (traxoprodil) produced an antidepressant response in depressed patients, although the onset of action was delayed (5 days) [77]. This could be due in part to the lower dose used to reduce the dissociative side effects. Studies of another GluN2B negative allosteric modulator, CERC-301 (MK-0657) have been inconsistent. Initial trials reported that CERC-301 produced a significant antidepressant response [only on the secondary efficacy measure] but a subsequent trial was negative [93]. While promising, further studies of these as well as novel GluN2B negative allosteric modulators are needed to validate this approach as comparable to the rapid and sustained actions of ketamine.

**NMDA receptor co-agonist glycine site modulators—**The glycine site is another important modulatory site on the NMDA receptor complex (Figure 1). Also referred to as the glycine B site to differentiate it from the strychnine-sensitive glycine site, binding of the co-agonist glycine or D-serine is required for glutamate-stimulation of the NMDA receptor complex by enhancing the affinity and efficacy of glutamate [24, 94]. In line with evidence of antidepressant actions of NMDA receptor blockade, there has been development of an agent, AV-101 (VistaGen) that leads to blockade of the glycine B co-agonist site on the NMDA

receptor complex. AV-101 is a prodrug, L-4-chlorokynurenine (4-CL-KYN), which is transported into the brain and converted in astrocytes into 7-chlorokynurenic acid, a potent antagonist of the glycine B coagonist site. Preclinical studies demonstrate that 4-CL-KYN administration results in a rapid and sustained antidepressant response in several different antidepressant models [95]. In addition, the antidepressant actions of 4-CL-KYN are blocked by pretreatment with an AMPA receptor antagonist. This agent did not have the side effect profile of ketamine, including rewarding (CPP), psychotomimetic (PPI), locomotor sensitization, or stereotypic behaviors [95]. AV-101 has a relatively good safety profile in humans [96] and phase 2 clinical trials in depressed patients are underway. AV-101 has been granted fast track-breakthrough status by the FDA.

Studies have also shown that D-cycloserine, a partial agonist of the glycine site at low doses but an antagonist at high doses produces antidepressant actions in rodents [97] and in clinical trials in depressed patients [98]. D-cycloserine has also been used to augment cognitive behavioral therapy in a number of different conditions, with some limited success [99]. This is based on evidence that D-cycloserine enhancement of NMDA receptor function can augment neuroplasticity required for behavioral flexibility in models of fear extinction [100]. Surprisingly, a glycine site agonist, D-serine is also reported to produce antidepressant actions [101]. In addition, sarcosine an inhibitor of the glycine transporter 1 produces rapid antidepressant responses by increasing glycine levels [102]. Moreover, there is also evidence that the antidepressant actions of sarcosine in rodent models require AMPA receptor activity and mTORC1 signaling [103]. It is unclear why both glycine site antagonists and agonist produce rapid antidepressant responses, but one possibility is that these agents act at different initial cellular targets. For example, it is possible that glycine site antagonists act in an indirect fashion via inhibition of NMDA receptor function on GABA interneurons, and thereby lead to an increase in glutamate transmission similar to ketamine. In contrast, glycine site agonists could act to directly enhance glutamate-NMDA receptor function on excitatory neurons. Further studies will be needed to examine these possibilities using cell specific knockdown approaches as described above.

**Rapastinel, an NMDA receptor positive allosteric modulator—**Rapastinel, formerly referred to as GLYX-13 is another interesting NMDA modulator reported to have rapid antidepressant actions but via a different mechanism of action. Rapastinel is a tetrapeptide that was initially thought to be a partial agonist of the glycine site, but more recent studies indicate that while it functions like a glycine site partial agonist it acts at an allosteric site on the GluN1 subunit [104]. Initial studies in rodent models demonstrate rapid antidepressant actions of rapastinel (i.v. administration) in the FST, NSF, learned helplessness, and CUS models, without the side effects of ketamine in PPI and CPP [105–107]. A phase 2 clinical trial also reports rapid antidepressant actions in depressed patients [108], and large phase 3 trials are currently underway. The FDA has granted fast track breakthrough status for rapastinel.

Rapastinel produces cellular effects that overlap with ketamine, including stimulation of mTORC1 signaling, increased BDNF release, and increased synapse formation in the mPFC [107]. The antidepressant actions of rapastinel are also blocked by pretreatment with an AMPA receptor antagonist, the mTORC1 inhibitor rapamycin, a BDNF neutralizing antibody, and

in BDNF Met mice [107]. However, in contrast to ketamine, rapastinel does not increase extracellular glutamate in the mPFC, which could account for the reduced side effects [109, 110]. However, rapastinel increases dopamine in the mPFC [35], which has also been observed with ketamine [35], suggesting that dopamine may be involved in the antidepressant actions of these agents.

Despite these convergent effects, it is surprising that an NMDA positive allosteric modulator would produce rapid antidepressant actions given the potent channel blocking properties of ketamine. As discussed for agonists and antagonists of the glycine co-agonist site this could occur via different initial cellular targets with the positive modulator acting on NMDA receptors on excitatory neurons and antagonists acting at NMDA receptors on GABA interneurons. Preliminary cell specific knockdown studies support this possibility; showing that knockdown of GluN2B on pyramidal neurons, but not on GABA interneurons in the mPFC blocks the antidepressant behavioral actions of rapastinel (Kato et al., unpublished). Additional studies are needed to further test this hypothesis and to more fully understand the cellular mechanisms underlying the actions of these agents.

**mGluR2/3 antagonists and allosteric modulators—**The antidepressant actions of ketamine are linked to the glutamate burst, which leads to activity dependent synaptic and behavioral responses. These findings indicate that other agents that transiently increase glutamate should also produce an antidepressant response. The metabotropic glutamate receptors 2/3 (mGluR2/3) are located on synaptic terminals and provide negative feedback inhibitory control of glutamate synaptic activity [16, 111]. Antagonists of the mGluR2/3 receptors increase extracellular glutamate in the mPFC [112, 113]. Several mGluR2/3 antagonists, including LY324,495 and MGS0039, are reported to produce rapid antidepressant actions in rodent models [114–117]. These agents increase mTORC1 signaling and the antidepressant behavioral actions are blocked by rapamycin or AMPA receptor blockade [114–116]. These agents also increase levels of synaptic proteins, suggesting an increase in synapse function [116].

In addition, development of selective mGluR2 and mGluR3 negative allosteric modulators has provided tools to examine the antidepressant actions of each subtype that are also located at postsynaptic sites and have different functions [118]. These studies demonstrate that a selective mGluR3, but not mGluR2 NAM produces antidepressant actions in a tail suspension test [119]. Further studies are needed to extend this work to additional antidepressant models. Despite the preclinical evidence, a recent phase II randomized study conducted by Roche reported that a mGluR2/3 negative allosteric modulator, RO4995819 (declogurant), failed to produce antidepressant effects in treatment-resistant MDD patients (clinicaltrials.gov/ct2/show/NCT01457677). Nevertheless, additional clinical trials using mGluR2/3 competitive antagonists as well as negative allosteric modulators, are warranted.

## Common/convergent effects of different classes of rapid acting antidepressants

These studies demonstrate that although rapid antidepressant actions are produced by multiple classes of agents, either antagonists or agonists at different NMDA receptor sites, there are some convergent, downstream mechanisms [13, 16]. As discussed for ketamine, this

includes activity dependent actions (i.e., effects are blocked by AMPA receptor antagonist), increased BDNF release and/or expression, activation of protein synthesis pathways (i.e., mTORC1 and eEF2 kinase), increased expression of synaptic proteins (GluA1, PSD95, and synapsin), and increased synaptic number and function in the mPFC (Figure 2, Table 1). It is notable that a completely different class of rapid acting antidepressant, rapastinel, has convergent effects on these pathways [107]. Rapid and sustained up-regulation of GluA1 and other synaptic proteins is consistent with the possibility that increased synapse formation and connectivity accounts for the rapid and sustained antidepressant actions of ketamine and other rapid acting agents.

Several of these agents, including ketamine, the selective GluN2B antagonist Ro-25–6981, the ketamine metabolite (2R,6R)-HNK, and scopolamine also cause a burst of glutamate that leads to BDNF release, mTORC1 signaling, and synaptic changes [36, 82, 120]. Rapastinel stands out as one of the few agents that does not cause an increase in glutamate, although its antidepressant actions are activity dependent [106]. The increase in levels of GluA1 in synaptic preparations is another common feature of these rapid acting agents (Figure 2, Table 1). One exception based on a recent report is the antidepressant actions of (S)-norketamine, which are not blocked by pretreatment with an AMPA receptor antagonist [54]; however, it is notable that (S)-norketamine reverses the deficit in GluA1 caused by chronic social defeat. To date all of the agents that have been tested demonstrate a requirement for BDNF release and or BDNF-TrkB signaling, including ketamine, (R)- and (S)-ketamine, (S)-norketamine, (2R, 6R)-HNK, repastinel, and scopolamine [34, 67, 107, 120, 121].

A role for mTORC1 signaling has also been demonstrated for many of these agents, including ketamine, (S)-ketamine, (S)-norketamine, (2R,6R)-HNK, rapastinel, and scopolamine [36, 82, 87, 107, 120]; (R)-ketamine is an exception [54]. This includes stimulation of mTORC1 signaling and blockade of the antidepressant actions of these agents by infusion of a selective mTORC1 inhibitor, rapamycin. This has led to the hypothesis that stimulation of mTORC1 signaling, synaptic protein synthesis, and synapse formation could be targeted as a novel antidepressant approach. This possibility is supported by a novel small molecule derivative of D-leucine, NV-5138 that stimulates mTORC1 signaling by blockade of an upstream inhibitor sestrin 2 [122]. A single dose of NV-5138 produces rapid, and sustained antidepressant effects in multiple rodent models, including the CUS anhedonia model [123]. Importantly, NV-5138 also increases the number and function of spine synapses in the mPFC, similar to ketamine, and the behavioral actions of NV-5138 require mTORC1 signaling and BDNF release. NV-5138 is also safe in rodents and phase 1 trials in man and phase 2 clinical studies in depression are being initiated.

Convergent mechanisms provide a framework for development of additional rapid acting agents, with greater efficacy and reduced side effects. Moreover, these convergent actions, particularly increased synthesis of synaptic proteins and increased number and function of synapses provides further evidence that these agents target a deficit in synaptic density and function that contributes to the underlying pathophysiology of depression. Further studies of the mechanisms underlying the decrease in synapse number in stress and depression could provide additional novel antidepressant targets. In addition, the time course for increased synapse number is similar to that for the therapeutic response to rapid acting agents (rapid

onset and sustained for approximately 7 days), and treatments that prolong this synaptic increase would be expected to also prolong the antidepressant behavioral response to these agents.

## Role of other neurotransmitter systems in the ketamine response

In addition to glutamate, ketamine also rapidly influences levels of other neurotransmitters and there is evidence that some of these systems are required for the antidepressant actions of ketamine. The initial study of glutamate also reported a rapid and transient burst of dopamine in the mPFC [35]. It's not clear if increased glutamate signaling stimulates dopamine release or if ketamine has direct effects on dopamine neurons, but in either case, dopamine could contribute to ketamine-induction of synapse formation and function in the mPFC. Dopamine D1 receptors can increase the insertion of AMPA-GluR1 receptors required for increased synaptic function [124, 125]. Repeated stress causes cognitive deficits via decreased activity of D1-dopamine receptor (Drd1) signaling and atrophy of pyramidal neurons in the mPFC [126, 127]. Previous work has demonstrated that systemic D1 receptor agonist administration produces antidepressant actions in the FST [128]; more recent studies also demonstrate that infusion of a D1, but not D2 receptor antagonist into the mPFC blocks the antidepressant behavioral actions of ketamine [129].

Recent studies have also demonstrated a role for the serotonin system in the actions of ketamine. The antidepressant behavioral effects of ketamine are blocked by administration of 5-HT depletion or by infusion of a 5-HT1A antagonist into the mPFC [120, 130, 131]. The antidepressant actions of mGluR2/3 antagonists also require intact 5-HT neurotransmission and 5-HT1A receptors [131]. Infusion of a selective 5-HT1A agonist into the mPFC, but not systemic administration is sufficient to produce a rapid antidepressant response [120].

**Role of opiates in ketamine response—**Clinical studies have reported inconsistent antidepressant actions of memantine and AZD6765 [26–29]. This could be due to differences in the blocking and trapping activity of these agents, but these findings have also led to the hypothesis that actions at other non-NMDA sites mediate the antidepressant effects of ketamine. In particular, a recent study has investigated the possibility that ketamine acts via opiate receptors to produce an antidepressant response [30]. Ketamine has actions at the μ- and κ-opiate receptors [132], and there is evidence that the analgesic actions of ketamine are blocked by naltrexone [133]. To directly test this hypothesis, a double-blind crossover study was conducted in which depressed patients were pretreated with naltrexone before one of two ketamine treatments [30]. The results demonstrate a significant, near complete blockade of the antidepressant actions of ketamine that were sustained throughout the treatment period.

This interesting and important study indicates a key role for opiate receptors in the actions of ketamine. However, the number of patients was relatively small (n = 7), and another small study (5 patients) reports that naltrexone has no effect on the rapid antidepressant actions of ketamine [134]. Caution is also needed when interpreting these data. Ketamine has relatively low affinity for μ- and κ-opiate receptors and acts primarily as an antagonist, raising a question about direct effects [132]. Another possibility is that ketamine stimulates the release of endogenous opiates, similar to the increased release of glutamate and dopamine, and

thereby indirectly stimulates opiate receptors. It is also interesting to note that naltrexone blocks the placebo response in MDD patients [135], and it is possible that under certain conditions and/or in studies with small patient numbers that this could contribute, in part to the actions of naltrexone. Additional clinical studies are needed to further explore the role of opiate systems in the antidepressant actions of ketamine and to identify the underlying mechanisms in preclinical models.

**Role of GABA systems in depression and treatment response—**Brain imaging and postmortem studies consistently report altered levels of GABA and GABA interneuron markers in the brains of depressed patients [121, 136, 137]. This is particularly true for the somatostatin (SST) subtype of GABA interneurons with evidence of decreased levels of SST in postmortem depressed patients [138] and in animals submitted to CUS [139]. Sst deletion mutant mice also display depressive behaviors [140]. There are also preclinical studies reporting decreased parvalbumin interneurons after chronic stress exposure [141]. This has led Sibille and colleagues to the development of selective $GABA_AR$ receptor agonists for the treatment of depression. They have focused on the GABA-A α5 because the forebrain distribution of this subtype and have reported that α5-subtype agonists have antidepressant as well as cognitive enhancing effects [142]. This is consistent with other reports that genetic enhancement of GABA function produces antidepressant actions in rodent models [143].

Studies of postpartum depression also provide evidence for GABA involvement. Levels of allopregnanolone, a neuroactive steroid and $GABA_AR$ positive allosteric modulator, undergoes large fluctuations, with high levels during pregnancy and a precipitous drop at birth that is associated with postpartum depression [144, 145]. Allopregnanolone, a metabolite of progesterone, is a positive modulator of most $GABA_AR$ subtypes, including the extrasynaptic δ-subunit [146, 147]. This has to led testing of an allopregnanolone preparation, referred to as brexanolone as a novel treatment for postpartum depression with promising clinical results [148]. In addition, additional analogues have been developed, including SAGE-217 and shown to have rapid antidepressant efficacy in a general population of depressed patients, both males and females (unpublished). Because of these promising reports the FDA has granted brexanolone and SAGE-217 breakthrough status for the treatment of depression.

The reduction in GABA levels and function in depression appears to contradict evidence that the actions of ketamine are mediated by blockade of NMDA receptors on GABA interneurons, resulting in disinhibition of glutamate signaling (Figure 2). Further support of the disinhibition hypothesis is evidence that $GABA_AR$ α5 antagonists also cause rapid antidepressant actions, presumably via similar disinhibition of glutamate signaling [149, 150]. However, ketamine inhibition of GABA/SST interneurons is rapid and transient and leads to subsequent adaptive changes that mediate the antidepressant response. In addition to evidence of synaptic changes of excitatory neurons, new evidence demonstrates that ketamine increases GABA interneuron function, including increased levels of GAD65/67, vGAT, and gephyrin (Figure 2) [121]. These studies indicate that ketamine, via a burst of glutamate, resets both excitatory and inhibitory neurotransmitter tone in the mPFC, thereby correcting deficits in both systems. Further studies are needed to determine if GABA or

glutamate neurons are more vulnerable to stress-mediated functional deficits and if one of these systems is more critical for the rapid antidepressant actions of ketamine.

## Brain regions and circuits underlying the actions of rapid acting agents

Brain imaging studies demonstrate that ketamine administration results in rapid effects on BOLD in a number of different brain regions, including subregions of PFC, hippocampus, thalamus, and other areas [151, 152]. Further fMRI studies demonstrate that ketamine increases global connectivity, and that these effects are associated with depressive symptoms and treatment response [153, 154]. A recent fMRI study also demonstrates that ketamine alters connectivity and the balance of the default mode network (DMN), salience network (SAL), and central executive network (CEN) in support of the triple network hypothesis of depression [155]. The results show that connectivity in the DMN, which is hyperactive and responsible for introspection and rumination in depression, is normalized by ketamine up to 2 days after ketamine administration [155].

Preclinical studies examining the brain regions that mediate the actions of ketamine report that ketamine infusion into the mPFC is sufficient to produce rapid and sustained antidepressant actions in several rodent models, and that the actions of systemic ketamine are blocked by neuronal silencing of the mPFC [57]. Optogenetic stimulation of mPFC pyramidal neurons also produces rapid and long-lasting antidepressant actions similar to ketamine [57]. The antidepressant actions of ketamine are also blocked by infusions into the mPFC of rapamycin [36], or an anti-BDNF neutralizing antibody [67]. Lodge and colleagues also report that neuronal silencing of the ventral hippocampus blocks the antidepressant actions of ketamine and that optogenetic inhibition of the ventral hippocampal to mPFC pathway blocks the actions of ketamine in the FST, but only if inhibition occurs at the time of behavioral testing[156].

There is also evidence for a role of the lateral habenula in the actions of ketamine. The lateral habenula has connections with the mesolimbic dopamine pathway and when activated negatively regulates reward related behaviors. Hu and colleagues demonstrate that two different chronic stress models, a congenital learned helplessness model and chronic restraint stress increase burst firing of neurons in the lateral habenula, leading to inhibition of ventral tegmental dopamine neurons, as well as neurons in the dorsal raphe [157]. This study also shows that burst firing is driven by enhanced NMDA receptor activity and low voltage sensitive T-type Ca2+ channels. Importantly, they also show that acute ketamine administration rapidly reverses the burst firing of lateral habenula neurons in both models to produce rapid antidepressant actions. The authors point out that these findings are relevant to the rapid actions of ketamine but the role of lateral habenula in the sustained actions of ketamine were not examined.

## Summary and Conclusions

The discovery of ketamine has provided a critical rapid acting therapeutic option with improved efficacy even in treatment resistant depressed patients, paving the road for development of new rapid acting agents with fewer side effects. This includes agents that sustain the synaptic actions of ketamine, which would be predicted to further increase the

durability of therapeutic actions of ketamine. Most drug development programs are focused on the glutamate system and known NMDA receptor sites, but the discovery of allosteric modulators (e.g., rapastinel) offers the potential for additional agents that act at a wide array of novel allosteric sites on the NMDA receptor complex (Figure 1). Mechanistic studies of ketamine and other rapid acting agents also demonstrate convergence at downstream sites, including BDNF release, TrkB-mTORC1 signaling, and increased synapse formation (Figure 2) (Table 1). Most of these studies have examined single dose ketamine, and it will be important in future studies to determine the effects of repeated dosing schedules that could produce more sustained synaptic effects, as well as side effects. These studies indicate that additional novel agents could be developed for other targets that indirectly influence synapse formation and synaptic plasticity (e.g., NV-5138). These positive findings with ketamine and additional putative rapid acting agents that show promise in the clinic are stimulating renewed interest and investment by the pharmaceutical industry in the development of antidepressants and other medications for psychiatric illnesses. Importantly, evidence that ketamine and other rapid acting agents increase synapse formation demonstrates the ability of these agents to reverse, in part the underlying pathophysiology of depression and stress related illnesses.

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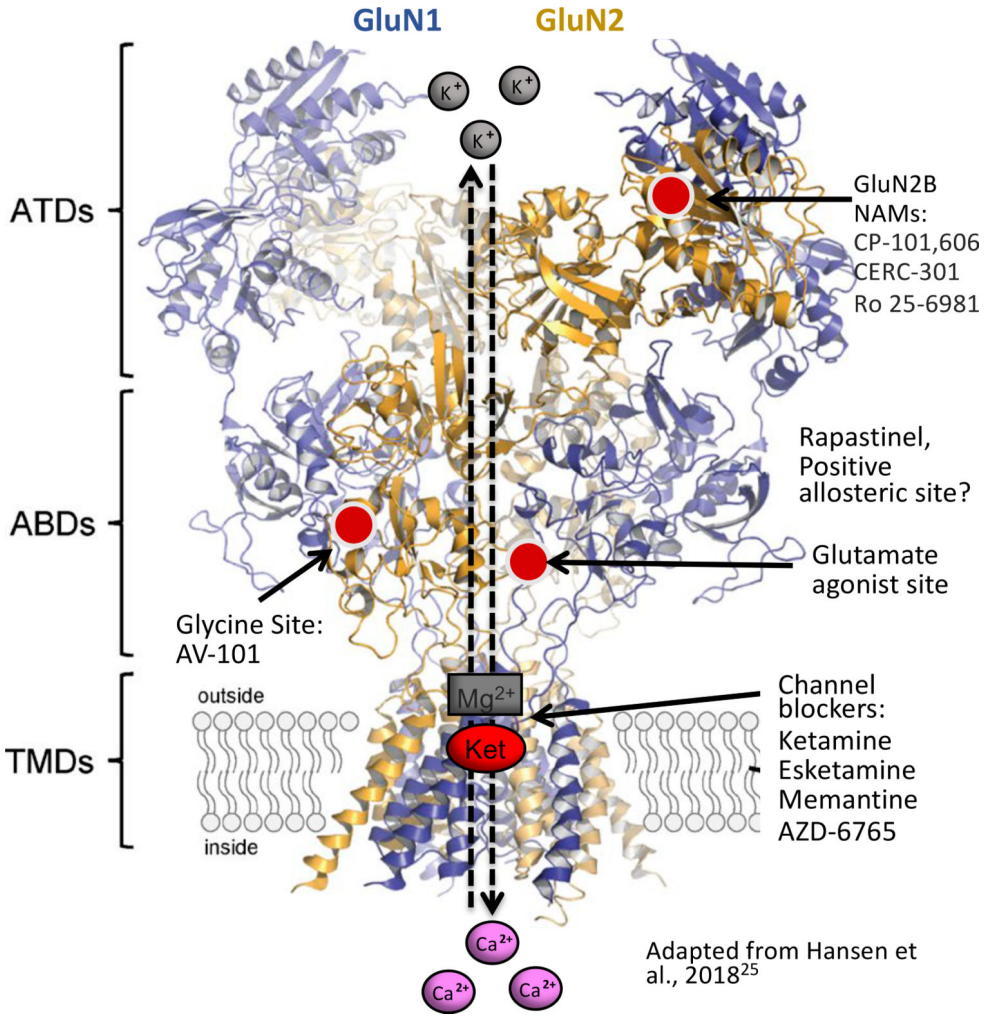


**Figure 1. Model of the NMDA receptor complex and target sites of rapid acting antidepressants.**

(A) Structure of NMDA receptor complex based on crystallography of reconstituted GluN1 (blue) and GluN2B (gold) subunits (adapted from Hansen et al., 2018)[25]. View is from the side and depicts the amino terminal domains (ATDs), glutamate agonist binding domain (ABDs), and transmembrane domains (TMDs). Sites of GluN2B inhibitor ifenprodil (CP101,606), competitive antagonist, and channel blockers are also shown. The NMDA receptor complex forms a pore that gates Ca2+ entry, which is required for intracellular signaling and synaptic plasticity. At resting Mg2+ is bound in and blocks the pore, and thereby blocks Ca2+ flux as well as entry and binding of channel blockers. Upon neuronal activation, typically by AMPA receptor dependent depolarization, the Mg2+ block is removed allowing entry of Ca2+. This open pore state is also accessible to ketamine and other channel blockers, which enter and block Ca2+ influx. The binding affinity, blocking, and trapping within the pore differ for the various NMDA channel blockers, as well as ketamine stereoisomers and metabolites, which could account, in part for the different therapeutic efficacy of these agents, as well as the side effects. Other regulatory sites on the NMDA receptor complex include a glycine co-agonist binding site on the GluN1 subunit that enhances NMDA receptor function; AV-101, in clinical trials for treatment of

depression, is an antagonist of the glycine co-agonist site. Rapastinel is a positive allosteric modulator of the NMDA receptor complex. The selective GluN2B negative allosteric modulators have also demonstrated antidepressant efficacy in preclinical studies, as well as clinical trials with mixed results. These agents include CP-101,606, CERC-301, and Ro 25–6981. It is currently unknown what the initial target is for the metabolite (*2R,6R*)-HNK.

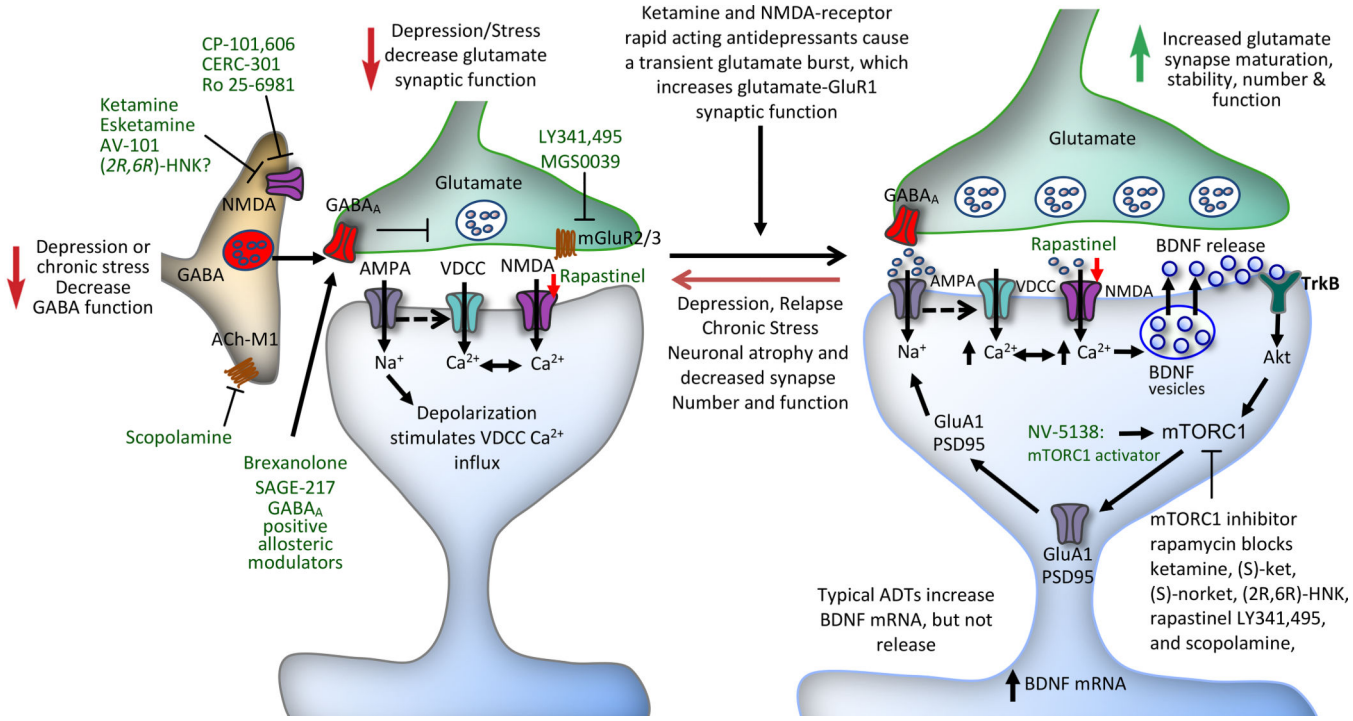


**Figure 2. Synaptic model for the initial cellular target sites for different types of of rapid acting antidepressants and convergent synaptic changes.**

Preclinical and clinical studies demonstrate that chronic stress and depression cause neuronal atrophy and decreased synapse number in the mPFC, as well as hippocampus that are associated with depressive behaviors in rodent models and symptoms in patients. This includes evidence of reductions in both glutamate and GABA neuronal function. In contrast, rapid acting antidepressants, notably ketamine rapidly increase synapse number and function and reverse the synaptic deficits caused chronic stress. The synaptic actions of ketamine, as well as several other channel blockers (i.e., Esketamine), negative allosteric modulators (i.e., Ro 25–6981), ketamine stereoisomers and metabolites (i.e., (S)-ketamine, (S)-norketamine, *2R,6R*)-HNK), and muscarinic receptor antagonists (i.e., scopolamine) are activity dependent and cause a burst of glutamate via blockade of receptors on tonic firing GABA interneurons, resulting in disinhibtion of glutamate transmission. The mGluR2/3 antagonists (i.e., LY341,495 and MGS0039) also cause an increase in glutamate via blockade of presynaptic autoreceptors that provide negative feedback regulation. The burst of glutamate causes activity dependent release of BDNF, stimulation of TrkB-Akt and mTORC1 signaling; these pathways lead to rapid induction of synaptic protein synthesis that is required for new synapse formation. Agents like rapastinel, which acts as a glycine like partial agonist, may increase synapse formation by enhancing NMDA function directly on pyramidal neurons and thereby increasing BDNF release and downstream mTORC1 signaling. A requirement for mTORC1 has been demonstrated for several rapid acting agents (i.e., blockade by the mTORC1 inhibitor rapamycin). Further support for mTORC1 is provided by evidence that a small molecule activator of mTORC1 also produces rapid synaptic and antidepressant behavioral responses. Note that while chronic administration of typical monoaminergic antidepressants increases BDNF, this is limited to expression and not

activity dependent release as observed with ketamine. Recent clinical studies also demonstrate that the GABA-A positive allosteric modulating agents, notably the neuroactive steroid allopregnanolone (referred to as brexanolone) and related compound SAGE-217 also produce rapid antidepressant responses in postpartum as well as general depression. The intersection of these agents with the mechanisms underlying the rapid response to glutamatergic agents remains to be identified.

**Table 1.**

Neurobiological mechanisms for different rapid acting antidepressants.

| | GluA1, AMPA-R Dependent ADT Behavior | Glutamate Burst | Increased mTORC1, rapamycin sensitive | BDNF release, BDNF dependent ADT behavior | Increased synapse function and density | GABA/GAD neurons are Initial cell trigger |
|---|---|---|---|---|---|---|
| R,S-Ketamine (NMDA antagonist) | ↑ GluA1, Blocked by NBQX | ↑ mPFC | ↑ mTORC1, rapamyc sensitive | ↑ Release, ADT beh Blocked by BDNF nAb | ↑ Increased EPSCs & spines in mPFC | ↑ Blocked, NR2B kd Sst/Gad |
| Scopolamine (ACh-muscarinic antagonist) | ↑ GluA1, Blocked by NBQX | ↑ mPFC | ↑ mTORC1, rapamyc sensitive | ↑ Release, ADT beh Blocked by BDNF nAb | ↑ Increased EPSCs & spines in mPFC | ↑ Blocked, AChM1 kd Sst/Gad |
| Rapastinel (positive allosteric modulator) | ↑ GluA1, Blocked by NBQX | No glutamate Increase Banerjee et al, 2016 ACNP | ↑ mTORC1, rapamyc sensitive | ↑ Release, ADT beh Blocked by BDNF nAb | ↑ Increased EPSCs & spines in mPFC | Blocked by NR2B kd on pyramidal Camk2 cells |
| LY341495 (mGlu2/3 antagonist) | ↑ GluA1, Blocked by NBQX | ↑ mPFC | ↑ mTORC1, rapamyc sensitive | ↑ BDNF release | Not tested | Not tested |
| Ro 25-6981 (NR2B NAM) | ↑ GluA1, Blocked by NBQX | ↑ mPFC glut cycling | ↑ mTORC1, rapamyc sensitive | Not tested | Not tested | Not tested |
| (2R,6R)-HNK (Ketamine metabolite) | ↑ GluA1, Blocked by NBQX | ↑ mPFC | ↑ mTORC1, rapamyc sensitive | ↑ Release, ADT beh Blocked by BDNF nAb | ↑ Increased EPSCs in mPFC | ↑ Blocked, NR2B kd Sst/Gad |
| S-ketamine (stereoisomer) | ↑ GluA1, Blocked by NBQX | Not tested | ↑ mTORC1, rapamyc sensitive | Not tested | Not tested | Not tested |

Included are several different rapid acting agents for which the neurobiological mechanisms that have been extensively studied. Only R,S- and S-ketamine have been tested in clinical trials and both are reported to have consistent antidepressant actions in depressed patients; both produce dissociative and psychotomimetic effects. R,S-ketamine also has abuse potential; there are no reports as of yet on the abuse potential of S-ketamine. R,S- and S-ketamine, as well as scopolamine also produce effects on prepulse inhibition and/or conditioned place preference in rodent models, while the other glutamatergic

agents listed, including rapastinel and (2R,6R)-HNK, have no effects. All of these agents have convergent effects on induction of GluA1-synaptic function and the antidepressant. Agents tested also increase synapse number and function, including R,S-ketamine, scopolamine, and Rapastinel; (2R,6R)-HNK increases synaptic function but not density. Behavioral actions are blocked by an AMPA receptor antagonist, NBQX. Most of these agents also cause a burst of glutamate in the mPFC, with the exception of rapastinel. All of the agents tested also increase BDNF release in primary cultured neurons and/or the antidepressant behavioral actions are blocked by infusion of a function blocking antibody into the mPFC or in BDNF mutant mice. These agents also increase mTORC1 signaling and the behavioral effects are blocked by rapamycin. The initial cellular trigger has been tested for some of these agents, with the actions of ketamine and scopolamine blocked by knockdown of GluN2B or ACh-M1 on Gad- or Sst-interneurons, and rapastinel blocked by knockdown of GluN2B on Camk2 pyramidal neurons in the mPFC.