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

Ronald S. Duman, Ryota Shinohara, Manoela V. Fogaça, Brendan Hare
Department of Psychiatry, Yale University School of Medicine

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 affects 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.
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. 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. 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. In addition, ketamine has proven effective for reducing bipolar depression and suicidal ideation. The rapid and sustained antidepressant actions of ketamine have now been replicated in multiple studies, even in treatment resistant patients. 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. 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. In addition, there have been reports that ketamine metabolites and enantiomers have antidepressant actions in rodent models with fewer side effects.

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. 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). 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). 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. There are two other classes of glutamate ionotropic receptors, AMPA and kainate, that gate Na+ and mediate fast excitatory transmission. 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).

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. Clinical trials with memantine in depressed patients have been negative, suggesting that memantine has channel blocking properties that differ from ketamine. Another agent, AZD6765 (lanicemine) was initially reported to produce rapid antidepressant actions in depressed patients but subsequent studies failed to support this conclusion. 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, and/or that actions at other targets play an important role in the antidepressant actions of ketamine. 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. 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. These characteristics 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.

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, but not sustained response in rodent models, 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). 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, indicating that the diverse ligands act differently depending on the composition of the NMDA receptor.

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. 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.

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. This is consistent with other reports that ketamine increases levels of synaptic proteins. This effect was observed as early as 2 hours after ketamine dosing, consistent with the onset of the therapeutic actions of ketamine. 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)\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{46}. Conditions that cause loss of plasticity can lead to cognitive deficits, habit related disorders, drug abuse, and major depression\textsuperscript{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)\textsuperscript{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\textsuperscript{49}. We have reported that ketamine rapidly increases mTORC1 signaling in the mPFC, within 30 to 60 minutes after dosing (Figure 2)\textsuperscript{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\textsuperscript{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\textsuperscript{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. 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. 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. In addition, the behavioral actions of ketamine are activity dependent (i.e., blocked by an AMPA receptor antagonist). 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. 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. Expression of BDNF is decreased by chronic stress exposure in rodent models and is reduced in depressed postmortem hippocampus and PFC. BDNF plays an important role in synaptic plasticity; 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. 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.

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. 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). In addition, studies in cultured primary cortical neurons demonstrate that ketamine stimulates BDNF release, which is blocked by AMPA receptor inhibition. 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. 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. 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. 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. The first, related to the rapid paradoxical burst of glutamate, 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.

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. 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.

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. A very recent study reports a similar blockade of inhibitory activity using a novel synaptic imaging approach.

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. Preliminary studies indicate that knockdown of GluN2B in GABA but not glutamate neurons in the mPFC blocks the antidepressant behavioral actions of ketamine. 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 its 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. 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. 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 and the antidepressant actions of (2R,6R)-HNK are blocked by pretreatment with an AMPA receptor antagonist. (2R,6R)-HNK also increases eEF2 kinase activity; 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. 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) and in a rat learned helplessness model, and that a high concentration of (2R,6R)-HNK (50 μM) blocks NMDA receptors.

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.

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). 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. 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. 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. 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. AV-101 has a relatively good safety profile in humans 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 and in clinical trials in depressed patients. D-cycloserine has also been used to augment cognitive behavioral therapy in a number of different conditions, with some limited success. 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. Surprisingly, a glycine site agonist, D-serine is also reported to produce antidepressant actions. In addition, sarcosine an inhibitor of the glycine transporter 1 produces rapid antidepressant responses by increasing glycine levels. Moreover, there is also evidence that the antidepressant actions of sarcosine in rodent models require AMPA receptor activity and mTORC1 signaling. 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. 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. A phase 2 clinical trial also reports rapid antidepressant actions in depressed patients, 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. 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. However, in contrast to ketamine, rapastinel does not increase extracellular glutamate in the mPFC, which could account for the reduced side effects. However, rapastinel increases dopamine in the mPFC, which has also been observed with ketamine, 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. Antagonists of the mGluR2/3 receptors increase extracellular glutamate in the mPFC. Several mGluR2/3 antagonists, including LY324,495 and MGS0039, are reported to produce rapid antidepressant actions in rodent models. These agents increase mTORC1 signaling and the antidepressant behavioral actions are blocked by rapamycin or AMPA receptor blockade. These agents also increase levels of synaptic proteins, suggesting an increase in synapse function.

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. These studies demonstrate that a selective mGluR3, but not mGluR2 NAM produces antidepressant actions in a tail suspension test. 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. 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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, rapastinel, and scopolamine\textsuperscript{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\textsuperscript{36, 82, 87, 107, 120}; (R)-ketamine is an exception\textsuperscript{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\textsuperscript{122}. A single dose of NV-5138 produces rapid, and sustained antidepressant effects in multiple rodent models, including the CUS anhedonia model\textsuperscript{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. 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. Repeated stress causes cognitive deficits via decreased activity of D1-dopamine receptor (Drd1) signaling and atrophy of pyramidal neurons in the mPFC. Previous work has demonstrated that systemic D1 receptor agonist administration produces antidepressant actions in the FST; 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.

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. The antidepressant actions of mGluR2/3 antagonists also require intact 5-HT neurotransmission and 5-HT1A receptors. Infusion of a selective 5-HT1A agonist into the mPFC, but not systemic administration is sufficient to produce a rapid antidepressant response.

**Role of opiates in ketamine response**—Clinical studies have reported inconsistent antidepressant actions of memantine and AZD6765. 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. Ketamine has actions at the μ- and κ-opiate receptors, and there is evidence that the analgesic actions of ketamine are blocked by naltrexone. 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. 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. 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. 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, 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. This is particularly true for the somatostatin (SST) subtype of GABA interneurons with evidence of decreased levels of SST in postmortem depressed patients and in animals submitted to CUS. Sst deletion mutant mice also display depressive behaviors. There are also preclinical studies reporting decreased parvalbumin interneurons after chronic stress exposure. This has led Sibille and colleagues to the development of selective GABA\(\alpha\)5 receptor agonists for the treatment of depression. They have focused on the GABA-A\(\alpha\)5 because the forebrain distribution of this subtype and have reported that \(\alpha\)5-subtype agonists have antidepressant as well as cognitive enhancing effects. This is consistent with other reports that genetic enhancement of GABA function produces antidepressant actions in rodent models.

Studies of postpartum depression also provide evidence for GABA involvement. Levels of allopregnanolone, a neuroactive steroid and GABA\(\alpha\)R positive allosteric modulator, undergoes large fluctuations, with high levels during pregnancy and a precipitous drop at birth that is associated with postpartum depression. Allopregnanolone, a metabolite of progesterone, is a positive modulator of most GABA\(\alpha\)R subtypes, including the extrasynaptic \(\delta\)-subunit. This has led to testing of an allopregnanolone preparation, referred to as brexanolone as a novel treatment for postpartum depression with promising clinical results. 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. Further support of the disinhibition hypothesis is evidence that GABA\(\alpha\)R \(\alpha\)5 antagonists also cause rapid antidepressant actions, presumably via similar disinhibition of glutamate signaling. 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. 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. Further fMRI studies demonstrate that ketamine increases global connectivity, and that these effects are associated with depressive symptoms and treatment response. 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. 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.

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. Optogenetic stimulation of mPFC pyramidal neurons also produces rapid and long-lasting antidepressant actions similar to ketamine. The antidepressant actions of ketamine are also blocked by infusions into the mPFC of rapamycin, or an anti-BDNF neutralizing antibody. 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.

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. 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.

Acknowledgements

This research was supported by NIMH Grants MH045481 and MH093897 and the State of CT.

References

1. Kessler R The costs of depression. Psychiatr Clin North Am 2012; 35(1): 1–14. [PubMed: 22370487]
2. Murray CJ, Atkinson C, Bhalla K, Birbeck G, Burststein R, Chou D et al. The state of US health, 1990–2010: burden of diseases, injuries, and risk factors. JAMA 2013; 310(6): 591–608. [PubMed: 23842577]
3. WHO. WHO | Depression [Internet]. Depression 2017.
4. Trivedi M, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psych 2006; 163(1): 28–40.
5. Curtin SC, Warner M, Hedegaard H. Increase in Suicide in the United States, 1999–2014. NCHS Data Brief 2016; (241): 1–8.
6. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 2000; 47(4): 351–354. [PubMed: 10686270]
7. Wilkinson S, Ballard E, Bloch M, Mathew S, Murrough J, Feder A et al. The Effect of a Single Dose of Intravenous Ketamine on Suicidal Ideation: A Systematic Review and Individual Participant Data Meta-Analysis. Am J Psychiatry 2018; 175(2): 150–158. [PubMed: 28969441]
8. Diazgranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S et al. A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Arch Gen Psychiatry 2010; 67(8): 793–802. [PubMed: 20679587]
11. Newport D, Carpenter L, McDonald W, Potash J, Tohen M, Nemeroff C. Ketamine and Other NMDA Antagonists: Early Clinical Trials and Possible Mechanisms in Depression. Am J Psych 2015; 172(10).

12. Sanacora G, Frye MA, McDonald W, Mathew SJ, Turner MS, Schatzberg AF et al. A Consensus Statement on the Use of Ketamine in the Treatment of Mood Disorders. JAMA Psychiatry 2017; 74(4): 399–405. [PubMed: 28249076]

13. Zanos P, Gould TD. Mechanisms of ketamine action as an antidepressant. Mol Psychiatry 2018; 23(4): 801–811. [PubMed: 29532791]

14. Moghaddam B, Krystal JH. Capturing the angel in “angel dust”: twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. Schizophr Bull 2012; 38(5): 942–949. [PubMed: 22899397]

15. Henter ID, de Sousa RT, Zarate CA Jr. Glutamatergic Modulators in Depression. Harv Rev Psychiatry 2018.

16. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 2016; 22(3): 238–249. [PubMed: 26937618]

17. Krystal JH, Sanacora G, Duman RS. Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. Biol Psychiatry 2013; 73(12): 1133–1141. [PubMed: 23726151]

18. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 2016; 533(7604): 481–486. [PubMed: 27144355]

19. Yang C, Qu Y, Abe M, Nozawa D, Chaki S, Hashimoto K. (R)-Ketamine Shows Greater Potency and Longer Lasting Antidepressant Effects Than Its Metabolite (2R,6R)-Hydroxynorketamine. Biol Psych 2017; 85(5): e43–e44.

20. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006; 59(12): 1116–1127. [PubMed: 16631126]

21. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361(6407): 31–39. [PubMed: 8421494]

22. Nicoll RA, Malenka RC. Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 1995; 377(6545): 115–118. [PubMed: 7675078]

23. Ogden KK, Traynelis SF. New advances in NMDA receptor pharmacology. Trends Pharmacol Sci 2011; 32(12): 726–733. [PubMed: 21996280]

24. Hansen KB, Yi F, Perszyk RE, Furukawa H, Wollmuth LP, Gibb AJ et al. Structure, function, and allosteric modulation of NMDA receptors. J Gen Physiol 2018; 150(8): 1081–1105. [PubMed: 30037851]

25. Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ et al. Memantine in moderate-to-severe Alzheimer’s disease. N Engl J Med 2003; 348(14): 1333–1341. [PubMed: 12672860]

26. Zarate CA Jr., Singh JB, Quiroz JA, De Jesus G, Denicoff KK, Luckenbaugh DA et al. A double-blind, placebo-controlled study of memantine in the treatment of major depression. Am J Psychiatry 2006; 163(1): 153–155. [PubMed: 16390905]

27. Sanacora G, Smith MA, Pathak S, Su HL, Boeijinga PH, McCarthy DJ et al. Lanicemine: a low-trapping NMDA channel blocker produces sustained antidepressant efficacy with minimal psychotomimetic adverse effects. Mol Psychiatry 2014; 19(9): 978–985. [PubMed: 24126931]

28. Zarate CA Jr., Mathews D, Ibrahim L, Chaves JF, Marquardt C, Ukok I et al. A randomized trial of a low-trapping nonselective N-methyl-D-aspartate channel blocker in major depression. Biol Psychiatry 2013; 74(4): 257–264. [PubMed: 23206319]

29. Sanacora G, Johnson MR, Khan A, Atkinson SD, Riesenbarg RR, Schronen JP et al. Adjunctive Lanicemine (AZD6765) in Patients with Major Depressive Disorder and History of Inadequate Response to Antidepressants: A Randomized, Placebo-Controlled Study. Neuropsychopharmacology 2017; 42(4): 844–853. [PubMed: 27681442]

30. Williams NR, Heifets BD, Blasey C, Sudheimer K, Pannu J, Pankow H et al. Attenuation of Antidepressant Effects of Ketamine by Opioid Receptor Antagonism. Am J Psychiatry 2018: appi201818020138.
31. Mealing GA, Lanthorn TH, Murray CL, Small DL, Morley P. Differences in degree of trapping of low-affinity uncompetitive N-methyl-D-aspartic acid receptor antagonists with similar kinetics of block. J Pharmacol Exp Ther 1999; 288(1): 204–210. [PubMed: 9862772]

32. Blanpied TA, Boeckman FA, Aizenman E, Johnson JW. Trapping channel block of NMDA-activated responses by amantadine and memantine. J Neurophysiol 1997; 77(1): 309–323. [PubMed: 9120573]

33. Maeng S, Zarate CA Jr., Du J, Schloesser RJ, McCammon J, Chen G et al. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol Psychiatry 2008; 63(4): 349–352. [PubMed: 17643398]

34. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF et al. NMDA receptor blockade at rest triggers rapid behavioural anatidepressant responses. Nature 2011; 475: 91–95. [PubMed: 21677641]

35. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997; 17(8): 2921–2927. [PubMed: 9092613]

36. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 2010; 329(5994): 959–964. [PubMed: 20724638]

37. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science 2012; 338(6103): 68–72. [PubMed: 23042884]

38. McEwen BS, Morrison JH. The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. Neuron 2013; 79(1): 16–29. [PubMed: 23849196]

39. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN et al. Mechanisms of stress in the brain. Nat Neurosci 2015; 18(10): 1353–1363. [PubMed: 26404710]

40. MacQueen G, Frodl T. The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry 2011; 16(3): 252–264. [PubMed: 20661246]

41. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol Psychiatry 2011; 69(8): 754–761. [PubMed: 21292242]

42. Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK. Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. Biol Psychiatry 2012; 71(11): 996–1005. [PubMed: 22036038]

43. Liu RJ, Aghajanian GK. Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. Proc Natl Acad Sci U S A 2008; 105(1): 359–364. [PubMed: 18172209]

44. Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR et al. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. Cereb Cortex 2006; 16(3): 313–320. [PubMed: 15901656]

45. Chen MK, Mecca AP, Naganawa M, Finnema SJ, Toyonaga T, Lin SF et al. Assessing Synaptic Density in Alzheimer Disease With Synaptic Vesicle Glycoprotein 2A Positron Emission Tomographic Imaging. JAMA Neurol 2018; 75(10): 1215–1224. [PubMed: 30014145]

46. Citri A, Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology 2008; 33(1): 18–41. [PubMed: 17728696]

47. Huber KM, Klann E, Costa-Mattioli M, Zukin RS. Dysregulation of Mammalian Target of Rapamycin Signaling in Mouse Models of Autism. J Neurosci 2015; 35(41): 13836–13842. [PubMed: 26468183]

48. Richter JD, Bassell GJ, Klann E. Dysregulation and restoration of translational homeostasis in fragile X syndrome. Nat Rev Neurosci 2015; 16(10): 595–605. [PubMed: 26350240]

49. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci 2010; 33(2): 67–75. [PubMed: 19963289]
50. Carrier N, Kabbaj M. Sex differences in the antidepressant-like effects of ketamine. Neuropharmacology 2013; 70: 27–34. [PubMed: 23337256]
51. Miller OH, Yang L, Wang CC, Hargroder EA, Zhang Y, Delprie E et al. GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. Elife 2014; 3: e03581. [PubMed: 25340958]
52. Pazini F, Cunha M, Rosa J, Colla A, Lieberknecht V, Oliveira A et al. Creatine, Similar to Ketamine, Counteracts Depressive-Like Behavior Induced by Corticosterone via PI3K/Akt/mTOR Pathway. Molecular Neurobiology 2016; 53(10): 6818–6834.
53. Harraz M, Tyagi R, Cortés P, Snyder S. Antidepressant action of ketamine via mTOR is mediated by inhibition of nitricergic Rheb degradation. Mol Psych 2016; 21(3): 313–319.
54. Yang C, Kobayashi S, Nakao K, Dong C, Han M, Qu Y et al. AMPA Receptor Activation-Independent Antidepressant Actions of Ketamine Metabolite (S)-Norketamine. Biol Psychiatry 2018; 84(8): 591–600. [PubMed: 29945718]
55. Zhou W, Wang N, Yang C, Li XM, Zhou ZQ, Yang JJ. Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. European psychiatry : the journal of the Association of European Psychiatrists 2014; 29(7): 419–423. [PubMed: 24321772]
56. Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM. Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. Neuron 2007; 55(4): 648–661. [PubMed: 17698016]
57. Fuchikami M, Thomas A, Liu R, Kohleb ES, Land BB, DiLeone RJ et al. Optogenetic stimulation of infralimbic PFC reproduces ketamine’s rapid and sustained antidepressant actions. Proceedings of the National Academy of Sciences 2015; 112(26): 8106–8111.
58. Zagrebelsky M, Korte M. Form follows function: BDNF and its involvement in sculpting the function and structure of synapses. Neuropharmacology 2014; 76 Pt C: 628–638. [PubMed: 23752094]
59. Hill JL, Martinowich K. Activity-dependent signaling: influence on plasticity in circuits controlling fear-related behavior. Curr Opin Neurobiol 2016; 36: 59–65. [PubMed: 26485574]
60. Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 1995; 15(3 Pt 1): 1768–1777. [PubMed: 7891134]
61. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 2005; 136(1–2): 29–37. [PubMed: 15893584]
62. Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide victims. Archives of General Psychiatry 2003; 60(8): 804–815. [PubMed: 12912764]
63. Chen B, Dowlatabadi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001; 50(4): 260–265. [PubMed: 11522260]
64. Zakharenko SS, Patterson SL, Dragatsis I, Zeitlin SO, Siegelbaum SA, Kandel ER et al. Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1-CA3 synapses. Neuron 2003; 39(6): 975–990. [PubMed: 12971897]
65. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003; 112(2): 257–269. [PubMed: 12553913]
66. Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Xiao CJ et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006; 314(5796): 140–143. [PubMed: 17023662]
67. Lepack A, Fuchikami M, Dwyer J, Banos M, Aghajanian G, Duman R. BDNF release is required for the behavioral actions of ketamine. Int J Neuropsychopharmacol 2014; 18(1).
69. Nibuaya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995; 15(11): 7539–7547. [PubMed: 7472505]

70. Laje G, Lally N, Mathews D, Brutsche N, Chemerinski A, Akula N et al. Brain-Derived Neurotrophic Factor Val66Met Polymorphism and Antidepressant Efficacy of Ketamine in Depressed Patients. Biological psychiatry 2012; 72(11): e27–e28. [PubMed: 22771240]

71. Su TP, Chen MH, Li CT, Lin WC, Hong CJ, Gueorguieva R et al. Dose-Related Effects of Adjunctive Ketamine in Taiwanese Patients with Treatment-Resistant Depression. Neuropsychopharmacology 2017; 42(13): 2482–2492. [PubMed: 28492279]

72. Miller OH, Moran JT, Hall BJ. Two cellular hypotheses explaining the initiation of ketamine’s antidepressant actions: Direct inhibition and disinhibition. Neuropharmacology 2016; 100: 17–26. [PubMed: 26211972]

73. Duman RS. Ketamine and rapid-acting antidepressants: a new era in the battle against depression and suicide. F1000Research 2018; 7.

74. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci 2007; 27(43): 11496–11500. [PubMed: 17959792]

75. Widman AJ, McMahon LL. Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. Proc Natl Acad Sci U S A 2018; 115(13): E3007–E3016. [PubMed: 29531088]

76. AJ W, LL M. Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. Proc Natl Acad Sci U S A 2018; 115(13): E3007–E3016. [PubMed: 29531088]

77. Preskorn S, Baker B, Kolluri S, Menniti FS, Krams M, Landen JW. An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101, 606, in patients with treatment-refractory major depressive disorder. J Clin Psychopharmacol 2008; 28: 631–637. [PubMed: 19011431]

78. Gerhard DM, Duman RS. Role of GABAergic interneuron GluN2B subunits on the antidepressant actions of ketamine in male and female mice. Society for Neuroscience: San Diego, California, 2018.

79. Furey ML, Drevets WC. Antidepressant efficacy of the antimuscarinic drug scopolamine: a randomized, placebo-controlled clinical trial. Arch Gen Psychiatry 2006; 63(10): 1121–1129. [PubMed: 17015814]

80. Drevets WC, Furey ML. Replication of scopolamine’s antidepressant efficacy in major depressive disorder: a randomized, placebo-controlled clinical trial. Biol Psychiatry 2010; 67(5): 432–438. [PubMed: 20074703]

81. Park L, Furey M, Nugent AC, Farmer C, Ellis J, Szczepanik J et al. Neurophysiological Changes Associated with Antidepressant Response to Ketamine Not Observed in a Negative Trial of Scopolamine in Major Depressive Disorder. Int J Neuropsychopharmacol 2019; 22(1): 10–18. [PubMed: 30184133]

82. Voleti B, Navarraia A, Liu RJ, Banasr M, Li N, Terwilliger R et al. Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. Biol Psychiatry 2013; 74(10): 742–749. [PubMed: 23751205]

83. Wohleb ES, Wu M, Gerhard DM, Taylor S, Picciotto M, Alreja M et al. M1-type muscarinic acetylcholine receptors on prefrontal cortex interneurons mediate the rapid antidepressant effects of scopolamin. J Clin Invest 2016; Under revision.

84. Pozzi L, Pollak Dorocic I, Wang X, Carlen M, Meletis K. Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. PLoS One 2014; 9(1): e83879. [PubMed: 24454710]

85. Wang CC, Held RG, Chang SC, Yang L, Delpire E, Ghosh A et al. A critical role for GluN2B-containing NMDA receptors in corticidal development and function. Neuron 2011; 72(5): 789–805. [PubMed: 22153375]
86. Yang C, Shirayama Y, Zhang Jc, Ren Q, Yao W, Ma M et al. R-ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects. Translational Psychiatry 2015; 5(9).
87. Yang C, Ren Q, Qu Y, Zhang J, Ma M, Dong C et al. Mechanistic Target of Rapamycin-Independent Antidepressant Effects of (R)-Ketamine in a Social Defeat Stress Model. Biol Psych 2018; 83(1).
88. Pham TH, Defaix C, Xu X, Deng SX, Fabresse N, Alvarez JC et al. Common Neurotransmission Recruited in (R,S)-Ketamine and (2R,6R)-Hydroxynorketamine-Induced Sustained Antidepressant-like Effects. Biol Psychiatry 2018; 84(1): e3–e6. [PubMed: 29174592]
89. Fukumoto K, Fogaca M, Liu R, Duman C, Kato T, Li X et al. Activity dependent BDNF signaling is required for the antidepressant actions of (2R, 6R)-Hydroxynorketamine. PNAS 2018, in revision; in revision.
90. Zhang K, Toki H, Fujita Y, Ma M, Chang L, Qu Y et al. Lack of deuterium isotope effects in the antidepressant effects of (R)-ketamine in a chronic social defeat stress model. Psychopharmacology 2018; 235(11): 3177–3185. [PubMed: 30215218]
91. Shirayama Y, Hashimoto K. Lack of Antidepressant Effects of (2R,6R)-Hydroxynorketamine in a Rat Learned Helplessness Model: Comparison with (R)-Ketamine. Int J Neuropsychopharmacol 2018; 21(1): 84–88. [PubMed: 29155993]
92. Suzuki K, Nosyreva E, Hunt K, Kavalai E, Monteggia L. Effects of a ketamine metabolite on synaptic NMDAR function. Nature 2017; 546(7659).
93. Ibrahim L, Granados ND, Jolkovskyy L, Brutsche N, Luckenbaugh D, Herring W et al. A Randomized, placebo-controlled, crossover pilot trial of the oral selective NR2B antagonist MK-0657 in patients with treatment-resistant major depressive disorder. J Clin Pharmacol 2012; 32(4): 1231–1242.
94. Clements JD, Westbrook GL. Activation kinetics reveal the number of glutamate and glycine binding sites on the N-methyl-D-aspartate receptor. Neuron 1991; 7(4): 605–613. [PubMed: 1681832]
95. Zanos P, Piantadosi SC, Wu HQ, Pribut HJ, Dell MJ, Can A et al. The Prodrug 4-Chlorokynurenine Causes Ketamine-Like Antidepressant Effects, but Not Side Effects, by NMDA/GlycineB-Site Inhibition. J Pharmacol Exp Ther 2015; 355(1): 76–85. [PubMed: 26265321]
96. Wallace M, White A, Grako KA, Lane R, Cato AJ, Snodgrass HR. Randomized, double-blind, placebo-controlled, dose-escalation study: Investigation of the safety, pharmacokinetics, and antihyperalgesic activity of 1-4-chlorokynurenine in healthy volunteers. Scand J Pain 2017; 17: 243–251. [PubMed: 29229209]
97. Papp M, Moryl E. Antidepressant-like effects of l-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. Eur J Pharmacol 1996; 316(2–3): 145–151. [PubMed: 8982680]
98. Heresco-Levy U, Gelfin G, Bloch B, Levin R, Edelman S, Javitt DC et al. A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. Int J Neuropsychopharmacol 2013; 13(3): 501–506. [PubMed: 23174090]
99. Mataix-Cols D, Fernandez de la Cruz L, Monzani B, Rosenfield D, Andersson E, Perez-Vigil A et al. D-Cycloserine Augmentation of Exposure-Based Cognitive Behavior Therapy for Anxiety, Obsessive-Compulsive, and Posttraumatic Stress Disorders: A Systematic Review and Meta-analysis of Individual Participant Data. JAMA Psychiatry 2017; 74(5): 501–510. [PubMed: 28122091]
100. Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. Biol Psychiatry 2006; 60(4): 369–375. [PubMed: 16919524]
101. Malkesman O, Austin DR, Tragon T, Wang G, Rompala G, Hamidi AB et al. Acute D-serine treatment produces antidepressant-like effects in rodents. Int J Neuropsychopharmacol 2012; 15(8): 1135–1146. [PubMed: 21906419]
102. Huang CC, Wei IH, Huang CL, Chen KT, Tsai MH, Tsai P et al. Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. Biol Psychiatry 2013; 74(10): 734–741. [PubMed: 23562005]
103. Chen KT, Tsai MH, Wu CH, Jou MJ, Wei IH, Huang CC. AMPA Receptor-mTOR Activation is Required for the Antidepressant-Like Effects of Sarcosine during the Forced Swim Test in Rats: Insertion of AMPA Receptor may Play a Role. Front Behav Neurosci 2015; 9: 162. [PubMed: 26150775]

104. Moskal JR, Burgdorf JS, Stanton PK, Kroes RA, Disterhoft JF, Burch RM et al. The Development of Rapastinel (Formerly GLYX-13): A Rapid Acting and Long Lasting Antidepressant. Curr Neuropharmacol 2017; 15(1): 47–56. [PubMed: 26997507]

105. Burgdorf J, Zhang XL, Weiss C, Gross A, Boikess SR, Kroes RA et al. The long-lasting antidepressant effects of rapastinel (GLYX-13) are associated with a metaplasticity process in the medial prefrontal cortex and hippocampus. Neuroscience 2015; 308: 202–211. [PubMed: 26343295]

106. Burgdorf J, Zhang XL, Nicholson KL, Balster RL, Leander JD, Stanton PK et al. GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. Neuropsychopharmacology 2013; 38(5): 729–742. [PubMed: 23303054]

107. Liu RJ, Duman C, Kato T, Hare B, Lopresto D, Bang E et al. GLYX-13 Produces Rapid Antidepressant Responses with Key Synaptic and Behavioral Effects Distinct from Ketamine. Neuropsychopharmacology 2017; 42(6): 1231–1242. [PubMed: 27634355]

108. Preskorn S, Macaluso M, Mehra DO, Zammit G, Moskal JR, Burch RM et al. Randomized proof of concept trial of GLYX-13, an N-methyl-D-aspartate receptor glycine site partial agonist, in major depressive disorder nonresponsive to a previous antidepressant agent. Journal of psychiatric practice 2015; 21(2): 140–149. [PubMed: 25782764]

109. Rajagopal L, Huang M, Li J, He W, Soni D, Banerjee P et al. Rapastinel, a novel NMDA receptor modulator, produces prolonged rescue of subchronic phencyclidine - induced deficits in episodic memory as well as other beneficial effects on cognitive function in a rapamycin sensitive manner. Society for Neuroscience: Washington, DC, 2017.

110. (Glyx-13), A rapid acting antidepressant, does not increase extracellular levels of dopamine and glutamate in rat medial prefrontal cortex. Proceedings of the American College of Neuropsychopharmacology2016; Hollywood, Florida.

111. Abdallah CG, Sanacora G, Duman RS, Krystal JH. The neurobiology of depression, ketamine and rapid-acting antidepressants: Is it glutamate inhibition or activation? Pharmacol Ther 2018; 190: 148–158. [PubMed: 29803629]

112. Hascup ER, Hascup KN, Stephens M, Pomerleau F, Huettl P, Gratton A et al. Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. J Neurochem 2010; 115(6): 1608–1620. [PubMed: 20969570]

113. Xi ZX, Baker DA, Shen H, Carson DS, Kalivas PW. Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. J Pharmacol Exp Ther 2002; 300(1): 162–171. [PubMed: 11752112]

114. Koike H, Chaki S. Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. Behav Brain Res 2014; 271: 111–115. [PubMed: 24909673]

115. Koike H, Iijima M, Chaki S. Involvement of the mammalian target of rapamycin signaling in the antidepressant-like effect of group II metabotropic glutamate receptor antagonists. Neuropharmacology 2011; 61(8): 1419–1423. [PubMed: 21903115]

116. Dwyer JM, Lepack AE, Duman RS. mTOR activation is required for the antidepressant effects of mGluR2/3 blockade. Int J Neuropsychopharmacol 2012; 15(4): 429–434. [PubMed: 22114864]

117. Dwyer JM, Lepack AE, Duman RS. mGluR2/3 blockade produces rapid and long-lasting reversal of anhedonia caused by chronic stress exposure. J Mol Psychiatry 2013; 1(1): 15. [PubMed: 25408908]

118. Joffe ME, Conn PJ. Antidepressant potential of metabotropic glutamate receptor mGlu2 and mGlu3 negative allosteric modulators. Neuropsychopharmacology 2018.

119. Engers JL, Rodriguez AL, Konkol LC, Morrison RD, Thompson AD, Byers FW et al. Discovery of a Selective and CNS Penetrant Negative Allosteric Modulator of Metabotropic Glutamate
10. Fukumoto K, Iijima M, Funakoshi T, Chaki S. Role of 5-HT1A Receptor Stimulation in the Medial Prefrontal Cortex in the Sustained Antidepressant Effects of Ketamine. Int J Neuropsychopharmacol 2018; 21(4): 371–381. [PubMed: 29309585]

12. Ghosal S, Bang E, Yue W, Hare BD, Lepack AE, Girgenti MJ et al. Activity-Dependent Brain-Derived Neurotrophic Factor Release Is Required for the Rapid Antidepressant Actions of Scopolamine. Biol Psychiatry 2018; 83(1): 29–37. [PubMed: 28751069]

122. Efeyan A, Comb W, Sabatinii D. Nutrient-sensing mechanisms and pathways. Nature 2015; 517(7534): 302–310. [PubMed: 25592535]

123. Kato T, Fogaca M, Deyama S, Li X, Fukumoto K, Duman R. BDNF release and signaling are required for the antidepressant actions of GLYX-13. Mol Psych 2017.

124. Bjorkholm C, Marcus MM, Konradsson-Geuken A, Jardemark K, Svensson TH. The novel antipsychotic drug brexpiprazole, alone and in combination with escitalopram, facilitates prefrontal glutamatergic transmission via a dopamine D1 receptor-dependent mechanism. Eur Neuropsychopharmacol 2017; 27(4): 411–417. [PubMed: 28190661]

125. Sun X, Zhao Y, Wolf ME. Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. J Neurosci 2005; 25(32): 7342–7351. [PubMed: 16093384]

126. Arnsten AF. Stress weakens prefrontal networks: molecular insults to higher cognition. Nat Neurosci 2015; 18(10): 1376–1385. [PubMed: 26404712]

128. D’Aquila PS, Collu M, Pani L, Gessa GL, Serra G. Antidepressant-like effect of selective dopamine D1 receptor agonists in the behavioural despair animal model of depression. Eur J Pharmacol 1994; 262(1–2): 107–111. [PubMed: 7813561]

132. Hirota K, Okawa H, Appadu BL, Grandy DK, Devi LA, Lambert DG. Stereoselective interaction of ketamine with recombinant mu, kappa, and delta opioid receptors expressed in Chinese hamster ovary cells. Anesthesiology 1999; 90(1): 174–182. [PubMed: 9915326]

134. Yoon G, Petrakis IL, Krystal JH. Preliminary evidence against a role for opiate receptor signaling in the antidepressant effects of R/S-ketamine. JAMA Psychiatry 2018.

135. Sanacora G, Chen A, Shin K and RS Duman RS. Influence of ketamine on ECS induction of BDNF and sprouting in rat hippocampus. J Neurochem 1999: submitted.

Mol Psychiatry. Author manuscript; available in PMC 2019 September 21.
138. Fee C, Banasr M, Sibille E. Somatostatin-Positive Gamma-Aminobutyric Acid Interneuron Deficits in Depression: Cortical Microcircuit and Therapeutic Perspectives. Biol Psychiatry 2017; 82(8): 549–559. [PubMed: 28697889]

139. Banasr M, Lepack A, Fee C, Duric V, Maldonado-Aviles J, DiLeone R et al. Characterization of GABAergic marker expression in the chronic unpredictable stress model of depression. Chronic Stress (Thousand Oaks) 2017; 1.

140. Lin LC, Sibille E. Transcriptome changes induced by chronic psychosocial/environmental or neuroendocrine stressors reveal a selective cellular vulnerability of cortical somatostatin (SST) neurons, compared with pyramidal (PYR) neurons. Mol Psychiatry 2015; 20(3): 285. [PubMed: 25754192]

141. Czéh B, Vardy I, Varga Z, Febbraro F, Csabai D, Martis L-S et al. Long-Term Stress Disrupts the Structural and Functional Integrity of GABAergic Neuronal Networks in the Medial Prefrontal Cortex of Rats. Frontiers in cellular neuroscience 2018; 12(148).

142. Piantadosi SC, French BJ, Poe MM, Timic T, Markovic BD, Pabba M et al. Sex-Dependent Anti-Stress Effect of an alpha5 Subunit Containing GABA Receptor Positive Allosteric Modulator. Front Pharmacol 2016; 7: 446. [PubMed: 27920723]

143. Fuchs T, Jefferson SJ, Hooper A, Yee P-HP, Maguire J, Luscher B. Disinhibition of somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-like brain state. Molecular psychiatry 2017; 22(6): 920–930. [PubMed: 27821870]

144. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of gonadal steroids in women with a history of postpartum depression. Am J Psychiatry 2000; 157(6): 924–930. [PubMed: 10831472]

145. Schiller CE, Meltzer-Brody S, Rubinow DR. The role of reproductive hormones in postpartum depression. CNS Spectr 2015; 20(1): 48–59. [PubMed: 25263255]

146. MacKenzie G, Maguire J. Neurosteroids and GABAergic signaling in health and disease. Biomol Concepts 2013; 4(1): 29–42. [PubMed: 25436563]

147. Zorumski CF, Paul SM, Izumi Y, Covey DF, Mennerick S. Neurosteroids, stress and depression: potential therapeutic opportunities. Neuroscience and biobehavioral reviews 2013; 37(1): 109–122. [PubMed: 23085210]

148. Kanes S, Colquhoun H, Gunduz-Bruce H, Raines S, Arnold R, Schacterle A et al. Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. Lancet 2017; 390(10093): 480–489. [PubMed: 28619476]

149. Zanos P, Nelson ME, Highland JN, Krimmel SR, Georgiou P, Gould TD et al. A Negative Allosteric Modulator for alpha5 Subunit-Containing GABA Receptors Exerts a Rapid and Persistent Antidepressant-Like Action without the Side Effects of the NMDA Receptor Antagonist Ketamine in Mice. eNeuro 2017; 4(1).

150. Fischell J, Van Dyke AM, Kvarta MD, LeGates TA, Thompson SM. Rapid Antidepressant Action and Restoration of Excitatory Synaptic Strength After Chronic Stress by Negative Modulators of Alpha5-Containing GABAA Receptors. Neuropsychopharmacology 2015; 40(11): 2499–2509. [PubMed: 25900119]

151. De Simoni S, Schwarz AJ, O’Daly OG, Marquand AF, Brittain C, Gonzales C et al. Test-retest reliability of the BOLD pharmacological MRI response to ketamine in healthy volunteers. Neuroimage 2013; 64: 75–90. [PubMed: 23009959]

152. Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM. Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. Arch Gen Psychiatry 2008; 65(2): 154–164. [PubMed: 18250253]

153. Driesen NR, McCarthy G, Bhagwagar Z, Bloch M, Calhoun V, D’Souza DC et al. Relationship of resting brain hyperconnectivity and schizophrenia-like symptoms produced by the NMDA receptor antagonist ketamine in humans. Mol Psychiatry 2013; 18(11): 1199–1204. [PubMed: 23337947]

154. Abdallah CG, Averill LA, Collins KA, Geha P, Schwartz J, Averill C et al. Ketamine Treatment and Global Brain Connectivity in Major Depression. Neuropsychopharmacology 2017; 42(6): 1210–1219. [PubMed: 27604566]
155. Evans JW, Szczepanik J, Brutsche N, Park LT, Nugent AC, Zarate CA Jr. Default Mode Connectivity in Major Depressive Disorder Measured Up to 10 Days After Ketamine Administration. Biol Psychiatry 2018; 84(8): 582–590. [PubMed: 29580569]

156. Carreno FR, Donegan JJ, Boley AM, Shah A, DeGuzman M, Frazer A et al. Activation of a ventral hippocampus-medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. Mol Psychiatry 2016; 21(9): 1298–1308. [PubMed: 26619811]

157. Yang Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S et al. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. Nature 2018; 554(7692): 317–322. [PubMed: 29446381]
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)\textsuperscript{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 Ca\textsuperscript{2+} entry, which is required for intracellular signaling and synaptic plasticity. At resting Mg\textsuperscript{2+} is bound in and blocks the pore, and thereby blocks Ca\textsuperscript{2+} flux as well as entry and binding of channel blockers. Upon neuronal activation, typically by AMPA receptor dependent depolarization, the Mg\textsuperscript{2+} block is removed allowing entry of Ca\textsuperscript{2+}. This open pore state is also accessible to ketamine and other channel blockers, which enter and block Ca\textsuperscript{2+} 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.
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 disinhibition 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**       | GluA1, Blocked by NBQX               | mPFC            | mTORC1, rapamycin sensitive           | Release, ADT beh Blocked by BDNF nAb       | Increased EPSCs & spines in mPFC        | Blocked, NR2B kd Sst/Gad                  |
| (NMDA antagonist)      |                                      |                 |                                      |                                            |                                       |                                          |
| **Scopolamine**        | GluA1, Blocked by NBQX               | mPFC            | mTORC1, rapamycin sensitive           | Release, ADT beh Blocked by BDNF nAb       | Increased EPSCs & spines in mPFC        | Blocked, AChM1 kd Sst/Gad                 |
| (ACh-muscarinic        |                                      |                 |                                      |                                            |                                       |                                          |
| antagonist)            |                                      |                 |                                      |                                            |                                       |                                          |
| **Rapastinel**         | GluA1, Blocked by NBQX               | mPFC            | mTORC1, rapamycin sensitive           | Release, ADT beh Blocked by BDNF nAb       | Increased EPSCs & spines in mPFC        | Blocked by NR2B kd on pyramidal Camk2 cells |
| (positive allosteric   |                                      |                 |                                      |                                            |                                       |                                          |
| modulator)             |                                      |                 |                                      |                                            |                                       |                                          |
| **LY341495**           | GluA1, Blocked by NBQX               | mPFC            | mTORC1, rapamycin sensitive           | BDNF release                               | Not tested                             | Not tested                               |
| (mGlu2/3 antagonist)   |                                      |                 |                                      |                                            |                                       |                                          |
| **Ro 25-6981**         | GluA1, Blocked by NBQX               | mPFC glut       | mTORC1, rapamycin sensitive           | Not tested                                 | Not tested                             | Not tested                               |
| (NR2B NAM)             |                                      | cycling         |                                      |                                            |                                       |                                          |
| **(2R,6R)-HNK**        | GluA1, Blocked by NBQX               | mPFC            | mTORC1, rapamycin sensitive           | Release, ADT beh Blocked by BDNF nAb       | Increased EPSCs in mPFC                 | Blocked, NR2B kd Sst/Gad                  |
| (Ketamine metabolite)  |                                      |                 |                                      |                                            |                                       |                                          |
| **S-ketamine**         | GluA1, Blocked by NBQX               | Not tested      | mTORC1, rapamycin sensitive           | Not tested                                 | Not tested                             | Not tested                               |
| (stereoisomer)         |                                      |                 |                                      |                                            |                                       |                                          |

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.