New Targets for Rapid Antidepressant Action

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Abstract

Current therapeutic options for major depressive disorder (MDD) and bipolar disorder (BD) are associated with a lag of onset that can prolong distress and impairment for patients, and their antidepressant efficacy is often limited. All currently approved antidepressant medications for MDD act primarily through monoaminergic mechanisms. Glutamate is the major excitatory neurotransmitter in the central nervous system, and glutamate and its cognate receptors are implicated in the pathophysiology of MDD, and in the development of novel therapeutics for this disorder. The rapid and robust antidepressant effects of the N-methyl-D-aspartate (NMDA) antagonist ketamine were first observed in 2000. Since then, other NMDA receptor antagonists have been studied in MDD. Most have demonstrated relatively modest antidepressant effects compared to ketamine, but some have shown more favorable characteristics. This article reviews the clinical evidence supporting the use of novel glutamate receptor modulators with direct affinity for cognate receptors: 1) non-competitive NMDA receptor antagonists (ketamine, memantine, dextromethorphan, AZD6765); 2) subunit (GluN2B)-specific NMDA receptor antagonists (CP-101,606/traxoprodil, MK-0657); 3) NMDA receptor glycine-site partial agonists (GLYX-13); and 4) metabotropic glutamate receptor (mGluR) modulators (AZD2066, RO4917523/basimglurant). We also briefly discuss several other theoretical glutamate receptor targets with preclinical antidepressant-like efficacy that have yet to be studied clinically; these include \textalpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) agonists and mGluR2/3 negative allosteric modulators. The review also discusses other promising, non-glutamatergic targets for potential rapid antidepressant effects, including the cholinergic system (scopolamine), the opioid system (ALKS-5461), corticotropin releasing factor (CRF) receptor antagonists (CP-316,311), and others.
1. Introduction

Depression directly affects the brain and periphery and is associated with diverse other medical comorbidities due its systemic deleterious effects. The “monoamine hypothesis” of depression—which was developed after observing the pharmacological effects of early drugs for depression—is no longer the only model capable of explaining the mechanism of action of antidepressants or for studying the underlying pathophysiology of depressive episodes in mood disorders.

Currently available conventional antidepressants unfortunately have low rates of treatment response; while one-third of patients with depression will respond to their first antidepressant, approximately two-thirds will respond only after trying several classes of antidepressants (Trivedi et al., 2006). Furthermore, therapeutic approaches must be considered not only in the context of treating acute episodes, but for relapse prevention as well as intervention in the early phases of illness. With regard to conventional antidepressants, few targets besides the monoamines and the hypothalamic pituitary adrenal (HPA) stress axis have been identified as key candidates; nevertheless, the interaction between organs, proteins, hormones, and several comorbid diseases remains complex, and results of studies investigating these targets are preliminary. Thus, there is a strong need to identify and rapidly test novel antidepressants with different biological targets beyond the classic monoaminergic receptors and their downstream targets; these agents would also be expected to act faster in a larger percentage of individuals. However, in recent years the pharmaceutical industry has been investing less in psychiatry and mood disorders as a therapeutic area. This review discusses some of the striking recent advances in the development of novel, rapid-acting antidepressants as well as the potential issues and pitfalls related to research in this field. We also present an overview of the most promising targets and approaches as well as ideas for next steps for drug development.

2. Rapid Onset of Antidepressant Action

As noted above, currently available monoaminergic antidepressants are associated with a delayed onset of action of several weeks, a latency period that significantly increases risk of suicide and self-harm and is a key public health issue in psychiatric practice (Machado-Vieira et al., 2009c). This concept of a latency period before achieving antidepressant efficacy is widely accepted despite the fact that very few trials have evaluated efficacy outcomes on a daily basis during the first week of treatment with conventional antidepressants. High rate of placebo response has also been problematic when evaluating new antidepressants. As a result, much remains unknown about the actual timing of antidepressant efficacy (that is, early improvement) for any class of standard antidepressants (Katz et al., 2004; Machado-Vieira et al., 2010); most of these data come from post-hoc analyses.

Nevertheless, several clinical studies suggest that rapid antidepressant effects are achievable in humans. This lends an additional urgency to the development of new treatments for depression that target alternative neurobiological systems, particularly for those subgroups of patients who do not respond to any currently available pharmacological agents. New
therapeutics could significantly lower morbidity and mortality for both major depressive disorder (MDD) and bipolar disorder (BD) and commensurately minimize or prevent disruption to personal, family, and occupational life and functioning as well as lower risk of suicide. In addition, the neurobiological impact of cumulative exposure to depression would be minimized, which might result in less chronicity and fewer recurrences. It should also be noted that new insights into the potential association between early improvement and long-term outcomes would be helpful tools in clinical practice; knowledge gleaned from such studies could be used in the context of personalized medicine. Indeed, identifying new targets for rapid antidepressant efficacy seems to be a relevant approach not only in treatment-resistant cases but also for the initial treatment of patients who respond well to conventional monoaminergic antidepressants and are, as a result, expected to wait several weeks for therapeutic effects to manifest. Nevertheless, developing agents that exert rapid antidepressant effects remains difficult. Perhaps the most significant challenge is dealing with the gap between rapid antidepressant response, long-term treatment, and maintenance therapy after response and remission.

In the context of developing novel therapeutic targets for depression, glutamate and other ionic channel receptors seem to induce faster biological effects at intracellular downstream targets and currently represent the most promising targets for drug development. Rapid improvement is a key paradigm for achieving fast relief of symptoms and, in some cases, preventing new episodes when prodromal symptoms are observed; this paradigm is similar to that seen for other medical illnesses such as asthma, migraine, and atrial fibrillation. Below, we discuss the concept of rapid antidepressant action and present findings and perspectives related to modulation of the glutamatergic system by ketamine and other subunit-specific glutamate modulators. We also describe the molecular pathways and downstream-related targets associated with the regulation of rapid antidepressant action in diverse neurotransmitter and neuromodulatory systems.

3. Regulation of Glutamate Ionotropic Receptors (NMDA, AMPA) in the context of Rapid Antidepressant Effects: General Overview

Glutamate is the main excitatory neurotransmitter in the mammalian brain. Roughly one-third of central nervous system (CNS) neurons use glutamate and, in combination with other excitatory neurotransmitters, it plays a key role in memory, learning, and neuroplasticity (Machado-Vieira et al., 2012; Machado-Vieira et al., 2009b); broadly, the term neuroplasticity includes changes in gene regulation and intracellular signaling cascade, variations in neurotransmitter release, modifications of synaptic number and strength, modeling of dendritic and axonal architecture and, in some areas of the CNS, the generation of new neurons (Machado-Vieira et al., 2008). Glutamate is also crucial to dendritic spine formation remodeling, influencing the density and morphology of dendritic spines. Indeed, changes in glutamate levels could contribute to abnormalities in dendritic spines and may represent a therapeutic target for rapid-acting glutamate modulators.

Glutamate neurons are present in high densities in the cortex as well as in subcortical structures such as the cerebellum, hippocampus, thalamic nuclei, and caudate nucleus.
Glutamate is generated from α-ketoglutarate, an intermediate in the Krebs cycle, and is packed into secretory vesicles in the presynaptic neuron by a family of vesicular glutamate transporters. Alternative sources of glutamate include enzymatic reactions regulated by glutamate dehydrogenase (GDH) and aminotransferases; in particular, alanine-aminotransferase (ALAT), aspartate aminotransferase (AAT), and the branched chain aminotransferase (BCAA) are most likely to be involved in glutamate biosynthesis (Schousboe et al., 2013). Glutamate is subsequently released pre-synaptically into the synaptic cleft and activates both ionotropic and metabotropic glutamate receptors on astrocytes and in pre- and postsynaptic neurons. Glutamate receptor subtypes involve ligand-gated ion channels (N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors) as well as the eight G-protein coupled metabotropic receptors (mGluRs). Glutamate is not metabolized by any process; its concentrations are tightly regulated by glutamate reuptake transporters localized on neurons and glia (Danbolt, 2001).

The NMDA receptor is activated by glutamate in the presence of a co-agonist D-serine or glycine and blocked by extracellular magnesium. Only depolarization induced by AMPA receptor activation releases magnesium-induced blockade from the NMDA receptor pore, thus allowing the flow of other electrolytes (e.g., calcium) (Lai et al., 2014; Machado-Vieira et al., 2009b).

The NMDA channel includes a combination of GluN1, GluN2, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B receptor subunits. Two molecules of glycine and two of glutamate are required for ion channel activation. Other identified sites include the “s” and phencyclidine (PCP) sites. Several drugs that bind to the PCP site are defined as noncompetitive NMDA receptor antagonists. These include dizocilpine (MK-801), PCP, and ketamine. The AMPA channel is composed of the glutamate receptor GluA1, GluA2, GluA3, and GluA4 subunits, which have lower affinity for glutamate than NMDA receptors. Within the tripartite glutamate synapse and its circuitry (Machado-Vieira et al., 2009b), a complex and intricate dynamic interaction exists between ionotropic glutamate receptors and mGluRs with regard to the reuptake and transport of glutamate as well as the glutamate/glutamine recycling mechanism (Machado-Vieira et al., 2009b). Indeed, the glutamate system is far more complex than the monoaminergic system. Both ionotropic glutamate receptors and mGluRs have a wide range of effects, enzymes, downstream targets, and proposed biological models. This complexity is one of the main reasons why some glutamate modulators are so effective in treating mood disorders (eg, ketamine, lamotrigine), while others appear not to work (eg, memantine, riluzole).

4. AMPA and NMDA Receptors: Specific Findings in Mood Disorders Research

Preclinical evidence suggests that the glutamatergic system in general—and the NMDA and ionotropic receptors in the tripartite glutamatergic synapse in particular—may be central to both the pathophysiology of MDD and the mechanism of action of antidepressants (Skolnick, 1999, 2002; Skolnick et al., 1996). Most of the evidence pertaining to the
pathophysiology of mood disorders supports the presence of increased glutamate levels and activity in the brain and periphery (Zarate et al., 2010). Some researchers have hypothesized that NMDA could even represent a convergent mechanistic target for the antidepressant action of conventional antidepressants and mood stabilizers as well as novel experimental therapeutics, given that previous studies found that chronic treatment with various classes of antidepressant agents affected—predominantly by antagonizing—NMDA receptor function (Skolnick, 1999). Chronic and acute conventional antidepressants have also widely been reported to directly target NMDA receptors and dampen the presynaptic glutamate release induced by acute stress or in physiological circumstances.

In the search for novel, rapid-acting therapeutic targets, the major obstacles to success have included difficulty establishing the clinical validity of a particular target and the limited predictive value of pre-clinical models for mood disorders (Paul et al., 2010). Nevertheless, preclinical studies have noted several intriguing findings. For instance, chronic treatment with conventional antidepressants reduced the number of cortical β-adrenoreceptors (Koshikawa et al., 1989; Vetulani, 1984). In addition, the NMDA antagonists MK-801 (a non-competitive antagonist) and 1-aminocyclopropanecarboxylic acid (ACPC; a partial agonist at the glycine or co-activator site) both reduced $[^{3}H]$ dihydroalprenolol binding to β-cortical adrenoreceptors (Klimek and Papp, 1994; Paul et al., 1992). Imipramine had similar effects (Klimek and Papp, 1994). Glutamate microinjections in the prefrontal cortex (PFC) aggravated learned helplessness in rats one and 72 hours post-administration (Petty et al., 1985). Conversely, antidepressant administration affected NMDA binding profiles and receptor function (Mjellem et al., 1993). Chronic antidepressant administration also induced adaptive changes in ligand binding at the NMDA receptor glycine site (Nowak et al., 1993). In vitro, tricyclic antidepressants (TCAs) directly interacted with the NMDA receptor complex to block NMDA’s actions. One early study reported that, similar to zinc, imipramine and desipramine slowed the dissociation rate of $[^{3}H]$ MK-801 binding; zinc is thought to act noncompetitively at a site outside the channel (Reynolds and Miller, 1988). TCAs appear to be less potent when magnesium and L-glutamate are added (Sills and Loo, 1989); they also appear to be selective for the low-affinity state of the PCP binding site. Citalopram, fluoxetine, sertraline, and TCAs such as amitriptyline and imipramine all enhanced MK-801-induced locomotor effects (Maj et al., 1991).

Research using several animal models has also demonstrated that NMDA receptor antagonists induce antidepressant-like effects (Layer et al., 1995; Meloni et al., 1993; Moryl et al., 1993; Papp and Moryl, 1994; Przegalinski et al., 1997; Trullas and Skolnick, 1990). For instance, in male Wistar rats a single dose of the NMDA antagonist ketamine interfered with induction of behavioral despair for up to 10 days post-administration (Yilmaz et al., 2002). Studies from our laboratory found that in rats, a single dose of ketamine (2.5 mg/kg) resulted in sustained antidepressant effects lasting approximately one week (Maeng et al., 2008). Another study found that a single pretreatment dose with the NMDA antagonist MK-801 induced a lasting sensitivity to the second administration of the same agent four, seven, or 14 days later (O’Neill and Sanger, 1999). Interestingly, antidepressant-like behavioral responses were observed in mice lacking interneuronal NMDA receptors, supporting the notion that NMDA antagonism is not the only mechanism involved in ketamine’s rapid antidepressant effects (Pozzi et al., 2014). The role of glutamatergic...
dysfunction in depression is further supported by findings that repeated antidepressant administration regionally altered mRNA expression encoding multiple NMDA receptor subunits (Boyer et al., 1998) as well as radioligand binding to these receptors within particular areas of the CNS (Skolnick, 1999). Relatedly, enhanced glutamate levels and fewer hippocampal NMDA receptors were observed in postmortem studies of individuals with mood disorders (Hashimoto et al., 2007; Scarr et al., 2003). However, it should be noted that excessive glutamate levels (caused by stress) in MDD have been associated with decreased brain volume and plasticity (Sanacora et al., 2012): glutamate antagonists, including ketamine, could reverse these deleterious effects.

In preclinical studies, the rapid antidepressant effects of ketamine appeared to involve the activation of AMPA receptors. Increased glutamatergic activity seems key to this effect, given that AMPA receptor antagonists blocked ketamine’s antidepressant effects in preclinical studies (Autry et al., 2011; Duman and Aghajanian, 2012; Koike et al., 2011; Maeng et al., 2008). Specifically, pre-treatment with NBQX, an AMPA receptor antagonist, blocked ketamine’s molecular and behavioral effects (Maeng et al., 2008; Zhou et al., 2014). In support of this model, increased hippocampal AMPA/NMDA receptor density ratio was observed after ketamine treatment in rodents (Tizabi et al., 2012). In the same context, low-dose ketamine enhanced glutamate activity in the PFC, activating synaptic function. This, in turn, was proposed to activate AMPA signaling (Autry et al., 2011; Maeng et al., 2008). These data support the notion that ketamine exerts rapid antidepressant-like effects by enhancing AMPA relative to NMDA throughput in critical neuronal circuits and molecular pathways and targets. Given the supported key role for AMPA in the rapid efficacy of ketamine, several new pharmacological approaches were developed and tested that target AMPA receptor function and levels. These AMPAkines, also known as AMPA positive allosteric modulators, are described in Section 8.1.

5. Ketamine as a Proof of Concept Agent in Studies of Rapid Antidepressant Action: Biological Models

Ketamine is a noncompetitive antagonist; it binds within the ion channel and blocks the influx of diverse ions. Ketamine is called a “trapping blocker” of the NMDA channel. It acts as a non-competitive NMDA receptor antagonist, which means that it only blocks the receptor when the channel is open after activation. Evidence from different models suggests that several molecular mechanisms are associated with ketamine’s plasticity-inducing effects. For instance, studies of diverse proteins and intracellular signaling cascades suggest that increased neuroplasticity and synaptogenesis are key convergent downstream targets for rapid-acting agents. Links are also believed to exist between the neuroplasticity hypothesis of depression and the glutamate hypothesis of rapid antidepressant action. Improved neuronal plasticity (e.g. synaptogenesis, neurogenesis, dendritic remodeling, etc) appears to be a target for ketamine, and may reverse the deleterious effects of long-term depression such as reduced cellular resilience and stress in the hippocampus and PFC (Duman and Duman, 2015).
A full review of the evidence for ketamine’s antidepressant effects is beyond the scope of this manuscript. We refer interested readers to several excellent comprehensive reviews on ketamine trials in depression (Abdallah et al., 2015; Coyle and Laws, 2015). Instead, this manuscript will focus on the neurobiological basis of rapid antidepressant action as well as alternative targets with a focus on ketamine as a proof-of-concept agent.

The hypothesis of glutamate hyperfunction for mood disorders—which differs from the hypothesis of glutamate hypofunction in schizophrenia—posits that these elevated glutamate levels may revert to normal as a result of the increased extracellular glutamate levels observed after low-dose ketamine; this effect is thought to be due to the disinhibition of pyramidal neurons mediated by the NMDA antagonism of inhibitory interneurons (Ohgi et al., 2015). Ketamine selectively induces antagonistic effects in the presence of excessive NMDA activation (Mealing et al., 1999), with a consequent increase in extrasynaptic glutamate andactivation of plasticity pathways; notably, this should not be misinterpreted as glutamate hyperfunction. Indeed, transient increases in glutamate release may represent an upstream event; evidence from $^{13}$C-magnetic resonance spectroscopy (MRS) (Chowdhury et al., 2012) and microdialysis (Moghaddam et al., 1997) studies support the notion that ketamine dose-dependently affects glutamate efflux. Lower Glx (a combined measure of glutamate and glutamine) levels have also been identified in imaging studies in depression (Dutta et al., 2015), and these seem to be reversed by ketamine, which prevents overstimulation of NMDA receptor or glial dysfunction.

Some investigators have suggested that ketamine’s psychotomimetic effects may be related to its higher affinity for the NMDA receptor subunits GluN1 and GluN2 (Dutta et al., 2015). Interestingly, research shows that ketamine’s rapid antidepressant effects are strongly predicted by its dissociative side effects (Luckenbaugh et al., 2014); both of these support the role of GluN1 and GluN2a in ketamine’s rapid antidepressant efficacy. While ketamine’s psychotomimetic effects could briefly mimic antidepressant effects, the fact that ketamine’s antidepressant efficacy goes much beyond its short half-life suggests consistent downstream effects (beyond NMDA antagonism per se) that may explain its relatively sustained response. However, drugs with psychotomimetic side effects cannot be used in subjects with a history of psychosis (because of concerns that these may worsen or restart a psychotic state).

Although NMDA antagonism seems to mediate the antidepressants effects of ketamine, one key question remains. Why do other NMDA antagonists that also share comparable pharmacodynamics with ketamine not have similar rapid antidepressant efficacy (Emnett et al., 2013; Smith et al., 2013; Zarate et al., 2006)? Like ketamine, conventional antidepressants also reverse stress-induced reductions in dendritic arborization due to excessive glutamate levels and consequent neurotoxicity (Duman, 2009). Interestingly, early studies showed that conventional antidepressants decreased NMDA binding (Nowak et al., 1998; Paul et al., 1994; Skolnick et al., 1996). This further supports the notion that ketamine’s rapid antidepressant effects are due to more than simple NMDA antagonism.
6. Ketamine’s Effects on Synaptogenesis, mTOR, and Intracellular Signaling: Potential Therapeutic Implications

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cellular metabolism, growth, and survival as well as protein synthesis and transcription (Duman et al., 2012; Machado-Vieira et al., 2015). The mTOR pathway is altered in other illnesses such as obesity and diabetes, as well as in ageing and stress-related disorders (Zoncu et al., 2011). Reduced mTOR signaling has been observed in the PFC and periphery of subjects with mood disorders (Jernigan et al., 2011; Machado-Vieira et al., 2015), and ketamine has been shown to activate mTOR pathway-induced synaptogenesis, with consequent activation of other neuroprotective and neurotrophic downstream targets associated with mood disorders (eg, cyclic adenosine monophosphate response element-binding protein (CREB), brain derived neurotrophic factor (BDNF), and the Wnt pathway). Several case reports have also reported increased mTOR phosphorylation in peripheral cells after acute ketamine administration in individuals with MDD (Denk et al., 2011; Yang et al., 2013). Interestingly, mTOR inhibition by rapamycin reversed ketamine’s antidepressant effects in preclinical models (Yu et al., 2013). Ketamine also stimulated mTOR signaling; the consequent rapid antidepressant action was hypothesized to depend on AMPA receptor activation (Duman et al., 2012), supporting the notion that AMPA receptors play a crucial role in ketamine’s rapid antidepressant effects (see Section 4). In addition, ketamine also had antidepressant-like effects in preclinical models and increased hippocampal and prefrontal cortical mTOR levels; in contrast, pretreatment with the AMPA antagonist NBQX significantly reduced these antidepressant-like effects, and concomitantly decreased levels of mTOR and BDNF (Zhou et al., 2014).

Other researchers have proposed that increased extrasynaptic glutamate levels may activate its presynaptic receptors, thus limiting synaptic glutamate transmission. In turn, glutamate transmission may be even more disrupted by the induced loss in dendritic spines. In preclinical studies, ketamine was shown to selectively counteract these deleterious effects at the synaptosome level. Regarding the induction of synaptogenesis by ketamine, recent studies found that increased spine-remodeling and synaptic plasticity mediated the antidepressant-like effects of ketamine in preclinical models, particularly through actions at the mammalian target of rapamycin complex1 (mTORC1) signaling pathway (Li et al., 2010). However, translating these findings into potential drug development in humans is complicated because mTOR is a critical effector in cell-signaling pathways that are commonly deregulated in human cancers (Guertin and Sabatini, 2007). Thus, its activation may result in the overexpression of undesirable proteins and cascades.

Interestingly, the mTOR inhibitor rapamycin suppressed antidepressant response to ketamine. This action involved the blockade of p70S6 kinase activation and limited the expression of synaptic proteins (Chung et al., 1992; Heitman et al., 1991; Tizabi et al., 2012). In preclinical models, mTOR allowed synaptic protein translation by inhibiting the inhibitory 4E binding proteins (4E-BPs) and activating p70S6 kinase (Hoeffer and Klann, 2010; Livingstone et al., 2010). Upregulation of the synaptic plasticity markers activity-regulated cytoskeleton-associated protein (Arc), GluA1, postsynaptic density protein 95
(PSD95), and synapsin I were also observed with ketamine. Similarly, mTOR targets the suprachiasmatic nucleus (the central regulator of circadian rhythms to light in mammals) and was found to reset the circadian clock by modulating the circadian gene proteins PER1 and PER2 (Cao et al., 2010). In individuals who responded to ketamine, mTOR also positively correlated with its downstream effectors, glycogen synthase kinase-3beta (GSK-3β) and dephosphorylation of eukaryotic elongation factor 2 (e-EF2) (Denk et al., 2011; Dwyer and Duman, 2013; Yang et al., 2013). This effect has been also associated with increased BDNF synthesis (Shah et al., 2014). Overall, dendritic spine growth, spine enlargement, and increased post-synaptic density protein levels have been observed after ketamine treatment (Li et al., 2010; Malenka and Bear, 2004). All three of these effects are directly modulated by mTOR pathways, suggesting that long-term potentiation as well as postsynaptic activation of neuroplasticity-related signaling pathways are involved in ketamine’s rapid antidepressant effects, with consequent improvement in prefrontal synaptic connectivity.

7. Other Molecular Downstream Targets for Developing Rapid Antidepressant Treatments

As noted above, ketamine’s mechanism of action goes beyond simple NMDA antagonism or even activation of the mTOR pathway (Fig. 1). It directly affects other ionic channels such as voltage-operated calcium channels (VOCC), opioid receptors, and AMPA receptors as well as monoamine and muscarinic receptors (Hirot a and Lambert, 1996) and other intracellular signaling cascades associated with neuroplasticity. Other pathways associated with rapid antidepressant effects in preclinical models involve the depolarization of AMPA receptor activity to allow calcium influx and exocytosis of BDNF by activating VOCCs, thus activating downstream protein kinase B (PKB/AKT) and extracellular signal-related kinase (ERK) (Duman et al., 2012; Legutko et al., 2001). Interestingly, ketamine rapidly and transiently increased the phosphorylated and activated forms of ERK (including ERK1 and ERK2) and AKT growth factor signaling pathways (Hoeffer and Klann, 2010). Importantly, these pathways directly affect mTOR activity.

Ketamine has been also shown to regulate the neurotrophic factor BDNF (Garcia et al., 2008; Linden et al., 2000). Studies have shown that peripheral BDNF levels are increased or unaltered after ketamine treatment in patients with treatment-resistant MDD (Duncan et al., 2013; Machado-Vieira et al., 2009c). Reduced BDNF levels and expression were also found in the brain and periphery of subjects with mood disorders, and these effects seemed to be counteracted by antidepressants and mood stabilizers. One randomized trial found significantly higher plasma BDNF levels in ketamine responders than non-responders (Haile et al., 2014). Ketamine has been also shown to reduce depressive-like symptoms associated with enhanced hippocampal BDNF levels (Garcia et al., 2008). Finally, studies with knockout mice demonstrated that BDNF mediated ketamine’s antidepressant-like effects (Autry et al., 2011).

Ketamine also inhibits e-EF2 kinase, a calcium-dependent protein that regulates MAP kinase and mTOR pathways. e-EF2 plays a key role in regulating synaptic plasticity in association with a concomitant increase in BDNF synthesis (Monteggia et al., 2013). The differential
clinical profile when comparing efficacy in depression among diverse NMDA antagonists has stimulated further investigation of potential alternative biological effects between different glutamatergic modulators to better understand the diverse biological actions implied by specific efficacy and clinical patterns. In this context, and in contrast to ketamine, memantine did not block e-EF2 nor alter BDNF expression. This suggests that ketamine’s effects may be indirectly implicated in differential effects at neural glutamate levels as well as receptor trapping and binding (Gideons et al., 2014; Kotermanski et al., 2009). These effects seem to be related to AMPA activity and to surface expression in neurons that, in turn, stimulate quick e-EF2- and BDNF-dependent potentiation (Autry et al., 2011; Nosyreva et al., 2013).

Similarly, ketamine inhibited brain GSK-3 expression, and animals with a knock-in mutation that blocked GSK-3 phosphorylation displayed no antidepressant-like response to ketamine in the learned helplessness model (Beurel et al., 2011). Ketamine also regulates diverse genes in the circadian molecular machinery that has been shown to be disrupted in mood disorders and that is directly involved in the effects of GSK-3 (Zunszain et al., 2013). In rodent models of depression, ketamine also reversed dendritic atrophy by enhancing the number of dendritic spines and increasing hypocretin-induced post-synaptic excitatory currents (Li et al., 2011). In addition, lower spontaneous activity of gamma-aminobutyric acid (GABA)ergic interneurons and enhanced firing rate in glutamatergic pyramidal neurons in the PFC were described after ketamine use, suggesting that NMDA receptor antagonism blocks GABAergic activity with consequent increased glutamatergic transmission (Fig. 1).

Other preclinical studies using ketamine identified alternative targets (both in vitro and in vivo) that are potentially related to its efficacy. These include the opioid system (e.g., sigma receptors (Robson et al., 2012)), monoamine transporters (Nishimura et al., 1998), and nucleus accumbens and ventral pallidum serotonergic 5-HT1B receptors in primates (Yamanaka et al., 2014). With regard to the opioid system, an interaction was observed between ketamine and μ- and κ-opioid receptors (Hirota et al., 1999; Wong et al., 1996). In addition, a preliminary PET study showed lower right habenula, insula, ventrolateral PFC, and dorsolateral PFC (dlPFC) metabolism after ketamine infusion (Carlson et al., 2013); notably, in humans, PET assessment of cerebral glucose metabolism provides a relatively specific proxy measure of glutamatergic neurotransmission. Functional neuroimaging studies also suggested that the anterior cingulate cortex (ACC) may be key to ketamine’s rapid antidepressant effects (Zarate et al., 2013b), but most of these research avenues are unlikely to play a major role in target identification and drug development, in general due to the lack of receptor engagement studies and to scarce data comparing pre- vs post-treatment levels and associations with clinical outcomes.

Interestingly, preclinical studies suggest that serotonin receptors are a therapeutic target for ketamine’s effects (Gigliucci et al., 2013). For instance, ketamine’s antidepressant-like effects were abolished when a tryptophan hydroxylase inhibitor was used to yield serotonin-deprived rodents (Gigliucci et al., 2013).

A recent study found that higher baseline D-serine plasma levels predicted better response to ketamine in patients with treatment-resistant depression, which reinforces the potential role
of D-serine as a key regulator of response to ketamine and NMDA receptor antidepressant profile (Moaddel et al., 2015). This response seems selective to ketamine and perhaps other rapid-acting antidepressants, given that lower D- and L-serine levels were associated with reduced response to standard antidepressants (Maes et al., 1998). By reducing D-serine, ketamine decreased the activity of the NMDA receptor and, eventually, the related neuroinflammation.

8. Preclinical and Proof-of-Concept Clinical Trials with Receptor/Subunit Selective Glutamate Modulators

8.1. Ionotropic Glutamate Receptor Modulators

Early studies with ketamine (see (Niciu et al., 2014) for a recent review) inspired the pharmaceutical industry to develop similar glutamate modulators such as ketamine enantiomers. One key agent with this profile is esketamine, which acts primarily as a non-competitive NMDA receptor antagonist, but is also a dopamine reuptake inhibitor. Phase II studies of esketamine are underway using esketamine in intranasal spray form, a more feasible and rational route of administration when considering potential large-scale use (Singh et al., 2013).

Agents that target NMDA receptor subunits are also under development, and some pilot proof-of-concept trials have evaluated glutamatergic modulators (Table 1). These aim to test more specific targets within this widespread neurotransmitter system in an attempt to overcome the psychotomimetic effects associated with NMDA receptor antagonists as well as the need for IV access. Two GluN2B antagonists were tested in MDD: CP-101,606 and MK-0657. With regard to the GluN2B antagonist CP-101,606, a single infusion given as add-on therapy in individuals who did not respond to paroxetine induced an antidepressant response that lasted one week, though this response was not as robust as that of ketamine (Preskorn et al., 2008). In that study, patients were initially treated with six weeks of open-label paroxetine 40 mg/day. Non-responders (30 patients) were then randomized to IV CP-101,606 or placebo. A 60% response rate was seen for patients receiving CP-101,606 versus 20% for placebo, as assessed by the Hamilton Depression Rating Scale (HAM-D); 33% of patients met remission criteria by Day 5, and response was maintained by 78% of patients for at least one week, and by 42% of patients for 15 days after the initial dose. It should be noted that the dose was reduced to 0.5 mg/kg for 1.5 hours for half the patients because CP-101,606 caused a high number of dissociative effects at higher doses. Despite these promising preliminary findings, continued development of this compound was halted due to potential cardiovascular toxicity (specifically, QTc prolongation). Other promising subunit-specific glutamate modulators under evaluation in phase I and II trials are described in Table 1.

In monotherapy, GluN2B antagonist MK-0657 had no antidepressant efficacy in a pilot trial for individuals with treatment-resistant MDD (Ibrahim et al., 2012b). In that small, randomized, double-blind, placebo-controlled, crossover trial, oral MK-0657 was administered to individuals with treatment-resistant MDD for 12 days. Although no significant improvement was noted on the Montgomery-Asberg Depression Rating Scale
(MADRS)—the primary outcome measure—significant improvement was observed when symptoms were assessed using the HAM-D and the Beck Depression Inventory (BDI). Because only five patients were included in this analysis, additional studies with larger sample sizes are needed to definitively assess the efficacy of MK-0657. No dissociative effects were observed (Ibrahim et al., 2012b).

Another target within the NMDA receptor is the glycine site. GLYX-13 is a partial agonist at the NMDA receptor glycine binding site whose potential rapid antidepressant effects have been studied. In a phase II clinical trial (NCT01684163) as add-on treatment, a single intravenous dose of GLYX-13 had antidepressant effects within 24 hours that lasted up to seven days in MDD patients who had not responded to one or more antidepressant medications (Moskal et al., 2014). No dissociative effects were observed (Burch, 2012), suggesting that agents that target this site may induce rapid antidepressant effects without psychotomimetic effects.

AZD6765 (lanicemine) is another non-selective, non-competitive NMDA receptor antagonist with lower trapping than ketamine. Because ketamine has greater receptor affinity, AZD6765 may have reduced psychotomimetic or dissociative adverse effects while retaining antidepressant efficacy. Two small proof-of-concept studies with AZD6765 showed that a single infusion had rapid but not sustained antidepressant effects compared to placebo (Sanacora et al., 2014b; Zarate et al., 2013a); however, the antidepressant response was not as robust or sustained as that of ketamine. A subsequent, larger, six-week phase IIb trial evaluating repeated add-on AZD6765 IV infusions for three weeks (50 mg or 150 mg, 3x/week) found that it failed to separate from placebo, potentially due to the large placebo effect (Sanacora et al., 2014b). However, AZD6765 did not trigger dissociative symptoms (Sanacora et al., 2014a).

The cough suppressant dextromethorphan, a derivate of morphine with sedative and dissociative properties, is a non-selective, non-competitive NMDA receptor antagonist. It should be noted that other molecular mechanisms are also present in its mechanism of action, including sigma receptors, sigma-1, calcium channels, serotonin transporters, and muscarinic sites (Lauterbach, 2011). In animal models, dextromethorphan had antidepressant-like effects in the tail suspension test similar to those observed with both conventional and rapid-acting antidepressants such as imipramine and ketamine (Nguyen and Matsumoto, 2015). To date, no randomized controlled trials have explored dextromethorphan as monotherapy for the treatment of MDD. However, it was studied in a randomized, placebo-controlled trial as an add-on to valproic acid in BD (Lee et al., 2012); no significant group differences were seen between groups as assessed by either mean HAM-D or Young Mania Rating Scale (YMRS) scores. It has been tested in combination with quinidine (as Nuedexta) as a potential antidepressant with NMDA receptor antagonist properties. One case report found that Nuedexta had antidepressant effects in a single depressed patient with emotional lability (Messias and Everett, 2012). An open-label trial of Nuedexta in treatment-resistant MDD is ongoing (NCT01882829).

Memantine is another low-trapping NMDA receptor antagonist that showed no antidepressant response in an eight-week, placebo-controlled study in individuals with MDD.
(Zarate et al., 2006). The glutamate modulator riluzole was also hypothesized to have antidepressant properties via its ability to increase glial reuptake of glutamate and AMPA receptor trafficking. While an open-label clinical trial of riluzole in MDD and bipolar depression found that riluzole had significant antidepressant effects (Zarate et al., 2005), a larger study that used riluzole as maintenance therapy for 28 days after a single IV ketamine infusion found that riluzole had no antidepressant effects (Ibrahim et al., 2012a).

Other agents currently under development that target different receptors include AMPAkines, also known as AMPA receptor positive allosteric modulators. Notably, the activation of AMPA receptor signaling plays a key role in inducing neuroplasticity and activity-dependent BDNF release (Jourdi et al., 2009). For instance, the AMPA receptor potentiator LY392098 increased BDNF expression in neuronal cultures (Legutko et al., 2001). However, at the same time, direct stimulation of AMPA receptors can be neurotoxic (O’Neill et al., 2004). To overcome this potential issue, drug development in this field has focused on the use of AMPAkines, which potentiates currents mediated by AMPA receptors. Nevertheless, success in this area has been limited by low bioavailability and potential toxicity (Menniti et al., 2013). Preclinical studies found that these agents exhibit antidepressant-like efficacy (Bleakman et al., 2007; O’Neill and Witkin, 2007), and several AMPA modulators are being developed to treat MDD, including the AMPA agonist farmamaptor (CX-691/ORG 2448). While older AMPAkines (e.g., levetiracetam) have shown no evidence of efficacy (Saricicek et al., 2011), several more potent compounds have been developed; these include coluracetam (BCI-540), which is in clinical trials (Dutta et al., 2015). ORG-26576, an AMPA receptor positive allosteric modulator, has also been studied. A phase Ib safety and efficacy trial found that the maximum tolerated dose (400mg po bid) of ORG-26576 demonstrated preliminary antidepressant efficacy in a small cohort (n=30) (Nations et al., 2012). Among the three doses assessed (100 mg/bid, 400 mg/bid, and placebo), however, none of the arms statistically separated over the 28-day testing period. Nevertheless, the higher dose was associated with improved speed of information processing, improved executive functioning, increased growth hormone, and decreased cortisol; no effect was seen on prolactin or BDNF levels. As is true for other glutamate modulators, replication in larger cohorts will be critical to assess the overall safety, tolerability, and antidepressant efficacy of AMPAkines.

8.2. Metabotropic Glutamate Receptors (mGluRs)

mGluRs are G protein-coupled receptors that directly affect glutamate levels and synaptic plasticity and are linked to the pathophysiology and treatment of mood disorders (Machado-Vieira et al., 2009a). They are predominantly (but not exclusively) located presynaptically at glutamatergic synapses. With the exception of mGluR1/5, their activation generally decreases neurotransmitter release, thereby limiting excitotoxic damage. To achieve efficacy, presynaptic mGluR2 agonists seem to reduce excessive glutamate release, while mGluR2/3 antagonists seem to enhance synaptic glutamate levels, commensurately boosting AMPA receptor transmission and firing rates and extracellular monoamine levels. As seen with ketamine, mGluR2/3 antagonists also directly target mTOR signaling (Dwyer et al., 2012; Koike et al., 2011).
Several mGluR2/3 antagonists, as well as the negative allosteric modulator RO4995819, were found to have antidepressant-like efficacy in rodent models of depression (reviewed in (Chaki et al., 2013)). Specifically, animal studies have noted that the increased presynaptic glutamate release induced by mGluR antagonists produced ketamine-like effects (Dwyer et al., 2012; Karasawa et al., 2005). Positive allosteric modulators do not directly activate the receptor but potentiate the response of mGluRs to glutamate. The safety and tolerability of mGluR2/3 modulators has been studied in healthy volunteers (NCT01547703 and NCT01546051) but mGluR2/3 modulators have not yet been tested in clinical trials for the treatment of MDD.

The mGluR5s are expressed pre- and post-synaptically and are involved in AMPA receptor internalization, a key mechanism for modulating synaptic plasticity (Pilc et al., 2013). mGluR5s are also physically and physiologically interconnected with NMDA receptors. Several mGluR5 antagonists were found to display antidepressant-like effects in diverse behavioral models (Chaki et al., 2013). Notably, two mGluR5 antagonists were recently tested in clinical trials for treatment-resistant MDD: AZD2066 and the mGluR5 negative allosteric modulator basimglurant (RO4917523) (Quiroz et al., 2014). Basimglurant in particular has shown promising results in two clinical trials (NCT00809562, NCT01437657) (Roche group development pipeline, http://www.roche.com/irp150128-annex.pdf). In a nine-week, double-blind, placebo-controlled study in 333 individuals with treatment-resistant MDD, basimglurant (0.5 mg or 1.5/day adjunctive to ongoing treatment with serotonin-noradrenaline reuptake inhibitors (SNRIs) or selective serotonin reuptake inhibitors (SSRIs)) (Quiroz et al., 2014) had significant antidepressant effects; further studies in depressive disorders are underway. In contrast, AZD2066 (12–18 mg/day for six weeks) as monotherapy was no more effective in patients with MDD than placebo and the SNRI duloxetine (NCT01145755).

RG1578 (an mGluR2 negative allosteric modulator) has also been tested clinically. Though preliminary results were positive, final results were disappointing (Dale et al., 2015).

9. Therapeutic Targets for Rapid Antidepressant Efficacy beyond the Glutamatergic System

9.1. Sleep Deprivation

Rapid antidepressant effects have also been observed after a night of sleep deprivation (Bunney and Bunney, 2013), but few studies have shown a potential neurobiological basis for these effects. Approximately half of patients with either MDD or bipolar depression have been found to respond positively to one night of sleep deprivation (Wu and Bunney, 1990). Although some have argued that antidepressant response to sleep deprivation is only transient and therefore not a “real” antidepressant response, evidence suggests that the antidepressant effects of sleep deprivation can be sustained with other approaches including sleep phase advancement or medications such as lithium or conventional antidepressants (Wirz-Justice and Van den Hoofdakker, 1999). One study noted that disruption in central circadian clock genes such as BMAL1/CLOCK (NPAS2), which bind to enhancer boxes to
initiate the transcription of circadian genes, may be restored after a night of sleep deprivation (Bunney and Bunney, 2013).

9.2. The Cholinergic System

Another system currently under investigation as a target for rapid antidepressant effects is the cholinergic system (Drevets et al., 2013), with the underlying hypothesis being that overactive muscarinic cholinergic receptor function could lead to depression. In this context, several randomized, double-blind, placebo-controlled studies have been conducted with IV doses of the anticholinergic agent scopolamine as add-on or monotherapy in subjects with MDD or bipolar depression (Drevets et al., 2013; Jaffe et al., 2013; Khajavi et al., 2012). Scopolamine had rapid antidepressant effects equivalent to those of ketamine and, like ketamine, scopolamine’s antidepressant activity is thought to be mediated by the activation of neuroplasticity (Jaffe et al., 2013). In a pilot study designed to evaluate cognitive symptoms (particularly selective attention) in depression, Furey and Drevets (2006) observed a rapid and robust antidepressant response to scopolamine, especially with a dose of 4μg/Kg (compared to 3 and 2 μg/Kg). In this pilot, double-blind, crossover placebo-controlled study of patients with MDD (n=9) and bipolar depression (n=9), scopolamine showed increased antidepressant efficacy versus placebo for depressive and anxiety symptoms (Furey and Drevets, 2006). Interestingly, the antidepressant effects of scopolamine persisted for two weeks, and repeated dosing provided additional benefit and extended response. Limitations to the use of this agent include anticholinergic side effects and the risk of psychosis at higher doses (Khajavi et al., 2012), both of which may limit its broad clinical use. Furthermore, unlike ketamine, data from scopolamine trials have not been replicated by independent laboratories or in studies with inpatients using active placebo.

Assessing the effects of scopolamine in subjects with suicidal ideation, as well as the use of alternative routes of administration (e.g., intranasal, transdermal) are also necessary to confirm these preliminary results and expand the potential utility of this agent in mood disorders.

The potential mechanisms involved in scopolamine’s rapid antidepressant response include modulation of the cholinergic system by blocking the hypersensitivity of muscarinic receptors, with a concomitant acute increase in acetylcholine release that affects monoamine levels (particularly serotonin and dopamine). Other potential targets for the actions of scopolamine include the modulation of NMDA receptor activity, with increased NMDA receptor gene expression associated with muscarinic receptor stimulation (Liu et al., 2004). The activation of synaptic plasticity has also been proposed as an underlying biological effect for scopolamine’s rapid antidepressant activity. In this context, it was shown that scopolamine rapidly increases mTOR signaling and synaptogenesis (both number and function) in association with its rapid antidepressant-like effects (Voleti et al., 2013). Interestingly, scopolamine’s antidepressant-like effects were blocked by pretreatment with mTOR and AMPA antagonists (Voleti et al., 2013). Furthermore, scopolamine—as well as systemic administration of VU0255035, a selective M1-AChR antagonist—induced antidepressant-like effects associated with the activation of prefrontal cortex mTOR1 signaling (Navarria et al., 2015). Like ketamine, scopolamine administration acutely enhances extracellular glutamate concentrations in the striatum in rodents (Rawls and...
McGinty, 1998), which may activate synaptic plasticity pathways. Other cholinergic mechanisms have also been explored in the treatment of MDD. For instance, nicotinic antagonists and partial agonists have both been evaluated in proof-of-concept trials and showed limited efficacy (Vieta et al., 2014).

### 9.3. HPA Axis Hyperactivity

Other potential targets have been drawn from the “stress hypothesis of depression” that targets HPA axis hyperactivity. This altered pathway could be responsible for loss of synapses in the limbic system and impaired plasticity and cellular resilience (Machado-Vieira et al., 2014). In preclinical studies, stress-related dendritic remodeling was a key molecular effect of ketamine (Duman and Duman, 2015). Nevertheless, glucocorticoid mediation in the stress response may also be responsible for these key effects. Interestingly, glucocorticoids acutely increase glutamate release in the amygdala and PFC (Musazzi et al., 2013). Thus, chronic alterations in glutamate release induced by glucocorticoids/stress may dynamically affect dendritic remodeling and synaptic networks as well as extrasynaptic glutamate levels, but studies of the effects of chronic stress on glutamate levels remain limited.

In the search for effective novel antidepressants, trials have also examined corticotropin releasing factor (CRF) and glucocorticoid receptor antagonists, as well some neuropeptides such as neurokinin 1 (NK1), vasopressin, and orexin antagonists; results have been mixed and largely disappointing (Dale et al., 2015). Mifepristone, a synthetic glucocorticoid receptor antagonist, showed disappointing clinical results (http://www.in-pharmatechnologist.com/Regulatory-Safety/Sanofi-pulls-plug-on-four-Ph-III-drugs2009). An initial pilot open-label study with the CRF\textsubscript{1} receptor antagonist NBI-30775/R121919 showed positive results (Zobel et al., 2000), but these were not subsequently replicated in a phase II placebo-controlled, double-blind, randomized study with the CRF\textsubscript{1} receptor antagonist CP-316,311 (Binneman et al., 2008). NK1 and NK2 receptors are activated by substance P and neurokinin A, and their modulators have been tested for the treatment of depression. In two double-blind, placebo controlled, randomized, proof-of-concept studies, the NK1 receptor antagonists aprepitant (MK869) and L759274 reduced depressive symptoms (Kramer et al., 1998; Kramer et al., 2004); however, these results were not subsequently confirmed in five follow-up phase II studies (Keller et al., 2006) and the program was discontinued. Several other NK1 receptor antagonists have also been tested, but no positive results have yet been described (Ball et al., 2014).

### 9.4. The Opioid System

Another promising target for future studies is the opioid system. Stress-induced increases in dynorphin, the endogenous ligand for the kappa opioid receptor, induced dysphoria and anhedonia in both humans and rodents (Knoll and Carlezon Jr, 2010). ALKS-5461, a combination of buprenorphine (a partial \(\mu\)-opioid receptor agonist and kappa opioid receptor antagonist) and samidorphan (a \(\mu\)-opioid antagonist) showed positive results in phase II trials, and are currently in phase III trials as an add-on therapy for treatment-resistant MDD (http://phx.corporate-ir.net/phoenix.zhtml?c=92211&p=irol-corporateNewsArticle&ID=18258172013). In addition to this well-designed, placebo-
controlled, double-blind, crossover study, other observational, uncontrolled studies also found a significant and generally rapid elevation in mood in treatment-resistant MDD patients treated with μ-opioid agonists (Ehrich et al., 2015). However, it is not clear if these putative therapeutic effects are broader than the potential addictive effects of this class of agents.

9.5. The Melatonergic System

The neurohormone melatonin is secreted by the pineal gland and plays a crucial role in circadian rhythms and the sleep-wake cycle. The key target for melatonin is the suprachiasmatic nucleus (SCN), which is involved in diverse biological functions including regulation of sleep and circadian rhythms (Laposky et al., 2008; Wyatt et al., 2006), mood and behavior (Srinivasan et al., 2006), and adipokine levels (Espino et al., 2011; Koziorog et al., 2011; Nduhirabandi et al., 2011). Melatonin also has immunomodulatory, neuroprotective, antioxidant, and additional chronobiological effects (Cardinali et al., 2011; Pacchierotti et al., 2001; Pandi-Perumal et al., 2006; Reiter et al., 2009). It also seems to directly target synaptic plasticity by increasing hippocampal long-term potentiation (Pandi-Perumal et al., 2006).

The two melatonin receptors (MT1 and MT2) are high affinity G\textsubscript{i}/G\textsubscript{0} protein-coupled receptors with elevated expression in the brain; MT1 receptors are predominantly found in the pituitary gland and hypothalamus, and MT2 receptors are predominantly found in the retina. As noted above, the melatonergic system directly affects the circadian system and sleep-wake rhythms. Interestingly, studies have shown that during depressive and manic episodes, individuals with mood disorders have abnormal circadian rhythm and changes in sleep patterns; these abnormalities include sleep-wake irregularities (Gruber et al., 2009; Harvey, 2008; Salvatore et al., 2008), abnormal actimetric parameters (Millan et al., 2005), and circadian preference for evening (Ahn et al., 2008; Mansour et al., 2005; Wood et al., 2009). Supersensitivity to melatonin suppression by light has also been observed in BD subjects, monozygotic twins discordant for BD, and the non-affected offspring of individuals with BD, but these findings were not confirmed in a similar study evaluating euthymic BD subjects (Hallam et al., 2005c; Lewy et al., 1985; Lewy et al., 1981; Nurnberger et al., 2000). The evidence suggests that regulation of sleep homeostasis is critical to achieving and maintaining remission in subjects with mood disorders.

Interestingly, variations in the melatonin biosynthesis pathway have been shown in mood disorders. For instance, BD subjects had decreased melatonin levels in response to a light night (Nurnberger et al., 2000) as well as increased melatonin suppression to light (Nathan et al., 1999). These findings suggest a genetic trait marker in BD (Hallam et al., 2006; Hallam et al., 2005c; Kennedy et al., 1996; Lewy et al., 1985; Nurnberger et al., 1988; Whalley et al., 1991). A polymorphism in the GPR50 (H9, melatonin-related receptor) was associated with increased risk for BD, but this finding was not subsequently replicated (Alaerts et al., 2006; Thomson et al., 2005). Relatedly, abnormal melatonin secretion and biological clock setting in adapting circadian rhythms to social environment has been observed, which may induce mood episodes in individuals with BD (McClung, 2007).
Exogenous melatonin has been an effective primary therapeutic for primary insomnia and delayed sleep phase disorder. In addition, evidence suggests that treatment with melatonin agonists adjunctive to mood stabilizers may prove useful in treating sleep disorders and eventually preventing metabolic syndrome in patients with BD (Geoffrey et al., 2015; Romo-Nava et al., 2014). Lithium and valproate lowered melatonin light sensitivity in healthy volunteers (Hallam et al., 2005a; Hallam et al., 2005b). No controlled studies of melatonin in BD have been published, and mixed results were seen when melatonin was administered to BD subjects in preliminary open-label studies (Bersani and Garavani, 2000; Leibenluft et al., 1997).

With regard to specific therapeutics, the selective MT1 and MT2 receptor agonist ramelteon (8mg) (Neubauer, 2008) was approved in 2005 by the US Food and Drug Administration (FDA) to treat insomnia. One study found that in BD patients with euthymia and sleep disturbances, ramelteon adjunctive to mood stabilizers (8 mg/day, 23 weeks double-blind, n = 83) effectively maintained mood stabilization. Notably, the group taking ramelteon had half the relapse rate of those treated with placebo (Norris et al., 2013).

Agomelatine is a melatonin MT1 and MT2 receptor agonist and a 5-HT(2C) receptor antagonist (Bourin and Prica, 2009). In preclinical models of depression, agomelatine had significant antidepressant-like effects (Bertaina-Anglade et al., 2006; Millan et al., 2005; Papp et al., 2003), increased cellular proliferation, and increased synaptic levels of both norepinephrine and dopamine (Banasr et al., 2006; Van Oekelen et al., 2003).

In clinical studies, agomelatine was more effective and well-tolerated than placebo in three large, controlled, multi-centre clinical trials in MDD (Kennedy and Emsley, 2006; Loo et al., 2002; Montgomery and Kasper, 2007). In a recent six-week trial with agomelatine (25 mg/day), 81% of 21 patients with BD depression showed substantial antidepressant efficacy at study endpoint and 47% showed response during the first week of treatment (Calabrese et al., 2007). There were no dropouts secondary to adverse events, though three lithium-treated subjects had manic or hypomanic episodes during the optional extension period (Calabrese et al., 2007). In another 24-week randomized, double-blind, placebo-controlled trial for depression, agomelatine was more effective than placebo; in addition, a significantly lower relapse rate was observed with agomelatine compared to placebo during a six-month extension phase (Goodwin et al., 2009). A recent meta-analysis of 20 trials encompassing 7460 subjects in studies that used standard depression rating scales found that agomelatine was significantly more effective than placebo, with an effect size (standardized mean difference (SMD)) of 0.24 (95% confidence interval 0.12 to 0.35) and a relative risk of response of 1.25 (1.11 to 1.4). Overall, agomelatine was as effective as other antidepressants. Agomelatine also resynchronized altered circadian rhythms and had circadian phase-advancement properties (Armstrong et al., 1993; Nagayama, 1996; Redman and Francis, 1998).

Taken together, the evidence suggests that agomelatine may be an effective and safe antidepressant. Its neuroprotective and clinical effects may represent an important treatment approach for several medical conditions. However, use of melatonin in clinical practice is not as well developed as might be expected. Further studies are required to confirm these
promising findings and explore the role of melatonin agonists in the treatment of mood disorders.

9.6. Deep Brain Stimulation (DBS)

Preliminary and uncontrolled studies using deep brain stimulation (DBS) also found that it has rapid antidepressant effects in treatment-resistant MDD. The stimulation targets showing an association with rapid antidepressant effects included the subgenual cingulate gyrus, nucleus accumbens, anterior limb of the capsula interna (Bewernick et al., 2010; Lozano et al., 2008; Malone et al., 2009), and the medial forebrain bundle (Schlaepfer et al., 2013). However, it is too soon to make a definitive statement on potential targets for rapid antidepressant effects associated with this major invasive intervention.

9.7. Epigenetic Mechanisms

Interestingly, epigenetic mechanisms have also been associated with the pathophysiology of mood disorders and may represent potential targets for rapid antidepressant effects. While no selective CNS-specific molecule has been developed, the most promising target seems to be histone deacetylase (HDAC) inhibition and chromatin remodeling. For instance, elevated H3 acetylation and lower nucleus accumbens HDAC2 levels were observed in a postmortem study of patients with depression (Covington et al., 2009). In addition, HDAC2 inhibitors were found to have antidepressant-like effects and improve cognition in rodents (Covington et al., 2009; Covington et al., 2010; Day and Sweatt, 2012), which suggests that HDAC inhibitors could be further investigated as potential therapeutic targets in mood disorders (Machado-Vieira et al., 2011). L-acetylcarnitine (LAC), a well-tolerated acetylating agent, also displayed rapid (as early as day three of treatment) and long-lasting antidepressant-like effects via epigenetic regulation of mGluR2s in rodents by increasing acetylated H3K27 bound to the Grm2 promoter and the NF-κB-p65 subunit. These effects increased Grm2 gene transcription encoding for mGluR2s in the hippocampus and PFC.

9.8. Neuronal Plasticity

Neuronal plasticity may also represent a promising target for identifying molecules involved in the rapid improvement of depressive symptoms. For example, positive modulators of the tropomyosin receptor kinase B (TrkB) receptor, which regulates BDNF levels, are thought to induce antidepressant activity (Castren and Rantamaki, 2010; Rantamaki and Castren, 2008), although selectivity has been an issue. mTOR modulators and related molecules involved in the rapid action of ketamine and other proof-of-concept agents may also be promising, but the limitations of using potential agonists include the risk of cell proliferation (and eventually cancer) and inflammation (Johnson et al., 2013). Nevertheless, mTOR antagonists are FDA-approved for diverse types of cancer, and more than 1,300 trials with mTOR modulators are either under way or completed. The study of mTOR subunits may thus represent a valuable alternative, especially mTORC1.

9.9. Heterodimers

The study of heterodimers is another area of interest. A heterodimer is formed by two different macromolecules; this biologically active dimer derives from two or more different
monomers. Further studies in the area may help identify compounds that modulate specific receptors that interact with others. For instance, serotonergic 5-HT$_{2A}$ receptors form heterodimers with mGluR2s (Gonzalez-Maeso et al., 2008), a combination that may represent new avenues for studying the integration between receptor activity and coupling to multiple intracellular systems.

9.10. Neurotransmitters

Finally, several types of neurotransmitters have yet to be explored in psychiatric research. For instance, most amino acids are just now being explored; promising classes include peptides (eg, substance P, NK, somatostatin, neuropeptide Y) and gasotransmitters (e.g. nitric and nitrous oxide). Notably, a recent pilot proof-of-concept study found that the inhaled anesthetic gas nitrous oxide had rapid antidepressant effects in individuals with treatment-resistant MDD (Nagele et al., 2015). In addition to being relatively safe, nitrous oxide was associated with response rates of 20% compared to 5% in the placebo group. Moving forward, the main challenge will be to translate these findings into identifying clinically relevant therapeutic targets and surrogate outcomes for these potential new, improved therapeutics for depression.

9.11. Alternative Routes of Administration

Along a somewhat different line of inquiry, alternative routes of administration have also been investigated to ascertain whether ketamine’s rapid antidepressant effects could be sustained. For instance, in a double-blind, randomized, placebo-controlled trial of patients with treatment-resistant MDD, intranasal ketamine (50mg) showed similar efficacy as IV infusion 24 hours post-treatment (Lapidus et al., 2014). Interestingly, ketamine’s bioavailability administered via an intranasal route has been reported to be 25%–50% (Yanagihara et al., 2003). One open-label study observed that 0.25 mg/kg IM ketamine induced similar decreases in HAM-D score as IV ketamine in subjects with MDD (Chilukuri et al., 2014). Ketamine’s metabolites have also shown efficacy in preclinical models of depression, but their neurobiological basis seems to go beyond NMDA antagonism and targets the alpha-7 nicotinic receptor, a specific subtype of the nicotinic acetylcholine receptors. Hydroxynorketamine (HNK), a by-product of ketamine, also targets this receptor and may represent a relevant therapeutic target for rapid antidepressant effects (Singh et al., 2014).


As the above review has underscored, to date the most promising novel targets for achieving rapid antidepressant effects are the ionotropic glutamate receptors. However, to date, no subunit specific modulator has been shown to induce a robust and rapid antidepressant action. Moving forward, predicting response by identifying potential responders a priori will be key to the concept of personalized medicine. Towards this end, the search for the unique biosignatures of rapid-acting antidepressants whose validity has been tested in larger samples will require the clear evaluation of drug kinetics, ability to pass through the blood-brain barrier, and information regarding brain distribution. In the future, trials that use
enriched samples may be more efficient, as participants would be more likely to receive the potentially most effective agent available based on their biomarker signatures. A full understanding of the true clinical utility of this novel treatment approach and the key aspects of this concept for public health will be critical as we define our next steps in psychiatric research. Questions such as whether treatments should be of long or short duration, whether they should be add-on or monotherapy, what the best route of administration is, whether they should be tested in treatment-resistant cases only, and whether particular diagnostic subtypes should be emphasized will all be crucial to future investigations. The novel hypothesis that some receptor-specific antagonists could display efficacy in particular, well-defined subpopulations based on their biological profile and clinical dimensions is an important paradigm shift in the field of psychiatric research, as underscored by the new research domain criteria (RDoC) model (Cuthbert and Insel, 2013); briefly, RDoc is a research framework integrating new ways of studying mental disorders that draws from many disparate sources of information (from genomics to self-report) in order to better understand dimensions of functioning underlying the full range of human behavior. The recent shift toward RDoC symptom clusters may emphasize the potential validity of such studies and ultimately allow the identification of a subgroup of patients that may present with glutamate dysfunction-based depressive episodes.

To aid such research, one pathway of particular interest is the use of new positron emission tomography (PET) ligands that target glutamate and other systems. Given that alternative targets and approaches are particularly needed for treatment-resistant cases, and given that such cases may involve other pathways and neurotransmitter systems than in responders to monoaminergic antidepressants, it is promising to think that the field may move faster with the development of new target engagement approaches, particularly novel PET ligands. Indeed, specific PET ligands that target the glutamatergic system have recently been developed. For instance, $^{[11]}\text{C}]ABP688$ has shown high affinity for mGluR5, which may allow the monitoring of changes in glutamate levels (DeLorenzo et al., 2015). Studies using this ligand found lower levels of regional mGluR5 binding in the prefrontal cortex, the cingulate cortex, the insula, the thalamus, and the hippocampus in subjects with MDD compared to matched healthy controls (Deschwanden et al., 2011). In addition, a pharmacological challenge in rats with ceftriaxone, which activates the GLT-1 transporter (EAAT2), was found to reduce extracellular levels of glutamate, which regulated the availability of mGluR5 allosteric sites (Zimmer et al., 2015). Relatedly, alternative imaging methods such as using $^{13}\text{C-MRS}$ to measure glutamate cycling (Chowdhury et al., 2012) or functional magnetic resonance imaging (fMRI) studies of brain network connectivity could also be useful to assess target engagement and key downstream mechanisms associated with antidepressant response (Dawson et al., 2014; Driesen et al., 2013).

Certainly, future proof-of-concept studies with rapid-acting agents require the use of validated measures of target engagement, which will be key to defining dose/response and receptor binding. For instance, because there are no readily available NMDA receptor ligands in PET imaging studies, alternative approaches have been used. Other studies have proposed that electroencephalography (EEG) measures could be used to detect NMDA receptor target engagement (Hong et al., 2010; Kocsis et al., 2013). Receptor occupancy studies conducted in association with plasma drug levels will also be critical for defining
dose response parameters. Use of PET ligands to conduct target engagement or drug development studies is likely to hasten drug development in the field. On a related note, the early definition of dose response curves after the serendipitous identification of potential new approaches is also critical to identifying the most suitable effective dose associated with the lowest risk of dissociative or other adverse effects. It is interesting to note that this has not yet been done with ketamine.

It should also be noted that care is needed when testing downstream targets. For instance, clinical and preclinical studies have demonstrated that GSK-3B is directly regulated by lithium, and GSK3 antagonists have shown significant antidepressant effects in animal models. However, this target is so widespread in the brain and periphery that its molecular manipulation would likely induce systemic effects and, eventually, harmful adverse effects.

Once agents with rapid antidepressant effects are identified, strategies are also needed to maintain that efficacy, either by using multiple infusions or alternate routes of administration. Relatedly, the evaluation of therapeutic steady-state drug levels is a key factor; for instance IV infusion or intranasal use hasten steady-state levels and potentiate initial exposure, which may provide faster clinical efficacy. New treatments that work synergistically may be key to the post-ketamine long-term treatment phase.

Continued exploration of new, rapid-acting antidepressant agents is key to developing new treatments for mood disorders, and few would dispute that these treatments are urgently needed. Currently available antidepressants take weeks to achieve their full effects, which leaves patients vulnerable to devastating symptoms and higher risk of self-harm. As a result, any pharmacological strategy capable of exerting rapid and sustained antidepressant effects within hours or even days could substantially improve patients’ quality of life as well as public health. The evidence presented above underscores the recent developments in the search for novel therapeutics for mood disorders. These have revolutionized the field, challenged old paradigms and current limitations, and brought hope to those who must live with these devastating disorders.

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Funding for this work was supported by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH), by a NARSAD Independent Investigator to Dr. Zarate, and by a Brain and Behavior Mood Disorders Research Award to Dr. Zarate. These funding sources had no further role in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. Dr. Zarate is listed as a co-inventor on a patent application for the use of ketamine and its metabolites in major depression. He has assigned his rights in the patent to the U.S. government but will share a percentage of any royalties that may be received by the government. The remaining authors have no conflicts of interest to disclose, financial or otherwise.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ACPC</td>
<td>1-aminocyclopropanecarboxylic acid</td>
</tr>
<tr>
<td>AKT/PKB</td>
<td>protein kinase B</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>Arc</td>
<td>activity-regulated cytoskeleton-associated protein</td>
</tr>
<tr>
<td>BD</td>
<td>bipolar disorder</td>
</tr>
<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CREB</td>
<td>cyclic adenosine monophosphate response element-binding protein</td>
</tr>
<tr>
<td>CRF</td>
<td>corticotropin releasing factor</td>
</tr>
<tr>
<td>DBS</td>
<td>deep brain stimulation</td>
</tr>
<tr>
<td>dlPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalography</td>
</tr>
<tr>
<td>e-EF2</td>
<td>eukaryotic elongation factor 2</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-related kinase</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma aminobutyric acid</td>
</tr>
<tr>
<td>GluA1</td>
<td>AMPA receptor subunit 1</td>
</tr>
<tr>
<td>GSK-3B</td>
<td>glycogen synthase kinase 3B</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
</tr>
<tr>
<td>HNK</td>
<td>hydroxynorketamine</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic pituitary adrenal</td>
</tr>
<tr>
<td>IRS</td>
<td>insulin receptor substrate</td>
</tr>
<tr>
<td>LAC</td>
<td>L-acetylcarnitine</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery Asberg Depression Rating Scale</td>
</tr>
<tr>
<td>MDD</td>
<td>major depressive disorder</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>mGluR</td>
<td>metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>mTOR</td>
<td>mammalian target of rapamycin</td>
</tr>
<tr>
<td>NK1</td>
<td>neurokinin 1</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PCP</td>
<td>phencyclidine</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphoinositide-3 kinase</td>
</tr>
<tr>
<td>PSD95</td>
<td>postsynaptic density protein 95</td>
</tr>
<tr>
<td>RDoC</td>
<td>research domain criteria</td>
</tr>
<tr>
<td>SMD</td>
<td>standardized mean difference</td>
</tr>
<tr>
<td>SNRI</td>
<td>serotonin-noradrenaline reuptake inhibitor</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TCA</td>
<td>tricyclic antidepressant</td>
</tr>
<tr>
<td>TrkB</td>
<td>tropomyosin receptor kinase B</td>
</tr>
<tr>
<td>VOCC</td>
<td>voltage-operated calcium channels</td>
</tr>
</tbody>
</table>

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Fig. 1.
Schematic representation of postulated targets implicated in ketamine’s mechanism of rapid antidepressant action that are amenable to pharmacological manipulation: A) GABA interneuron inhibition that leads to increased glutamate transmission, B) enhanced AMPA throughput, and C) mTOR activation.

AKT3: protein kinase B3; AMPAR: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF: brain-derived neurotrophic factor; GABA: gamma aminobutyric acid; GluA1: AMPA receptor subunit 1; IRS: insulin receptor substrate; mTOR: mammalian target of rapamycin; NMDA: N-methyl-D-aspartate; PI3K: phosphoinositide-3 kinase; PSD95: postsynaptic density protein 95; TrkB: tropomyosin receptor kinase B; VOCC: voltage-operated calcium channel.
Table 1
The “Glutamate Storm” in Mood Disorders: Perspectives for the Development of New Glutamate Modulators for Depression

<table>
<thead>
<tr>
<th>Glutamate modulator</th>
<th>Mechanism of action</th>
<th>Development status</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esketamine</td>
<td>Non-competitive NMDA channel blocker</td>
<td>Positive results in phase II for depression</td>
<td><img src="image1" alt="Esketamine" /></td>
</tr>
<tr>
<td>MK-0657 (CERC-301)</td>
<td>GluN2B antagonist</td>
<td>Concluded phase II for MDD</td>
<td><img src="image2" alt="MK-0657" /></td>
</tr>
<tr>
<td>GLYX-13 (rapastinel)</td>
<td>Glycine site partial agonist</td>
<td>Starting phase III for treatment-resistant MDD</td>
<td><img src="image3" alt="GLYX-13" /></td>
</tr>
<tr>
<td>Org 26576</td>
<td>AMPA potentiator</td>
<td>Development halted after failed phase II in MDD</td>
<td>Not available</td>
</tr>
<tr>
<td>AZD6765 (Lanicemine)</td>
<td>Low-trapping NMDA antagonist</td>
<td>Positive results in phase II A/B for treatment-resistant MDD</td>
<td><img src="image4" alt="AZD6765" /></td>
</tr>
<tr>
<td>Basimglurant (RG-7090)</td>
<td>mGluR5 negative allosteric modulator</td>
<td>Under phase II for treatment-resistant MDD</td>
<td><img src="image5" alt="Basimglurant" /></td>
</tr>
<tr>
<td>NRX-1074 (Eliprodil)</td>
<td>NMDA glycine site partial agonist</td>
<td>Under phase II for MDD</td>
<td>Not available</td>
</tr>
<tr>
<td>EVT-101</td>
<td>Selective GluN2B antagonist</td>
<td>Completed phase I, no data available</td>
<td><img src="image6" alt="EVT-101" /></td>
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<tr>
<td>AV-101</td>
<td>Glycine receptor antagonist</td>
<td>Starting phase I B</td>
<td><img src="image7" alt="AV-101" /></td>
</tr>
<tr>
<td>Dextro-methorphan and quinidine (Nuedexta)</td>
<td>Non-competitive NMDA antagonist</td>
<td>FDA approved for PBA. Case reports in MDD</td>
<td><img src="image8" alt="Dextro-methorphan and quinidine" /></td>
</tr>
<tr>
<td>Acamprosate</td>
<td>NMDA and mGluR5 antagonist</td>
<td>Initial studies in MDD</td>
<td><img src="image9" alt="Acamprosate" /></td>
</tr>
</tbody>
</table>

NMDAR: N-methyl-D-aspartate receptor; GluN2B: NMDA receptor subunit, glutamate-binding site; mGluR5: metabotropic glutamate receptor 5; MDD: major depressive disorder; PBA: pseudobulbar affect.