Targeting glutamate signalling in depression: progress and prospects

James W. Murrough[1], Chadi G. Abdallah[2] and Sanjay J. Mathew[3]

Abstract | Major depressive disorder (MDD) is severely disabling, and current treatments have limited efficacy. The glutamate *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine was recently repurposed as a rapidly acting antidepressant, catalysing the vigorous investigation of glutamate-signalling modulators as novel therapeutic agents for depressive disorders. In this Review, we discuss the progress made in the development of such modulators for the treatment of depression, and examine recent preclinical and translational studies that have investigated the mechanisms of action of glutamate-targeting antidepressants. Fundamental questions remain regarding the future prospects of this line of drug development, including questions concerning safety and tolerability, efficacy, dose–response relationships and therapeutic mechanisms.

Synaptic plasticity
Activity- or experience-dependent changes in synaptic structure and function that are relatively long-lasting (that is, persisting beyond the initial electrochemical event).

[1]*Mood and Anxiety Disorders Program, Department of Psychiatry; Fishberg Department of Neuroscience; and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA.*
[2]*Clinical Neuroscience Division, VA National Center for PTSD; Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06511, USA.*
[3]*Mental Health Care Line, Michael E. DeBakey VA Medical Center; Menninger Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, Texas 77030, USA.*

Correspondence to J.W.M.
james.murrough@mssm.edu

doi:10.1038/nrd.2017.16
Published online 17 Mar 2017

Major depressive disorder (MDD) is among the most disabling medical illnesses worldwide and ranks first among all mental health, substance and neurological disorders in terms of disability-adjusted life years[1,2]. The consequences of untreated or partially treated depression are enormous for patients, their families, healthcare systems and society[3]. A substantial proportion of patients with MDD do not respond to current treatments, despite many antidepressant trials and augmentation strategies[4,5]: up to ~20% of patients with MDD have treatment-resistant depression (TRD; usually defined as a failure to respond to two or more antidepressant medication trials)[3]. Moreover, the peak efficacy of first-line antidepressants, such as serotonin-selective reuptake inhibitors (SSRIs), is delayed, with lag times of several weeks to months before benefit. Although the monoamine systems (including the serotonin, noradrenaline and dopamine systems) have long been the focus of depression research and treatment, there is now a general consensus that drug discovery must move beyond the monoamine systems to improve patient outcomes. In particular, the glutamate system has emerged as a vibrant area of investigation[6,7].

The ubiquity and complexity of the glutamate system poses considerable obstacles for drug discovery efforts, in large part owing to the potential for seizure induction and other tolerability-related issues. However, motivated by the recognition that glutamate and its specific receptor subtypes serve fundamental roles in the regulation of synaptic plasticity and affect basic human processes of mood, cognition, learning and reward, several early-stage clinical neuropsychiatric programmes have been initiated for compounds that target different components of the glutamate system. Such compounds include modulators of ionotropic receptors (including *N*-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs)), metabotropic glutamate receptor (mGluR) modulators, glycine transporter 1 (GlyT1) inhibitors and glutamate release inhibitors[8].

The NMDAR-blocking agent ketamine — an anaesthetic that has been available for human use since the 1960s — was reported in 2000 to induce profound clinical improvements in the core symptoms of depression within several hours of treatment[9]; this was an unexpected finding that was later hailed as "arguably the most important discovery [in mood disorders] in half a century" (REF. 10). Moreover, this discovery triggered vigorous research in both industry and academia to understand the role of glutamate signalling in depression pathophysiology and to develop novel treatments. Investigational drugs that target components of the glutamate system have begun to enter phase II and phase III trials. This Review evaluates the role of glutamate signalling in depression and discusses the potential of ketamine and other glutamate-signalling modulators as novel antidepressant agents. We critically review the limitations of existing studies of the clinical effects and hypothesized mechanisms of action of glutamate-based antidepressant candidates, and detail progress in this area. We evaluate evidence concerning the role of the NMDAR versus other molecular targets in the antidepressant mechanism of action of ketamine and other candidate glutamate modulators, and conclude with a discussion of the challenges of glutamate modulation for novel drug development and opportunities for new directions.

Box 1 | **Overview of glutamate signalling within the central nervous system**

The ligand-gated α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors conduct current primarily through the flux of Na^+ ions. AMPA receptors (AMPARs) are composed of glutamate receptor (GluR) subunits (GluR1–GluR4) that form tetramers to function in excitatory neurotransmission and in activity-dependent synaptic plasticity and synaptogenesis[149]. Glutamate binding to at least two of the receptor subunits triggers rapid channel opening, allowing depolarizing current carried mostly by Na^+ ions to enter the cell, followed by rapid channel closing and desensitization. AMPAR function is regulated through GluR1 subunit phosphorylation; this influences long-term potentiation (LTP) and subunit trafficking to and cycling from the plasma membrane, for example, processes that are important in synaptic plasticity. Kainate receptors are also tetramers and are composed of five possible subunits: GluR5, GluR6, GluR7, KA1 and KA2.

NMDARs (*N*-methyl-D-aspartate receptors) are tetrameric protein complexes and typically comprise two NR1 and two NR2 subunits (with NR3 occurring less frequently). NR2 subunits are classified as NR2A–NR2D, with NR2A and NR2B being the most common in the mammalian central nervous system. NMDAR function depends on the simultaneous binding of glutamate to each of two NR2 subunits and the co-agonist glycine on each of the NR1 subunits[150]. Separately, the NMDARs require depolarization-dependent displacement of Mg^{2+} from the channel pore; often the initial depolarization is dependent on AMPARs. This feature of NMDARs confers unique properties to these receptors, including functioning as a 'coincidence detector': amplifying excitatory transmission for converging inputs. In addition, NMDARs exhibit many modulatory binding sites, including polyamine and Zn^{2+} sites. Ca^{2+} influx through NMDARs leads to the activation of second-messenger systems that underlie alterations of synaptic plasticity. Activation of synaptic or extrasynaptic NMDARs promotes neurotrophic or apoptotic pathways, respectively, through brain-derived neurotrophic factor (BDNF) and other signalling pathways (see REF. 15).

In contrast to the fast-acting ionotropic AMPAR, kainate receptors and NMDARs, metabotropic GluRs (mGluRs) are seven-transmembrane domain G protein-coupled receptors (GPCRs) that mediate many cellular processes and slow-acting changes (including the modulation of presynaptic neurotransmitter release and postsynaptic responses) through G protein second messengers and downstream effector systems[151]. Classically, these receptors transduce extracellular signals through the cyclic AMP and phosphatidylinositol pathways, and are divided into three groups. Group I receptors, consisting of mGluR1 and mGluR5, are primarily localized to the postsynaptic membrane and transduce excitatory glutamate signalling through the activation of phospholipase C and the formation of inositol-1,4,5-trisphosphate and diacylglycerol. Activation of group I receptors tends to increase intracellular calcium signalling and augment NMDAR activity, and can increase the risk of excitotoxicity. Group II receptors (mGluR2 and mGluR3) and group III receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are generally localized presynaptically and decrease cAMP-dependent processes and thus serve a presynaptic inhibitory function; these receptors are generally associated with reduced NMDAR signalling and a reduction in the potential for excitotoxicity[150,151].

Glutamate and depression

Glutamate signalling in health and disease

Glutamate is the primary excitatory neurotransmitter in the central nervous system (CNS), and is released at synapses throughout the brain. It exerts both short-term changes in postsynaptic excitability and longer-term changes in synaptic strength and neuroplasticity through its regulation of second-messenger systems, and through downstream effects on the activity of various membrane-bound receptors, nuclear gene expression and translation. Glutamate binds to several different receptors, which can broadly be divided into ionotropic receptors (including NMDARs, AMPARs and kainate receptors) and mGluRs (BOX 1).

AMPARs and NMDARs are both Na^+-permeable and have major roles in activity-dependent synaptic plasticity[11,12], whereas NMDARs have a uniquely high permeability to Ca^{2+}. Long-term potentiation (LTP) is one well-characterized NMDAR- and AMPAR-dependent mechanism of synaptic plasticity[12]. In this process, AMPARs rapidly conduct depolarizing current through the plasma membrane, leading to the reversal of the Mg^{2+} occlusion of the NMDAR, the influx of Ca^{2+} through the NMDAR, and subsequent activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; also known as CAMK2A) and other downstream second-messenger systems that ultimately promote the trafficking and incorporation of AMPARs into the plasma membrane. Consistent with this model, the ratio of AMPARs to NMDARs seems to index LTP and probably other forms of synaptic plasticity.

NMDAR signalling can promote cell survival and neurotrophic functions or can activate cell death pathways, depending on the timing and duration of receptor activation, the location of the receptor, and the cellular and extracellular environment at the time of activation[13–16] (FIG. 1). Moderate levels of NMDAR activation and attendant Ca^{2+} influx promote neuroprotective signalling pathways, including activation of the RAS–mitogen-activated protein kinase (RAS–MAPK) pathway and cyclic AMP-responsive element-binding protein (CREB)-mediated induction of survival genes. For example, CREB promotes the expression of brain-derived neurotrophic factor (BDNF), which has a key role in neuroprotective and neurotrophic processes that are relevant to stress and mood disorders[17,18]. By contrast, abnormally elevated or misappropriated NMDAR signalling leads to deleterious effects on neurons (reviewed elsewhere[15]) (FIG. 1). Excessive glutamate release and overactivation of NMDARs has long been associated with a phenomenon known as excitotoxicity[19,20]. NMDAR-dependent neurotoxicity is implicated in several CNS disorders, including ischaemic stroke[21] and neurodegenerative disorders such as Parkinson disease, Alzheimer disease and Huntington disease[22].

Glutamate dysfunction in depression

Several lines of evidence implicate various aspects of the glutamate system in pathophysiological processes that are relevant to depressive disorders. For example, glutamate levels have been shown to be elevated in the plasma, cerebrospinal fluid and the brains of patients with depression[23], in neuroimaging and post-mortem studies (although unresolved inconsistencies concerning the affected regions and the direction of these changes remain), and in a small number of genetic studies, suggesting an association between glutamate-related gene variants and depression[24]. Moreover, a series of post-mortem studies has reported alterations in the expression or function of the NMDAR subunits in patients with MDD or bipolar disorder, and in victims of suicide[25–33] (TABLE 1).

Glial cells have an important role in the regulation of glutamate signalling, and may play a part in depression[34]. Glial cells clear glutamate from the synaptic cleft through their excitatory amino acid transporters (EAATs); synthesize and release the NMDAR co-agonist D-serine; metabolize glutamate to glutamine; synthesize and release trophic factors; and express group I and group

Long-term potentiation
A form of synaptic plasticity in which postsynaptic cellular responses are augmented as a function of recent neuronal activity.

Excitotoxicity
Neurotoxicity through a mechanism at least partially dependent on high Ca^{2+} influx and subsequent triggering of cell death mechanisms.

Bipolar disorder
A mood disorder that is characterized by episodes of depression alternating with episodes of mania or hypomania.

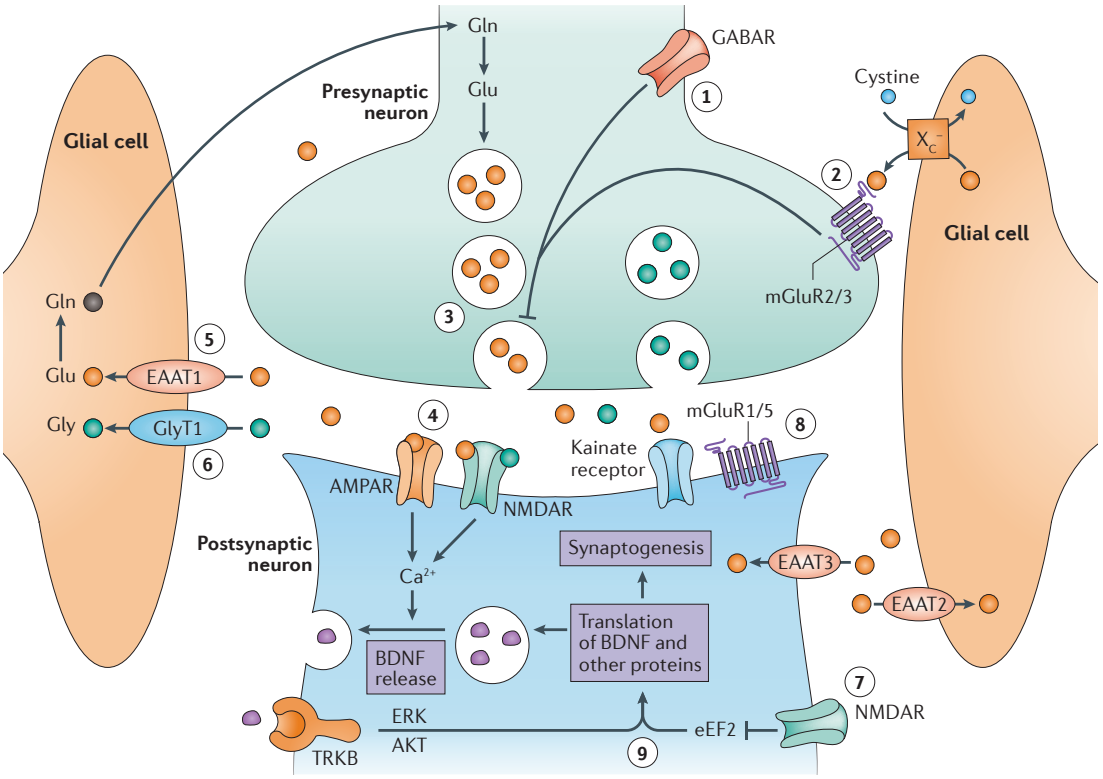

Figure 1 | **Glutamate signalling in health and disease**. This figure depicts the canonical tripartite synapse under normal physiological conditions, with sites and processes potentially affected by proposed therapeutic agents in mood disorders indicated by numbers. Glutamate (Glu) is the most abundant excitatory neurotransmitter in the brain and is crucial for information processing, memory and neuronal plasticity. It is produced in neurons through the conversion of glutamine (Gln). Depolarization within a presynaptic glutamatergic neuron (top) triggers the fusion of Glu-containing synaptic vesicles with the presynaptic membrane (a process that can be inhibited by the activation of inhibitory GABA receptors (GABARs)), the release of transmitter into the synaptic cleft and subsequent diffusion across the cleft and binding to various types of Glu receptors: the ionotropic receptors (*N*-methyl-D-aspartate receptors (NMDARs), kainate receptors and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs)) and metabotropic Glu receptors (mGluRs). Ionotropic Glu receptors are membrane-bound assemblies of oligomeric subunits and rapidly depolarize the postsynaptic membrane through the influx of cations (such as Na^+ and Ca^{2+}). AMPAR and kainate receptor activation results in the rapid depolarization of the neuronal membrane and requires Glu binding only, whereas NMDARs require initial depolarization as well as binding of both Glu and glycine (Gly). The mGluRs are transmembrane G protein-coupled receptors (GPCRs) and are divided into group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8). Group I mGluRs are located both presynaptically and postsynaptically and can activate the inositol-1,4,5-trisphosphate–calcium and diacylglycerol–protein kinase C cascades; group II receptors are most commonly localized presynaptically, inhibit adenylyl cyclase, and generally function to inhibit Glu and NMDAR signalling (not shown). NMDAR and AMPAR activation leads to synaptogenesis partly through the release of brain-derived neurotrophic factor (BDNF), which stimulates tropomyosin-related kinase B (TRKB) receptors and activates the AKT and extracellular signal-regulated kinase (ERK) signalling pathways. Notably, acute NMDAR blockade seems to stimulate a similar process through indirect pathways that may include the blockade of NMDARs on inhibitory GABAergic interneurons, thereby simulating synaptic NMDARs and AMPARs via endogenous Glu release. Glial cells play a crucial part in regulating intrasynaptic concentrations of Glu, including the uptake of Glu and Gly from the synapse through excitatory amino acid transporters (EAATs) and Gly transporters (GlyTs), respectively. Glial cells also modulate Glu neurotransmission through a cystine–Glu exchanger (X_C^-), releasing Glu in the extrasynaptic space in the vicinity of presynaptic mGluR2/3s. In depression, there are several changes in glutamatergic signalling that could potentially be targeted by modulators. Certain agents could aim to disinhibit glutamatergic neurons partly by reducing GABAergic inhibitory tone (process 1). mGluR2/3 antagonists may increase the presynaptic release of Glu (process 2), and have shown rapid synaptogenic and antidepressant effects in preclinical models. Agents that increase presynaptic vesicular Glu release (process 3) may have antidepressant potential. AMPAR potentiators have been investigated in depression (process 4), although their future is uncertain, owing to possible toxicity. Compounds that enhance EAAT function may mitigate the excitotoxic effects of stress and attendant excessive extrasynaptic Glu levels (process 5). The GlyT1 inhibitor sarcosine, which is also hypothesized to increase NMDAR signalling (process 6), has shown preliminary efficacy in patients with depression. Ketamine and other NMDAR antagonists are believed to exert neuroprotective effects by blocking extrasynaptic NMDARs (process 7). mGluR5 negative allosteric modulators or antagonists are believed to exert neuroprotective effects that are comparable to the blockade of extrasynaptic NMDARs, and may have antidepressant properties (process 8). Potentiation of the mechanistic target of rapamycin complex 1 (mTORC1; not shown) and other intracellular protein translation machinery seems to be a crucial downstream consequence of NMDAR blockade, and alternative approaches for affecting this pathway may represent novel antidepressant strategies (process 9). eEF2, eukaryotic elongation factor 2.

Glial cells
Non-neuronal central nervous system cells, including astrocytes and oligodendrocytes, that function to maintain homeostasis, support neurotransmission and neuronal health, and form myelin.

Table 1 | **Human post-mortem studies implicating the NMDA receptor in depression***

Tested measure	Sample	Brain region	Method	Results	Refs
NR1 subunit	Bipolar and depressive disorders	Temporal cortex	Western immunoblotting	↓NR1 density in bipolar disorder and depression	27
NR2C subunit	MDD	LC	Western immunoblotting	↑NR2C in MDD	28
NR1 and NR2A subunits	MDD	Lateral amygdala	Western immunoblotting	↑NR2A in MDD; no difference in NR1	30
NR1, NR2A and NR2B subunits	MDD	PFC	Western immunoblotting	↓NR2A and NR2B in MDD; no difference in NR1	31
NR2B and NR2C	MDD (most died by suicide)	LC	Gene expression	↑NR2B and NR2C in MDD	32
Multiple NMDAR- and glutamate-related genes	MDD	DLPFC	Gene expression	↑Expression of most glutamate-related genes in MDD (findings primarily driven by female patients)	33

DLPFC, dorsolateral prefrontal cortex; LC, locus coeruleus; MDD, major depressive disorder; NMDAR, *N*-methyl-D-aspartate receptor; PFC, prefrontal cortex; TRD, treatment-resistant depression. *Studies are listed chronologically. Note that depression is characterized by both upregulation and downregulation of NMDAR components, depending on the brain region and the study.

II mGluRs. A loss of glia has been reported in the prefrontal cortex (PFC) of people with mood disorders[34,35], and recent work suggests that chronic stress may lead to depression by impairing cortical astrocytes[36].

In animal studies, chronic stress — which is associated with depressive symptoms in animal models and in humans[37] — consistently leads to neuronal atrophy in the PFC and hippocampus and to a decrease in synaptic functioning. By modifying glutamate release and uptake, chronic stress affects the cortex by inducing a reduction in synaptic AMPAR and NMDAR availability; reductions in synapse density and diameter; and reductions in dendritic arborization and length (see REF. 38 for a review). Whereas acute stress reliably increases glutamate release, chronic stress leads to maladaptive changes within glutamate synapses, including reduced extracellular glutamate clearance by glia and the increased activation of extrasynaptic NR2B-containing NMDARs, potentially contributing to synaptic loss and the activation of cellular apoptotic pathways (FIG. 1). According to this model, the net effect of these changes is a disruption in cellular signalling and a reduction in cellular resilience within brain circuits that are crucial for mood regulation[39]. These microstructural and molecular changes are believed to underlie several gross abnormalities that have been found in the brains of individuals with MDD, including reductions in brain volume[40], changes in glutamate levels[41], and altered function and connectivity within brain networks[42].

Human neuroimaging approaches have also implicated the glutamate system in depression. Neurometabolites in the brain, including glutamate, glutamine, GABA and the combined glutamate plus glutamine signal known as Glx, can be quantified using proton magnetic resonance spectroscopy (^{1}H-MRS). Such studies have found that MDD is associated with reduced Glx levels in the PFC[43], and reduced GABA levels in the PFC[43] and occipital cortex[44], as well as elevated glutamate levels in the occipital cortex[45]. A human positron emission tomography (PET) study found lower regional binding of the mGluR5-selective radioligand [^{11}C]-ABP688 within the PFC and other regions; this finding was supported by a companion post-mortem study of PFC mGluR5 protein expression[46]. Most recently, a novel ^{13}C-MRS technique revealed that patients with MDD exhibited abnormally reduced mitochondrial energy production, but no change in the rate of glutamate–glutamine cycling[47].

Ketamine as an antidepressant

Ketamine is a non-competitive antagonist at the NMDAR, binding to a site within the channel. It also interacts with opioid and cholinergic receptors[48], although the contribution of these non-NMDAR-related interactions to the antidepressant mechanism of action of ketamine is unclear and represents an active area of study (see below). Following parenteral administration, the drug is rapidly distributed throughout the body and readily crosses the blood–brain barrier. Ketamine is commonly used clinically as a racemic mixture of (*R*)- and (*S*)-enantiomers ((*R*,*S*)-ketamine) to induce anaesthesia. Studies of ketamine for depression have predominantly focused on this racemic mixture, although the (*S*)-enantiomer is currently the focus of a pharmaceutical drug development effort. Recent preclinical work has also suggested that the (*R*)-enantiomer may have a favourable therapeutic and safety profile compared with either the racemic mix or the (*S*)-enantiomer[49–51] (see below).

In the first report of the rapid antidepressant effects of ketamine[9], a small group of patients with depression received a single low-dose intravenous (i.v.) infusion of ketamine or placebo (saline) (details in TABLE 2). Ketamine produced expected acute mental status changes, including psychosis-like effects, dissociation and a self-reported feeling of a 'high'. These acute effects peaked at the 40-minute infusion end point and then rapidly dissipated, whereas reductions in the core symptoms of depression, including sad mood, anhedonia, pessimism and indecision, occurred separately, hours to days after infusion. In a larger, hypothesis-driven study of patients with TRD,

Glutamate–glutamine cycling
Biochemical pathway that describes the uptake and conversion of glutamate to glutamine by astrocytes and the subsequent transfer of glutamine back to neurons for conversion to glutamate.

Enantiomers
Stereoisomers that are mirror images of each other.

ketamine produced a rapid antidepressant effect within hours of a single i.v. infusion that subsequently waned by 7 days post-treatment[52].

Although these early results were encouraging, small sample sizes or other methodological concerns served to limit their interpretation. In particular, the acute mental

Table 2 | **Randomized controlled clinical trials of ketamine in mood disorders***

Sample	Trial details (design, route, number of participants, comparator, outcome)	Dosing	Results	Refs
Unipolar depression				
MDD + BPD	• DB, CO • Single administration • i.v. • *n* = 9 • Saline • HDRS	0.5 mg per kg over 40 min	Decrease in depression severity within 72 hours following ketamine compared with placebo	9
TRD	• DB, CO • Single administration • i.v. • *n* = 18 • Saline • HDRS	0.5 mg per kg over 40 min	Greater response at 24 hours following ketamine (71% response) compared with placebo (0% response)	52
TRD	• DB, PA • Single administration • i.v. • *n* = 73 • Midazolam • MADRS	0.5 mg per kg over 40 min	Greater response in ketamine group (64%) compared with midazolam group (28%)	53
MDD	• DB, CO • Single administration • i.v. • *n* = 27 • Saline • MADRS	0.54 mg per kg over 30 min	Decrease in depression severity following ketamine compared with placebo; peak effect at 24 hours, although duration of effect unspecified	157
TRD	• DB, CO • Single administration • i.n. • *n* = 20 • Saline • MADRS	50 mg	Greater response following ketamine (44%) compared with placebo (6%)	65
MDD	• DB, PA‡ • Single administration • i.v. • *n* = 30 • Saline • MADRS	0.5 mg per kg over 40 min	Greater response at 4 weeks in ketamine-augmented group (92.3%) versus SSRI alone (57.1%)	64
TRD	• DB • Repeated administration (twice or three times weekly over 15 days) • i.v. • *n* = 67 • Saline • MADRS	0.5 mg per kg over 40 min	Twice and three times weekly maintained antidepressant efficacy over 15 days	63
Bipolar depression				
TRBPD	• DB, CO§ • Single administration • i.v. • *n* = 18 • Saline • MADRS	0.5 mg per kg over 40 min	Greater response following ketamine (71%) compared with placebo (6%)	158
TRBPD	• DB, CO§ • Single administration • i.v. • *n* = 15 • Saline • MADRS	0.5 mg per kg over 40 min	Greater response following ketamine (79%) compared with placebo (0%)	159

BPD, bipolar disorder; CO, crossover; DB, double-blind; HDRS, Hamilton Depression Rating Scale; i.n., intranasal; i.v., intravenous; MADRS, Montgomery–Åsberg Depression Rating Scale; MDD, major depressive disorder; PA, parallel arm; SSRI, serotonin-selective reuptake inhibitor; TRBPD, treatment-resistant bipolar disorder; TRD, treatment-resistant depression. *Studies are listed in chronological order within disorder. ‡Augmentation to SSRI. §Augmentation to mood stabilizer.

status changes associated with ketamine may affect the integrity of the study blind. To partially address this problem, a larger parallel-arm randomized controlled trial (RCT) compared ketamine with another anaesthetic agent — the benzodiazepine midazolam — in patients with TRD[53]. Consistent with previous studies, ketamine was superior to midazolam in rapidly reducing depression severity. Although midazolam is a seemingly preferable control to saline, there were still more robust blood pressure elevations and dissociative side effects in the ketamine group than in the midazolam group, highlighting the challenge in fully controlling for the effects of ketamine.

Nevertheless, to date, RCTs comparing ketamine with a control condition (usually saline) have reported consistent, rapid antidepressant effects in treatment-resistant unipolar and bipolar samples (recently reviewed elsewhere[54,55]) (TABLE 2). A meta-analysis of seven RCTs found that ketamine was ~9.9-fold more likely to induce an antidepressant response than the control, and ~14.5-fold more likely to induce the remission of depressive symptoms[54]. A separate meta-analysis, including eight RCTs, also reported a large antidepressant effect of ketamine compared with a control condition in patients with depression[55]. This report found that ketamine was more efficacious in unipolar depression than in bipolar depression, and had transient psychosis-like effects but no persisting adverse symptoms. A recent Cochrane Review found that ketamine treatment conferred a higher likelihood of response compared with placebo, although the evidence was limited by risk of bias and small samples[56]. Despite promising initial results, it should be emphasized that the total number of patients randomized in published controlled trials of ketamine for depression is low, and there is a considerable risk of bias stemming from methodological limitations, including uncertain masking of outcome assessments.

Several small open-label studies or case reports have examined the effect of repeated administration of ketamine over 2 weeks or more[57–62]. A recent industry-sponsored RCT examined a series of ketamine treatments administered i.v. two or three times weekly for 15 days[63]. Compared with placebo, both ketamine regimens maintained antidepressant efficacy over the 15-day assessment period. Another recent RCT found that daily escitalopram treatment plus a single dose of ketamine led to higher response rates than did daily escitalopram treatment plus saline[64] (TABLE 2). This study produced the first randomized controlled data to suggest that a single dose of ketamine can have an enduring effect on depression severity when used in combination with a conventional antidepressant agent.

There are currently several areas of active investigation concerning the use of ketamine for depression. For example, several groups have begun to explore intranasal (i.n.) or other delivery routes for ketamine in patients with depression as an alternative to i.v. administration[65–67]. Case reports and case series in the literature have also described the treatment of people with depression with oral, sublingual, intramuscular and transdermal ketamine[67]. The effect of ketamine on specific symptom dimensions within depression, and on specific underlying neurobehavioral constructs, represents an important research direction. Early evidence has provided some support for a specific effect of ketamine on anhedonia[68,69], fatigue[70] and suicidal ideation[71–74]. The effects of ketamine on cognition and cognitive control systems similarly represent an area of active research[75–78]. A single RCT compared ketamine with midazolam for the acute reduction of suicidal ideation[74]. No data currently exist concerning the potential anti-suicidal ideation effects of ketamine beyond a few days; clearly, the potential for ketamine or related compounds as rapid-acting therapies for suicidality represents an important area of research.

Most recently, an i.n. formulation of (*S*)-ketamine (also known as esketamine) is being developed as a treatment for TRD and depression-associated suicidality. Although only racemic ketamine is available in the United States, (*S*)-ketamine — which has a potency that is threefold to fourfold higher than (*R*)-ketamine — has regulatory approval as an anaesthetic agent in several European Union countries. A recent industry-sponsored double-blind placebo-controlled study in 30 patients with TRD found rapid antidepressant efficacy of a 40-minute i.v. infusion of (*S*)-ketamine (0.2 mg per kg or 0.4 mg per kg)[79]. No data concerning the tolerability or efficacy of i.n. (*S*)-ketamine have been published to date. To our knowledge, there are no published data on the effects of (*R*)-ketamine in humans.

Mechanisms of glutamate modulators

There is strong evidence that ketamine and other NMDAR antagonists show antidepressant properties in animal models[80,81] (BOX 2). Several lines of research, however, point to substantial complexity in the mechanism of action of these agents. For example, AMPAR blockade consistently prevents the antidepressant-like effects of ketamine and other putative NMDAR antagonists in animal models[49,80,82,83], and recent data suggest that ketamine may trigger an antidepressant effect through an active metabolite that acts in an NMDAR-independent manner[49]. From a clinical perspective, non-ketamine NMDAR antagonists such as memantine and some of the experimental agents have not yielded the robust antidepressant effects that are associated with ketamine, further leading the field to question whether NMDAR blockade really is the sole or primary cause of the antidepressant effects of ketamine (see below). Here, we attempt to bring together disparate findings in the literature and consider the evidence that there is a crucial role for synaptic plasticity in the mechanism of action of ketamine and other glutamatergic candidate antidepressants.

Findings from preclinical studies

NMDAR antagonism. Many studies show that non-selective NMDAR antagonists, as well as antagonists that are selective for NR2B-containing NMDARs, exert antidepressant behavioural effects in animal depression models, including the chronic variable stress and chronic social defeat stress models[10,80,82–84]. The activation of extrasynaptic NMDARs by extracellular glutamate is believed to be

Escitalopram
The (*S*)-stereoisomer of citalopram; a serotonin-selective reuptake inhibitor (SSRI) approved in the United States for the treatment of major depressive disorder and generalized anxiety disorder.

Chronic variable stress
Procedure used to model depression in rodents that typically consists of subjecting the animal to daily bouts of mild-to-moderate environmental stressors over several weeks.

Chronic social defeat stress
Procedure used to model depression in rodents that consists of exposing a target rodent to an aggressor daily for 10 days.

Box 2 | **Historical targeting of the NMDAR in depression**

An original series of experiments designed to assess the behavioural effects of *N*-methyl-D-aspartate receptor (NMDAR) antagonists in animal models of depression was published in 1990 (REF. 152). On the basis that exposure to inescapable shock produces both behavioural depression and impairments in hippocampal long-term potentiation (LTP), which is an NMDAR-dependent process, the authors hypothesized that NMDAR antagonists may represent a novel class of antidepressants[153,154].

Indeed, the same authors showed that the use-dependent NMDAR channel blocker dizocilpine (also known as MK-801), the competitive antagonist 2-amino-7-phosphonoheptanoic acid (AP-7) and the NMDAR glycine-site partial agonist 1-aminocyclopropanecarboxylic acid (ACPC) all dose-dependently reduced immobility in the forced swim test[152]. The same group subsequently showed that chronic, but not acute, administration of conventional antidepressants (including imipramine, the serotonin-selective reuptake inhibitor (SSRI) citalopram and electroconvulsive shock) produced dose-dependent and persistent changes in the binding profile of NMDARs[155]. On the basis of these findings, the authors proposed that adaptive changes in NMDARs may be a final common pathway for antidepressant action.

At the same time, other groups were testing the effects of NMDAR antagonists on the response to rewarding stimuli in the context of a chronic mild-stress paradigm, a depression model that is likely to have greater face validity for human depression[81,156]. In one example, ACPC reversed stress-induced deficits in sucrose preference in a manner similar to imipramine, but notably with a faster onset of action (within 2 weeks compared with 3–5 weeks for imipramine alone)[156]. The preclinical work reviewed above provides compelling evidence for the antidepressant effects of pharmacologically diverse functional NMDAR modulators across different stress models of depression.

important in excitotoxicity and in synaptic atrophy that is associated with depression and other neuropsychiatric disorders[6], leading to the hypothesis that the inhibition of NMDAR would promote synaptic formation and would reverse the detrimental effects of depression and stress. Consistent with this model, the activity-independent blockade of NMDAR by ketamine inhibits the phosphorylation of eukaryotic elongation factor 2 (eEF2), thus increasing BDNF expression and synaptic formation[83,85]. Particularly instructive to the question of differences in clinical efficacy between ketamine and memantine, recent studies have demonstrated that, whereas ketamine led to enhanced hippocampal translation of BDNF, which was required for its antidepressant effect, memantine had no effect on hippocampal BDNF and no antidepressant behavioural effects[85], possibly accounting for the differential clinical efficacies of these drugs.

As noted above, a recent study provided somewhat surprising evidence that ketamine could trigger an antidepressant effect independently of the NMDAR. The authors showed that higher levels of the ketamine metabolite (2*S*,6*S*;2*R*,6*R*)-hydroxynorketamine (HNK)[49] were associated with a stronger antidepressant effect in female mice, and that inhibition of the metabolism specifically of (*R*)-ketamine to (2*R*,6*R*)-HNK blocked this effect. Importantly, this study demonstrated that AMPAR activation is required for the antidepressant effects of ketamine and (2*R*,6*R*)-HNK, replicating the findings of previous reports (see below for additional discussion). If replicated, these findings have major implications for depression drug discovery that is focused on the glutamate system, and suggest that directly targeting the NMDAR may not be required.

AMPAR and NMDAR activation. An emerging common feature of rapidly acting antidepressants is their ability to directly, or indirectly, activate intrasynaptic AMPAR — and perhaps intrasynaptic NMDAR — signalling, and associated intracellular cascades. For example, the NMDAR antagonist Ro 25–6981 and the muscarinic cholinergic receptor (mAChR) antagonist scopolamine, which both have antidepressant effects, were found to induce a rapid increase in intrasynaptic glutamate neurotransmission[86], with presumed stimulation of both AMPARs and NMDARs. Moreover, AMPAR antagonism has been repeatedly shown to block the beneficial effects of ketamine and other putative rapid-acting antidepressants[49,80,82,83]. Notably, the NMDAR partial agonist GLYX-13 (Rapastinel; Allergan) (see below), the mGluR2 and mGluR3 antagonist LY341495, and the mAChR antagonist scopolamine were recently shown to partly depend on AMPAR activation for their antidepressant mechanism of action[87–89]. Separately, AMPAR activation was required to trigger the antidepressant effects of deep brain stimulation of the infralimbic prefrontal rat cortex[90]. Given that AMPAR activation is required for NMDAR activation, it is important to highlight that the AMPAR antagonism findings are not necessarily specific to AMPAR but rather underline the crucial role of synaptic AMPAR and NMDAR neurotransmission. Recent positive results from a clinical trial with a GlyT1 inhibitor, sarcosine, which potentiates NMDAR signalling, further highlight this mechanistic nuance (see below).

Role of synaptic plasticity. As described above, neuroplasticity pathways are altered in depression; for example, there are impairments in synaptic plasticity in the PFC and hippocampus[37]. Synaptic plasticity is necessary for the processing and storage of information and for adapting responses to future stimuli[91], and can occur locally (for example, in LTP and long-term depression (LTD)) or globally (as in synaptic scaling). Synaptic scaling may be particularly relevant to depression pathophysiology. Prolonged neuronal activation — for example, with chronic stress — precipitates an LTD-like downscaling of an entire affected brain region. By contrast, acute transient activation of synapses — as observed following low-dose ketamine administration in mice — induces global LTP-like upscaling, which enhances synaptic connectivity (for example, through AMPAR insertion and increased synaptic density)[92].

Sucrose preference
Procedure used to assess anhedonia or lack of response to pleasure in rodents that involves measuring the degree to which an animal preferentially selects a solution sweetened with sucrose over a non-sweet solution.

The tight coupling between glutamate signalling and mechanisms of synaptic plasticity provides a framework for understanding the molecular basis of the therapeutic action of glutamatergic antidepressants[37]. Ketamine increases the translation of synaptic proteins, including GluR1 (also known as GluA1) and postsynaptic density 95 (PSD95), through a BDNF–tropomyosin-related kinase B (TRKB)–AKT–mechanistic target of rapamycin complex 1 (mTORC1) pathway that is dependent on AMPAR activation[82]. Notably, however, some investigators have been unable to replicate the effects of ketamine on mTORC1-dependent synaptic protein translation[93], and the mechanism linking ketamine to the activation of synaptic plasticity pathways remains incompletely understood. Nevertheless, the mounting evidence that other putative rapidly acting antidepressants, including scopolamine and GLYX-13, trigger synaptic plasticity pathways lends support to the glutamate–synaptic plasticity mechanism of action framework as a useful conceptual model (FIGS 1,2).

Consistent with this model, ketamine inhibits glycogen synthase kinase 3 (GSK3) function and thus may disinhibit pro-plasticity pathways[94]. Moreover, ketamine synergizes with lithium and other GSK3 inhibitors in animal depression models[95]. In hippocampal neurons, ketamine increase AMPAR signalling by increasing GluR1 subunit trafficking to the cell surface in a GSK3-inhibition-dependent manner[96]. Finally, a small but growing body of literature suggests that ketamine may regulate immune cell signalling[97,98] (although see REF. 99 for contrary findings). Given that high levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF) negatively regulate synaptic plasticity pathways, a decrease in the levels of these cytokines may contribute to the pro-plasticity and antidepressant effects of ketamine.

Findings from human studies

As animal studies illuminate the molecular events underpinning the antidepressant mechanisms of ketamine and other glutamate modulators, translational and clinical studies will be required to validate these results in humans. Below, we briefly review studies of the effects of ketamine in humans as a prototypical modulator of glutamate signalling. It will be instructive to distinguish between immediate changes (that is, changes within 1 hour of treatment) and changes associated with neuroadaptive changes that emerge after the acute perturbations (that is, several hours, days and weeks after treatment).

Biomarkers related to the immediate effects of ketamine. Congruent with observations in animal models[86], ketamine transiently increases pooled ^{1}H-MRS measures of Glx and GABA in the PFC of individuals with MDD[100]. As described above, a transient elevation in synaptic glutamate concentrations is crucial to activitydependent stimulation of neuroplasticity pathways. Elevated glutamate release has similarly been linked to the dissociative or psychotomimetic effects of ketamine[101]. One study in people with depression found that the acute dissociative effects of ketamine correlated with its antidepressant activity[102] (but see also REF. 103). In a pharmaco-imaging study in healthy participants, ketamine decreased the blood oxygen level-dependent (BOLD) signal in the ventromedial PFC and subgenual anterior cingulate cortex (ACC), and increased the BOLD signal in the posterior cingulate cortex, thalamus and temporal cortex[104]. However, in patients with MDD, ketamine and the low-trapping NMDAR antagonist lanicemine both increased the BOLD signal in the subgenual ACC, and these increases were correlated with some measures of the antidepressant effects[105]. The extant data implicate transient potentiation of cortical glutamate signalling in both the antidepressant and the dissociative or psychotic effects of ketamine, raising the crucial question of whether the antidepressant efficacy of glutamate-based agents can be separated from the undesirable effects of ketamine.

Biomarkers related to the sustained effects of ketamine. A recent ^{1}H-MRS study in healthy volunteers treated with ketamine found sustained increases in the glutamine/glutamate ratio within the perigenual ACC at 24 hours[106]. Using task-based fMRI, our group recently reported that caudate responses to positive emotional stimuli (happy human faces) are smaller in patients with TRD than in healthy participants but are normalized 24 hours after ketamine infusion[107]. The antidepressant response to ketamine was positively correlated with increases in connectivity of the caudate with other brain regions, including the ACC. Separately, in healthy volunteers, ketamine blunted the responses of the amygdala to negative and neutral (but not positive) affective stimuli[108]. Using global brain connectivity (GBC), our group recently demonstrated that the PFC of patients with depression shows widespread reduced functional connectivity[109], which is partially reversed 24 hours after ketamine treatment[110]. The positive effect of ketamine on PFC connectivity in humans may represent a surrogate marker of changes in synaptic connectivity observed at the molecular level in animal models. Additional translational studies using multimodal imaging techniques in humans and animals are required to validate these and other fMRI-based surrogate markers of depression pathophysiology.

Recent studies of *in vivo* metabolism in participants with mood disorders paint a mixed picture. A PET study in unmedicated patients with TRD found that, after ketamine treatment, regional metabolism decreased in the right PFC, habenula and insula, and increased in several primary sensory cortical areas[111]. Improvement in depression severity correlated with the increase in metabolism in the temporal cortex and with the decrease in metabolism in the parahippocampal gyrus. In patients with bipolar disorder, improvement in depression correlated with increased metabolism within the ventral striatum in a region-of-interest analysis and with metabolism in the subgenual ACC in a whole-brain analysis[112]. Thus, ketamine may trigger antidepressant effects partly through changes in brain metabolism within prefrontal and medial temporal regions.

Global brain connectivity
A seed-free, whole-brain approach to resting-state functional magnetic resonance imaging connectivity analysis.

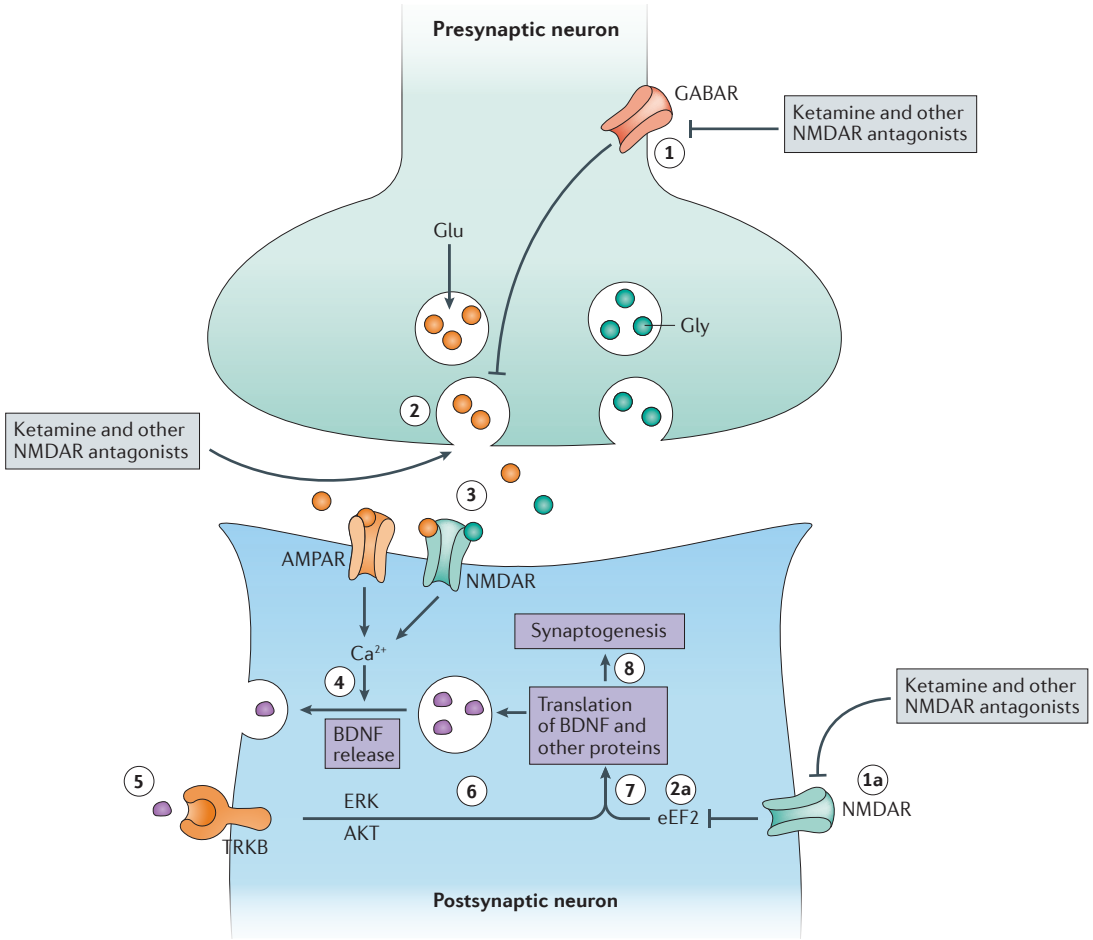

Figure 2 | **The antidepressant mechanism of action of NMDAR modulators.** In animal models, low-dose ketamine and other *N*-methyl-D-aspartate receptor (NMDAR) modulators seem to lead to antidepressant-like behaviour through two main pathways that ultimately serve to facilitate synaptic plasticity and restore homeostasis within glutamatergic synapses and circuits: promoting the effects of glutamate (Glu) signalling (labelled 1–8) and inhibiting the negative consequences of toxic Glu signalling (labelled 1a, 2a, 7 and 8). In the healthy Glu system, signalling pathways linking NMDAR modulation to increases in α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and structural proteins include activity-dependent release of brain-derived neurotrophic factor (BDNF), activation of extracellular signal-regulated kinase (ERK), AKT and the mechanistic target of rapamycin complex 1 (mTORC1) pathways (not shown), and suppression of eukaryotic elongation factor 2 (eEF2) kinase. Chronic mild stress (which is associated with the onset of depressive-like behaviour in animal models) is associated with a reduction of neurotrophic support and BDNF signalling within cortical and hippocampal glutamatergic synapses, and this stress-related reduction is rapidly reversed by low-dose (but not high-dose) ketamine. Ketamine increases the levels of the key synaptic proteins that are involved in synaptic plasticity and long-term potentiation (LTP)-like processes, including the AMPAR subunit GluR1 and structural proteins such as postsynaptic density 95 (PSD95), through a number of processes. Data suggest that low-dose ketamine preferentially inhibits NMDAR on a subpopulation of interneurons, indirectly leading to a decrease in the activation of inhibitory GABA receptors (GABARs) on prefrontal pyramidal neurons (process 1); precipitates a surge in Glu release (process 2); activates intrasynaptic NMDARs and AMPARs (process 3) (AMPARs may also be activated by the ketamine metabolite (2*R*,6*R*)-HNK); increases intracellular Ca^{2+} concentration and enhances BDNF release (process 4); activates tropomyosin-related kinase B (TRKB) (process 5); increases mTORC1 signalling through ERK–AKT pathways (process 6); induces BDNF and synaptic protein translation (process 7); and promotes the formation of new synapses and increases synaptic strength, spine diameter and density (process 8). Ketamine also blocks extrasynaptic NMDARs at rest (process 1a), leading to the disinhibition of eEF2 (process 2a), which in turn promotes processes 7 and 8, described above. Whereas ketamine has been shown to robustly modulate both the promotion and the inhibition of Glu signalling, other NMDAR antagonists and modulators may have more-restricted effects, possibly explaining the variability of their efficacy and onset of action compared with ketamine. For example, NMDAR enhancers (D-cycloserine, GLYX-13, NRX-1074 and sarcosine) are thought to activate processes 3–8, with no demonstrated effects on processes 1a and 2a. In contrast to ketamine, the NMDAR antagonist memantine does not inhibit NMDAR at rest, disinhibit eEF2 or promote processes 7 and 8 (REF. 85). In addition, we are not aware of evidence showing a memantine-induced surge in extracellular Glu levels. Thus, it appears that NMDAR antagonism per se is not sufficient to replicate the robust rapid effects of low-dose ketamine, but rather a specific modulation of NMDAR pathways is needed. Gly, glycine.

Finally, several studies have aimed to investigate the role of BDNF in the antidepressant effects of ketamine in humans, although these studies are methodologically limited to investigations of levels of circulating BDNF, rather than BDNF levels in the CNS. An earlier study failed to show an effect of ketamine on blood-derived BDNF levels[113], whereas a more recent study showed that individuals with TRD who responded to ketamine exhibited higher levels of plasma BDNF than did non-responders[114]. A separate study found that plasma BDNF levels increased 4 hours after ketamine administration, and that slow wave activity — a candidate surrogate measure of synaptic plasticity[115] — increased during sleep on the first night following treatment[116]; both of these changes were proportional to the antidepressant response. Consistent with these data, a preliminary report on the role of the Val66Met single-nucleotide polymorphism of *BDNF* shows that patients with depression who are homozygous for the Val variant exhibit an enhanced antidepressant response to ketamine[117]. Given the state of the current literature, the link between BNDF function and the mechanism of action of ketamine in humans remains tentative.

Other glutamate modulators in development

Below, we consider candidate glutamate modulating compounds currently in human testing for depressive disorders (TABLE 3). Several compounds that represent important milestones in drug development in this area but that are not currently being pursued will not be considered in detail, including traxoprodil (CP-101,606)[118] and lanicemine (AZD6765)[119].

D-Cycloserine

D-Cycloserine (DCS) is an antituberculosis drug that was reported to have antidepressant-like effects as early as the 1950s[120]. At low doses, DCS acts as a partial agonist at the glycine site of the NMDAR, whereas at high doses (>750 mg) it seems to behave as a functional NMDAR antagonist. A single-site 6-week trial of gradually titrated high-dose DCS as an adjunct to standard antidepressant medication therapy in patients with TRD reported a superior benefit of DCS compared with placebo[121]. A recent small open-label study reported good tolerability of 8 weeks of high-dose DCS (titrated to 1,000 mg per day) in patients with treatment-resistant bipolar depression following ketamine treatment[122].

GLYX-13 and NRX-1074

GLYX-13 is a tetrapeptide (Thr-Pro-Pro-Thr) that seems to function as a partial agonist at the glycine-binding site of the NMDAR, although the precise manner by which the compound modulates NMDAR functioning is not fully known. GLYX-13 enhances LTP *in vitro* and has concentration-dependent effects on NMDAR currents: at low concentrations, the compound enhances NMDAR-mediated excitatory postsynaptic potentials, whereas at high concentrations of GLYX-13 these potentials are attenuated[123]. GLYX-13 has antidepressant-like activity in several preclinical behavioural assays, including learned helplessness, the forced swimming test and novelty-induced hypophagia[124].

A recent company-sponsored RCT reported promising, although mixed, effects of a single i.v. infusion of GLYX-13 compared with placebo in 120 patients with MDD[125]. Of the four doses studied, the two middle doses, but not the highest or lowest doses, had a significantly greater antidepressant effect than placebo. The proportion of people who experienced improvement of symptoms did not differ from the placebo group in any of the dose groups. The compound was well tolerated and did not show any evidence of increasing psychotomimetic symptoms. The reason for the lack of efficacy for the lowest and highest doses is unclear, although it might reflect a similar inverted U-shape dose–response relationship for GLYX-13 to those of other NMDAR modulators in preclinical models[6]. Encouragingly, GLYX-13 was granted Fast Track status by the US Food and Drug Administration (FDA) for the treatment of MDD in 2014.

NRX-1074 is an orally active, and purportedly higher potency, analogue of GLYX-13. The oral formulation of NRX-1074 is anticipated to advance to phase II testing in the near future.

CERC-301 (MK-0657)

A small proof-of-concept study of the oral NR2B-selective antagonist MK-0657 (now known as CERC-301) was conducted in patients with TRD[126]. MK-0657 (4–8 mg per day) was administered orally for 12 days and showed favourable tolerability but a mixed efficacy profile. After acquiring the drug from Merck & Co., Cerecor initiated a randomized, double-blind placebo-controlled trial to evaluate the adjunctive antidepressant effects of two doses of CERC-301 (NCT02459236), which received Fast Track designation from the FDA in 2013 for the treatment of MDD. The trial design included two intermittent dose administrations 7 days apart, to test the hypothesis that NMDAR antagonism is more effective for depression when administered intermittently, rather than on a daily basis. The outcomes of the study are anticipated in the near future.

AV-101 (4-chlorokynurenine (4-Cl-KYN))

7-Chlorokynurenic acid (7-Cl-KYNA) is a potent and selective antagonist at the obligatory glycine-binding site on NMDAR NR1 subunits and has been used to probe NMDAR function[127]. 7-Cl-KYNA has low brain penetrance, but the pro-drug 4-chlorokynurenine (4-Cl-KYN; now known as AV-101) readily crosses the blood–brain barrier and is converted to 7-Cl-KYNA in astrocytes. Recent preclinical studies have demonstrated antidepressant-like effects of 4-Cl-KYN in several mouse models[128]. 4-Cl-KYN had dose-dependent antidepressant-like effects in the forced-swim and tail-suspension tests in a manner similar to ketamine. Pretreatment with glycine blocked the antidepressant effects of 4-Cl-KYN, supporting the idea that the active molecule 7-Cl-KYNA may bind to the glycine site. No data for 4-Cl-KYN in humans have been published to date.

Dextromethorphan-containing compounds

Dextromethorphan is a non-selective NMDAR antagonist and the active ingredient in several over-the-counter cough suppressants. Avanir Pharmaceuticals, which was

Learned helplessness
Behavioural pattern that occurs when animals are repeatedly exposed to aversive stimuli that cannot be controlled or from which the animal cannot escape.

Forced swimming test
Behavioural despair test in which the degree to which a rodent swims when placed in a cylinder filled with water from which it cannot escape is taken as a measure of antidepressant activity.

Table 3 | **Investigational strategies targeting the glutamate system for depression***

Compound (alternative name)	Pharmacology	Route	Sponsor	Phase	Comments	ClinicalTrials.gov Identifier	Refs
AXS-05 (dextromethorphan plus bupropion)	Non-selective NMDAR antagonist (dextromethorphan component)	Oral	Axsome Therapeutics	III	No human studies published to date	NCT02741791	–
D-Cycloserine	Glycine-site partial NMDAR agonist	Oral	–	–	Augmentation of exposure-based CBT	NCT02376257	121
(S)-Ketamine	Non-selective, non-competitive NMDAR antagonist	i.v. or i.n.	Janssen Pharmaceuticals	III	Single RCT of i.v. (S)-ketamine in depression published to date	• NCT02417064 • NCT02493868 • NCT02497287 • NCT02418585 • NCT02422186	79
CERC-301 (MK-0657)	NR2B-selective NMDAR antagonist	Oral	Cerecor	II	Tests intermittent dose strategy	NCT02459236	126
Rapastinel (GLYX-13)	Glycine-site partial NMDAR agonist	i.v.	Allergan	II	Single published study shows preliminary efficacy	NCT01684163	125
NRX-1074	Glycine-site partial NMDAR agonist	Oral	Allergan	II	i.v. dose–response study in MDD completed	NCT02067793	–
AVP-786	Non-selective NMDAR antagonist; also modulates sigma-1 receptors	Oral	Avanir Pharmaceuticals/ Otsuka Pharmaceutical	II	No human studies published to date	NCT02153502	–
AV-101 (4-chlorokynurenine)	Glycine-site NMDAR antagonist	Oral	VistaGen Therapeutics	II	No human studies published to date	NCT02484456	128
Diazoxide	Increases expression of glutamate transporter EAAT2; allosteric modulator of AMPARs and kainate receptors	Oral	NIMH	II	Non-diuretic vasodilator, acts as K^+ channel activator	NCT02049385	–
Basimglurant	mGluR5 negative allosteric modulator	Oral	Hoffmann-La Roche	Ib	Phase IIb study did not separate from placebo on primary outcome; study drug did separate from placebo on some secondary outcomes	NCT02433093	138

AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; CBT, cognitive behavioural therapy; EAAT, excitatory amino acid transporter; i.n., intranasal; i.v., intravenous; MDD, major depressive disorder; mGluR5, metabotropic glutamate receptor type 5; NIMH, US National Institute of Mental Health; NMDAR, *N*-methyl-D-aspartate receptor; RCT, randomized controlled trial. *Development programmes and compounds listed are active in clinical trials as of 15 June 2016.

recently acquired by Otsuka Pharmaceutical, sells a combination product that contains dextromethorphan plus quinidine approved for the treatment of pseudobulbar affect. The quinidine increases the bioavailability of dextromethorphan by inhibiting the primary pathway for dextromethorphan metabolism in the liver. AVP-786 represents a next-generation combination, as it contains a deuterium-modified form of dextromethorphan to enhance its pharmacokinetic profile[129]. In 2014, Avanir Pharmaceuticals announced the launch of a phase II programme for AVP-786 in MDD. A separate pilot study of dextromethorphan plus quinidine in patients with TRD is currently underway. Finally, phase III trials of AXS-05 (manufactured by Axsome Therapeutics), an oral combination of dextromethorphan plus bupropion (which is a noradrenaline- and dopamine-reuptake inhibitor), began in 2016.

Sarcosine (N-methylglycine)

Sarcosine is a naturally occurring GlyT1 inhibitor that inhibits glycine reuptake from the synaptic cleft, thus raising synaptic glycine levels and increasing NMDAR activity. A small RCT randomized 40 patients with MDD to 6 weeks of sarcosine or citalopram treatment; sarcosine led to superior reductions in depressive symptoms compared with citalopram[130]. Although preliminary, these results raise the intriguing possibility that potentiators of NMDARs, as well as antagonists, may have antidepressant properties in certain contexts.

AMPAR modulators

As described above, AMPARs have a key role in synaptic plasticity, and early studies identified potentiation of AMPAR signalling as an obligatory component of the antidepressant-like effects of ketamine in animal models[49,80,82]. The development of positive allosteric modulators of the AMPAR for CNS disorders, including depression, has been a major focus of drug development over the past 10 years[131,132]. To date, the published human data on an AMPAR potentiator in depression are limited to two early-phase studies[133,134]. These reports describe promising initial results examining the safety and preliminary efficacy and biomarker end points for Org 26576 in patients with MDD[134]. Results from additional planned

Pseudobulbar affect
Type of affect characterized by episodes of uncontrollable crying or laughing and which typically occurs secondary to a neurological injury.

or ongoing clinical trials investigating AMPAR modulators are awaited[132], although no trials were listed on ClinicalTrials.gov as of February 2017.

Metabotropic glutamate receptor modulators

Antidepressant drug development focusing on mGluRs is in relatively early stages, but may represent a particularly promising avenue. mGluR2/3 and mGluR5 antagonists have so far received the most attention, and both classes of compounds have demonstrated rapid, ketamine-like antidepressant effects in preclinical models[135–137]. As noted above, mGluR2/3 antagonists seem to mimic the ability of ketamine to trigger a stimulatory glutamate release at cortical synapses[135] but do so by inhibiting presynaptic mGluR2/3 autoreceptors. Although a phase I study of the mGluR2/3 antagonist BCI-632 has completed, data had not been published at the time of writing. A recently completed clinical trial of the selective mGluR5 negative allosteric modulator basimglurant (Hoffman-La Roche) tested as adjunctive therapy to standard antidepressant medications in a phase IIb trial in MDD failed to separate from placebo on its primary end point[138]. An ongoing phase Ib study is testing additional higher doses, as it seemed that the higher dose in the phase IIb study had antidepressant efficacy on several secondary measures.

Prospects and hurdles

We highlight below the key outstanding issues related to the development of ketamine and other glutamate modulators for use in depression, including aspects of patient selection, clinical trial design and safety.

Patient selection

A considerable challenge to developing therapies for depression concerns the heterogeneity in its pathophysiology and in mechanisms of resistance to medications. Most studies that involve mechanistically novel agents have selected patients who have failed to respond to one or more trials of conventional monoamine-based antidepressants. However, identifying the sample solely by the number of antidepressant medication failures may not effectively constrain heterogeneity. For example, prior studies suggest that treatment outcome may vary as a function of demographic and symptom characteristics[139], history of trauma[140], neurocircuit function[141] and genotype[142].

Dosing and clinical trial design

Nearly all clinical trials of parenteral ketamine in depression have used a dose of 0.5 mg per kg infused over 40 minutes. The US National Institute of Mental Health's ketamine dose-finding study in TRD comparing four dose regimens is expected to complete in 2017. A less well-studied but equally crucial component of dose optimization concerns dosing frequency, as some NMDAR modulators might be more optimally administered intermittently rather than daily.

A considerable design hurdle concerns the potential for elevated expectancy effects with parenteral drug administration that contribute to an exaggerated placebo response. All controlled ketamine trials to date have used inert saline as the placebo condition, with the exception of one[59] that used midazolam to provide a plausible (though imperfect) psychoactive control condition. Studies of ketamine and NMDAR modulators with unwanted acute psychotomimetic and haemodynamic effects suffer a potential bias in the form of functional unblinding. Keeping efficacy raters masked to acute changes in haemodynamic parameters will be essential. One study of lanicemine found that neither clinicians nor patients were likely to correctly guess whether they were receiving the active drug or the inactive placebo[143], suggesting that NMDAR modulators with less dramatic acute effects are less likely to compromise the blind.

In addition, the standard outcome scales for conventional antidepressant trials may lack sensitivity for detecting rapid changes in mood, as these scales were developed to detect changes in depressive symptoms over a 7-day time frame. Whether shorter versions of these instruments are more likely to show sensitivity to changes in core components of depression for rapid-onset antidepressant medications is under study.

Safety

Despite its favourable safety profile in well-controlled medical settings, there are important concerns regarding the toxicity of ketamine at high doses and for prolonged periods. Preclinical studies have documented neurotoxic effects of ketamine and NMDAR modulators when administered at high doses, or during specific developmental periods. Ketamine is recreationally abused as a club drug and is listed in Schedule 3 by the FDA (indicating that the drug has accepted medical uses but has the potential for abuse). In the United Kingdom, increasing restrictions have made it more cumbersome to conduct experimental research with ketamine, which was classified as Class B in 2014 (in the United Kingdom, controlled drugs are scheduled as Class A, B or C according to their potential for harm, with Class A reserved for drugs deemed to be associated with the greatest harm).

In an analysis of three clinical trials of ketamine for depression, we did not find evidence of negative psychological or medical sequelae, or substance abuse-related emergencies, although the patients included in this analysis were exposed only to low doses and to short courses of the drug (no more than six infusions over 2 weeks)[103]. In addition to neuropsychiatric and cognitive effects, potential adverse effects of chronic exposure to ketamine include hypertension, tachycardia and cystitis. These data highlight the crucial role of dose and frequency in determining the safety or toxicity of ketamine. The large gaps in our current knowledge of the safety or efficacy of ketamine and of other NMDAR antagonists for the treatment of depression should dissuade widespread clinical use until more data are obtained.

Conclusions

We have reviewed the history, rationale and efficacy of glutamate-modulating agents in the treatment of depression. Substantial progress has been made over the

past decade in the identification and characterization of ketamine as a prototype rapid-acting antidepressant, and insights from translational ketamine studies have yielded compelling hypotheses about the neurobiology and treatment of these common and often disabling conditions. Notably, however, there is a near absence of studies of ketamine in depression that examine its safety or efficacy beyond a single treatment administration. This large gap in the literature represents a crucial unmet research need and precludes an informed risk–benefit analysis of the clinical use of ketamine for the treatment of depression. Moreover, it remains unclear whether NMDAR engagement is a necessary or sufficient mechanistic step for ketamine-like drugs to trigger a clinical effect in patients with depression. For example, data exist that suggest that ketamine-like drugs may have antidepressant properties partly by regulating monoamine signalling[144], opioid signalling[145], inflammatory systems[146,147] or even epigenetic mechanisms[148]. The contribution of these processes to the antidepressant effects of glutamate-based drugs requires further study.

More broadly, many challenges clearly remain for this area of drug development, and no glutamate modulator is currently approved for the treatment of depression worldwide. The depression field continues to be challenged by a lack of definitive diagnostic and treatment-related biomarkers, as well as an exclusive reliance on the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) for case identification. The nascent clinical trial literature, even in treatment-resistant populations, continues to be plagued by elevated placebo response rates and a paucity of data on long-term outcomes. An important unknown is the relationship between glutamate modulation and conventional pharmacotherapeutic, neurostimulatory and psychotherapeutic approaches for depression, which may shed light on the strengths and limitations of this pharmacological approach.

1. Collins, P. Y. *et al.* Grand challenges in global mental health. *Nature* **475**, 27–30 (2011).
2. Whiteford, H. A. *et al.* Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* **382**, 1575–1586 (2013).
3. Mrazek, D. A., Hornberger, J. C., Altar, C. A. & Degtiar, I. A review of the clinical, economic, and societal burden of treatment-resistant depression: 1996–2013. *Psychiatr. Serv.* **65**, 977–987 (2014).
4. Rush, A. J. *et al.* Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am. J. Psychiatry* **163**, 1905–1917 (2006).
5. Trivedi, M. H. *et al.* Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* **163**, 28–40 (2006).
6. Abdallah, C. G., Sanacora, G., Duman, R. S. & Krystal, J. H. Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. *Annu. Rev. Med.* **66**, 509–523 (2015).
7. Papakostas, G. I. & Ionescu, D. F. Towards new mechanisms: an update on therapeutics for treatment-resistant major depressive disorder. *Mol. Psychiatry* **20**, 1142–1150 (2015).
This review provides a concise overview of new directions in treatment development for TRD, including treatments targeting the glutamate system, the opioid system, inflammatory signalling systems and others.
8. Mathew, S. J. Glycine transporter-I inhibitors: a new class of antidepressant? *Biol. Psychiatry* **74**, 710–711 (2013).
9. Berman, R. M. *et al.* Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* **47**, 351–354 (2000).
10. Duman, R. S. & Aghajanian, G. K. Synaptic dysfunction in depression: potential therapeutic targets. *Science* **338**, 68–72 (2012).
This review integrates preclinical research related to the effects of both stress and antidepressant treatment on synaptic plasticity, and proposes normalization of synaptic function as a final common pathway for depression treatment.
11. Morris, R. G., Anderson, E., Lynch, G. S. & Baudry, M. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature* **319**, 774–776 (1986).
12. Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* **340**, 474–476 (1989).
13. Hardingham, G. E., Fukunaga, Y. & Bading, H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* **5**, 405–414 (2002).
This report describes opposing effects of synaptic compared with extrasynaptic NMDARs on *BDNF* expression and cell survival: the activation of synaptic NMDARs led to upregulation of *BDNF* expression and the activation of extra-synaptic NMDARs reduced *BDNF* expression.
14. Stanika, R. I. *et al.* Coupling diverse routes of calcium entry to mitochondrial dysfunction and glutamate excitotoxicity. *Proc. Natl Acad. Sci. USA* **106**, 9854–9859 (2009).
15. Hardingham, G. E. & Bading, H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* **11**, 682–696 (2010).
16. Papouin, T. & Oliet, S. H. Organization, control and function of extrasynaptic NMDA receptors. *Phil. Trans. R. Soc. B* **369**, 20130601 (2014).
17. Duman, R. S. & Monteggia, L. M. A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* **59**, 1116–1127 (2006).
18. Krishnan, V. & Nestler, E. J. The molecular neurobiology of depression. *Nature* **455**, 894–902 (2008).
19. Olney, J. W., Labruyere, J. & Price, M. T. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* **244**, 1360–1362 (1989).
20. Olney, J. W. *et al.* NMDA antagonist neurotoxicity: mechanism and prevention. *Science* **254**, 1515–1518 (1991).
21. Camacho, A. & Massieu, L. Role of glutamate transporters in the clearance and release of glutamate during ischemia and its relation to neuronal death. *Arch. Med. Res.* **37**, 11–18 (2006).
22. Fan, M. M. & Raymond, L. A. *N*-Methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog. Neurobiol.* **81**, 272–293 (2007).
23. Sanacora, G., Zarate, C. A., Krystal, J. H. & Manji, H. K. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat. Rev. Drug Discov.* **7**, 426–437 (2008).
This comprehensive review brings together multiple lines of evidence for the dysfunction of the glutamate system in mood disorders, including genetic, post-mortem and *in vivo* neuroimaging data.
24. Smoller, J. W. The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology* **41**, 297–319 (2016).
25. Nowak, G., Ordway, G. A. & Paul, I. A. Alterations in the *N*-methyl-D-aspartate (NMDA) receptor complex in the frontal cortex of suicide victims. *Brain Res.* **675**, 157–164 (1995).
26. Scarr, E., Pavey, G., Sundram, S., MacKinnon, A. & Dean, B. Decreased hippocampal NMDA, but not kainate or AMPA receptors in bipolar disorder. *Bipolar Disord.* **5**, 257–264 (2003).
27. Nudmamud-Thanoi, S. & Reynolds, G. P. The NR1 subunit of the glutamate/NMDA receptor in the superior temporal cortex in schizophrenia and affective disorders. *Neurosci. Lett.* **372**, 173–177 (2004).
28. Karolewicz, B., Stockmeier, C. A. & Ordway, G. A. Elevated levels of the NR2C subunit of the NMDA receptor in the locus coeruleus in depression. *Neuropsychopharmacology* **30**, 1557–1567 (2005).
29. McCullumsmith, R. E. *et al.* Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. *Brain Res.* **1127**, 108–118 (2007).
30. Karolewicz, B. *et al.* Elevated levels of NR2A and PSD-95 in the lateral amygdala in depression. *Int. J. Neuropsychopharmacol.* **12**, 143–153 (2009).
31. Feyissa, A. M., Chandran, A., Stockmeier, C. A. & Karolewicz, B. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **33**, 70–75 (2009).
32. Chandley, M. J. *et al.* Elevated gene expression of glutamate receptors in noradrenergic neurons from the locus coeruleus in major depression. *Int. J. Neuropsychopharmacol.* **17**, 1569–1578 (2014).
33. Gray, A. L., Hyde, T. M., Deep-Soboslay, A., Kleinman, J. E. & Sodhi, M. S. Sex differences in glutamate receptor gene expression in major depression and suicide. *Mol. Psychiatry* **20**, 1057–1068 (2015).
34. Sanacora, G. & Banasr, M. From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry* **73**, 1172–1179 (2013).
35. Ongur, D., Drevets, W. C. & Price, J. L. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc. Natl Acad. Sci. USA* **95**, 13290–13295 (1998).
This study provides an early report of the loss of glial cells within the PFC of patients with mood disorders, consistent with the hypothesized glutamate abnormalities in these disorders.
36. Banasr, M. *et al.* Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* **15**, 501–511 (2010).
37. Duman, R. S., Aghajanian, G. K., Sanacora, G. & Krystal, J. H. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat. Med.* **22**, 238–249 (2016).

38. Popoli, M., Yan, Z., McEwen, B. S. & Sanacora, G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat. Rev. Neurosci.* **13**, 22–37 (2011).
This comprehensive review summarizes the diverse mechanisms by which glutamate signalling is influenced by stress and by the functioning of the glucocorticoid system.
39. Manji, H. K. *et al.* Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol. Psychiatry* **53**, 707–742 (2003).
40. Kempton, M. J. *et al.* Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder. *Arch. Gen. Psychiatry* **68**, 675–690 (2011).
41. Yuksel, C. & Ongur, D. Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biol. Psychiatry* **68**, 785–794 (2010).
42. Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D. & Pizzagalli, D. A. Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. *JAMA Psychiatry* **72**, 603–611 (2015).
This recent meta-analysis provides a comprehensive examination of resting-state network alterations reported in patients with depression.
43. Hasler, G. *et al.* Reduced prefrontal glutamate/glutamine and γ-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch. Gen. Psychiatry* **64**, 193–200 (2007).
44. Sanacora, G. *et al.* Reduced cortical γ-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch. Gen. Psychiatry* **56**, 1043–1047 (1999).
45. Sanacora, G. *et al.* Subtype-specific alterations of γ-aminobutyric acid and glutamate in patients with major depression. *Arch. Gen. Psychiatry* **61**, 705–713 (2004).
46. Deschwanden, A. *et al.* Reduced metabotropic glutamate receptor 5 density in major depression determined by [^{11}C]ABP688 PET and postmortem study. *Am. J. Psychiatry* **168**, 727–734 (2011).
47. Abdallah, C. G. *et al.* Glutamate metabolism in major depressive disorder. *Am. J. Psychiatry* **171**, 1320–1327 (2014).
48. Hirota, K. & Lambert, D. G. Ketamine: its mechanism(s) of action and unusual clinical uses. *Br. J. Anaesth.* **77**, 441–444 (1996).
49. Zanos, P. *et al.* NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* **533**, 481–486 (2016).
This recent preclinical study showed that the metabolism of racemic ketamine to (2*R*,6*R*)-HNK is necessary for the antidepressant effects of ketamine, and that this metabolite itself shows antidepressant-like effects in mice that seem to be independent of the NMDAR.
50. Zhang, J. C., Li, S. X. & Hashimoto, K. *R* (–)-ketamine shows greater potency and longer lasting antidepressant effects than *S* (+)-ketamine. *Pharmacol. Biochem. Behav.* **116**, 137–141 (2014).
51. Yang, C. *et al. R*-Ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects. *Transl Psychiatry* **5**, e632 (2015).
52. Zarate, C. A. Jr *et al.* A randomized trial of an *N*-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* **63**, 856–864 (2006).
53. Murrough, J. W. *et al.* Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry* **170**, 1134–1142 (2013).
This first multi-site RCT using a psychoactive control condition demonstrated a rapid antidepressant effect of ketamine in patients with TRD.
54. Newport, D. J. *et al.* Ketamine and other NMDA antagonists: early clinical trials and possible mechanisms in depression. *Am. J. Psychiatry* **172**, 950–966 (2015).
55. McGirr, A. *et al.* A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. *Psychol. Med.* **45**, 693–704 (2015).
56. Caddy, C. *et al.* Ketamine and other glutamate receptor modulators for depression in adults. *Cochrane Database Syst. Rev.* **9**, CD011612 (2015).
57. aan het Rot, M. *et al.* Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol. Psychiatry* **67**, 139–145 (2010).
58. Murrough, J. W. *et al.* Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol. Psychiatry* **74**, 250–256 (2013).
59. Shiroma, P. R. *et al.* Augmentation of response and remission to serial intravenous subanesthetic ketamine in treatment resistant depression. *J. Affect. Disord.* **155**, 123–129 (2014).
60. Rasmussen, K. G. *et al.* Serial infusions of low-dose ketamine for major depression. *J. Psychopharmacol.* **27**, 444–450 (2013).
61. Segmiller, F. *et al.* Repeated S-ketamine infusions in therapy resistant depression: a case series. *J. Clin. Pharmacol.* **53**, 996–998 (2013).
62. Cusin, C. *et al.* Ketamine augmentation for outpatients with treatment-resistant depression: preliminary evidence for two-step intravenous dose escalation. *Aust. N. Z. J. Psychiatry* **51**, 55–64 (2017).
63. Singh, J. B. *et al.* A double-blind, randomized, placebo-controlled, dose-frequency study of intravenous ketamine in patients with treatment-resistant depression. *Am. J. Psychiatry* **173**, 816–826 (2016).
This is the first RCT of repeated ketamine dosing in patients with TRD, in which two- and three-times weekly treatment schedules over up to 4 weeks showed comparable efficacy that was superior to placebo.
64. Hu, Y. D. *et al.* Single i.v. ketamine augmentation of newly initiated escitalopram for major depression: results from a randomized, placebo-controlled 4-week study. *Psychol. Med.* **46**, 623–635 (2016).
65. Lapidus, K. A. *et al.* A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biol. Psychiatry* **76**, 970–976 (2014).
66. Loo, C. K. *et al.* Placebo-controlled pilot trial testing dose titration and intravenous, intramuscular and subcutaneous routes for ketamine in depression. *Acta Psychiatr. Scand.* **134**, 48–56 (2016).
67. Schoevers, R. A., Chaves, T. V., Balukova, S. M., Rot, M. A. & Kortekaas, R. Oral ketamine for the treatment of pain and treatment-resistant depression. *Br. J. Psychiatry* **208**, 108–113 (2016).
68. Lally, N. *et al.* Anti-anhedonic effect of ketamine and its neural correlates in treatment-resistant bipolar depression. *Transl Psychiatry* **4**, e469 (2014).
69. Lally, N. *et al.* Neural correlates of change in major depressive disorder anhedonia following open-label ketamine. *J. Psychopharmacol.* **29**, 596–607 (2015).
70. Saligan, L. N., Luckenbaugh, D. A., Slonena, E. E., Machado-Vieira, R. & Zarate, C. A. Jr. An assessment of the anti-fatigue effects of ketamine from a double-blind, placebo-controlled, crossover study in bipolar disorder. *J. Affect. Disord.* **194**, 115–119 (2016).
71. Price, R. B., Nock, M. K., Charney, D. S. & Mathew, S. J. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol. Psychiatry* **66**, 522–526 (2009).
This early study reported rapid effects of ketamine on patient reports of suicidal thinking, as well as on implicit cognitive measures that had been previously linked to suicide risk.
72. Price, R. B. *et al.* Effects of ketamine on explicit and implicit suicidal cognition: a randomized controlled trial in treatment-resistant depression. *Depress. Anxiety* **31**, 335–343 (2014).
73. Ballard, E. D. *et al.* Improvement in suicidal ideation after ketamine infusion: relationship to reductions in depression and anxiety. *J. Psychiatr. Res.* **58**, 161–166 (2014).
74. Murrough, J. W. *et al.* Ketamine for rapid reduction of suicidal ideation: a randomized controlled trial. *Psychol. Med.* **45**, 3571–3580 (2015).
75. Murrough, J. W. *et al.* Neurocognitive effects of ketamine in treatment-resistant major depression: association with antidepressant response. *Psychopharmacology (Berl.)* **231**, 481–488 (2014).
76. Murrough, J. W. *et al.* Neurocognitive effects of ketamine and association with antidepressant response in individuals with treatment-resistant depression: a randomized controlled trial. *Neuropsychopharmacology* **40**, 1084–1090 (2015).
77. Shiroma, P. R. *et al.* Neurocognitive performance and serial intravenous subanesthetic ketamine in treatment-resistant depression. *Int. J. Neuropsychopharmacol.* **17**, 1805–1813 (2014).
78. Lee, Y. *et al.* A new perspective on the anti-suicide effects with ketamine treatment: a procognitive effect. *J. Clin. Psychopharmacol.* **36**, 50–56 (2016).
79. Singh, J. B. *et al.* Intravenous esketamine in adult treatment-resistant depression: a double-blind, double-randomization, placebo-controlled study. *Biol. Psychiatry* **80**, 424–431 (2015).
80. Maeng, S. *et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry* **63**, 349–352 (2008).
81. Papp, M. & Moryl, E. Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. *Eur. J. Pharmacol.* **263**, 1–7 (1994).
82. Li, N. *et al.* mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959–964 (2010).
This landmark report demonstrates rapid-onset antidepressant effects of ketamine dependent on the activation of the mTOR pathway and increased number and function of new spine synapses in the PFC of rats.
83. Autry, A. E. *et al.* NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* **475**, 91–95 (2011).
This important preclinical study showed that ketamine and other NMDAR antagonists produce fast-acting antidepressant-like effects in mouse models that depend on the rapid synthesis of BDNF via a mechanism that includes the deactivation of eEF2 kinase.
84. Donahue, R. J., Muschamp, J. W., Russo, S. J., Nestler, E. J. & Carlezon, W. A. Jr. Effects of striatal ΔFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol. Psychiatry* **76**, 550–558 (2014).
85. Gideons, E. S., Kavalali, E. T. & Monteggia, L. M. Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proc. Natl Acad. Sci. USA* **111**, 8649–8654 (2014).
86. Chowdhury, G. M. *et al.* Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects. *Mol. Psychiatry* **22**, 120–126 (2016).
87. Karasawa, J., Shimazaki, T., Kawashima, N. & Chaki, S. AMPA receptor stimulation mediates the antidepressant-like effect of a group II metabotropic glutamate receptor antagonist. *Brain Res.* **1042**, 92–98 (2005).
88. Voleti, B. *et al.* Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. *Biol. Psychiatry* **74**, 742–749 (2013).
89. Liu, R. J. *et al.* GLYX-13 produces rapid antidepressant responses with key synaptic and behavioral effects distinct from ketamine. *Neuropsychopharmacology* http://dx.doi.org/10.1038/npp.2016.202 (2016).
90. Jimenez-Sanchez, L. *et al.* Activation of AMPA receptors mediates the antidepressant action of deep brain stimulation of the infralimbic prefrontal cortex. *Cereb. Cortex* **26**, 2778–2789 (2016).
91. Citri, A. & Malenka, R. C. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology* **33**, 18–41 (2008).
92. Turrigiano, G. Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annu. Rev. Neurosci.* **34**, 89–103 (2011).
93. Popp, S. *et al.* In search of the mechanisms of ketamine's antidepressant effects: how robust is the evidence behind the mTor activation hypothesis. *F1000Res.* **5**, 634 (2016).
94. Beurel, E., Song, L. & Jope, R. S. Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. *Mol. Psychiatry* **16**, 1068–1070 (2011).
95. Liu, R. J. *et al.* GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. *Neuropsychopharmacology* **38**, 2268–2277 (2013).
96. Beurel, E., Grieco, S. F., Amadei, C., Downey, K. & Jope, R. S. Ketamine-induced inhibition of glycogen synthase kinase-3 contributes to the augmentation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor signaling. *Bipolar Disord.* **18**, 473–480 (2016).
97. Yang, J. J. *et al.* Serum interleukin-6 is a predictive biomarker for ketamine's antidepressant effect in treatment-resistant patients with major depression. *Biol. Psychiatry* **77**, e19–e20 (2015).

98. Wesseling, H., Rahmoune, H., Tricklebank, M., Guest, P. C. & Bahn, S. A targeted multiplexed proteomic investigation identifies ketamine-induced changes in immune markers in rat serum and expression changes in protein kinases/phosphatases in rat brain. *J. Proteome Res.* **14**, 411–421 (2015).
99. Park, M. *et al.* Change in cytokine levels is not associated with rapid antidepressant response to ketamine in treatment-resistant depression. *J. Psychiatr. Res.* **84**, 113–118 (2016).
100. Milak, M. S. *et al.* A pilot *in vivo* proton magnetic resonance spectroscopy study of amino acid neurotransmitter response to ketamine treatment of major depressive disorder. *Mol. Psychiatry* **21**, 320–327 (2015).
This *in vivo* ^{1}H-MRS study demonstrated that ketamine administration is associated with a transient rise in Glx and GABA levels in patients with depression.
101. Stone, J. M. *et al.* Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Mol. Psychiatry* **17**, 664–665 (2012).
102. Luckenbaugh, D. A. *et al.* Do the dissociative side effects of ketamine mediate its antidepressant effects? *J. Affect. Disord.* **159**, 56–61 (2014).
103. Wan, L. L. Ketamine safety and tolerability in clinical trials for treatment-resistant depression. *J. Clin. Psychiatry* **76**, 247–252 (2015).
104. Deakin, J. F. *et al.* Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. *Arch. Gen. Psychiatry* **65**, 154–164 (2008).
105. Downey, D. *et al.* Comparing the actions of lanicemine and ketamine in depression: key role of the anterior cingulate. *Eur. Neuropsychopharmacol.* **26**, 994–1003 (2016).
106. Li, M. *et al.* Temporal dynamics of antidepressant ketamine effects on glutamine cycling follow regional fingerprints of AMPA and NMDA receptor densities. *Neuropsychopharmacology* http://dx.doi.org/10.1038/npp.2016.184 (2016).
107. Murrough, J. W. *et al.* Regulation of neural responses to emotion perception by ketamine in individuals with treatment-resistant major depressive disorder. *Transl Psychiatry* **5**, e509 (2015).
108. Scheidegger, M. *et al.* Ketamine administration reduces amygdalo-hippocampal reactivity to emotional stimulation. *Hum. Brain Mapp.* **37**, 1941–1952 (2016).
109. Murrough, J. W. *et al.* Reduced global functional connectivity of the medial prefrontal cortex in major depressive disorder. *Hum. Brain Mapp.* **37**, 3214–3223 (2016).
110. Abdallah, C. G. *et al.* Ketamine treatment and global brain connectivity in major depression. *Neuropsychopharmacology* http://dx.doi.org/10.1038/npp.2016.186 (2016).
111. Carlson, P. J. *et al.* Neural correlates of rapid antidepressant response to ketamine in treatment-resistant unipolar depression: a preliminary positron emission tomography study. *Biol. Psychiatry* **73**, 1213–1221 (2013).
112. Nugent, A. C. *et al.* Neural correlates of rapid antidepressant response to ketamine in bipolar disorder. *Bipolar Disord.* **16**, 119–128 (2014).
113. Machado-Vieira, R. *et al.* Brain-derived neurotrophic factor and initial antidepressant response to an *N*-methyl-D-aspartate antagonist. *J. Clin. Psychiatry* **70**, 1662–1666 (2009).
114. Haile, C. N. *et al.* Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. *Int. J. Neuropsychopharmacol.* **17**, 331–336 (2013).
115. Esser, S. K., Hill, S. L. & Tononi, G. Sleep homeostasis and cortical synchronization: I. modeling the effects of synaptic strength on sleep slow waves. *Sleep* **30**, 1617–1630 (2007).
116. Duncan, W. C. *et al.* Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. *Int. J. Neuropsychopharmacol.* **16**, 301–311 (2013).
117. Laje, G. *et al.* Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. *Biol. Psychiatry* **72**, e27–28 (2012).
118. Preskorn, S. H. *et al.* 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.* **28**, 631–637 (2008).
119. Sanacora, G. *et al.* Lanicemine: a low-trapping NMDA channel blocker produces sustained antidepressant efficacy with minimal psychotomimetic adverse effects. *Mol. Psychiatry* **19**, 978–985 (2014).
120. Crane, G. E. Cyloserine as an antidepressant agent. *Am. J. Psychiatry* **115**, 1025–1026 (1959).
121. Heresco-Levy, U. *et al.* A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. *Int. J. Neuropsychopharmacol.* **16**, 501–506 (2013).
122. Kantrowitz, J. T., Halberstam, B. & Gangwisch, J. Single-dose ketamine followed by daily D-cycloserine in treatment-resistant bipolar depression. *J. Clin. Psychiatry* **76**, 737–738 (2015).
123. Zhang, X. L., Sullivan, J. A., Moskal, J. R. & Stanton, P. K. A. NMDA receptor glycine site partial agonist, GLYX-13, simultaneously enhances LTP and reduces LTD at Schaffer collateral-CA1 synapses in hippocampus. *Neuropharmacology* **55**, 1238–1250 (2008).
124. Burgdorf, J. *et al.* GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. *Neuropsychopharmacology* **38**, 729–742 (2013).
125. Preskorn, S. *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. *J. Psychiatr. Pract.* **21**, 140–149 (2015).
126. Ibrahim, L. *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. Psychopharmacol.* **32**, 551–557 (2012).
127. Kemp, J. A. *et al.* 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. *Proc. Natl Acad. Sci. USA* **85**, 6547–6550 (1988).
128. Zanos, P. *et al.* The prodrug 4-chlorokynurenine causes ketamine-like antidepressant effects, but not side effects, by NMDA/glycine$_B$-site inhibition. *J. Pharmacol. Exp. Ther.* **355**, 76–85 (2015).
129. Mullard, A. Deuterated drugs draw heavier backing. *Nat. Rev. Drug Discov.* **15**, 219–221 (2016).
130. Huang, C. C. *et al.* Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression. *Biol. Psychiatry* **74**, 734–741 (2013).
131. Durr, K. L. *et al.* Structure and dynamics of AMPA receptor GluA2 in resting, pre-open, and desensitized states. *Cell* **158**, 778–792 (2014).
132. Ward, S. E., Bax, B. D. & Harries, M. Challenges for and current status of research into positive modulators of AMPA receptors. *Br. J. Pharmacol.* **160**, 181–190 (2010).
133. Nations, K. R. *et al.* Maximum tolerated dose evaluation of the AMPA modulator Org 26576 in healthy volunteers and depressed patients: a summary and method analysis of bridging research in support of phase II dose selection. *Drugs R. D.* **12**, 127–139 (2012).
134. Nations, K. R. *et al.* Examination of Org 26576, an AMPA receptor positive allosteric modulator, in patients diagnosed with major depressive disorder: an exploratory, randomized, double-blind, placebo-controlled trial. *J. Psychopharmacol.* **26**, 1525–1539 (2012).
135. Dwyer, J. M., Lepack, A. E. & Duman, R. S. mGluR2/3 blockade produces rapid and long-lasting reversal of anhedonia caused by chronic stress exposure. *J. Mol. Psychiatry* **1**, 15 (2013).
136. Witkin, J. M. *et al.* The rapidly acting antidepressant ketamine and the mGlu2/3 receptor antagonist LY341495 rapidly engage dopaminergic mood circuits. *J. Pharmacol. Exp. Ther.* **358**, 71–82 (2016).
137. Hughes, Z. A. *et al.* Negative allosteric modulation of metabotropic glutamate receptor 5 results in broad spectrum activity relevant to treatment resistant depression. *Neuropharmacology* **66**, 202–214 (2013).
138. Quiroz, J. A. *et al.* Efficacy and safety of basimglurant as adjunctive therapy for major depression: a randomized clinical trial. *JAMA Psychiatry* **73**, 675–684 (2016).
139. Jain, F. A., Hunter, A. M., Brooks, J. O. III & Leuchter, A. F. Predictive socioeconomic and clinical profiles of antidepressant response and remission. *Depress. Anxiety* **30**, 624–630 (2013).
140. Miller, S. *et al.* Cognition-childhood maltreatment interactions in the prediction of antidepressant outcomes in major depressive disorder patients: results from the iSPOT-D trial. *Depress. Anxiety* **32**, 594–604 (2015).
141. Williams, L. M. *et al.* Amygdala reactivity to emotional faces in the prediction of general and medication-specific responses to antidepressant treatment in the randomized iSPOT-D trial. *Neuropsychopharmacology* **40**, 2398–2408 (2015).
142. Breitenstein, B. *et al. ABCB1* gene variants and antidepressant treatment outcome: a meta-analysis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **168B**, 274–283 (2015).
143. Zarate, C. A. Jr *et al.* A randomized trial of a low-trapping nonselective *N*-methyl-D-aspartate channel blocker in major depression. *Biol. Psychiatry* **74**, 257–264 (2013).
144. Tan, S., Lam, W. P., Wai, M. S., Yu, W. H. & Yew, D. T. Chronic ketamine administration modulates midbrain dopamine system in mice. *PLoS ONE* **7**, e43947 (2012).
145. Williams, N. R. & Schatzberg, A. F. NMDA antagonist treatment of depression. *Curr. Opin. Neurobiol.* **36**, 112–117 (2016).
146. Miller, A. H. Conceptual confluence: the kynurenine pathway as a common target for ketamine and the convergence of the inflammation and glutamate hypotheses of depression. *Neuropsychopharmacology* **38**, 1607–1608 (2013).
147. Dale, O., Somogyi, A. A., Li, Y., Sullivan, T. & Shavit, Y. Does intraoperative ketamine attenuate inflammatory reactivity following surgery? A systematic review and meta-analysis. *Anesth. Analg.* **115**, 934–943 (2012).
148. Choi, M. *et al.* Ketamine produces antidepressant-like effects through phosphorylation-dependent nuclear export of histone deacetylase 5 (HDAC5) in rats. *Proc. Natl Acad. Sci. USA* **112**, 15755–15760 (2015).
149. Hayashi, Y. *et al.* Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262–2267 (2000).
150. Kew, J. N. & Kemp, J. A. Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl.)* **179**, 4–29 (2005).
151. Schoepp, D. D. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* **299**, 12–20 (2001).
152. Trullas, R. & Skolnick, P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur. J. Pharmacol.* **185**, 1–10 (1990).
This early preclinical report demonstrates proof-of-principle that NMDAR antagonists may represent a new class of antidepressants.
153. Shors, T. J., Seib, T. B., Levine, S. & Thompson, R. F. Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science* **244**, 224–226 (1989).
154. Skolnick, P., Popik, P. & Trullas, R. Glutamate-based antidepressants: 20 years on. *Trends Pharmacol. Sci.* **30**, 563–569 (2009).
155. Skolnick, P. *et al.* Adaptation of *N*-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry* **29**, 23–26 (1996).
156. Papp, M. & Moryl, E. Antidepressant-like effects of 1-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. *Eur. J. Pharmacol.* **316**, 145–151 (1996).
157. Sos, P. *et al.* Relationship of ketamine's antidepressant and psychotomimetic effects in unipolar depression. *Neuro Endocrinol. Lett.* **34**, 287–293 (2013).
158. Diazgranados, N. *et al.* A randomized add-on trial of an *N*-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch. Gen. Psychiatry* **67**, 793–802 (2010).
159. Zarate, C. A. Jr *et al.* Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol. Psychiatry* **71**, 939–946 (2012).

Competing interests statement
The authors declare competing interests: see Web version for details.

FURTHER INFORMATION
ClinicalTrials.gov: http://clinicaltrials.gov/
ALL LINKS ARE ACTIVE IN THE ONLINE PDF