Antidepressant mechanisms of ketamine: Focus on GABAergic inhibition

Bernhard Luscher[a,b,c,*], **Mengyang Feng**[a,c], **Sarah J. Jefferson**[a,c,†]

[a]Department of Biology, Pennsylvania State University, University Park, PA, United States
[b]Department of Biochemistry & Molecular Biology, Pennsylvania State University, University Park, PA, United States
[c]Center for Molecular Investigation of Neurological Disorders (CMIND), The Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, United States
[*]Corresponding author: e-mail address: BXL25@psu.edu

Contents

[†] Current address: College of Medicine, Pennsylvania State University, Hershey, PA, United States.

Advances in Pharmacology
ISSN 1054-3589
https://doi.org/10.1016/bs.apha.2020.03.002

Abstract

There has been much recent progress in understanding of the mechanism of ketamine's rapid and enduring antidepressant effects. Here we review recent insights from clinical and preclinical studies, with special emphasis of ketamine-induced changes in GABAergic synaptic transmission that are considered essential for its antidepressant therapeutic effects. Subanesthetic ketamine is now understood to exert its initial action by selectively blocking a subset of NMDA receptors on GABAergic interneurons, which results in disinhibition of glutamatergic target neurons, a surge in extracellular glutamate and correspondingly elevated glutamatergic synaptic transmission. This surge in glutamate appears to be corroborated by the rapid metabolism of ketamine into hydroxynorketamine, which acts at presynaptic sites to disinhibit the release of glutamate. Preclinical studies indicate that glutamate-induced activity triggers the release of BDNF, followed by transient activation of the mTOR pathway and increased expression of synaptic proteins, along with functional strengthening of glutamatergic synapses. This drug-on phase lasts for approximately 2 h and is followed by a period of days characterized by structural maturation of newly formed glutamatergic synapses and prominently enhanced GABAergic synaptic inhibition. Evidence from mouse models with constitutive antidepressant-like phenotypes suggests that this phase involves strengthened inhibition of dendrites by somatostatin-positive GABAergic interneurons and correspondingly reduced NMDA receptor-mediated Ca^{2+} entry into dendrites, which activates an intracellular signaling cascade that converges with the mTOR pathway onto increased activity of the eukaryotic elongation factor eEF2 and enhanced translation of dendritic mRNAs. Newly synthesized proteins such as BDNF may be important for the prolonged therapeutic effects of ketamine.

Abbreviations

3α,5α-THP	3α,5α-tetrahydro-progesterone
3α,5β-THP	3α,5β-tetrahydro-progesterone
3α,5β-THDOC	3α,5β-tetrahydrodeoxycorticosterone
5-HT	5-hydroxytryptamine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ARC	activity-regulated cytoskeleton-associated protein
BDNF	brain-derived neurotrophic factor
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
eEF2	eukaryotic elongation factor 2
eEF2K	eukaryotic elongation factor 2 kinase

FS	fast spiking
GABA	γ-aminobutyric acid
$GABA_A$ receptor	ionotropic GABA receptor
GAD	glutamic acid decarboxylase
GluN1	NMDA-receptor subunit 1
GluN2	NMDA-receptor subunit 2
GSK-3β	glycogen synthase kinase-3β
HK	hydroxyketamine
HNK	hydroxynorketamine
HPA	hypothalamus pituitary adrenal
IP3	inositol 1,4,5-trisphosphate
IPSC	inhibitory postsynaptic current
KCC2	potassium chloride cotransporter 2
LTP	long term potentiation
MDD	major depressive disorder
mGluR2	metabotropic glutamate receptor 2
mPFC	medial prefrontal cortex
mTOR	mammalian target of rapamycin
NMDA	*N*-methyl-D-aspartate
OLM	oriens lacunosum moleculare
PSD-95	postsynaptic density protein of 95KD
PTSD	posttraumatic stress disorder
PV	parvalbumin
SST	somatostatin
TrkB	tropomyosin receptor kinase B
VGAT	vesicular GABA transporter

1. Introduction

Major Depressive Disorder (MDD) is the leading cause of ill health and disability with more than 300 million afflicted people worldwide and a cost to the global economy of a trillion US dollars every year (WHO, 2017). Conventional antidepressants that target monoaminergic neurotransmitter systems are widely available but often ineffective and they suffer from a pronounced delay in therapeutic efficacy of weeks to months. Accordingly, patients who are resistant to treatment often end up in a lengthy and futile pursuit of treatment while at high risk of self-harm and suicidal behavior (Jick, Kaye, & Jick, 2004). However, much hope has emerged in recent years from ketamine, which following administration of a single subanesthetic dose has rapid antidepressant effects that are

significant already within a couple hours and last for up to 1 week even in otherwise treatment-resistant patients (Fava et al., 2019; Lapidus et al., 2014; Murrough et al., 2013; Singh et al., 2016; Zarate et al., 2006).

The mechanism underlying ketamine's antidepressant effects is strikingly different from that of conventional antidepressants. This is evidenced by the rapid onset of action and by the therapeutic benefits being observed almost exclusively after the drug has been eliminated from the brain (referred to here as the "drug-off" situation) (Berman et al., 2000; Zarate et al., 2006). Other telling features of ketamine's mechanism observed in patients and animal models include the inverted U-shaped dose-response curve (Fava et al., 2019; Kim & Monteggia, 2020; Moghaddam, Adams, Verma, & Daly, 1997; Su et al., 2017) and the rapid yet transient increase in extracellular glutamate (Lorrain, Baccei, Bristow, Anderson, & Varney, 2003; Moghaddam et al., 1997; Rotroff et al., 2016) that then triggers a wave of synaptogenesis that reverses a functional deficit in neural connectivity associated with depression (Abdallah Averill, Collins et al., 2017; Abdallah, Averill, Salas et al., 2017; Chowdhury et al., 2017; Evans et al., 2018; Kraguljac et al., 2017; Li et al., 2010; Moda-Sava et al., 2019). Preclinical studies further indicate that ketamine-induced synaptogenesis and the antidepressant behavioral responses are critically dependent on the function of AMPA receptors and a neural activity-induced increase in the synthesis and release of neurotrophic factors such as BDNF (Autry et al., 2011; Deyama, Bang, Kato, Li, & Duman, 2019; Li et al., 2010).

Ketamine represents a racemic mixture of equal parts of R-(−)-ketamine (arketamine) and S-(+)-ketamine (esketamine). Racemic R/S-ketamine was first approved by the United States Food and Drug Administration (FDA) in 1970 as a rapidly acting dissociative anesthetic, analgesic, sedative and amnesic and remains on the World Health Organization's List of Essential Medicines (Green et al., 1998; Reich & Silvay, 1989). Ketamine acts as a noncompetitive antagonist and open channel blocker of NMDA-type glutamate-gated cation channels (NMDA receptors) (Hirota & Lambert, 1996). That is, ketamine requires membrane depolarization-mediated removal of a Mg^{2+} ion from the channel for access to the channel pore. Notably, ketamine has lower affinity (>10 μM) at multiple other receptors and neurotransmitter transporters that may contribute to its therapeutic and side effects at anesthetic concentrations (Tyler, Yourish, Ionescu, & Haggarty, 2017). However, there is no evidence that these targets are relevant at subanesthetic doses. Nevertheless, even at subanesthetic doses, ketamine exhibits dissociative and psychotomimetic

properties and abuse potential reminiscent of other NMDA receptor antagonists (Krystal et al., 1994; Sassano-Higgins, Baron, Juarez, Esmaili, & Gold, 2016; Short, Fong, Galvez, Shelker, & Loo, 2018; Thomson, West, & Lodge, 1985). These features confirm NMDA receptors as key targets but also limit adoption of ketamine as an antidepressant outside of the clinic. Therefore, a detailed understanding of ketamine's mechanism of action is fundamentally important for the design of superior agents that act similarly but with fewer side effects and with potential for wider adoption. In addition, studies of ketamine's mechanism of action have provided pivotal new key insights into the pathophysiology of depressive disorders.

Initial exploratory clinical studies of ketamine's antidepressant properties were conducted off-label with an intravenous 40 min infusion of racemic ketamine (0.5 mg/kg) (Berman et al., 2000; Zarate et al., 2006). Then, in March 2019, the (S)-enantiomer esketamine (Spravato) was approved by the FDA as a nasal spray specifically for treatment-resistant depression (Canuso et al., 2018; Popova et al., 2019; Singh et al., 2016; U.S. Food and Drug Administration, 2019). As might be expected of an NMDA receptor antagonist, ketamine has profound effects on glutamatergic neurotransmission, which has bolstered the view that depressive disorders reflect dysregulation of glutamatergic transmission (Musazzi, Treccani, & Popoli, 2012; Paul & Skolnick, 2003; Sanacora, Treccani, & Popoli, 2012; Thompson et al., 2015). However, it is important to note that ketamine's action as an NMDA receptor antagonist by itself says little about the nature and cause of dysregulated glutamate function. Changes in glutamatergic transmission are implicated in a wide range of neuropsychiatric and neurological conditions and they do not occur in isolation. Rather they involve upstream and/or downstream changes in other neurotransmitter systems that may be more specifically associated with depressive disorders. In particular, changes in glutamatergic transmission and NMDA receptor activity are in a tight bidirectionally inverse relationship with changes in GABAergic inhibitory transmission. While diverse NMDA receptor antagonists have shown antidepressant-like properties in preclinical tests (Trullas & Skolnick, 1990), ketamine to this day remains the only such agent that shows robust and sustained antidepressant activity in patients (Gould, Zarate, & Thompson, 2019; Newport et al., 2015). Collectively, this suggests that NMDA receptors are not the only target relevant for ketamine's antidepressant action. Indeed, as detailed below, ketamine is rapidly metabolized into multiple compounds, including some that appear to contribute to its antidepressant mechanism through other targets. Moreover, there is rapidly

emerging evidence that ketamine has prominent effects not only on glutamatergic but also GABAergic synaptic transmission and other neurotransmitter systems. Here we review the mechanisms underlying ketamine-induced synaptic plasticity and antidepressant behavioral consequences with special emphasis of the role of GABAergic synaptic transmission and inferences that can be drawn from these observations to explain the pathophysiology of MDD.

2. Molecular targets of subanesthetic ketamine and its metabolites

Preclinical studies in rodents indicate that both R- and S-ketamine exert antidepressant-like effects (Xiong et al., 2019). Moreover, some studies in rats suggest that R-ketamine has greater potency and longer lasting antidepressant effects than S-ketamine (Fukumoto et al., 2017; Yang et al., 2017; Zanos et al., 2016; Zhang, Li, & Hashimoto, 2014). However, this is contrasted by ketamine's anesthetic effects in patients, where S-ketamine is about four times more potent than R-ketamine in its electroencephalographic response (Oye, Paulsen, & Maurset, 1992; Vollenweider, Leenders, Oye, Hell, & Angst, 1997), a feature that is observed independent of stereoselective differences in drug metabolism (White et al., 1985). The antidepressant properties of R-ketamine in patients have so far not been explored.

There is substantial preclinical evidence indicating that antagonism of NMDA receptors by ketamine is sufficient and necessary to induce its antidepressant effects (Li et al., 2010; Miller et al., 2014; Miller, Bruns, Ben Ammar, Mueggler, & Hall, 2017; Preskorn et al., 2008). However, while agents that block NMDA receptors more specifically than ketamine showed promise in preclinical models, they have all failed so far in clinical studies (Gould et al., 2019). Consistent with NMDA receptors being insufficient as targets, there is evidence that ketamine's effects are corroborated by some of its metabolites. Thus, understanding of ketamine's mechanism requires a detailed consideration of its metabolism. The two isomers of racemic ketamine are rapidly and stereoselectively metabolized by multiple cytochrome P450 enzymes to norketamine and dehydronorketamine, followed by hydroxylation to multiple variant hydroxyketamines (HKs) and hydroxynorketamines (HNKs) (Adams, Baillie, Trevor, & Castagnoli, 1981; Desta et al., 2012; Zarate et al., 2012). In particular, (*2S.6S,2R,6R*)-HNK is among the key metabolites detected in plasma of patients for up

to 3 days after infusion of an antidepressant dose of ketamine (Zhao et al., 2012). Moreover, antidepressant responses to ketamine in patients are positively correlated with plasma levels of (*2S.6S,2R,6R*)-HNK, suggesting that this is an active metabolite (Zarate et al., 2012).

Studies in mice suggest that metabolism of ketamine to (*2S,6S,2R,6R*)-HNK contributes to its antidepressant activity and that ketamine may act in part as a prodrug of HNK (Chou et al., 2018; Highland et al., 2018; Pham et al., 2018; Zanos et al., 2016). They also indicate that HNK triggers many of the same downstream mechanisms as ketamine (Fukumoto et al., 2019; Yao, Skiteva, Zhang, Svenningsson, & Chergui, 2018) and that, at the relevant concentrations, HNK functions independently of NMDA receptors (Morris et al., 2017; Zanos et al., 2016) (however, see Suzuki, Nosyreva, Hunt, Kavalali, & Monteggia, 2017). Notably, and consistent with an NMDA receptor-independent mechanism, preclinical tests indicate that (*2S,6S,2R,6R*)-HNK lacks unwanted side effects and therefore may be more broadly useful as an antidepressant than ketamine (Highland et al., 2018; Zanos et al., 2016). Recent preclinical data indicate that (*2S.6S,2R,6R*)-HNK replicates the effect of ketamine on glutamate release and does so through mGluR2 by disinhibiting the release of glutamate from select subsets of synapses (Lorrain et al., 2003; Riggs et al., 2020; Zanos et al., 2019). However, the direct molecular target(s) of (*2S,6S,2R,6R*)-HNK remain unknown and its contribution to ketamine's potent antidepressant effects in patients has yet to be demonstrated. Importantly, studies with rats, unlike mice, have consistently failed to replicate HNK's antidepressant behavioral effects (Lumsden et al., 2019; Shirayama & Hashimoto, 2017, 2018; Yamaguchi et al., 2018; Zhang et al., 2018). An important caveat of many of these preclinical studies is that they are often conducted with physiologically normal animals that are not supposed to show antidepressant responses, as further discussed below. Ultimately, clinical studies may be needed to help solve this puzzle.

3. Insights from ketamine indicate a key role for reduced GABAergic inhibition in the pathophysiology of major depression

3.1 Antidepressant efficacy of ketamine is controlled by imbalances between neural excitation and inhibition

The mechanisms underlying the therapeutic effects of antidepressants are intrinsically linked to the pathophysiological changes of depressive disorders.

For example, the therapeutic benefits of conventional antidepressants compared with placebo are known to increase with the severity of depressive symptoms and are minimal or absent in patients with mild or moderate symptom (Fournier et al., 2010). Similarly, healthy human subjects treated with ketamine show transient increases in depressive-like symptoms rather than anything resembling the antidepressant responses seen in patients (Nugent et al., 2019). These observations are consistent with the concept, based on preclinical studies, that the antidepressant behavioral and neurophysiological effects of ketamine are enhanced by chronic neural excitation:inhibition imbalances and that these effects include reversal of homeostatic synaptic adaptations caused by such imbalances (Fig. 1). Specifically, mice with modest genetic defects in GABAergic synaptic

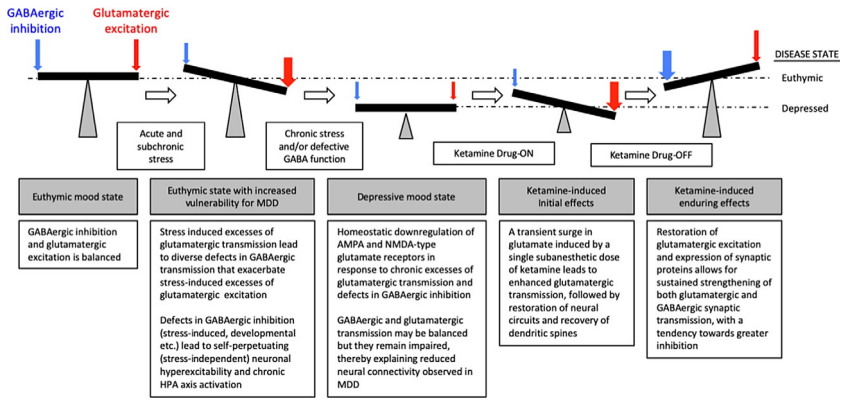

Fig. 1 Homeostatic regulation of GABAergic and glutamatergic synapses underlying depressive brain states and reversal by ketamine. Depressive brain states are proposed to be caused by homeostatic downregulation of glutamatergic synapses due to a chronic (endogenous or stress-induced) E:I imbalance and corresponding excessive activation of AMPA and NMDA type glutamate receptors (Ren et al., 2016; Yuen et al., 2012), along with degradation of gephyrin (Ghosal et al., 2020; Tyagarajan et al., 2013) and NMDA receptor-mediated dispersal of $GABA_A$ receptors from synapses (Bannai et al., 2009, 2015; Muir et al., 2010) and diverse other stress-induced defects in GABAergic transmission (see text). In depression, synaptic excitation and inhibition may be balanced but the strength of both types of synapses is reduced, resulting in reduced functional connectivity of neurons and networks (Li et al., 2010, 2011; Ren et al., 2016). The ketamine-induced surge of glutamate triggers a wave of synaptogenesis that leads to functional restoration of both glutamatergic and GABAergic synapses (Ren et al., 2016). The antidepressant brain state may involve a tendency toward greater inhibition as evidenced by ex vivo recording of brain slices from ketamine treated $GABA_A$ receptor $\gamma 2^{+/-}$ mice (Ren et al., 2016) and by ketamine's ability to ameliorate refractory status epilepticus (Synowiec et al., 2013).

inhibition ($GABA_A$ receptor γ2 subunit heterozygous mice, $\gamma 2^{+/-}$ mice), which suffer from a genetically defined, constitutive postsynaptic defect in GABAergic inhibition and corresponding neuronal hyperexcitability, exhibit behavioral, cognitive, neuroendocrine, neuroanatomical and synaptic functional phenotypes relevant for depression (Luscher & Fuchs, 2015) and they reliably show a more prominent antidepressant-like response to ketamine than normal controls (Ren et al., 2016). This same model is also more sensitive than genetically normal controls to chronic treatment with the conventional antidepressants desipramine and fluoxetine, and it reproduces the anxiogenic effects of subchronic fluoxetine seen in patients (Shen et al., 2010). Importantly, many of these findings have been confirmed in chronic stress-based rodent models. That is, mice subjected to chronic uncontrollable stress develop diverse defects in GABAergic inhibition (Banasr et al., 2017; Czeh et al., 2005, 2015; Ghosal et al., 2020; Hewitt, Wamsteeker, Kurz, & Bains, 2009; Hu, Zhang, Czeh, Flugge, & Zhang, 2010; MacKenzie & Maguire, 2015; Varga, Csabai, Miseta, Wiborg, & Czeh, 2017) and they tend to show more robust molecular and behavioral responses to ketamine than unstressed controls (Ghosal et al., 2020). Therefore, robust antidepressant drug responses depend on a chronic imbalance of neural excitation and inhibition, and this relationship is observed across multiple, mechanistically distinct drug classes and different animal models. By extension, depressive disorders are caused by chronic excesses of neural excitation and/or deficits in neural inhibition (Luscher & Fuchs, 2013; Luscher, Shen, & Sahir, 2011).

3.2 Chronic imbalances of neural excitation and inhibition lead to homeostatic downregulation of glutamatergic synapses that compromises normal neuronal communication

The source of imbalance between neural excitation and inhibition may be genetic (or epigenetic and developmental) in nature or involve chronic environmental stress-mediated increases in glutamate release (Musazzi, Tornese, Sala, & Popoli, 2017; Treccani et al., 2014), consistent with the well-established role of stress as a primary vulnerability factor for depressive disorders (Pittenger & Duman, 2008). Indeed, external stress and genetic defects in GABAergic inhibition have in common that they lead to chronically increased glutamatergic tone and to comparable homeostatic-like downregulation of cell surface AMPA and NMDA receptors, corresponding impairment of glutamatergic synapses, and reduced neural connectivity (Csabai, Wiborg, & Czeh, 2018; Kallarackal et al., 2013;

Moda-Sava et al., 2019; Ren et al., 2016; Yuen et al., 2012) (Fig. 1). The function of glutamatergic synapses in genetic or chronic stress-based models is restored to control levels within 24 h of an antidepressant dose of ketamine, indicating that ketamine acts to reverse homeostatic reductions in the function of glutamatergic synapses (Li et al., 2011; Ren et al., 2016) (Fig. 1). Moreover, these changes in the medial prefrontal cortex (mPFC) appear sufficient to elicit the behavioral effects of ketamine since optogenetic stimulation of this brain area has antidepressant effects similar to ketamine, and ketamine-induced neuronal activity and spine remodeling in this area is essential for ketamine's behavioral consequences (Fuchikami et al., 2015; Hare et al., 2019; Moda-Sava et al., 2019). The time course of ketamine-induced initial restoration of neural communication matches that of initial restoration of normal behavior (Moda-Sava et al., 2019). By contrast, the restoration of dendritic spines, which serves as a morphological index of functionally mature glutamatergic synapses, occurs on a slower time course and is required for maintenance of at least some of the restored behaviors (Moda-Sava et al., 2019). Collectively, these preclinical studies, along with altered expression of synapse-related gene products and reduced synapse density in postmortem brain of depressed patients (Holmes et al., 2019; Kang et al., 2012), define depressive disorders as synaptopathologies that are caused by chronic excitation:inhibition imbalances and reversible by ketamine-induced synaptogenesis (Fig. 1).

3.3 Chronic imbalances between neural excitation and inhibition lead to defects in GABAergic inhibition that delimit spontaneous recovery from transient excesses in excitation

In the absence of other impairments, above chronic stress-induced adaptations of glutamatergic transmission should be homeostatic in nature and protect neurons and circuits from runaway excitation, and they should reverse on their own as soon as the external sources of stress subside. However, chronic stress also leads to a variety of defects in GABAergic synaptic transmission, which is relevant for depression as evidenced by reduced GABAergic inhibition in patients (Bhagwagar et al., 2007; Gabbay et al., 2012; Hasler et al., 2007; Levinson et al., 2010; Price et al., 2009; Sanacora et al., 2004; Sibille, Morris, Kota, & Lewis, 2011; Tripp, Kota, Lewis, & Sibille, 2011; Voineskos et al., 2019) (for review see Luscher & Fuchs, 2015). We propose that it is these defects in neural inhibition that prevent the spontaneous recovery of homeostatically downregulated

glutamatergic synapses (Fig. 1). Defects in GABAergic inhibition in chronic stress-based rodent models of depression include reduced expression of (i) glutamic acid decarboxylase (GAD), the principal enzyme involved in GABA synthesis (Banasr et al., 2017), (ii) the vesicular GABA transporter (VGAT) required for normal synaptic release of GABA (Ghosal et al., 2020), (iii) gephyrin, the principal postsynaptic scaffold protein controlling the postsynaptic clustering and function of GABA receptors (Essrich, Lorez, Benson, Fritschy, & Luscher, 1998; Ghosal et al., 2020) and (iv) diverse marker proteins selectively expressed in specific subsets of GABAergic interneurons that indicate that these cells are especially vulnerable to stress (Banasr et al., 2017; Czeh et al., 2015, 2018; Lin & Sibille, 2015; Ma et al., 2016; Veeraiah et al., 2014). Functionally, chronic stress leads to reduced function of GABAergic synapses (Czeh et al., 2018; Ghosal et al., 2020) and a depolarizing shift in the chloride reversal potential (E_{GABA}) that results in loss of GABAergic inhibitory drive (Hewitt et al., 2009; MacKenzie & Maguire, 2015). Lastly, chronic isolation stress of rats results in reduced expression of genes involved in the synthesis of neurosteroids such as allopregnanolone [3α,5α-tetrahydro-progesterone (3α,5α-THP)], pregnanolone [3α,5β-tetrahydro-progesterone (3α,5β-THP) and allotetrahydro-deoxycorticosterone [3α,5β-tetrahydro-deoxycorticosterone (3α,5β-THDOC)] (Agis-Balboa et al., 2007). Low levels of these neurosteroids are implicated in the pathogenesis of MDD (Murugan, Jakka, Namani, Mujumdar, & Radhakrishnan, 2019; Schule et al., 2006; Uzunova et al., 1998) and posttraumatic stress disorder (PTSD) (Pineles et al., 2018; Rasmusson et al., 2019, 2006; Uzunova et al., 1998), and they are normalized by antidepressant drug treatment (Uzunova et al., 1998). Neurosteroids are synthesized in the brain by GABAergic and glutamatergic neurons (Agis-Balboa et al., 2006) and contribute to neural inhibition directly as a positive allosteric modulator of $GABA_A$ receptors (Chen et al., 2018) and indirectly as ligands of metabotropic receptors that signal to increase the cell surface expression of $GABA_A$ receptors (Abramian et al., 2014; Modgil et al., 2017; Parakala et al., 2019). Importantly, even modest defects in GABAergic inhibition (i.e., $GABA_A$ receptor $\gamma 2^{+/-}$ mice are missing one of 38 genetic loci encoding subunits for $GABA_A$ receptors) are known to facilitate stress axis activation (Earnheart et al., 2007; Melon, Hooper, Yang, Moss, & Maguire, 2018; Shen et al., 2010). Thus, chronic stress-induced excesses in glutamatergic tone become self-reinforcing via GABAergic deficit-induced increases in glutamatergic tone and activation of the HPA axis (Luscher & Fuchs, 2015; Luscher & Mohler, 2019).

In this way, external chronic stress-induced defects in GABAergic inhibition lead to 'internal stress' and prevent spontaneous recovery of downregulated glutamatergic synapses even after the external sources of stress have disappeared.

4. GABAergic interneurons serve as the initial cellular targets of subanesthetic ketamine

The earliest biological effect of treatment with a subanesthetic dose of ketamine reported in both rats (10–30 mg/kg) (Chowdhury et al., 2017; Moghaddam et al., 1997) and patients (0.5 mg/kg) (Lorrain et al., 2003) is the rapid and transient surge in extracellular glutamate. In rodents this response lasts for maximally 100 min (Chowdhury et al., 2017; Moghaddam et al., 1997), is paralleled by increased neuronal activity (elevated expression of activity-regulated cytoskeleton-associated protein, ARC) and rapidly followed by an increase in the synaptosomal expression of AMPA receptors (first detected at 2 h) and enhanced glutamatergic synaptic transmission (Li et al., 2010). Importantly, no increase in extracellular glutamate is observed with partially or fully anesthetic doses of ketamine (50–200 mg/kg in rats) (Moghaddam et al., 1997) or after pretreatment with an AMPA receptor antagonist (Maeng et al., 2008), and these same conditions also interfere with the antidepressant behavioral effects of ketamine (Kim & Monteggia, 2020; Li et al., 2010; Maeng et al., 2008). Thus, the initial swell in glutamate represents an essential trigger for both the initial (drug-on) and the enduring (drug-off) antidepressant effects of ketamine (Fig. 2). Blockade of even the earliest events of this mechanism by anesthetic concentrations of ketamine points to a key role also for the subset of NMDA receptors that are *not* blocked by subanesthetic ketamine (i.e., receptors localized on pyramidal cells, as detailed in Sections 4.2 and 4.3), at a time when the drug is still present in the brain.

4.1 Direct hypothesis of ketamine-induced synaptic plasticity

Two contrasting hypotheses were initially proposed to underlie the surge in glutamate and neural activity induced by ketamine (Miller, Moran, & Hall, 2016). A first proposed mechanism, referred to as the direct hypothesis, predicts that ketamine-induced formation of glutamatergic synapses on pyramidal cell dendrites involves temporary antagonism of NMDA receptors on these very same neurons. This mechanism has received little recent support as there is no clear rationale for how it could trigger the above

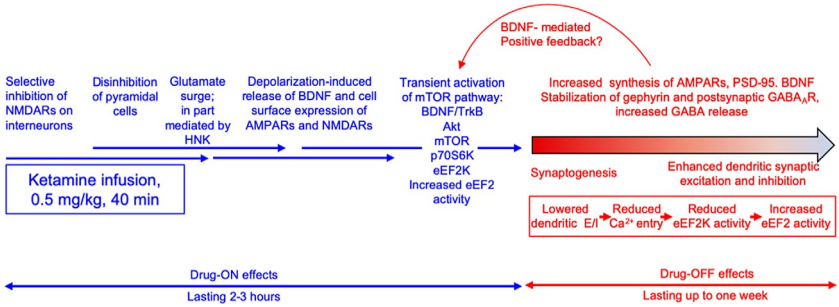

Fig. 2 Sequence of events underlying ketamine-induced antidepressant activity. A subanesthetic dose of ketamine leads to selective inhibition of NMDA receptors on GABAergic interneurons, followed by disinhibition of pyramidal cells and corresponding surge of extracellular glutamate (Gerhard et al., 2020) that further facilitates network activity, along with enhanced secretion of BDNF, increased expression of AMPA receptors (Li et al., 2010) and cell surface trafficking of NMDA receptors (Ren et al., 2016) and enhancement of AMPA receptor and NMDA receptor-mediated synaptic transmission (Ren et al., 2016). AMPA receptor-mediated synaptic transmission facilitates BDNF signaling through its receptor TrkB and leads to transient activation of Akt and the mTOR pathway (Li et al., 2010; Zhou et al., 2014) and activation of eEF2 as a primary effector (Adaikkan, Taha, Barrera, David, & Rosenblum, 2018; Autry et al., 2011). Due to lack of evidence for seizures and evidence from analyses of primary cultured neurons that the formation of glutamatergic synapses is preceded by the formation of functional GABAergic synapses (Deng et al., 2007) we infer that GABAergic inhibition is enhanced in parallel. These events occur in rapid succession and are all observed in the ketamine drug-on situation. They are followed by a second and more sustained drug-off phase of synaptogenesis that includes the structural maturation (or regeneration) of dendritic spines (Moda-Sava et al., 2019) and a reduced synaptic excitation:inhibition ratio at dendrites. Increased dendritic inhibition by SST^+ interneurons appears to reduce NMDA receptor-mediated Ca^{2+} influx into spines and dendrites and leads to reduced activity of Ca^{2+}/calmodulin-dependent eEF2K and reduced phosphorylation and increased activation of eEF2, as modeled in mice with constitutively enhanced dendritic GABAergic inhibition (Fuchs et al., 2017) (see also Chiu et al., 2013). This phase appears to be modeled also in mice lacking GluN2B NMDA receptors selectively in pyramidal cells, which leads to constitutively increased expression of BDNF and activation of mTOR, consistent with a positive feedback loop whereby dendritically synthesized and released BDNF leads to increased activation of the mTOR pathway and constitutively increased eEF2 activity and dendritic translation (Miller et al., 2014).

mentioned rapid surge in glutamate, which is now understood to be essential for ketamine-induced plasticity and behavior. However, the selective suppression of NMDA receptor activity on pyramidal cells appears to be relevant to the delayed consequences of ketamine treatment that operate in the drug-off situation and appears to involve enhanced GABAergic inhibition of dendrites, as discussed in Section 5.2.

4.2 Indirect or disinhibitory hypothesis of ketamine-induced synaptic plasticity

The second mechanism for ketamine-induced plasticity is known as the disinhibitory hypothesis and has recently received substantial experimental support (Gerhard, Wohleb, & Duman, 2016; Miller et al., 2016) (Fig. 2). It implies that the induction of synaptogenesis on dendrites of pyramidal cells involves inhibition of NMDA receptors localized on GABAergic interneurons and is based on the rationale that GABAergic interneurons on average are more active and their plasma membrane more depolarized than pyramidal cells. Ketamine therefore should more easily enter and block the channel pore of NMDA receptors on interneurons than pyramidal cells. This hypothesis is also consistent with and supported by the above-mentioned inverted U-shaped dose-response curve of ketamine. That is, disinhibition-induced plasticity of pyramidal cells should be blocked if NMDA receptors are inhibited on all neurons. Predating this hypothesis, Wang, Ren, Zhang, and Zhao (2005) had shown that transient blockade of GABAergic inhibition in brain slices of the anterior cingulate cortex of mice can trigger the formation of new glutamatergic synapses. Moreover, Homayoun and Moghaddam (2007) had shown that a subanesthetic dose of ketamine rapidly and selectively suppresses the activity of fast spiking (FS) interneurons, and that this initial effect is followed closely by increased activity of principal neurons. The authors had inferred that the FS subset of interneurons were the subtype of interneurons responsible for disinhibition of pyramidal cells but it turns out this was premature. FS cells are also known as parvalbumin (PV)-positive interneurons, which constitute about 40% of all GABAergic interneurons of the neocortex and preferentially target the soma of principal cells. The remaining 60% of cortical GABAergic interneurons are evenly split between those that express somatostatin (SST) or the 5-HT3a receptor as a marker protein (Rudy, Fishell, Lee, & Hjerling-Leffler, 2011). In particular, SST neurons are of interest because SST protein levels are downregulated in postmortem brain of depressed patients and because these cells exhibit increased vulnerability to chronic stress in mice (Fee, Banasr, & Sibille, 2017; Girgenti et al., 2019; Lin & Sibille, 2015).

In further support of the disinhibitory hypothesis, Widman and McMahon (2018) recently showed that ex vivo perfusion of hippocampal brain slices with subsaturating concentrations of ketamine results in the selective activation of pyramidal cells. Similarly, Gerhard et al. (2020) perfused prefrontal cortical slices with low concentrations of ketamine and

showed that this leads to a rapid reduction of the frequency of GABAergic synaptic inputs to pyramidal cells, while increasing the frequency of excitatory synaptic currents recorded from these cells. Thus, ketamine-induced inhibition of interneurons results in disinhibition of principal cells, and the transiently increased activity of these cells leads to the transient surge in extracellular glutamate. Interestingly, higher ketamine concentrations reduce the frequency of both types of inputs to glutamatergic cells, which is consistent with earlier findings that only low concentrations of ketamine relevant for antidepressant effects leads to disinhibition of principal neurons (Gerhard et al., 2020; Homayoun & Moghaddam, 2007). Importantly, this mechanism appears to be enhanced by HNK-mediated disinhibition of glutamate release from glutamatergic terminals (Riggs et al., 2020; Zanos et al., 2019). Moreover, one would readily predict that this ketamine-induced glutamate surge is facilitated by genetic or stress induced defects in GABAergic inhibition, as evidenced in animal models of depression (Garcia et al., 2009; Ghosal et al., 2020; Li et al., 2011; Ren et al., 2016).

4.3 NMDA receptors on somatostatin- and parvalbumin-positive GABAergic interneurons serve as the initial targets of ketamine

Following up on the above experiments, Gerhard et al. (2020) next used viral knock down in the mPFC and global Cre-mediated deletion of GluN2B NMDA receptors in different cell types of mice to determine the cellular targets for ketamine-induced antidepressant behavioral effects. NMDA receptors are heterotetrameric complexes containing two obligatory GluN1 subunits together with two variable GlunN2 subunits encoded by four separate genes (GluN2A, B, C, D), and GluN2B-specific antagonists have shown antidepressant effects similar to ketamine in animals and clinical trials (Maeng et al., 2008; Preskorn et al., 2008). Gerhard et al. (2020) found that knockdown of GluN2B on GABAergic interneurons (GAD^+) or more selectively on SST^+ interneurons results in measurable disinhibition of pyramidal cells as evidenced by reduced inhibitory and increased excitatory synaptic inputs in slice recordings. Moreover, knockdown of GluN2B on GABAergic interneurons but not pyramidal cells of the mPFC had anxiolytic and antidepressant-like behavioral consequences and interfered with or occluded the behavioral effects of ketamine, which is consistent with NMDA receptors on GABAergic cells serving as initial targets of ketamine.

However, these results appear in conflict with earlier findings by Miller et al. (2014), who analyzed mice with Cre-mediated deletion of GluN2B

from pyramidal cells across the entire cortex. These mice exhibit a constitutive antidepressant-like behavioral phenotype that occludes the behavioral effects of ketamine, similar to the behavioral phenotype seen by Gerhard et al. upon deletion of GluN2B deletion from mPFC interneurons. There is no straight forward explanation for these discrepancies except that the Miller et al. (2014) study used a partially anesthetic (50 mg/kg) dose of ketamine that has been shown to preclude induction of a glutamate surge (Moghaddam et al., 1997). Moreover, behavioral tests were conducted in the drug-on condition (30 min after drug injection) and thus may have been compromised by psychotomimetic and locomotor drug effects. Less likely, it is possible that NMDA receptors of glutamatergic neurons are dispensable in the mPFC (assessed by Gerhard et al., 2020) but essential in projection areas of mPFC neurons that were included in the manipulations by Miller et al. (2014). In addition, while NMDA receptors on interneurons appear to serve as initial targets, their counterparts on pyramidal cells in other parts of the cortex may be essential for initial activity-induced plasticity immediately downstream of the glutamate surge. The effect by Miller et al. seen on pyramidal cells at the 30 min time point post drug injection would unlikely be evident at the 24 h time point assessed by Gerhard et al.

In an attempt to further delineate the initial target of ketamine to specific interneuron types, Gerhard et al. (2020) applied their GluN2B knockdown strategy selectively to PV^+ and SST^+ interneurons. SST neurons are more heterogeneous than FS interneurons and include low threshold spiking, regular spiking and burst firing variants. They are less active on average than FS neurons but more active than pyramidal cells in an animal at rest and therefore should support ketamine-induced disinhibition of pyramidal cells similar to FS neurons. Using ex vivo slice recordings from male mice, Gerhard et al. found that deletion or knockdown of NMDA receptors from SST neurons reduces the frequency of inhibitory inputs to pyramidal cells while increasing excitatory synaptic inputs, which is consistent with NMDA receptors on SST neurons serving as targets for ketamine-mediated disinhibition of principal cells. However, in female mice the excitatory synaptic inputs were either reduced or unchanged, which is in keeping with evidence for sex differences of SST cells (Girgenti et al., 2019). Similar to manipulation of all GABAergic cells, regionally restricted viral-mediated knockdown of GluN2B in the mPFC or global conditional deletion of GluN2B from SST^+ cells prevented or occluded the behavioral responses to ketamine. Lastly, Ali et al. (2020) showed in awake mice that ketamine acutely suppresses the activity of SST^+ interneurons and facilitates Ca^{2+} spikes on distal

apical dendrites of pyramidal cells, juxtaposed to SST axon terminals. Somewhat unexpectedly, however, Gerhard et al. (2020) found that GluN2B deletion from PV^+ cells produced behavioral outcomes similar to above experiments targeting SST^+ cells, suggesting that ketamine-mediated-inhibition of PV^+ cells might contribute to the rapid disinhibition of pyramidal cells and antidepressant behavioral phenotypes. Importantly, PV^+ cells show prominent innervation of each other and also of SST^+ cells, suggesting that the circuit-wide consequences of GluN2B deletion from PV^+ cells are more complex than apparent from these studies. Nevertheless, the findings by Gerhard et al. (2020) distinguish the mechanism of ketamine from that of scopolamine, which triggers disinhibition of pyramidal cells and antidepressant effects in mice by antagonizing M1-type muscarinic acetylcholine receptors selectively on SST cells (Wohleb et al., 2016).

5. The mechanism of ketamine-induced plasticity includes a sustained increase in GABAergic synaptic inhibition at dendrites

5.1 Ketamine-induced antidepressant effects are associated with sustained pre- and postsynaptic enhancement of GABAergic synaptic inhibition

Within 2 h of drug administration, ketamine leads to increased synaptosomal accumulation of AMPA receptors and the subsynaptic scaffolding protein PSD95, measurable for at least 3 days and accompanied by corresponding enhancement of glutamatergic synaptic transmission (Li et al., 2010, 2011; Miller et al., 2014) (Fig. 2). In $GABA_A$ receptor deficient mice, ketamine also restores the compromised cell surface trafficking and postsynaptic function of NMDA receptors and neuroligin-1, a synaptic cell adhesion protein required for normal postsynaptic accumulation of NMDA receptors and presynaptic functional maturation of glutamatergic synapses (Budreck et al., 2013; Ren et al., 2016). Given that depressive disorders may be caused by chronic stress and elevated glutamatergic tone, which are known to reduce seizure thresholds (Becker et al., 2015; MacKenzie & Maguire, 2015), a mechanism involving selective enhancement of glutamatergic synapses seems ill-conceived and predictive of seizures rather than restoration of normal function. Indeed, there is evidence that ketamine at doses comparable to those used for depression can treat refractory status epilepticus (Synowiec et al., 2013). Ketamine also fails to trigger seizures in $GABA_A$ receptor

$\gamma 2^{+/-}$ mice (Z. Ren, S. J. J. and B.L, unpublished), despite their reduced seizure threshold (Reid et al., 2013). Instead, measurements in the $\gamma 2^{+/-}$ model (24 h post drug injection) show that ketamine restores the number and function of GABAergic inhibitory synapses recorded from mPFC pyramidal cells, along with restoration of glutamatergic synaptic transmission (Ren et al., 2016). Ketamine fully restores the amplitude of miniature inhibitory synaptic currents (IPSCs, measured at the 24 h time point), indicating that it restores the postsynaptic accumulation and function of inhibitory synapses, despite the genetically delimited expression of postsynaptic $GABA_A$ receptors in this model. In addition, ketamine greatly increases the GABA release probability to levels significantly above control levels and indicative of an overall shift of the synaptic excitation:inhibition ratio toward increased inhibition (Ren et al., 2016). No such drug effects are observed in wild-type control animals. The latter reinforces the notion that ketamine's mechanism depends on a basal imbalance between synaptic excitation and inhibition. Importantly, at the 24 h time point post-injection, ketamine similarly restores the function of mPFC GABAergic synapses that were compromised by exposure of animals to chronic stress (Ghosal et al., 2020), indicating that the effects of ketamine on inhibitory synapses are not model-specific. Strengthening of GABAergic synapses along with glutamatergic synapses appears to ensure that the synaptic excitation:inhibition balance at dendrites of pyramidal cells is maintained or even shifted toward greater inhibition (Figs. 1 and 2). It may further contribute to stress resilience and is likely to be critical for the extended duration of ketamine-induced antidepressant effects, especially in situations in which the source of the initial excitation:inhibition imbalance persists. However, the exact time course of ketamine-induced potentiation of synaptic inhibition vs excitation remains to be determined.

The molecular mechanisms underlying ketamine-induced potentiation of GABAergic inputs to pyramidal cells have not specifically been investigated. However, it is likely that the initial trigger of inhibitory synapse formation is the same as that for glutamatergic synapses. Disinhibition of neural networks by injection of the $GABA_A$ receptor antagonist bicucullin results in the formation of both GABAergic and glutamatergic synapses (Schlosser, ten Bruggencate, & Sutor, 1999). Moreover, a number of additional inferences can be made from experiments addressing NMDA receptor-mediated regulation of inhibitory synapses in cultured neurons and brain slices. These studies indicate that excessive activation of NMDA receptors, assumed to occur during high levels of stress, triggers Ca^{2+}/calcineurin-mediated

dephosphorylation of postsynaptic $GABA_A$ receptors and weakening of inhibitory synapses through enhanced diffusional dispersal of $GABA_A$ receptors (Bannai et al., 2009, 2015; Muir et al., 2010). In addition, excessive NMDA receptor-mediated Ca^{2+} influx leads to the internalization of the chloride transporter KCC2 from the cell surface, which reduces the GABAergic inhibitory drive (Lee, Deeb, Walker, Davies, & Moss, 2011). This mechanism also operates during the aforementioned chronic stress-induced downregulation of KCC2 (Hewitt et al., 2009; MacKenzie & Maguire, 2015).

Contrary to strong activation of NMDA receptors with glutamate, more limited activation of NMDA receptors (mimicked by exposure of cultures or brain slices to NMDA) that may be observed following ketamine-induced GABAergic inhibition of dendrites (Ren et al., 2016), strengthens inhibitory postsynapses through a Ca^{2+}/calmodulin-dependent kinase (CaMK)II-dependent increase in the translocation of $GABA_A$ receptors to the cell surface (Marsden, Beattie, Friedenthal, & Carroll, 2007). In support of this mechanism, SST^+ neuron-mediated GABAergic inhibition of NMDA receptor-mediated Ca^{2+} entry into dendrites triggers a positive feedback mechanism that leads to functional potentiation of these very same GABAergic inputs to pyramidal cell dendrites (Chiu et al., 2018). Notably, SSTCre:$\gamma 2^{f/f}$ mice show enhanced GABAergic inhibition of pyramidal cells due to increased excitability of SST^+ interneurons and reflected by an increase in the frequency of spontaneous IPSCs that is presynaptic activity-dependent, as expected. However, the amplitude of miniature IPCS recorded from hippocampal pyramidal cells was also enhanced, pointing to a inhibitory input-induced mechanism of postsynaptic plasticity that is consistent with that described by Chiu et al. (2018).

Above activity-dependent bidirectional regulation of the strength of GABAergic synapses is at least in part mediated by changes in the phosphorylation of the $GABA_A$ receptor clustering protein, gephyrin (reviewed by Fritschy, Harvey, & Schwarz, 2008; Higley, 2014) and this appears to be relevant for ketamine's mechanism of action. Chronic stress results in loss of gephyrin that is restored 1 day after an antidepressant dose of ketamine (Ghosal et al., 2020). A similar loss of gephyrin and restoration by ketamine has been reported for $GABA_A$ receptor deficient primary cultured neurons (Ren et al., 2016). Restoration of postsynaptic gephyrin may involve NMDA receptor and CaMKII-mediated phosphorylation of gephyrin (Flores et al., 2015). This NMDA receptor-dependent mechanism has been proposed to ensure that learning-associated long term potentiation (LTP) of

excitatory synapses is balanced by strengthened inhibitory synapses. This same mechanism could conceivably help balance excitation and inhibition in the context of ketamine-induced synaptogenesis.

The strength of GABAergic synapses is also regulated by glycogen synthase kinase-3β (GSK-3β). GSK-3β-mediated phosphorylation of gephyrin triggers calpain-mediated degradation of gephyrin. Conversely, pharmacological inhibition of GSK-3β with the mood stabilizer Li^+ or ablation of the GSK-3β phosphorylation sites on gephyrin lead to stabilization of gephyrin and strengthening of GABAergic synaptic inhibition (Tyagarajan et al., 2011). Similar to Li^+, ketamine mediated activation of the mTOR pathway leads to inhibition of GSK-3β (Wuchter et al., 2012; Zhou et al., 2014). Such inhibition of GSK-3β is required for ketamine-induced synaptic plasticity and antidepressant behavioral effects (Beurel, Grieco, Amadei, Downey, & Jope, 2016; Beurel, Song, & Jope, 2011; Liu et al., 2013). Conversely, elevated GSK-3β activity and loss of GABAergic inhibition are strongly implicated in the etiology of depressive disorders (Jope, 2011; Luscher & Fuchs, 2015; Luscher et al., 2011). Collectively, these data indicate that ketamine-induced enhancement of GABAergic inhibition involves inhibition of GSK-3β and corresponding stabilization of gephyrin. Conversely, chronic stress- or excitation:inhibition imbalance-induced downregulation of gephyrin and GABAergic synapses and the ensuing depressive-like phenotypes (Ghosal et al., 2020; Ren et al., 2016) may involve elevated GSK-3β activity and increased calpain-mediated degradation of gephyrin.

5.2 Genetic enhancement of GABAergic inhibition at pyramidal cell dendrites mimics the lasting antidepressant behavioral and biochemical consequences of ketamine in the drug-off situation

The above observations raise the possibility that sustained strengthening of GABAergic synaptic inhibition by itself may be sufficient to elicit antidepressant effects. We had addressed this question in mice by genetically disinhibiting SST^+ GABAergic interneurons (Fuchs et al., 2017). SST neurons are heterogeneous in nature but include Martinotti cells of the neocortex and oriens lacunosum moleculare (OLM) interneurons in the hippocampus as major subpopulations that preferentially target the distal apical dendrites of pyramidal cells. They provide firing rate-dependent feedforward inhibition to pyramidal cells of the neocortex and hippocampus and are specifically tuned to balance neural excitation with inhibition during

periods of sustained activation of neural circuits (Kapfer, Glickfeld, Atallah, & Scanziani, 2007; Pouille & Scanziani, 2004; Silberberg & Markram, 2007; Tan, Hu, Huang, & Agmon, 2008). To increase the excitability of SST cells, we chose to disinhibit them by cell type-specific deletion of $GABA_A$ receptors (SSTCre:$\gamma 2^{f/f}$ mice). As predicted, SSTCre:$\gamma 2^{f/f}$ mice exhibited increased SST cell excitability and an anxiolytic and antidepressant-like phenotype in multiple anxiety- and depression-related behavioral tests. Moreover, brain extracts from the mPFC and hippocampus of these mice showed reduced inhibitory phosphorylation (and thus activation) of eEF2 similar to ketamine-treated mice in hippocampus (Fuchs et al., 2017). Notably, deletion of GluN2B from pyramidal cells produced a comparable antidepressant phenotype (Miller et al., 2017, 2014), which suggests that in SSTCre:$\gamma 2^{f/f}$ mice, enhanced inhibitory inputs delimits NMDA receptor function of glutamatergic spine synapses, as also evidenced by slice recordings (Chiu et al., 2013).

Reduced eEF2 phosphorylation may be thought of as a biochemical end point or effector of ketamine treatment that indicates enhanced translation of dendritic transcripts such as BDNF (Miller et al., 2014; Sutton, Taylor, Ito, Pham, & Schuman, 2007). Ketamine-induced transient disinhibition of pyramidal cells is known to result in a burst of AMPA-receptor-mediated membrane depolarization, which appears to trigger enhanced exocytosis of BDNF (Lepack, Bang, Lee, Dwyer, & Duman, 2016). Studies in mice further indicated that the effect of ketamine is blocked by the mTOR inhibitor, rapamycin, suggesting that BDNF-mediated activation of TrkB leads to activation of AKT and the mTOR pathway (Li et al., 2010; Miller et al., 2014), which then results in increased inhibitory phosphorylation of eEF2 kinase (eEF2K) and reduced inhibitory phosphorylation (and thus activation) of eEF2 (Adaikkan et al., 2018; Autry et al., 2011) (Fig. 2). Preclinical studies further indicate that activation of eEF2 leads to increased translation of dendritic mRNAs encoding AMPA receptors, PSD95 and BDNF, suggesting more sustained dendritic release of BDNF (Adaikkan et al., 2018; Li et al., 2010; Miller et al., 2014; Zhou, Wang, et al., 2014). Notably, however, the role of mTOR remains controversial with at least two groups failing to replicate corresponding preclinical findings (Autry et al., 2011; Zanos et al., 2016). Moreover, a recent clinical study found that pretreatment with the mTOR inhibitor rapamycin increased the response and remission rates of ketamine treatment, rather than blocking its effects (Abdallah et al., 2020). It is possible that the rapamycin concentration used in these clinical studies was insufficient to completely block

mTOR activation Nevertheless, based on current understanding from preclinical experiments, maintenance of the antidepressant effects in the drug off situation appear to involve keeping eEF2 in a dephosphorylated active state, as seen in pyramidal cell GluN2B knockout mice (Miller et al., 2014) (Fig. 2). Importantly, dephosphorylated eEF2 is not only a product of mTOR activation; eEF2 phosphorylation is also regulated independent of mTOR as a function of NMDA receptor activity and changes in the synaptic excitation:inhibition ratio (Fuchs et al., 2017; Sutton et al., 2007) (Fig. 2). That is, intrinsic action potential-mediated network activity promotes a relatively dephosphorylated and active state of eEF2 that is associated with enhanced dendritic translation. By contrast, activation of NMDA receptors by spontaneous neurotransmitter release that is observed in the absence of neural activity promotes the phosphorylation and inactivation of eEF2 (Sutton et al., 2007). Curiously, spontaneous neurotransmitter release-mediated activation of NMDA receptors can also be blocked by ketamine in vitro (Autry et al., 2011; Nosyreva et al., 2013). However, this mechanism cannot account for NMDA receptor inhibition in the ketamine drug-off situation and therefore cannot possibly contribute to the delayed antidepressant effects of ketamine.

6. Conclusion

In summary, the data support the following sequence of events. Ketamine initially acts directly to inhibit NMDA receptors on interneurons (Gerhard et al., 2020), which leads to a surge in glutamate (Moghaddam et al., 1997), increased activation of pyramidal cells (Gerhard et al., 2020), increased Ca^{2+}-mediated release of BDNF and transient activation of the mTOR pathway (Li et al., 2010; Miller et al., 2014), followed by a wave of synaptogenesis and strengthening of glutamatergic and even more so GABAergic synaptic transmission (Li et al., 2010; Miller et al., 2014; Ren et al., 2016) (Fig. 2). Increased GABAergic inhibition of pyramidal cell dendrites is long lasting (days) (Ren et al., 2016) and appears to suppress untimely or excessive NMDA receptor activity-mediated Ca^{2+} entry into dendrites of pyramidal cells (Fuchs et al., 2017; Sutton et al., 2007). This secondary effect appears to depend mainly on GABAergic inputs from SST^{+} cells, which are tailored to control NMDA receptor mediated Ca^{2+} entry into dendritic spines (Chiu et al., 2013). Reduced NMDA receptor-mediated Ca^{2+} entry leads to reduced Ca^{2+}/calmodulin-mediated activation of eEF2K and reduced phosphorylation/increased activation of

eEF2 (Fuchs et al., 2017). Unlike the initial direct and transient inactivation of NMDA receptors by ketamine on interneurons, this secondary effect is observed in the ketamine drug-off situation. It is constitutively active in SSTCre:$\gamma 2^{f/f}$ mice, which show an antidepressive-like phenotype associated with constitutive reductions in eEF2 phosphorylation (increased activation of eEF2) but unaltered mTOR (Fuchs et al., 2017). This same pathway appears constitutively active also in pyramidal cell-specific GluN2B knockout mice. Compared to the SSTCre:$\gamma 2^{f/f}$ model, knockout of GluN2B in pyramidal cells appears to result in greater activation of eEF2, constitutively increased BDNF synthesis and constitutive activation of the mTOR pathway (Miller et al., 2014) (Fig. 2).

We have seen remarkable progress in recent years toward understanding ketamine's mechanism of antidepressant effects. At the same time, many of the finer details remain unresolved. In particular, a key question remains whether HNK-mediated activation of glutamate release is sufficient to trigger the sequelae of synaptic plasticity needed for antidepressant effects in patients and whether its contributions to the glutamate surge are essential. The answers to these questions will help predict the therapeutic potential of alternative drugs that target glutamatergic transmission. Another interesting question is the relative timing of ketamine-induced formation of glutamatergic vs GABAergic synapses on pyramidal cells. The notion that ketamine may ameliorate refractory status epilepticus (Synowiec et al., 2013) suggests that GABAergic synapses emerge in parallel with or even before glutamatergic synapses. This prediction is also supported by analyses of primary cultured neurons that show that the novo formation of glutamatergic synapses is preceded by the formation of functional GABAergic synapses (Deng et al., 2007) as well as by genetic evidence showing that BDNF serves as a key regulator for the formation of inhibitory synapse, more so than excitatory synapses (Lu, Wang, & Nose, 2009; Seil, 2016). Some evidence suggest that ketamine leads to long lasting increases in the GABA release probability 24 h post drug injection (Ren et al., 2016). It will be interesting to examine whether this involves HNK-mediated inhibition of mGluR2, analogous to HNK's effects at glutamatergic synapses (Riggs et al., 2020; Zanos et al., 2019), or whether the effect is limited entirely to the ketamine- and HNK-off situation. A third area that remains largely unexplored is ketamine's effects on the activity of interneurons and the strength of inhibitory and excitatory synaptic inputs to SST^+ and PV^+ cells. We look forward to a day when we will have a complete and time-resolved understanding of ketamine's actions on all of these different neurons and synapses.

Conflict of interest

The authors declare there are no conflicts of interest.

Reference

Abdallah, C. G., Averill, L. A., Collins, K. A., Geha, P., Schwartz, J., Averill, C., et al. (2017). Ketamine treatment and global brain connectivity in major depression. *Neuropsychopharmacology*, *42*(6), 1210–1219. https://doi.org/10.1038/npp.2016.186.

Abdallah, C. G., Averill, L. A., Gueorguieva, R., Goktas, S., Purohit, P., Ranganathan, M., et al. (2020). Modulation of the antidepressant effects of ketamine by the mTORC1 inhibitor rapamycin. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-020-0644-9 [Epub ahead of print].

Abdallah, C. G., Averill, C. L., Salas, R., Averill, L. A., Baldwin, P. R., Krystal, J. H., et al. (2017). Prefrontal connectivity and glutamate transmission: Relevance to depression pathophysiology and ketamine treatment. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *2*(7), 566–574. https://doi.org/10.1016/j.bpsc.2017.04.006.

Abramian, A. M., Comenencia-Ortiz, E., Modgil, A., Vien, T. N., Nakamura, Y., Moore, Y. E., et al. (2014). Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic GABAA receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(19), 7132–7137. https://doi.org/10.1073/pnas.1403285111.

Adaikkan, C., Taha, E., Barrera, I., David, O., & Rosenblum, K. (2018). Calcium/calmodulin-dependent protein kinase II and eukaryotic elongation factor 2 kinase pathways mediate the antidepressant action of ketamine. *Biological Psychiatry*, *84*(1), 65–75. https://doi.org/10.1016/j.biopsych.2017.11.028.

Adams, J. D., Jr., Baillie, T. A., Trevor, A. J., & Castagnoli, N., Jr. (1981). Studies on the biotransformation of ketamine. 1-Identification of metabolites produced in vitro from rat liver microsomal preparations. *Biomedical Mass Spectrometry*, *8*(11), 527–538. https://doi.org/10.1002/bms.1200081103.

Agis-Balboa, R. C., Pinna, G., Pibiri, F., Kadriu, B., Costa, E., & Guidotti, A. (2007). Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(47), 18736–18741. https://doi.org/10.1073/pnas.0709419104.

Agis-Balboa, R. C., Pinna, G., Zhubi, A., Maloku, E., Veldic, M., Costa, E., et al. (2006). Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(39), 14602–14607. https://doi.org/10.1073/pnas.0606544103.

Ali, F., Gerhard, D. M., Sweasy, K., Pothula, S., Pittenger, C., Duman, R. S., et al. (2020). Ketamine disinhibits dendrites and enhances calcium signals in prefrontal dendritic spines. *Nature Communications*, *11*, 72. https://doi.org/10.1038/s41467-019-13809-8.

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., et al. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, *475*(7354), 91–95. https://doi.org/10.1038/nature10130.

Banasr, M., Lepack, A., Fee, C., Duric, V., Maldonado-Aviles, J., DiLeone, R., et al. (2017). Characterization of GABAergic marker expression in the chronic unpredictable stress model of depression. *Chronic Stress (Thousand Oaks)*, *1*, 1–13. https://doi.org/10.1177/2470547017720459.

Bannai, H., Levi, S., Schweizer, C., Inoue, T., Launey, T., Racine, V., et al. (2009). Activity-dependent tuning of inhibitory neurotransmission based on GABAAR diffusion dynamics. *Neuron*, *62*(5), 670–682. https://doi.org/10.1016/j.neuron.2009.04.023.

Bannai, H., Niwa, F., Sherwood, M. W., Shrivastava, A. N., Arizono, M., Miyamoto, A., et al. (2015). Bidirectional control of synaptic GABAAR clustering by glutamate and calcium. *Cell Reports*, *13*(12), 2768–2780. https://doi.org/10.1016/j.celrep.2015.12.002.

Becker, C., Bouvier, E., Ghestem, A., Siyoucef, S., Claverie, D., Camus, F., et al. (2015). Predicting and treating stress-induced vulnerability to epilepsy and depression. *Annals of Neurology*, *78*(1), 128–136. https://doi.org/10.1002/ana.24414.

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., et al. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351–354. https://doi.org/10.1016/s0006-3223(99)00230-9.

Beurel, E., Grieco, S. F., Amadei, C., Downey, K., & Jope, R. S. (2016). Ketamine-induced inhibition of glycogen synthase kinase-3 contributes to the augmentation of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor signaling. *Bipolar Disorders*, *18*(6), 473–480. https://doi.org/10.1111/bdi.12436.

Beurel, E., Song, L., & Jope, R. S. (2011). Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. *Molecular Psychiatry*, *16*(11), 1068–1070. https://doi.org/10.1038/mp.2011.47.

Bhagwagar, Z., Wylezinska, M., Jezzard, P., Evans, J., Ashworth, F., Sule, A., et al. (2007). Reduction in occipital cortex gamma-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. *Biological Psychiatry*, *61*(6), 806–812. https://doi.org/10.1016/j.biopsych.2006.08.048.

Budreck, E. C., Kwon, O. B., Jung, J. H., Baudouin, S., Thommen, A., Kim, H. S., et al. (2013). Neuroligin-1 controls synaptic abundance of NMDA-type glutamate receptors through extracellular coupling. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(2), 725–730. https://doi.org/10.1073/pnas.1214718110.

Canuso, C. M., Singh, J. B., Fedgchin, M., Alphs, L., Lane, R., Lim, P., et al. (2018). Efficacy and safety of intranasal Esketamine for the rapid reduction of symptoms of depression and suicidality in patients at imminent risk for suicide: Results of a double-blind, randomized, placebo-controlled study. *The American Journal of Psychiatry*, *175*(7), 620–630. https://doi.org/10.1176/appi.ajp.2018.17060720.

Chen, Q., Wells, M. M., Arjunan, P., Tillman, T. S., Cohen, A. E., Xu, Y., et al. (2018). Structural basis of neurosteroid anesthetic action on GABAA receptors. *Nature Communications*, *9*(1), 3972. https://doi.org/10.1038/s41467-018-06361-4.

Chiu, C. Q., Lur, G., Morse, T. M., Carnevale, N. T., Ellis-Davies, G. C., & Higley, M. J. (2013). Compartmentalization of GABAergic inhibition by dendritic spines. *Science*, *340*(6133), 759–762. https://doi.org/10.1126/science.1234274.

Chiu, C. Q., Martenson, J. S., Yamazaki, M., Natsume, R., Sakimura, K., Tomita, S., et al. (2018). Input-specific NMDAR-dependent potentiation of dendritic GABAergic inhibition. *Neuron*, *97*(2), 368–377, e363. https://doi.org/10.1016/j.neuron.2017.12.032.

Chou, D., Peng, H. Y., Lin, T. B., Lai, C. Y., Hsieh, M. C., Wen, Y. C., et al. (2018). (2R,6R)-hydroxynorketamine rescues chronic stress-induced depression-like behavior through its actions in the midbrain periaqueductal gray. *Neuropharmacology*, *139*, 1–12. https://doi.org/10.1016/j.neuropharm.2018.06.033.

Chowdhury, G. M., Zhang, J., Thomas, M., Banasr, M., Ma, X., Pittman, B., et al. (2017). Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects. *Molecular Psychiatry*, *22*(1), 120–126. https://doi.org/10.1038/mp.2016.34.

Csabai, D., Wiborg, O., & Czeh, B. (2018). Reduced synapse and axon numbers in the prefrontal cortex of rats subjected to a chronic stress model for depression. *Frontiers in Cellular Neuroscience*, *12*, 24. https://doi.org/10.3389/fncel.2018.00024.

Czeh, B., Simon, M., van der Hart, M. G., Schmelting, B., Hesselink, M. B., & Fuchs, E. (2005). Chronic stress decreases the number of parvalbumin-immunoreactive

interneurons in the hippocampus: Prevention by treatment with a substance P receptor (NK1) antagonist. *Neuropsychopharmacology*, *30*(1), 67–79. https://doi.org/10.1038/sj.npp.1300581.

Czeh, B., Vardya, I., Varga, Z., Febbraro, F., Csabai, D., Martis, L. S., et al. (2018). Long-term stress disrupts the structural and functional integrity of GABAergic neuronal networks in the medial prefrontal cortex of rats. *Frontiers in Cellular Neuroscience*, *12*, 148. https://doi.org/10.3389/fncel.2018.00148.

Czeh, B., Varga, Z. K., Henningsen, K., Kovacs, G. L., Miseta, A., & Wiborg, O. (2015). Chronic stress reduces the number of GABAergic interneurons in the adult rat hippocampus, dorsal-ventral and region-specific differences. *Hippocampus*, *25*(3), 393–405. https://doi.org/10.1002/hipo.22382.

Deng, L., Yao, J., Fang, C., Dong, N., Luscher, B., & Chen, G. (2007). Sequential postsynaptic maturation governs the temporal order of GABAergic and glutamatergic synaptogenesis in rat embryonic cultures. *Journal of Neuroscience*, *27*(40), 10860–10869. https://doi.org/10.1523/JNEUROSCI.2744-07.2007.

Desta, Z., Moaddel, R., Ogburn, E. T., Xu, C., Ramamoorthy, A., Venkata, S. L., et al. (2012). Stereoselective and regiospecific hydroxylation of ketamine and norketamine. *Xenobiotica*, *42*(11), 1076–1087. https://doi.org/10.3109/00498254.2012.685777.

Deyama, S., Bang, E., Kato, T., Li, X. Y., & Duman, R. S. (2019). Neurotrophic and antidepressant actions of brain-derived Neurotrophic factor require vascular endothelial growth factor. *Biological Psychiatry*, *86*(2), 143–152. https://doi.org/10.1016/j.biopsych.2018.12.014.

Earnheart, J. C., Schweizer, C., Crestani, F., Iwasato, T., Itohara, S., Mohler, H., et al. (2007). GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of trait anxiety and depression states. *Journal of Neuroscience*, *27*(14), 3845–3854. https://doi.org/10.1523/JNEUROSCI.3609-06.2007.

Essrich, C., Lorez, M., Benson, J. A., Fritschy, J. M., & Luscher, B. (1998). Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. *Nature Neuroscience*, *1*(7), 563–571. https://doi.org/10.1038/2798.

Evans, J. W., Szczepanik, J., Brutsche, N., Park, L. T., Nugent, A. C., & Zarate, C. A., Jr. (2018). Default mode connectivity in major depressive disorder measured up to 10 days after ketamine administration. *Biological Psychiatry*, *84*(8), 582–590. https://doi.org/10.1016/j.biopsych.2018.01.027.

Fava, M., Freeman, M. P., Flynn, M., Judge, H., Hoeppner, B. B., Cusin, C., et al. (2019). Double-blind, placebo-controlled, dose-ranging trial of intravenous ketamine as adjunctive therapy in treatment-resistant depression (TRD). *Molecular Psychiatry*. https://doi.org/10.1038/s41380-018-0311-2.

Fee, C., Banasr, M., & Sibille, E. (2017). Somatostatin-positive gamma-aminobutyric acid interneuron deficits in depression: Cortical microcircuit and therapeutic perspectives. *Biological Psychiatry*, *82*(8), 549–559. https://doi.org/10.1016/j.biopsych.2017.05.024.

Flores, C. E., Nikonenko, I., Mendez, P., Fritschy, J. M., Tyagarajan, S. K., & Muller, D. (2015). Activity-dependent inhibitory synapse remodeling through gephyrin phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(1), E65–E72. https://doi.org/10.1073/pnas.1411170112.

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., et al. (2010). Antidepressant drug effects and depression severity: A patient-level meta-analysis. *JAMA*, *303*(1), 47–53. https://doi.org/10.1001/jama.2009.1943.

Fritschy, J. M., Harvey, R. J., & Schwarz, G. (2008). Gephyrin: Where do we stand, where do we go? *Trends in Neurosciences*, *31*(5), 257–264. https://doi.org/10.1016/j.tins.2008.02.006.

Fuchikami, M., Thomas, A., Liu, R., Wohleb, E. S., Land, B. B., DiLeone, R. J., et al. (2015). Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8106–8111. https://doi.org/10.1073/pnas.1414728112.

Fuchs, T., Jefferson, S. J., Hooper, A., Yee, P. H., Maguire, J., & Luscher, B. (2017). Disinhibition of somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-like brain state. *Molecular Psychiatry*, *22*(6), 920–930. https://doi.org/10.1038/mp.2016.188.

Fukumoto, K., Fogaca, M. V., Liu, R. J., Duman, C., Kato, T., Li, X. Y., et al. (2019). Activity-dependent brain-derived neurotrophic factor signaling is required for the antidepressant actions of (2R,6R)-hydroxynorketamine. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(1), 297–302. https://doi.org/10.1073/pnas.1814709116.

Fukumoto, K., Toki, H., Iijima, M., Hashihayata, T., Yamaguchi, J. I., Hashimoto, K., et al. (2017). Antidepressant potential of (R)-ketamine in rodent models: Comparison with (S)-ketamine. *The Journal of Pharmacology and Experimental Therapeutics*, *361*(1), 9–16. https://doi.org/10.1124/jpet.116.239228.

Gabbay, V., Mao, X., Klein, R. G., Ely, B. A., Babb, J. S., Panzer, A. M., et al. (2012). Anterior cingulate cortex gamma-aminobutyric acid in depressed adolescents: Relationship to anhedonia. *Archives of General Psychiatry*, *69*(2), 139–149. https://doi.org/10.1001/archgenpsychiatry.2011.131.

Garcia, L. S., Comim, C. M., Valvassori, S. S., Reus, G. Z., Stertz, L., Kapczinski, F., et al. (2009). Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*(3), 450–455. https://doi.org/10.1016/j.pnpbp.2009.01.004.

Gerhard, D. M., Pothula, S., Liu, R. J., Wu, M., Li, X. Y., Girgenti, M. J., et al. (2020). GABA interneurons are the cellular trigger for ketamine's rapid antidepressant actions. *The Journal of Clinical Investigation*, *130*, 1336–1349. https://doi.org/10.1172/JCI130808.

Gerhard, D. M., Wohleb, E. S., & Duman, R. S. (2016). Emerging treatment mechanisms for depression: Focus on glutamate and synaptic plasticity. *Drug Discovery Today*, *21*(3), 454–464. https://doi.org/10.1016/j.drudis.2016.01.016.

Ghosal, S., Duman, C. H., Liu, R. J., Wu, M., Terwilliger, R., Girgenti, M. J., et al. (2020). Ketamine rapidly reverses stress-induced impairments in GABAergic transmission in the prefrontal cortex in male rodents. *Neurobiology of Disease*, *134*, 104669. https://doi.org/10.1016/j.nbd.2019.104669.

Girgenti, M. J., Wohleb, E. S., Mehta, S., Ghosal, S., Fogaca, M. V., & Duman, R. S. (2019). Prefrontal cortex interneurons display dynamic sex-specific stress-induced transcriptomes. *Translational Psychiatry*, *9*(1), 292. https://doi.org/10.1038/s41398-019-0642-z.

Gould, T. D., Zarate, C. A., Jr., & Thompson, S. M. (2019). Molecular pharmacology and neurobiology of rapid-acting antidepressants. *Annual Review of Pharmacology and Toxicology*, *59*, 213–236. https://doi.org/10.1146/annurev-pharmtox-010617-052811.

Green, S. M., Rothrock, S. G., Lynch, E. L., Ho, M., Harris, T., Hestdalen, R., et al. (1998). Intramuscular ketamine for pediatric sedation in the emergency department: Safety profile in 1,022 cases. *Annals of Emergency Medicine*, *31*(6), 688–697. https://doi.org/10.1016/s0196-0644(98)70226-4.

Hare, B. D., Shinohara, R., Liu, R. J., Pothula, S., DiLeone, R. J., & Duman, R. S. (2019). Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. *Nature Communications*, *10*(1), 223. https://doi.org/10.1038/s41467-018-08168-9.

Hasler, G., van der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., & Drevets, W. C. (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Archives of General Psychiatry*, *64*(2), 193–200. https://doi.org/10.1001/archpsyc.64.2.193.

Hewitt, S. A., Wamsteeker, J. I., Kurz, E. U., & Bains, J. S. (2009). Altered chloride homeostasis removes synaptic inhibitory constraint of the stress axis. *Nature Neuroscience*, *12*(4), 438–443. https://doi.org/10.1038/nn.2274.

Highland, J. N., Morris, P. J., Zanos, P., Lovett, J., Ghosh, S., Wang, A. Q., et al. (2018). Mouse, rat, and dog bioavailability and mouse oral antidepressant efficacy of (2R,6R)-hydroxynorketamine. *Journal of Psychopharmacology*, *33*(1), 12–24, 269881118812095. https://doi.org/10.1177/0269881118812095.

Higley, M. J. (2014). Localized GABAergic inhibition of dendritic Ca(2+) signalling. *Nature Reviews Neuroscience*, *15*(9), 567–572. https://doi.org/10.1038/nrn3803.

Hirota, K., & Lambert, D. G. (1996). Ketamine: Its mechanism(s) of action and unusual clinical uses. *British Journal of Anaesthesia*, 77(4), 441–444. https://doi.org/10.1093/bja/77.4.441.

Holmes, S. E., Scheinost, D., Finnema, S. J., Naganawa, M., Davis, M. T., Della Gioia, N., et al. (2019). Lower synaptic density is associated with depression severity and network alterations. *Nature Communications*, *10*(1), 1529. https://doi.org/10.1038/s41467-019-09562-7.

Homayoun, H., & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *Journal of Neuroscience*, *27*(43), 11496–11500. https://doi.org/10.1523/JNEUROSCI.2213-07.2007.

Hu, W., Zhang, M., Czeh, B., Flugge, G., & Zhang, W. (2010). Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. *Neuropsychopharmacology*, *35*(8), 1693–1707. https://doi.org/10.1038/npp.2010.31.

Jick, H., Kaye, J. A., & Jick, S. S. (2004). Antidepressants and the risk of suicidal behaviors. *JAMA*, *292*(3), 338–343. https://doi.org/10.1001/jama.292.3.338.

Jope, R. S. (2011). Glycogen synthase kinase-3 in the etiology and treatment of mood disorders. *Frontiers in Molecular Neuroscience*, *4*, 16. https://doi.org/10.3389/fnmol.2011.00016.

Kallarackal, A. J., Kvarta, M. D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A. M., et al. (2013). Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. *Journal of Neuroscience*, *33*(40), 15669–15674. https://doi.org/10.1523/JNEUROSCI.2588-13.2013.

Kang, H. J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C. A., Licznerski, P., et al. (2012). Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nature Medicine*, *18*(9), 1413–1417. https://doi.org/10.1038/nm.2886.

Kapfer, C., Glickfeld, L. L., Atallah, B. V., & Scanziani, M. (2007). Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. *Nature Neuroscience*, *10*(6), 743–753. https://doi.org/10.1038/nn1909.

Kim, J. W., & Monteggia, L. M. (2020). Increasing doses of ketamine curtail antidepressant responses and suppress associated synaptic signaling pathways. *Behavioural Brain Research*, *380*, 112378. https://doi.org/10.1016/j.bbr.2019.112378.

Kraguljac, N. V., Frolich, M. A., Tran, S., White, D. M., Nichols, N., Barton-McArdle, A., et al. (2017). Ketamine modulates hippocampal neurochemistry and functional connectivity: A combined magnetic resonance spectroscopy and resting-state fMRI study in healthy volunteers. *Molecular Psychiatry*, *22*(4), 562–569. https://doi.org/10.1038/mp.2016.122.

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., et al. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of General Psychiatry*, *51*(3), 199–214. https://doi.org/10.1001/archpsyc.1994.03950030035004.

Lapidus, K. A., Levitch, C. F., Perez, A. M., Brallier, J. W., Parides, M. K., Soleimani, L., et al. (2014). A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biological Psychiatry*, *76*(12), 970–976. https://doi.org/10.1016/j.biopsych.2014.03.026.

Lee, H. H., Deeb, T. Z., Walker, J. A., Davies, P. A., & Moss, S. J. (2011). NMDA receptor activity downregulates KCC2 resulting in depolarizing GABAA receptor-mediated currents. *Nature Neuroscience*, *14*(6), 736–743. https://doi.org/10.1038/nn.2806.

Lepack, A. E., Bang, E., Lee, B., Dwyer, J. M., & Duman, R. S. (2016). Fast-acting antidepressants rapidly stimulate ERK signaling and BDNF release in primary neuronal cultures. *Neuropharmacology*, *111*, 242–252. https://doi.org/10.1016/j.neuropharm.2016.09.011.

Levinson, A. J., Fitzgerald, P. B., Favalli, G., Blumberger, D. M., Daigle, M., & Daskalakis, Z. J. (2010). Evidence of cortical inhibitory deficits in major depressive disorder. *Biological Psychiatry*, *67*(5), 458–464. https://doi.org/10.1016/j.biopsych.2009.09.025.

Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., et al. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, *329*(5994), 959–964. https://doi.org/10.1126/science.1190287.

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., et al. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry*, *69*(8), 754–761. https://doi.org/10.1016/j.biopsych.2010.12.015.

Lin, L. C., & Sibille, E. (2015). Somatostatin, neuronal vulnerability and behavioral emotionality. *Molecular Psychiatry*, *20*(3), 377–387. https://doi.org/10.1038/mp.2014.184.

Liu, R. J., Fuchikami, M., Dwyer, J. M., Lepack, A. E., Duman, R. S., & Aghajanian, G. K. (2013). GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. *Neuropsychopharmacology*, *38*(11), 2268–2277. https://doi.org/10.1038/npp.2013.128.

Lorrain, D. S., Baccei, C. S., Bristow, L. J., Anderson, J. J., & Varney, M. A. (2003). Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: Modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience*, *117*(3), 697–706. https://doi.org/10.1016/s0306-4522(02)00652-8.

Lu, B., Wang, K. H., & Nose, A. (2009). Molecular mechanisms underlying neural circuit formation. *Current Opinion in Neurobiology*, *19*(2), 162–167. https://doi.org/10.1016/j.conb.2009.04.004.

Lumsden, E. W., Troppoli, T. A., Myers, S. J., Zanos, P., Aracava, Y., Kehr, J., et al. (2019). Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5160–5169. https://doi.org/10.1073/pnas.1816071116.

Luscher, B., & Fuchs, T. (2013). Adult hippocampal neurogenesis in the absence of serotonin (Commentary on Diaz et al.). *The European Journal of Neuroscience*, *38*(5), 2649. https://doi.org/10.1111/ejn.12335.

Luscher, B., & Fuchs, T. (2015). GABAergic control of depression-related brain states. *Advances in Pharmacology*, *73*, 97–144. https://doi.org/10.1016/bs.apha.2014.11.003.

Luscher, B., & Mohler, H. (2019). Brexanolone, a neurosteroid antidepressant, vindicates the GABAergic deficit hypothesis of depression and may foster resilience. *F1000Res*, *8*, 751. https://doi.org/10.12688/f1000research.18758.1.

Luscher, B., Shen, Q., & Sahir, N. (2011). The GABAergic deficit hypothesis of major depressive disorder. *Molecular Psychiatry*, *16*(4), 383–406. https://doi.org/10.1038/mp.2010.120.

Ma, K., Xu, A., Cui, S., Sun, M. R., Xue, Y. C., & Wang, J. H. (2016). Impaired GABA synthesis, uptake and release are associated with depression-like behaviors induced by chronic mild stress. *Translational Psychiatry*, *6*(10), e910. https://doi.org/10.1038/tp.2016.181.

MacKenzie, G., & Maguire, J. (2015). Chronic stress shifts the GABA reversal potential in the hippocampus and increases seizure susceptibility. *Epilepsy Research*, *109*, 13–27. https://doi.org/10.1016/j.eplepsyres.2014.10.003.

Maeng, S., Zarate, C. A., Jr., Du, J., Schloesser, R. J., McCammon, J., Chen, G., et al. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry*, *63*(4), 349–352. https://doi.org/10.1016/j.biopsych.2007.05.028.

Marsden, K. C., Beattie, J. B., Friedenthal, J., & Carroll, R. C. (2007). NMDA receptor activation potentiates inhibitory transmission through GABA receptor-associated protein-dependent exocytosis of GABA(A) receptors. *Journal of Neuroscience*, *27*(52), 14326–14337. https://doi.org/10.1523/JNEUROSCI.4433-07.2007.

Melon, L. C., Hooper, A., Yang, X., Moss, S. J., & Maguire, J. (2018). Inability to suppress the stress-induced activation of the HPA axis during the peripartum period engenders deficits in postpartum behaviors in mice. *Psychoneuroendocrinology*, *90*, 182–193. https://doi.org/10.1016/j.psyneuen.2017.12.003.

Miller, O. H., Bruns, A., Ben Ammar, I., Mueggler, T., & Hall, B. J. (2017). Synaptic regulation of a thalamocortical circuit controls depression-related behavior. *Cell Reports*, *20*(8), 1867–1880. https://doi.org/10.1016/j.celrep.2017.08.002.

Miller, O. H., Moran, J. T., & Hall, B. J. (2016). Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. *Neuropharmacology*, *100*, 17–26. https://doi.org/10.1016/j.neuropharm.2015.07.028.

Miller, O. H., Yang, L., Wang, C. C., Hargroder, E. A., Zhang, Y., Delpire, E., et al. (2014). GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. *eLife*, *3*, e03581. https://doi.org/10.7554/eLife.03581.

Moda-Sava, R. N., Murdock, M. H., Parekh, P. K., Fetcho, R. N., Huang, B. S., Huynh, T. N., et al. (2019). Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science*, *364*(6436). https://doi.org/10.1126/science.aat8078.

Modgil, A., Parakala, M. L., Ackley, M. A., Doherty, J. J., Moss, S. J., & Davies, P. A. (2017). Endogenous and synthetic neuroactive steroids evoke sustained increases in the efficacy of GABAergic inhibition via a protein kinase C-dependent mechanism. *Neuropharmacology*, *113*(Pt. A), 314–322. https://doi.org/10.1016/j.neuropharm.2016.10.010.

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *Journal of Neuroscience*, *17*(8), 2921–2927. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9092613.

Morris, P. J., Moaddel, R., Zanos, P., Moore, C. E., Gould, T. D., Zarate, C. A., Jr., et al. (2017). Synthesis and N-methyl-D-aspartate (NMDA) receptor activity of ketamine metabolites. *Organic Letters*, *19*(17), 4572–4575. https://doi.org/10.1021/acs.orglett.7b02177.

Muir, J., Arancibia-Carcamo, I. L., MacAskill, A. F., Smith, K. R., Griffin, L. D., & Kittler, J. T. (2010). NMDA receptors regulate GABAA receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the gamma2 subunit. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(38), 16679–16684. https://doi.org/10.1073/pnas.1000589107.

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: A two-site randomized controlled trial. *The American Journal of Psychiatry*, *170*(10), 1134–1142. https://doi.org/10.1176/appi.ajp.2013.13030392.

Murugan, S., Jakka, P., Namani, S., Mujumdar, V., & Radhakrishnan, G. (2019). The neurosteroid pregnenolone promotes degradation of key proteins in the innate immune signaling to suppress inflammation. *The Journal of Biological Chemistry*, *294*(12), 4596–4607. https://doi.org/10.1074/jbc.RA118.005543.

Musazzi, L., Tornese, P., Sala, N., & Popoli, M. (2017). Acute stress is not acute: Sustained enhancement of glutamate release after acute stress involves readily releasable pool size and synapsin I activation. *Molecular Psychiatry*, *22*(9), 1226–1227. https://doi.org/10.1038/mp.2016.175.

Musazzi, L., Treccani, G., & Popoli, M. (2012). Glutamate hypothesis of depression and its consequences for antidepressant treatments. *Expert Review of Neurotherapeutics*, *12*(10), 1169–1172. https://doi.org/10.1586/ern.12.96.

Newport, D. J., Carpenter, L. L., McDonald, W. M., Potash, J. B., Tohen, M., Nemeroff, C. B., et al. (2015). Ketamine and other NMDA antagonists: Early clinical trials and possible mechanisms in depression. *The American Journal of Psychiatry*, *172*(10), 950–966. https://doi.org/10.1176/appi.ajp.2015.15040465.

Nosyreva, E., Szabla, K., Autry, A. E., Ryazanov, A. G., Monteggia, L. M., & Kavalali, E. T. (2013). Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *Journal of Neuroscience*, *33*(16), 6990–7002. https://doi.org/10.1523/JNEUROSCI.4998-12.2013.

Nugent, A. C., Ballard, E. D., Gould, T. D., Park, L. T., Moaddel, R., Brutsche, N. E., et al. (2019). Ketamine has distinct electrophysiological and behavioral effects in depressed and healthy subjects. *Molecular Psychiatry*, *24*(7), 1040–1052. https://doi.org/10.1038/s41380-018-0028-2.

Oye, I., Paulsen, O., & Maurset, A. (1992). Effects of ketamine on sensory perception: Evidence for a role of N-methyl-D-aspartate receptors. *The Journal of Pharmacology and Experimental Therapeutics*, *260*(3), 1209–1213. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1312163.

Parakala, M. L., Zhang, Y., Modgil, A., Chadchankar, J., Vien, T. N., Ackley, M. A., et al. (2019). Metabotropic, but not allosteric, effects of neurosteroids on GABAergic inhibition depend on the phosphorylation of GABAA receptors. *The Journal of Biological Chemistry*, *294*(32), 12220–12230. https://doi.org/10.1074/jbc.RA119.008875.

Paul, I. A., & Skolnick, P. (2003). Glutamate and depression: Clinical and preclinical studies. *Annals of the New York Academy of Sciences*, *1003*, 250–272. https://doi.org/10.1196/annals.1300.016.

Pham, T. H., Defaix, C., Xu, X., Deng, S. X., Fabresse, N., Alvarez, J. C., et al. (2018). Common neurotransmission recruited in (R,S)-ketamine and (2R,6R)-Hydroxynorketamine-induced sustained antidepressant-like effects. *Biological Psychiatry*, *84*(1), e3–e6. https://doi.org/10.1016/j.biopsych.2017.10.020.

Pineles, S. L., Nillni, Y. I., Pinna, G., Irvine, J., Webb, A., Arditte Hall, K. A., et al. (2018). PTSD in women is associated with a block in conversion of progesterone to the GABAergic neurosteroids allopregnanolone and pregnanolone measured in plasma. *Psychoneuroendocrinology*, *93*, 133–141. https://doi.org/10.1016/j.psyneuen.2018.04.024.

Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology*, *33*(1), 88–109. https://doi.org/10.1038/sj.npp.1301574.

Popova, V., Daly, E. J., Trivedi, M., Cooper, K., Lane, R., Lim, P., et al. (2019). Efficacy and safety of flexibly dosed Esketamine nasal spray combined with a newly initiated oral antidepressant in treatment-resistant depression: A randomized double-blind active-controlled study. *The American Journal of Psychiatry*, *176*(6), 428–438. https://doi.org/10.1176/appi.ajp.2019.19020172.

Pouille, F., & Scanziani, M. (2004). Routing of spike series by dynamic circuits in the hippocampus. *Nature*, *429*(6993), 717–723. https://doi.org/10.1038/nature02615.

Preskorn, S. H., Baker, B., Kolluri, S., Menniti, F. S., Krams, M., & Landen, J. W. (2008). 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. *Journal of Clinical Psychopharmacology*, *28*(6), 631–637. https://doi.org/10.1097/JCP.0b013e31818a6cea.

Price, R. B., Shungu, D. C., Mao, X., Nestadt, P., Kelly, C., Collins, K. A., et al. (2009). Amino acid neurotransmitters assessed by proton magnetic resonance spectroscopy: Relationship to treatment resistance in major depressive disorder. *Biological Psychiatry*, *65*(9), 792–800. https://doi.org/10.1016/j.biopsych.2008.10.025.

Rasmusson, A. M., King, M. W., Valovski, I., Gregor, K., Scioli-Salter, E., Pineles, S. L., et al. (2019). Relationships between cerebrospinal fluid GABAergic neurosteroid levels and symptom severity in men with PTSD. *Psychoneuroendocrinology*, *102*, 95–104. https://doi.org/10.1016/j.psyneuen.2018.11.027.

Rasmusson, A. M., Pinna, G., Paliwal, P., Weisman, D., Gottschalk, C., Charney, D., et al. (2006). Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. *Biological Psychiatry*, *60*(7), 704–713. https://doi.org/10.1016/j.biopsych.2006.03.026.

Reich, D. L., & Silvay, G. (1989). Ketamine: An update on the first twenty-five years of clinical experience. *Canadian Journal of Anaesthesia*, *36*(2), 186–197. https://doi.org/10.1007/BF03011442.

Reid, C. A., Kim, T., Phillips, A. M., Low, J., Berkovic, S. F., Luscher, B., et al. (2013). Multiple molecular mechanisms for a single GABAA mutation in epilepsy. *Neurology*, *80*(11), 1003–1008. https://doi.org/10.1212/WNL.0b013e3182872867.

Ren, Z., Pribiag, H., Jefferson, S. J., Shorey, M., Fuchs, T., Stellwagen, D., et al. (2016). Bidirectional homeostatic regulation of a depression-related brain state by gamma-aminobutyric acidergic deficits and ketamine treatment. *Biological Psychiatry*, *80*(6), 457–468. https://doi.org/10.1016/j.biopsych.2016.02.009.

Riggs, L. M., Aracava, Y., Zanos, P., Fischell, J., Albuquerque, E. X., Pereira, E. F. R., et al. (2020). (2R,6R)-hydroxynorketamine rapidly potentiates hippocampal glutamatergic transmission through a synapse-specific presynaptic mechanism. *Neuropsychopharmacology*, *45*(2), 426–436. https://doi.org/10.1038/s41386-019-0443-3.

Rotroff, D. M., Corum, D. G., Motsinger-Reif, A., Fiehn, O., Bottrel, N., Drevets, W. C., et al. (2016). Metabolomic signatures of drug response phenotypes for ketamine and esketamine in subjects with refractory major depressive disorder: New mechanistic insights for rapid acting antidepressants. *Translational Psychiatry*, *6*(9), e894. https://doi.org/10.1038/tp.2016.145.

Rudy, B., Fishell, G., Lee, S., & Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental Neurobiology*, *71*(1), 45–61. https://doi.org/10.1002/dneu.20853.

Sanacora, G., Gueorguieva, R., Epperson, C. N., Wu, Y. T., Appel, M., Rothman, D. L., et al. (2004). Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Archives of General Psychiatry*, *61*(7), 705–713. https://doi.org/10.1001/archpsyc.61.7.705.

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, *62*(1), 63–77. https://doi.org/10.1016/j.neuropharm.2011.07.036.

Sassano-Higgins, S., Baron, D., Juarez, G., Esmaili, N., & Gold, M. (2016). A review of ketamine abuse and diversion. *Depression and Anxiety*, *33*(8), 718–727. https://doi.org/10.1002/da.22536.

Schlosser, B., ten Bruggencate, G., & Sutor, B. (1999). Local disinhibition of neocortical neuronal circuits causes augmentation of glutamatergic and GABAergic synaptic transmission in the rat neostriatum in vitro. *Experimental Neurology*, *157*(1), 180–193. https://doi.org/10.1006/exnr.1999.7039.

Schule, C., Romeo, E., Uzunov, D. P., Eser, D., di Michele, F., Baghai, T. C., et al. (2006). Influence of mirtazapine on plasma concentrations of neuroactive steroids in major depression and on 3alpha-hydroxysteroid dehydrogenase activity. *Molecular Psychiatry*, *11*(3), 261–272. https://doi.org/10.1038/sj.mp.4001782.

Seil, F. J. (2016). Activity-dependent inhibitory synaptogenesis in cerebellar cultures. *Brain Plasticity*, *1*(2), 207–214. https://doi.org/10.3233/BPL-160026.

Shen, Q., Lal, R., Luellen, B. A., Earnheart, J. C., Andrews, A. M., & Luscher, B. (2010). Gamma-aminobutyric acid-type A receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression. *Biological Psychiatry*, *68*(6), 512–520. https://doi.org/10.1016/j.biopsych.2010.04.024.

Shirayama, Y., & Hashimoto, K. (2017). Effects of a single bilateral infusion of R-ketamine in the rat brain regions of a learned helplessness model of depression. *European Archives of Psychiatry and Clinical Neuroscience*, *267*(2), 177–182. https://doi.org/10.1007/s00406-016-0718-1.

Shirayama, Y., & Hashimoto, K. (2018). Lack of antidepressant effects of (2R,6R)-hydroxynorketamine in a rat learned helplessness model: Comparison with (R)-ketamine. *The International Journal of Neuropsychopharmacology*, *21*(1), 84–88. https://doi.org/10.1093/ijnp/pyx108.

Short, B., Fong, J., Galvez, V., Shelker, W., & Loo, C. K. (2018). Side-effects associated with ketamine use in depression: A systematic review. *Lancet Psychiatry*, *5*(1), 65–78. https://doi.org/10.1016/S2215-0366(17)30272-9.

Sibille, E., Morris, H. M., Kota, R. S., & Lewis, D. A. (2011). GABA-related transcripts in the dorsolateral prefrontal cortex in mood disorders. *The International Journal of Neuropsychopharmacology*, *14*(6), 721–734. https://doi.org/10.1017/S1461145710001616.

Silberberg, G., & Markram, H. (2007). Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron*, *53*(5), 735–746. https://doi.org/10.1016/j.neuron.2007.02.012.

Singh, J. B., Fedgchin, M., Daly, E. J., De Boer, P., Cooper, K., Lim, P., et al. (2016). A double-blind, randomized, placebo-controlled, dose-frequency study of intravenous ketamine in patients with treatment-resistant depression. *The American Journal of Psychiatry*, *173*(8), 816–826. https://doi.org/10.1176/appi.ajp.2016.16010037.

Singh, J. B., Fedgchin, M., Daly, E., Xi, L., Melman, C., De Bruecker, G., et al. (2016). Intravenous Esketamine in adult treatment-resistant depression: A double-blind, double-randomization, placebo-controlled study. *Biological Psychiatry*, *80*(6), 424–431. https://doi.org/10.1016/j.biopsych.2015.10.018.

Su, T. P., Chen, M. H., Li, C. T., Lin, W. C., Hong, C. J., Gueorguieva, R., et al. (2017). Dose-related effects of adjunctive ketamine in Taiwanese patients with treatment-resistant depression. *Neuropsychopharmacology*, *42*(13), 2482–2492. https://doi.org/10.1038/npp.2017.94.

Sutton, M. A., Taylor, A. M., Ito, H. T., Pham, A., & Schuman, E. M. (2007). Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron*, *55*(4), 648–661. https://doi.org/10.1016/j.neuron.2007.07.030.

Suzuki, K., Nosyreva, E., Hunt, K. W., Kavalali, E. T., & Monteggia, L. M. (2017). Effects of a ketamine metabolite on synaptic NMDAR function. *Nature*, *546*(7659), E1–E3. https://doi.org/10.1038/nature22084.

Synowiec, A. S., Singh, D. S., Yenugadhati, V., Valeriano, J. P., Schramke, C. J., & Kelly, K. M. (2013). Ketamine use in the treatment of refractory status epilepticus. *Epilepsy Research*, *105*(1–2), 183–188. https://doi.org/10.1016/j.eplepsyres.2013.01.007.

Tan, Z., Hu, H., Huang, Z. J., & Agmon, A. (2008). Robust but delayed thalamocortical activation of dendritic-targeting inhibitory interneurons. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(6), 2187–2192. https://doi.org/10.1073/pnas.0710628105.

Thompson, S. M., Kallarackal, A. J., Kvarta, M. D., Van Dyke, A. M., LeGates, T. A., & Cai, X. (2015). An excitatory synapse hypothesis of depression. *Trends in Neurosciences*, *38*(5), 279–294. https://doi.org/10.1016/j.tins.2015.03.003.

Thomson, A. M., West, D. C., & Lodge, D. (1985). An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: A site of action of ketamine? *Nature*, *313*(6002), 479–481. https://doi.org/10.1038/313479a0.

Treccani, G., Musazzi, L., Perego, C., Milanese, M., Nava, N., Bonifacino, T., et al. (2014). Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Molecular Psychiatry*, *19*(4), 433–443. https://doi.org/10.1038/mp.2014.5.

Tripp, A., Kota, R. S., Lewis, D. A., & Sibille, E. (2011). Reduced somatostatin in subgenual anterior cingulate cortex in major depression. *Neurobiology of Disease*, *42*(1), 116–124. https://doi.org/10.1016/j.nbd.2011.01.014.

Trullas, R., & Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European Journal of Pharmacology*, *185*(1), 1–10. https://doi.org/10.1016/0014-2999(90)90204-J.

Tyagarajan, S. K., Ghosh, H., Yevenes, G. E., Imanishi, S. Y., Zeilhofer, H. U., Gerrits, B., et al. (2013). Extracellular signal-regulated kinase and glycogen synthase kinase 3beta regulate gephyrin postsynaptic aggregation and GABAergic synaptic function in a calpain-dependent mechanism. *The Journal of Biological Chemistry*, *288*(14), 9634–9647. https://doi.org/10.1074/jbc.M112.442616.

Tyagarajan, S. K., Ghosh, H., Yevenes, G. E., Nikonenko, I., Ebeling, C., Schwerdel, C., et al. (2011). Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(1), 379–384. https://doi.org/10.1073/pnas.1011824108.

Tyler, M. W., Yourish, H. B., Ionescu, D. F., & Haggarty, S. J. (2017). Classics in chemical neuroscience: Ketamine. *ACS Chemical Neuroscience*, *8*(6), 1122–1134. https://doi.org/10.1021/acschemneuro.7b00074.

U.S. Food and Drug Administration. (2019). *FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic [Press release]*. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified.

Uzunova, V., Sheline, Y., Davis, J. M., Rasmusson, A., Uzunov, D. P., Costa, E., et al. (1998). Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(6), 3239–3244. https://doi.org/10.1073/pnas.95.6.3239.

Varga, Z., Csabai, D., Miseta, A., Wiborg, O., & Czeh, B. (2017). Chronic stress affects the number of GABAergic neurons in the orbitofrontal cortex of rats. *Behavioural Brain Research*, *316*, 104–114. https://doi.org/10.1016/j.bbr.2016.08.030.

Veeraiah, P., Noronha, J. M., Maitra, S., Bagga, P., Khandelwal, N., Chakravarty, S., et al. (2014). Dysfunctional glutamatergic and gamma-aminobutyric acidergic activities in prefrontal cortex of mice in social defeat model of depression. *Biological Psychiatry*, *76*(3), 231–238. https://doi.org/10.1016/j.biopsych.2013.09.024.

Voineskos, D., Blumberger, D. M., Zomorrodi, R., Rogasch, N. C., Farzan, F., Foussias, G., et al. (2019). Altered transcranial magnetic stimulation-electroencephalographic markers of inhibition and excitation in the dorsolateral prefrontal cortex in major depressive disorder. *Biological Psychiatry*, *85*(6), 477–486. https://doi.org/10.1016/j.biopsych.2018.09.032.

Vollenweider, F. X., Leenders, K. L., Oye, I., Hell, D., & Angst, J. (1997). Differential psychopathology and patterns of cerebral glucose utilisation produced by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET). *European Neuropsychopharmacology*, 7(1), 25–38. https://doi.org/10.1016/S0924-977x(96)00042-9.

Wang, H., Ren, W. H., Zhang, Y. Q., & Zhao, Z. Q. (2005). GABAergic disinhibition facilitates polysynaptic excitatory transmission in rat anterior cingulate cortex. *Biochemical and Biophysical Research Communications*, *338*(3), 1634–1639. https://doi.org/10.1016/j.bbrc.2005.10.132.

White, P. F., Schuttler, J., Shafer, A., Stanski, D. R., Horai, Y., & Trevor, A. J. (1985). Comparative pharmacology of the ketamine isomers. Studies in volunteers. *British Journal of Anaesthesia*, *57*(2), 197–203. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3970799.

WHO. (2017). *"Depression: let's talk" says WHO, as depression tops list of causes of ill health*. Retrieved from http://www.who.int/mediacentre/news/releases/2017/world-health-day/en/.

Widman, A. J., & McMahon, L. L. (2018). Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(13), E3007–E3016. https://doi.org/10.1073/pnas.1718883115.

Wohleb, E. S., Wu, M., Gerhard, D. M., Taylor, S. R., Picciotto, M. R., Alreja, M., et al. (2016). GABA interneurons mediate the rapid antidepressant-like effects of scopolamine. *The Journal of Clinical Investigation*, *126*(7), 2482–2494. https://doi.org/10.1172/JCI85033.

Wuchter, J., Beuter, S., Treindl, F., Herrmann, T., Zeck, G., Templin, M. F., et al. (2012). A comprehensive small interfering RNA screen identifies signaling pathways required for gephyrin clustering. *Journal of Neuroscience*, *32*(42), 14821–14834. https://doi.org/10.1523/JNEUROSCI.1261-12.2012.

Xiong, Z., Fujita, Y., Zhang, K., Pu, Y., Chang, L., Ma, M., et al. (2019). Beneficial effects of (R)-ketamine, but not its metabolite (2R,6R)-hydroxynorketamine, in the depression-like phenotype, inflammatory bone markers, and bone mineral density in a chronic social defeat stress model. *Behavioural Brain Research*, *368*, 111904. https://doi.org/10.1016/j.bbr.2019.111904.

Yamaguchi, J. I., Toki, H., Qu, Y., Yang, C., Koike, H., Hashimoto, K., et al. (2018). (2R,6R)-Hydroxynorketamine is not essential for the antidepressant actions of (R)-ketamine in mice. *Neuropsychopharmacology*, *43*(9), 1900–1907. https://doi.org/10.1038/s41386-018-0084-y.

Yang, C., Qu, Y., Abe, M., Nozawa, D., Chaki, S., & Hashimoto, K. (2017). (R)-ketamine shows greater potency and longer lasting antidepressant effects than its metabolite (2R,6R)-hydroxynorketamine. *Biological Psychiatry*, *82*(5), e43–e44. https://doi.org/10.1016/j.biopsych.2016.12.020.

Yao, N., Skiteva, O., Zhang, X., Svenningsson, P., & Chergui, K. (2018). Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit. *Molecular Psychiatry*, *23*(10), 2066–2077. https://doi.org/10.1038/mp.2017.239.

Yuen, E. Y., Wei, J., Liu, W., Zhong, P., Li, X., & Yan, Z. (2012). Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*, *73*(5), 962–977. https://doi.org/10.1016/j.neuron.2011.12.033.

Zanos, P., Highland, J. N., Stewart, B. W., Georgiou, P., Jenne, C. E., Lovett, J., et al. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(13), 6441–6450. https://doi.org/10.1073/pnas.1819540116.

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481–486. https://doi.org/10.1038/nature17998.

Zarate, C. A., Jr., Brutsche, N., Laje, G., Luckenbaugh, D. A., Venkata, S. L., Ramamoorthy, A., et al. (2012). Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. *Biological Psychiatry*, *72*(4), 331–338. https://doi.org/10.1016/j.biopsych.2012.03.004.

Zarate, C. A., Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*, *63*(8), 856–864. https://doi.org/10.1001/archpsyc.63.8.856.

Zhang, J. C., Li, S. X., & Hashimoto, K. (2014). R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology, Biochemistry, and Behavior*, *116*, 137–141. https://doi.org/10.1016/j.pbb.2013.11.033.

Zhang, K., Toki, H., Fujita, Y., Ma, M., Chang, L., Qu, Y., et al. (2018). Lack of deuterium isotope effects in the antidepressant effects of (R)-ketamine in a chronic social defeat stress model. *Psychopharmacology*, *235*(11), 3177–3185. https://doi.org/10.1007/s00213-018-5017-2.

Zhao, X., Venkata, S. L., Moaddel, R., Luckenbaugh, D. A., Brutsche, N. E., Ibrahim, L., et al. (2012). Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. *British Journal of Clinical Pharmacology*, *74*(2), 304–314. https://doi.org/10.1111/j.1365-2125.2012.04198.x.

Zhou, W., Dong, L., Wang, N., Shi, J. Y., Yang, J. J., Zuo, Z. Y., et al. (2014). Akt mediates GSK-3beta phosphorylation in the rat prefrontal cortex during the process of ketamine exerting rapid antidepressant actions. *Neuroimmunomodulation*, *21*(4), 183–188. https://doi.org/10.1159/000356517.

Zhou, W., Wang, N., Yang, C., Li, X. M., Zhou, Z. Q., & Yang, J. J. (2014). Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. *European Psychiatry*, *29*(7), 419–423. https://doi.org/10.1016/j.eurpsy.2013.10.005.