ELSEVIER

Novel treatments like ketamine produce rapid and sustained antidepressant effects via targeting the glutamatergic system of the PFC. Receptor selectivity and local PFC microcircuitry provide new avenues for the development of safer and more efficacious agents.

Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity

Q1 **Danielle M. Gerhard[1], Eric S. Wohleb[2] and Ronald S. Duman[2]**

Q2 [1] Department of Psychology, Yale University, New Haven, CT 06511, United States
Q3 [2] Molecular Psychiatry, Yale University, New Haven, CT 06711, United States

Major depression is a chronic and debilitating illness that effects approximately 1 in 5 people, but currently available treatments are limited by low rates of efficacy, therapeutic time lag, and undesirable side effects. Recent efforts have been directed towards investigating rapid-acting agents that reverse the behavioral and neuronal deficits of chronic stress and depression, notably the glutamate NMDA receptor antagonist ketamine. The cellular mechanisms underlying the rapid antidepressant actions of ketamine and related agents are discussed, as well as novel, selective glutamatergic receptor targets that are safer and have fewer side effects.

Introduction

Q4 Major Depressive Disorder (MDD) is a leading cause of disability worldwide and in the United States alone has a lifetime prevalence of 17% [1]. Current estimates by the World Health Organization caution that MDD will be the second leading cause of disability by 2020 [2]. In addition to the increasing prevalence and associated behavioral sequelae, MDD exacts a very large economic burden on society, with an estimated cost of $210.5 billion annually as a result of treatment and workplace related expenses [3]. Furthermore, twice as many people die by suicide each year than by homicide, with 23% of suicide victims on an antidepressant treatment at the time of death, demonstrating a lack of efficacy [4].

These statistics are compounded by the limitations of currently available antidepressants, including a significant time lag for treatment response and modest rates of efficacy. Current agents, notably the serotonin (5-HT) selective reuptake inhibitor (SSRI) antidepressants, require several weeks to months of administration before a therapeutic response is observed. Moreover, only one-third of patients will respond to the first antidepressant prescribed and another third will only respond following multiple trials that can take many months to years. Importantly, approximately one-third of individuals diagnosed with depression fail to respond to two or more first line antidepressant treatments and are consequently characterized as having treatment resistant depression (TRD) [5].

Given the extensive personal and economic consequences and anticipated rise in rates of MDD, more efficacious and rapid-acting treatments are sorely needed. Current pharmacological

Corresponding author: Duman, R.S. (ronald.duman@yale.edu)

1359-6446/© 2016 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.drudis.2016.01.016

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

treatments, while effective for some, are largely inadequate and are associated with undesirable side effects. One logical step towards the development of effectual treatments is to better understand the etiology of the disease. Much of the work has focused on deficits in monoamine neurotransmitter systems, including 5-HT and norepinephrine, and is based largely on the discovery that drugs that block the metabolism or reuptake of monoamines have clinical efficacy [6]. However, the therapeutic limitations of these agents, combined with a lack of evidence to support a monoamine deficiency hypothesis, have lead to new avenues of investigation.

While the underlying etiology and pathophysiology of depression remain incomplete, clinical and basic research studies are beginning to provide evidence that depression is associated with atrophy of neurons in cortical and limbic brain regions that control mood and emotion [7,8]. In addition, the discovery that antagonists of the N-methyl-D-aspartate (NMDA) receptor, notably ketamine, produce rapid improvement in depressive symptoms (within hours), even in TRD patients, has shifted efforts towards novel agents targeting the glutamatergic system. Importantly, basic research studies demonstrate that ketamine rapidly increases synaptic connections in the PFC and reverses the deficits caused by chronic stress [9,10]. This pioneering work on ketamine, a nonselective NMDA receptor antagonist, has launched investigations into a variety of rapid agents that act at different NMDA sites or within the glutamate system. The ultimate goal in the development of these agents is reversal of the stress-induced cellular and molecular deficits, most notably the atrophy of neurons, caused by stress and depression.

There are other systems that are negatively affected by depression, including disruption of metabolic and immune/inflammatory pathways. However, the focus of the current review is on emerging treatments targeting NMDA receptors and the glutamate neurotransmitter system that reverse stress-induced behavioral, molecular, and structural deficits of MDD. We discuss the evidence for neuronal atrophy as a pathophysiological marker of MDD, how rapid acting agents such as ketamine and other NMDA modulating agents reverse these deficits, and how these agents differ from conventional treatments. Furthermore, we explore how the rapid antidepressant actions of these drugs, in particular induction of glutamate transmission, may be mediated through disinhibition of GABAergic interneurons in the PFC. These discoveries highlight a new era of promise for the development of more effective and fast-acting agents with fewer side effects.

Currently available treatments for depression

During the 1950s, the field of psychopharmacology experienced an explosion in the development of drugs for the treatment of illnesses, providing psychiatrists with new tools to complement traditional therapy techniques. The first drugs to be identified as antidepressants were monoaminergic agents and consequently the disease was investigated as a deficiency of monoamine neurotransmitters, particularly 5-HT, norepinephrine, and dopamine. Drugs used to specifically treat clinical depression came about fortuitously following the discovery of the tricyclic antidepressant (TCA) reuptake inhibitors and the monoamine oxidase inhibitors (MAOIs). The monoamine hypothesis of depression evolved from the observation that these drugs, effective in treating depression, increase levels of serotonin and norepinephrine. Unfortunately, as a result of their non-selective binding profile, the TCAs have undesirable side effects, including drowsiness and sedation, memory and cognitive impairments, dry mouth, and increased heart rate [11]. Additionally, although MAOIs are effective, their use is highly uncommon because of the potentially fatal reaction of MAOIs with foods high in tyramine (fermented foods) resulting in increased blood pressure, heart attack and stroke.

To improve the side effect profile, drug development focused on more selective reuptake inhibitors, leading to several SSRIs reaching the market, notably fluoxetine (Prozac). These agents are better tolerated as expected, but in addition to the therapeutic time lag, side effects include acute nausea and headaches as well as chronic sexual dysfunction, weight gain, and diminished REM sleep [12,13]. Furthermore, when treatment is stopped patients can suffer from discontinuation syndrome, characterized by insomnia, headaches, nausea and irritability [14]. There have been several other promising drug development programs based on preclinical studies and target development that have failed in human clinical trials, including corticotrophin releasing factor receptor-1 (CRF-R1) and substance P antagonists [15,16]. These failures highlight the difficulty and limitations of preclinical rodent models used for validating antidepressant drug targets, as well as the difficulties of conducting large-scale clinical trials.

Challenges for MDD drug development

There are several major hurdles in the development of therapeutic agents for MDD. One major impediment is heterogeneity, as MDD is a syndrome that is widely believed to have multiple subtypes and causes. Genetic heritability is relatively low (approximately 40 percent), and environmental factors, notably stress or trauma, are often associated with depression and interact with genetic vulnerability [17]. Another problem is the lack of a good biomarker of depression or treatment response. Further characterization of MDD and subtype specific pathophysiology, as well as development of biomarkers, will lead to treatments targeting selected abnormalities that are more efficacious and have fewer side effects. Another problem facing researchers is the placebo effect when testing new drugs. A meta-analysis of published clinical trials of treatments for MDD found that placebo responses are highly variable between studies and generally show substantial effects on measures of depression. Furthermore, placebo effects have been on the rise since the 1980s making it more and more difficult to identify agents that are significantly better than placebo [18].

Pathophysiological consequences of stress and depression

The adverse effects of MDD extend well beyond perceptible behavioral deficits, as decades of research have begun to elucidate the cellular and molecular changes that contribute to the underlying pathophysiology of depression and stress related illnesses. Clinical and pre-clinical studies have focused on the neural circuits that are altered following prolonged bouts of stress and depression. Although not exclusive, the prefrontal cortex (PFC), hippocampus, and amygdala comprise what is commonly referred to as a depression circuit [19]. These studies have contributed to further elucidation of MDD pathophysiology and can help direct the rational

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

design of novel antidepressants that correct the disrupted synaptic and circuit level alterations.

Stress and depression cause neuronal atrophy

Brain neuroimaging studies have shown a reduction in blood flow and glucose metabolism, and thus activity, in the PFC and parts of the hippocampus, which can partially be attributed to reduction in volume possibly as a result of neuronal atrophy in these regions [20]. Furthermore, the volume reduction is inversely correlated with the number of prior depressive episodes [21]. As there is a reduction in activity of the PFC and hippocampus, top-down control of the amygdala, which regulates fear, anxiety, and mood, is dampened [8,22,23]. Reduced volume of PFC and hippocampus could contribute to impairments in executive function and memory often observed in MDD patients [8,24] (Fig. 1). Although postmortem studies are limited, there is one report of decreased synapse number in the dorsal lateral PFC of MDD subjects [20].

Preclinical studies in rodent models demonstrate that chronic stress can result in symptoms of depression, including helplessness/despair and anhedonia, as seen in the forced-swim test (FST) and sucrose preference test (SPT), respectively [10]. The behavioral deficits of chronic stress are associated with reduced number and function of spine synapses in the medial PFC (mPFC) [10,25–27] (Fig. 1). Similar effects have been observed in CA3 pyramidal cells of the hippocampus following chronic stress [28]. Evidence of a direct link between PFC synapse number and behavior is provided by a recent report that an inhibitor of mTORC1 signaling (REDD1) that decreases synapse number causes helpless and anhedonic behavior in rodents [29]. Together, these human and rodent studies of PFC and hippocampus indicate that depression can be viewed as a mild neurodegenerative disease, characterized by neuronal atrophy and that treatments should target neural repair systems and reversal of the observed atrophy [30].

Role of BDNF in stress and depression

Chronic stress models of depression in rodents report reduced levels of BDNF mRNA and protein, particularly in the dentate gyrus of the hippocampus, and chronic antidepressant treatment reverses these effects [31,32]. Central or peripheral administration of BDNF has been shown to produce antidepressant-like effects, providing further support for the neurogenic hypothesis and the role of BDNF in the pathophysiology of depression [33]. In addition, over-expression of BDNF in the hippocampus prevents the development of depressive-like behaviors following chronic stress while knockdown of BDNF during adolescence is sufficient to cause depressive-like behaviors and prolonged increases in corticosterone release [34].

Individual differences in stress resilience and susceptibility have been linked to the expression and BDNF and genetic polymorphisms, particularly the Val/Met polymorphism at codon 66 (Val66Met). The Met allele blocks the processing and activity dependent release of BDNF [35] and is associated with increased vulnerability to stress and depression. Specifically, Met carriers exhibit a reduction in hippocampal volume that is comparable to

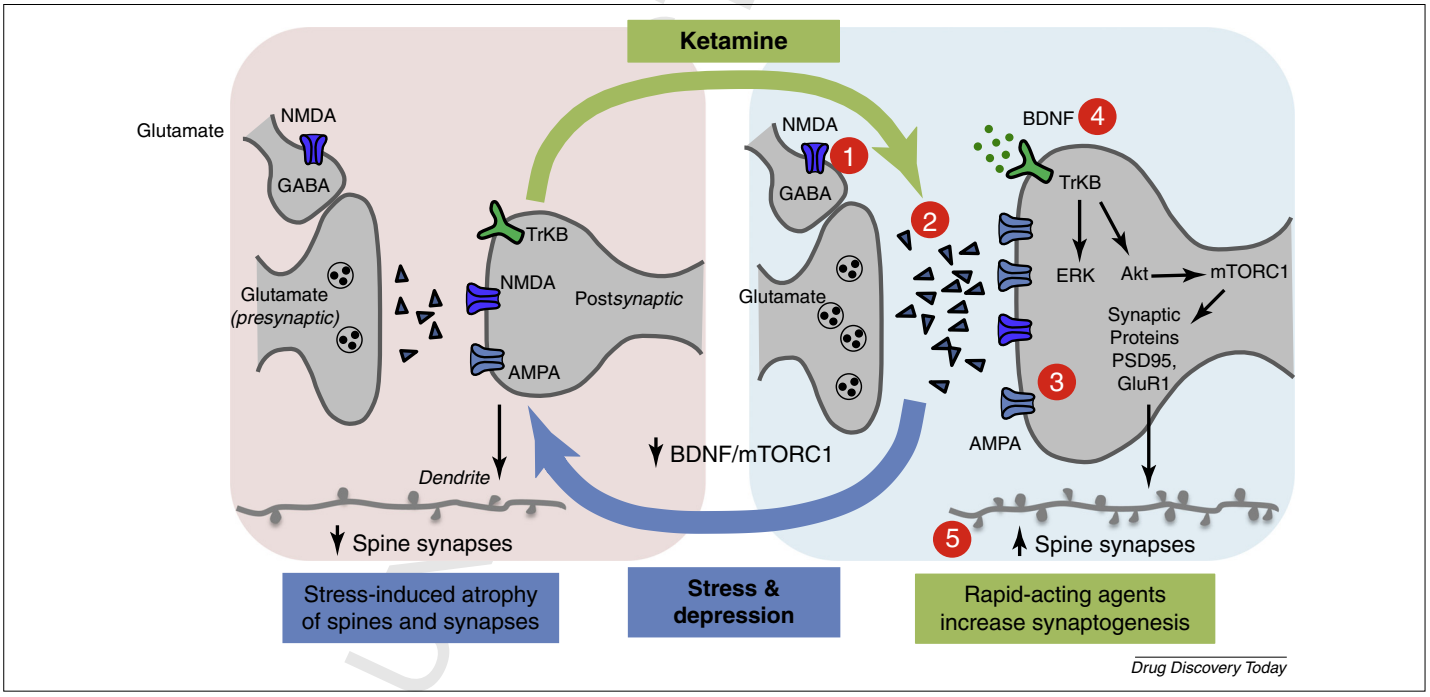

FIGURE 1

Stress and depression decrease, while rapid-acting antidepressants increase, synaptogenesis. Prolonged stress and depression is known to cause atrophy of spine and synapses in brain regions implicated in depression, notably the prefrontal cortex and hippocampus. Preclinical studies suggest that this is a result of reduced production and release of brain derived neurotrophic factor (BDNF). Rapid acting antidepressants (e.g., ketamine and scopolamine) reverse these stress-induced deficits by inducing a cascade of glutamate neurotransmitter signaling events. (1) Ketamine is thought to act via blockade of N-methyl-D-aspartate (NMDA) receptors located on inhibitory λ-aminobutyric acid (GABA)-ergic neurons. (2) This causes disinhibition of the pyramidal cells, resulting in a burst of glutamate transmission. (3) Increased glutamate release activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, resulting in depolarization and calcium influx. (4) Depolarization of the cell induces release of BDNF and activation of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. (5) Stimulation of mTORC1 increases the synthesis of synaptic proteins that results in increased number and function of spine synapses.

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

depressed patients not receiving treatment [36] as well as reduced activity in the ventromedial prefrontal cortex and heightened activity in the amygdala [37]. When compared with Val/Val homozygotes, Met-allele carriers exhibit heightened levels of anxiety and depression [38]. BDNF Val/Met transgenic mice have provided additional insight into the role of BDNF in the development and treatment of depression. Rodents carrying the Met-allele show reduced spine and dendrite complexity in the hippocampus and prefrontal cortex [39,40], as well as diminished synaptic plasticity in the infralimbic medial PFC and reduced dendritic secretion of BDNF in the hippocampus [35,41]. Additionally, SSRI treatment does not increase BDNF in Met/Met mice [42]. Together, these pre-clinical and clinical findings over the past decade have established support for a role of BDNF in the etiology and treatment of depression.

In contrast to the effects seen in the PFC and hippocampus, the neurons in the basolateral nucleus of the amygdala undergo hypertrophy following chronic stress, which could contribute to loss of emotional control and reduced motivation in individuals suffering from depression [43]. Disruption of these and other cortical and limbic structures demonstrate that depression is not a disorder confined to a specific brain region but is rather owing to dysfunctional neural circuitries controlling mood, cognition, memory and reward [19]. Although depression should be considered a system-wide disorder, the PFC is an ideal target for treatments as it receives and sends substantial projections throughout the brain to both cortical and subcortical regions that have been implicated in depression.

Discovery of the rapid antidepressant actions of ketamine

The limitations of SSRIs and other antidepressants provided the impetus for the development of novel strategies for the treatment of MDD. The seminal findings of Berman and colleagues in the early 2000s showing the rapid-acting (within hours) and sustained (up to one week) antidepressant effects following intravenous administration of low dose ketamine, an NMDA receptor antagonist, has prompted pre-clinical investigations into the mechanisms underlying the actions of this agent [44]. Subsequent replication studies have also highlighted ketamine's efficacy in patients suffering from TRD as well as rapidly reducing suicidal ideations [45,46]. These findings, by a mechanism completely different from traditional monoaminergic agents, represent the most significant advance in the field of antidepressant drug development in over six decades.

Ketamine is dissociative anesthetic used for surgical procedures in young and old humans, as well as veterinary medicine. It is also a drug of abuse and repeated, daily use of high doses can cause cognitive and memory impairments as well as schizotypal symptoms and neurotoxicity [47]. It should be noted that the antidepressant effects of ketamine have been reported to begin after the acute psychotomimetic, dissociative and euphoric effects have subsided, suggesting that the antidepressant effects are not just a result of acute elevated mood [44]. Because of the abuse potential and undesirable side effects of ketamine, investigations into additional, safe agents have been underway. In addition, studies to determine a therapeutically effective and safe dosing regiment for ketamine in addition to agents that could sustain the effects of acute ketamine treatment are being conducted.

Mechanisms underlying rapid-acting antidepressants

On-going efforts to develop more efficacious treatments strive to mimic the rapid-acting molecular and cellular effects of agents like ketamine. Because the monumental discovery of the antidepressant properties of acute, sub-anesthetic ketamine treatment, researchers have been trying to determine the molecular changes mediating the rapid-acting time course of ketamine and similar compounds. Acute ketamine treatment stimulates a rapid cascade of molecular and cellular events that underlie the long-lasting synaptic and behavioral responses.

First, ketamine acts via blockade of NMDA receptors located on GABAergic interneurons, resulting in disinhibition of glutamate neurons. These neurons are thought to be more sensitive to low doses of ketamine because they are tonically firing and thereby have open channels free for ketamine to enter. The increase in glutamate transmission results in a burst from pyramidal neurons in the prefrontal cortex caused depolarization of postsynaptic [48,49]. These effects are specific to sub-anesthetic doses of ketamine as no changes in glutamate release are observed at anesthetic doses [48,50]. Third, increased glutamate activates AMPA receptors, resulting in depolarization of the cell and an influx of calcium through L-type voltage gated calcium channels (VDCC). Studies using AMPA receptor and VDCC antagonists have shown that activation of these postsynaptic receptors and channels is necessary for the rapid-acting behavioral, molecular and structural effects of ketamine [9,51,52]. Fourth, depolarization of the cell induces release of BDNF and activation of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway [9]. The mTORC1 signaling pathway regulates protein translation following alterations in neuronal activity contributing to synaptic plasticity [53]. Fifth, these molecular signaling cascades are believed to underlie changes in PFC pyramidal neurons following ketamine treatment, including enhanced number and function of dendritic spines [9,54]. Ketamine administration rapidly increases phosphorylation of mTORC1 signaling proteins in the PFC and the behavioral effects of ketamine are blocked by pretreatment of the selective mTORC1 inhibitor rapamycin, providing further support for the role of mTORC1 in the rapid-acting antidepressant effects of ketamine [9,55] (Fig. 1). In addition, a recent study has demonstrated that expression of signaling proteins that increase or decrease mTORC1 signaling in the medial PFC produce antidepressant or pro-depressive actions, respectively [29,56].

While classical antidepressants eventually result in an increase in BDNF levels, they require chronic activation of second messenger systems and thus produce subtle, indirect effects spine number and function. Notably, classical antidepressants do not cause a burst of glutamate that is required for activity dependent release of BDNF (Fig. 1). Levels of synaptic proteins, which presumably correlate with synapse formation, are increased in the prefrontal cortex within 2 hours of ketamine administration [9]. Furthermore, acute ketamine treatment is capable of rapidly reversing behavioral (anhedonia) and synaptic deficits resulting from exposure to chronic unpredictable stress for 3 weeks [10]. The molecular and synaptic changes following ketamine have largely been investigated in the PFC yet the exact mechanism of action of ketamine

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

is still unclear. Understanding the microcircuitry of the PC can lend insight into the mechanisms of ketamine.

Current drugs under investigation: glutamatergic agents

The shift in our understanding of depression as a disorder of synaptic deficits has transformed the modus operandi of drug development. No longer designed to directly increase monoamines, treatments are now being designed to increase synaptic plasticity and to oppose the cellular effects of stress and depression. Ketamine, an open-channel blocker of the NMDA receptor, was the first rapid-acting agent discovered and many agents targeting the NMDA receptor complex have emerged since then. While glutamatergic agents targeting the ionotropic NMDA receptor have shown promise, other classes of drugs that influence glutamate transmission have also received attention, including NMDA receptor subtypes and presynaptic metabotropic glutamate 2/3 receptors (mGlu2/3). In addition, clinical studies have found that scopolamine, a nonselective muscarinic receptor antagonist, also produces rapid antidepressant actions in depressed patients and preclinical studies have demonstrated a role for increased glutamate transmission [54,57].

All of the ionotropic, glutamatergic agents target the NMDA receptor, however, they differ in the location of binding on the channel receptor complex (Fig. 2). NMDA receptors have a heterotetramer composition of two obligatory GluN1 and two GluN2 subunits surrounding an ion-permeating channel that is blocked by magnesium in a voltage-dependent manner; once magnesium is removed open channel blockers like ketamine bind within the channel pore and block ion flow [58]. GluN1 subunits contain glycine-binding sites, are present in almost all neurons throughout development, and lack variability as they are produced by only one gene. By contrast, the GluN2 subunits are generated by four different genes (GluN2A, B, C, & D), produce unique physiological, biochemical, a pathophysiological properties, and have divergent regional localization, making them an ideal target in the development of selective therapeutic agents [59,60]. In the adult rat, GluN2 C is primarily expressed in the cerebellum, GluN2D is very low-expressing in midbrain structures, and GluN2A and GluN2B are expressed throughout the forebrain. The extracellular region contains an allosteric regulatory domain where selective agents bind [58]. Depending on where an agent binds to the NMDA receptor complex there is a distinctive physiological, and thus therapeutic response and side effect profile. Furthermore, it is believed that this determines whether or not a particular agent is effective as a rapid-acting antidepressant.

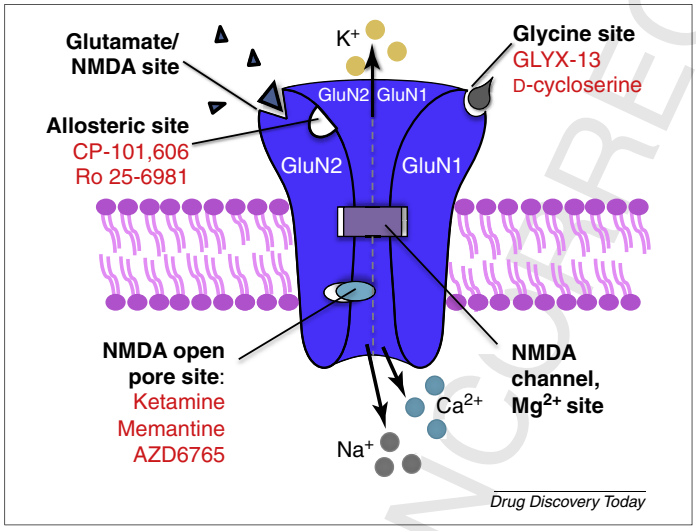

FIGURE 2

NMDA receptor targets for rapid acting antidepressants. Agents targeting ionotropic N-methyl-D-aspartate receptors have shown promise in both preclinical and clinical studies for their rapid-acting antidepressant effects. Differing therapeutic effects of these drugs may be explained by their binding sites on NMDARs. NMDA receptors are heterotetramers with two GluN1 and two GluN2 subunits surrounding an ion permeating channel that is blocked by Mg^{2+}; this Mg^{2+} block is removed upon depolarization, which then allows ketamine and other channel blockers like memantine and AZD6765 to enter the pore. The glycine-site is another modulatory site located on the extracellular GluN1 subunit; D-cycloserine binds at this site and GLYX-13 nearby. Selective GluN2B antagonists CP-101,606 and Ro-25-6981 bind to an extracellular allosteric site on the GluN2B subunit.

Open-channel Blockers

Although ketamine is one of the most widely investigated agents, other nonselective NMDA antagonists have been investigated as potential treatments. AZD6765 (lanicemine), originally designed for the treatment of stroke, has surfaced as a potential rapid acting agent. A clinical investigation in patients with TRD found that one third of the patients exhibited an antidepressant response in under two hours of treatment initiation [61]. Furthermore, patients only reported minor side effects, including dizziness and headaches without the psychotomimetic or dissociative effects characteristic of ketamine. Although the effects were rapid, they were also short-lived, lasting only approximately one hour. A more recent large clinical study found that only with repeated dosing over a three week period (3-day intervals) did the drug result in significantly improved depression scores when compared to placebo controls and this difference was sustained for two weeks after the last infusion [62]. However, there are several limitations to this study, including a large placebo response and required repeated dosing. Another study of repeated AZD6765 treatment has been conducted (not published), but did not find a significant antidepressant response, due in part to a large placebo response. A subgroup of TRD patients, with lower placebo response, did show a significant antidepressant response in this study (Dr. Sanacora, personal communication).

Another nonselective NMDA antagonist that has been examined for the treatment of depression is memantine (Namenda). Memantine is an approved drug used as a treatment for Alzheimer's Disease but owing to its NMDA antagonist properties, it has been considered as a treatment for MDD. Unfortunately, clinical investigations have not been fruitful, failing to find any significant effects of memantine above placebo [63]. A recent study links memantine's lack of efficacy to its inability to inhibit phosphorylation of eukaryotic elongation factor 2 or increase the subsequent increase in BDNF expression, both of which occur with ketamine [64]. Attempts to isolate the antidepressant effects from the psychotomimetic effects have shifted research towards selective NMDA receptor antagonists, specifically the NMDA receptor subtype 2B (GluN2B) antagonists (Fig. 2).

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

Channel blocking and drug efficacy

Open channel blockers like memantine have shown promise as a clinical treatment for delaying the progress of Alzheimer's Disease as a result of their neuroprotective effects in the presence of increased amyloid-beta plaque formation and resulting glutamate excitotoxicity [65]. With respect to depression, one question that has been raised is why one channel blocker is shown to be more effective as a rapid-acting antidepressant over another? Ketamine, a high trapping agent (86%), or an agent that has a greater tendency to be trapped within the channel pore after the drug has been removed and reapplied, shows both strong rapid-acting antidepressant effects as well as psychotomimetic effects [62]. AZD6765, a lower-trapping agent (54%) shows minimal psychotomimetic effects but only very transient antidepressant effects with a single dose [61,62]. Another low-trapping agent, memantine (71%), dissociates quickly from the receptor and has been shown to have fewer aversive side effects as compared to ketamine but has not proven to be an effective rapid-acting antidepressant [63,66]. This has led to a second question asking whether or not it is possible to separate antidepressant efficacy from the psychomimetic effects?

Selective GluN2B antagonists

NMDA receptors are localized at both synaptic and extrasynaptic sites that have differential physiological effects, including excitotoxicity at the extrasynaptic site, and it is still unknown whether the therapeutic actions of ketamine result from blockade at a specific synaptic location. GluN2B subunits are located at extrasynaptic, as well as synaptic NMDA receptors sites, and have been shown to play a role in excitotoxity, resulting in targeting of GluN2B for the treatment of stroke induced damage [67]. Interestingly, GluN2B has been the focus of depression drug development studies following a clinical report that a selective GluN2B antagonist produces a relatively rapid antidepressant response. Preskorn and colleagues conducted a double-blind placebo-controlled clinical study of the GluN2B selective antagonist CP-101,606 (traxoprodil) and found antidepressant responses 5 days after drug administration, including in patients with TRD [68]. Furthermore, compared to ketamine, the psychotomimetic effects of CP-101,606 appear to be diminished.

Following these clinical findings, preclinical studies of another GluN2B antagonist, Ro-25-6891 have been conducted and compared with ketamine (Fig. 2). Ro-25-6891administration rapidly increases mTORC1 signaling within 1 hr and elevates synaptic proteins in the PFC within 6 hr after dosing [9]. Ro-25-6891 also produces antidepressant responses in the forced swim and novelty suppressed feeding tests that are blocked with the selective mTORC1 inhibitor rapamycin. Another study reports that a single dose of Ro-25-6891 produces a rapid reversal of depressive-like behaviors resulting from chronic unpredictable stress, as seen in the sucrose preference test and novelty-suppressed feeding test [10]; these effects are also blocked by rapamycin.

Another GluN2B selective antagonist, MK-0657 (CERC-301), has recently been studied as an oral treatment option for TRD. A pilot study found that following 12 days of oral MK-0657 monotherapy regimen patients reported a reduction in depressive symptoms [69]. However, the small sample size is a limiting factor and thus further studies into MK-0657 are necessary. Additional GluN2B selective antagonists are currently under investigation, including another oral treatment, TXT-0300 (Traxion Therapeutics) as well as an EVT-100 series (EvoTec). Together, these preclinical and clinical studies, combined with reduced side effect profile, provide good evidence for further development of GluN2B antagonists as rapid acting antidepressants (Fig. 2).

Glycine-site modulators of the NMDAR

The binding site for the co-agonist glycine is located on GluN2 subunits, making it a unique target for the treatment of depression as GluN2 subunits have been under investigation. One compound thought to act at the glycine site is GLYX-13 (rapastinel), a tetrapeptide and functional partial glycine site agonist, specifically GluN2B containing NMDARs [70]. An initial study by Burgdorf and colleagues reported positive effects of GLYX-13 on hippocampal-dependent learning tasks (trace conditioning, Morris water maze) and enhanced long-term potentiation (LTP), a model of synaptic plasticity, in hippocampal slices [71]. Recent preclinical work by the group has explored the antidepressant models and found that a single dose of GLYX-13 is sufficient to produce a rapid antidepressant response, including reversal of anhedonia resulting from chronic unpredictable stress exposure [72]. GLYX-13 is also reported to increase spine synapse formation in the PFC [73].

Phase II trials in depressed patients have also demonstrated that GLYX-13 produces rapid antidepressant action [74]. Using a double-blind, randomized placebo controlled design, Preskorn and colleagues show that a single intravenous injection of GLYX-13 reduced depressive symptoms, as measured by the Hamilton Depression Rating Scale-17, within 2 hours. These effects were maintained for an average of 7 days. There are current plans for a Phase 3 study as well as trials to investigate the efficacy and safety of a multiple dosing regime. Additional preclinical and clinical studies are required to determine the exact mechanism of action of GLYX-13 as well as its full therapeutic potential.

An NMDAR glycine-site partial agonist with antidepressant potential is D-cycloserine (DCS) (Fig. 2). Originally used as an antibiotic to treat tuberculosis, it was observed that DCS improved insomnia, lethargy, and appetite in patients [75]. A 6-week study using DCS as an adjuvant to antidepressant therapy failed to show a significant improvement in depressive symptoms [76]. However, a recent, small-scale clinical trial in 22 treatment-resistant patients using a similar experimental design but a higher dose of DCS showed improvement in depressive symptoms over placebo [77]. Additional larger scale clinical studies are required to investigate the antidepressant potential of DCS as a supplement to traditional antidepressant therapy.

Recently, the NMDA glycine$_B$-site antagonist and prodrug 4-Chlorokynurenine (4-Cl-KYN) and has shown promise as a rapid acting antidepressant [78]. Similar to ketamine, a single treatment with 4-Cl-KYN is sufficient to produce antidepressant-like effects in the FST, novelty-suppressed feeding test (NSFT), and learned helplessness test. Furthermore, these effects were blocked by pretreatment with either glycine or the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX). However, in contrast to ketamine, 4-Cl-KYN did not induce stereotypic behaviors. Phase II clinical trials are currently underway on 4-Cl-KYN (AV-101, VistaGen Therapeutics)

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

to investigate the efficacy and safety for people with MDD (ClinicalTrials.gov identifier: NCT02484456).

mGlu receptor antagonists

Ketamine and the other rapid-acting compounds discussed thus far produce their rapid-acting effects by targeting fast-acting ionotropic glutamate receptors. The metabotropic glutamate receptors (G-protein coupled) have also been examined as potential therapeutic targets for depression. There are 8 subtypes of mGlu receptors ($mGlu_1$–$mGlu_8$). Drugs acting at $mGlu_{2/3}$, $mGlu_5$, and $mGlu_7$ receptors have shown therapeutic potential in pre-clinical models of depression [79–81].

Support for targeting $mGlu_{2/3}$ receptors comes from a study using microelectrode arrays of glutamate in the PFC following local application of different drugs known to bind to glutamate receptors. While the $mGlu_{2/3}$ agonist LY379268 decreases extracellular glutamate release, the antagonist LY341495 significantly increases glutamate release [82]. The ability of these agents to regulate glutamate is thought to occur via regulation of presynaptic autoreceptors located on glutamatergic terminals (Fig. 3). Similar to the actions of ketamine, the antidepressant effects LY341495 as well as another $mGluR_{2/3}$ antagonist MGS0039 require activation of the mTORC1 pathway [55,83]. LY341495 also rapidly reverses the anhedonia caused by chronic stress exposure, a rigorous rodent test of rapid antidepressant actions [84]. Together these preclinical studies provide strong evidence of the therapeutic potential of mGlu2/3 receptor antagonists.

A recent study investigated the effects of the $mGlu_5$ antagonist MTEP and the $mGlu_7$ agonist AMN082 and found that while both drugs produced antidepressant-like behavioral effects in the FST 60 minutes following administration, the behavioral effects were no longer present at 23 hours. Furthermore, the study found that the behavioral effects of MTEP seen at 60 minutes were not dependent on the mTORC1 pathway while the effects of AMN082 were dependent on mTORC1 but not on AMPA receptor activation (as seen with ketamine and other rapid-acting antidepressants) [85]. The investigations into the mGluR modulators are still early in the experimental phases and additional studies are necessary to better understand the potential of targeting the metabotropic receptors in the treatment of depression.

Scopolamine

Another potential rapid antidepressant drug is the non-selective muscarinic acetylcholine receptor antagonist, scopolamine. Clinical studies have reported that intravenous infusion of a very low dose of scopolamine (4 μg/kg) initiated rapid antidepressant effects (within 3–5 days after administration) and 3 doses over 5–7 days produces long-lasting improvements in mood (12–16 days after administration) [57,86]. In addition, a follow-up study indicated that females show a significantly greater antidepressant and antianxiety effect following scopolamine treatment compared to males [87]. Interestingly the antidepressant effects of scopolamine appear to be increased with repeated administration in patients with no prior antidepressant

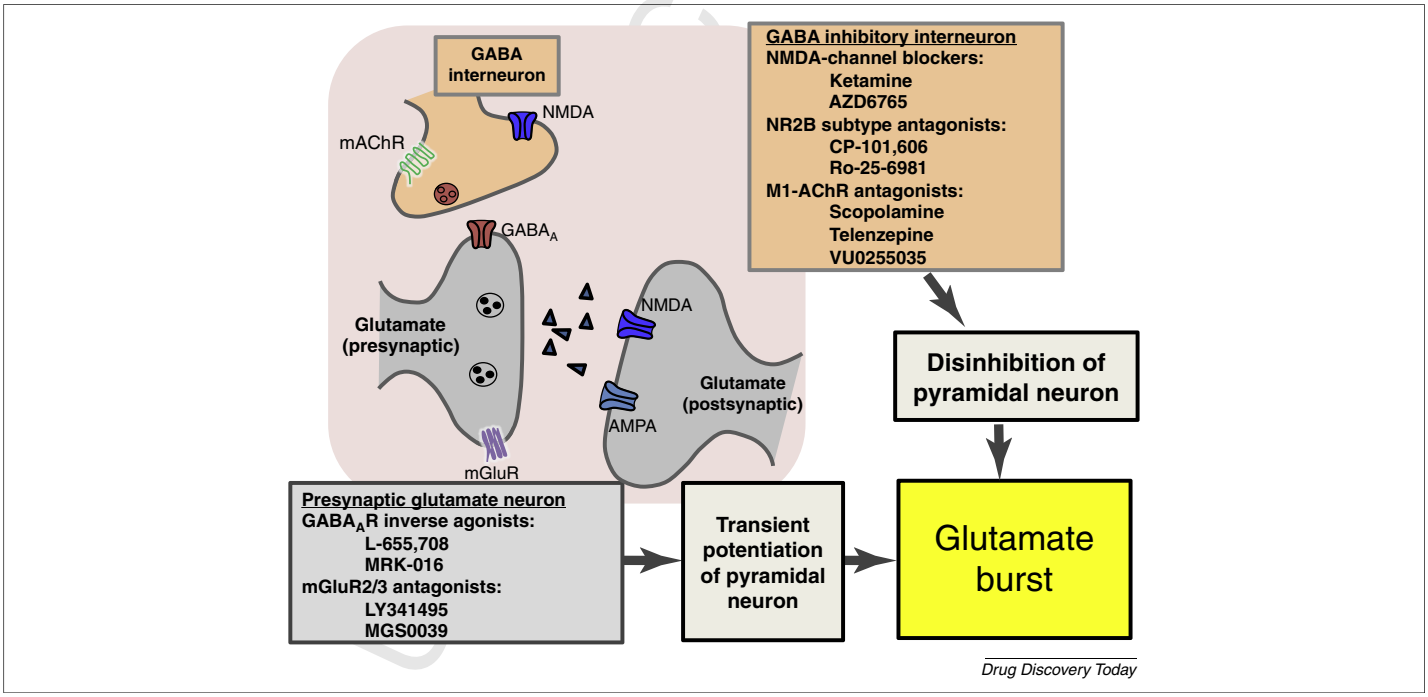

FIGURE 3

Receptor targets for rapid-acting antidepressants. Pre-clinical studies have shown that low dose ketamine produces a rapid glutamate burst from pyramidal cells in the prefrontal cortex. This is hypothesized to result from disinhibition of λ-aminobutyric acid (GABA)-ergic interneurons that control the activity/depolarization of glutamatergic pyramidal neurons. In addition to ketamine, agents hypothesized to antagonize presynaptic GABAergic interneurons include other nonselective NMDA receptor antagonists like AZD6765, the selective GluN2B antagonists Ro-25-6891 and CP-101,606, the muscarinic acetylcholine receptor antagonist scopolamine, and the selective M1 antagonists telenzepine and VU0255035. Another route investigated to produce a rapid glutamate burst is via transient potentiation of pyramidal neurons. Drugs believed to directly target pre-synaptic glutamatergic pyramidal neurons include the $GABA_A$ receptor inverse agonists L-655,708 and MRK-016 and the $mGluR_{2/3}$ antagonists LY341495 and MGS0039.

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

treatment history although treatment-resistant patients still show significant reductions in depressive symptoms [88]. Moreover, an early study using other routes (i.e., intramuscular) and dosing regimens suggest that scopolamine can produce antidepressant effects within several hours after drug treatment [89].

Corresponding to these clinical findings, animal models show that, like ketamine, scopolamine caused rapid antidepressant responses through increased glutamate neurotransmission and activation of mTORC1 signaling in the PFC [9,54]. The increase in glutamate transmission is thought to occur via blockade of muscarinic receptors located on GABAergic interneurons in the medial PFC, similar to the initial cellular target underlying the actions of ketamine. Indeed, there is functional and immunohistochemical evidence that interneurons expression muscarinic receptors [90]. Based on these preclinical studies it is evident that scopolamine can produce an antidepressant effect through neurophysiological pathways that increase PFC function which would effectively reverse the pathophysiology reported in depression.

While scopolamine acts as a non-selective muscarinic acetylcholine receptor antagonist it may elicit antidepressant effects through interactions with specific subtypes of muscarinic acetylcholine receptors. Indeed seminal studies revealed that blockade of specific mACh receptor subtypes recapitulate the molecular and behavioral effects of scopolamine. For example, selective antagonism of M1-ACh receptors with telenzepine produced antidepressant responses [54]. Consistent with this study, a recent report showed that the M1-AChR antagonist, VU0255035, cause a robust antidepressant response in rats that coincided with mTORC1 signaling in the PFC [91]. Furthermore, repeated administration of scopolamine and VU0255035 reduced anhedonia in CUS-exposed rats [91]. A separate study using higher doses of scopolamine indicated that mutant mice lacking M1- or M2-ACh receptor do not show antidepressant responses to scopolamine [92]. In this context it is relevant to note that prior studies with other partially selective M1-AChR compounds (i.e., biperiden) report antidepressant effects in severely depressed, but not moderately depressed patients [89,93]. There is also evidence that selective blockade of M4 receptor antagonists produce antidepressant actions (Dr. Jones, Vanderbilt). In the end, both clinical and preclinical studies indicate that antagonism of specific muscarinic acetylcholine receptors lead to the molecular and behavioral responses reported following scopolamine administration (Fig. 3).

GABA-A inverse agonist: new approach to increase glutamate transmission

Another approach to increase glutamate transmission, particularly when considering the disinhibition model (Fig. 1) is to block GABA-A receptors. This approach is not without limitations as GABA-A receptor blockade can cause seizures. However, a recent study has used a selective GABA-A alpha 5 inverse agonist to produce a more specific, forebrain blockade of GABA-A receptors and thereby reduce the potential for seizure genic side effects [94]. The choice of selective GABA-A alpha-5 agents is based on the expression of this subunit in hippocampus and forebrain cortical regions. The authors tested a GABA-A alpha-5 selective inverse agonist, L-655,708 (FG-8094), which has approximately 30-fold selectivity for alpha-5 over other GABA-A receptors containing alpha-1, alpha-2, or alpha-3. The results indicate that the compound is a partial allosteric modulator at GABA-A receptors containing all the alpha subunits previously mentioned, with comparable efficacy albeit with higher affinity for alpha-5 [95,96]. Therefore to further examine the efficacy of targeting alpha-5 subunit, they tested another apha-5 agent, MRK-16. The results demonstrate that a single dose of either L-655,708 or MRK-016 significantly reverses the deficits in sucrose preference and social interaction caused by exposure to chronic stress (restraint or unpredictable stress). Additional studies of synaptic plasticity were conducted with L-655,708, and demonstrate that L-655,708 reverses the decrease in excitatory transmission caused by chronic restraint stress, as well as the reduction of GluA1 in the hippocampus.

These findings identify GABA-A alpha-5 as a potential target for novel rapid acting antidepressants, and demonstrate a novel approach for producing a burst of glutamate that results in a rapid antidepressant response (Fig. 3). Further studies will be required to determine the therapeutic efficacy and safety of these agents.

Future studies

Although the discovery of ketamine represents a major advance for the treatment of mood disorders there are still significant limitations to overcome before ketamine and other novel glutamatergic agents are accessible to the general population. Even at the low therapeutic doses used in a clinical setting, ketamine can produce euphoric and/or psychotomimetic effects. Furthermore, ketamine, or 'Special K', is a drug of abuse and prolonged use can cause neuronal damage. For instance, individuals with daily use of high doses of ketamine have cortical atrophy and pre-clinical studies have shown that repeated ketamine treatment produces neurotoxic effects [44,97].

Despite the potential for abuse and toxicity, ketamine represents a compelling therapeutic agent as well as prototype for drug discovery and clinical studies of ketamine are currently underway. For example, a recent study has demonstrated the therapeutic efficacy of nasal ketamine and development of this route of administration has been fast tracked by the FDA [98]. Janssen Research & Development, LLC is currently sponsoring a Phase II clinical trial to evaluate the safety and efficacy of intranasal esketamine, the S(+) enantiomer of ketamine (ClinicalTrials.gov Identifier: NCT01998958). Additional clinical trials to define the effective therapeutic dose of ketamine are also being conducted (ClinicalTrials.gov identifier: NCT01920555), as well as investigations are to determine the safety and efficacy of repeated dosing regimens [99]. Furthermore, the use of lithium to sustain the rapid-acting effects of ketamine is currently being investigated in Phase II clinical studies (ClinicalTrials.gov identifier: NCT01880593).

The development of safer and more effective agents will also rely on a better understanding of the mechanisms underlying the rapid-actions of ketamine. A question of major interest is the mechanisms, in particular the initial cellular target that 'triggers' the cascade of signaling, that underlies the rapid antidepressant actions of ketamine. One hypothesis is that ketamine directly targets NMDA receptors on the pyramidal neurons to cause a homeostatic response in the absence of neuronal activity [52]. Another growing hypothesis, as discussed in this review, is

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

that ketamine first acts on the inhibitory interneurons resulting in disinhibition of pyramidal neurons and a burst of glutamate. During basal, resting state conditions, pyramidal cell firing is inhibited by presynaptic tonic firing GABA neurons [100]. This would remove the magnesium block of NMDA receptors, making GABA neurons more sensitive to low-dose ketamine (Fig. 1). Preclinical studies that selectively delete or knockdown NMDA receptor subunits in specific neuronal subtypes would provide insight into these hypotheses (e.g. targeting GluN2B containing NMDA receptors on GABAergic or glutamatergic neurons). In support of this approach, a study investigating the selective GluN2B antagonist ifenprodil revealed that approximately 50% of NMDA receptors on interneurons contain GluN2B subunits [101]. Similar studies are underway investigating the role of different muscarinic acetylcholine receptors on interneurons in the rapid-acting antidepressant actions of scopolamine.

Studies are still needed to examine the cellular actions of stress and depression on interneuron subtypes in the PFC, as well as the influence of ketamine on synaptic plasticity of these neurons. Identification of the cellular targets of ketamine and other rapid-acting agents could reveal additional receptors that regulate GABA firing and thus glutamate transmission, which could potentially lead to better antidepressant agents. In addition, further studies of the molecular pathways that control subtypes of GABA, as well as glutamate neurons in the PFC in response to stress could lead to a better understanding of the pathophysiology of depression. Finally, understanding developmental shifts in receptor expression across cell types could be important in development of novel treatments. These findings may provide context for mental health disorders across the lifespan, including neurodevelopment and neurodegeneration, leading to more effect treatment of heterogeneous populations.

References

1 Kessler, R.C. *et al.* (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 617–627

2 Reddy, M.S. (2010) Depression: the disorder and the burden. *Indian J. Psychol. Med.* 32, 1–2

3 Greenberg, P.E. *et al.* (2015) The economic burden of adults with major depressive disorder in the United States (2005 and 2010). *J. Clin. Psychiatry* 76, 155–162

4 Karch, D.L. *et al.* (2012) Surveillance for violent deaths—National Violent Death Reporting System, 16 states, 2009. *MMWR Surveill. Summ.* 61, 1–43

5 Trivedi, M.H. *et al.* (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28–40

6 Undurraga, J. and Baldessarini, R.J. (2012) Randomized, placebo-controlled trials of antidepressants for acute major depression: thirty-year meta-analytic review. *Neuropsychopharmacology* 37, 851–864

7 Duman, R.S. and Aghajanian, G.K. (2012) Synaptic dysfunction in depression: potential therapeutic targets. *Science* 338, 68–72

8 Savitz, J. and Drevets, W.C. (2009) Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neurosci. Biobehav. Rev.* 33, 699–771

9 Li, N. *et al.* (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* 329, 959–964

10 Li, N. *et al.* (2011) Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol. Psychiatry* 69, 754–761

11 Harrigan, R.A. and Brady, W.J. (1999) ECG abnormalities in tricyclic antidepressant ingestion. *Am. J. Emerg. Med.* 17, 387–393

12 Sharpley, A.L. and Cowen, P.J. (1995) Effect of pharmacologic treatments on the sleep of depressed patients. *Biol. Psychiatry* 37, 85–98

13 Sussman, N. *et al.* (2001) Effects of nefazodone on body weight: a pooled analysis of selective serotonin reuptake inhibitor- and imipramine-controlled trials. *J. Clin. Psychiatry* 62, 256–260

14 Birmes, P. *et al.* (2003) Serotonin syndrome: a brief review. *CMAJ* 168, 1439–1442

15 Keller, M. *et al.* (2006) Lack of efficacy of the substance p (neurokinin1 receptor) antagonist aprepitant in the treatment of major depressive disorder. *Biol. Psychiatry* 59, 216–223

16 Zorrilla, E.P. and Koob, G.F. (2010) Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discov. Today* 371–383

17 Sullivan, P.F. *et al.* (2000) Genetic epidemiology of major depression: review and meta-analysis. *Am. J. Psychiatry* 157, 1552–1562

18 Walsh, B.T. *et al.* (2002) Placebo response in studies of major depression: variable, substantial, and growing. *JAMA* 1840–1847

19 Mayberg, H.S. (1997) Limbic-cortical dysregulation: a proposed model of depression. *J. Neuropsychiatry Clin. Neurosci.* 9, 471–481

20 Kang, H.J. *et al.* (2012) Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nat. Med.* 18, 1413–1417

21 Treadway, M.T. *et al.* (2015) Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. *Biol. Psychiatry* 77, 285–294

22 Drevets, W.C. *et al.* (2002) Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol levels. *Pharmacol. Biochem. Behav.* 71, 431–447

23 Anand, A. *et al.* (2005) Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. *Biol. Psychiatry* 57, 1079–1088

24 Drevets, W.C. *et al.* (2008) Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118

25 Shansky, R.M. and Morrison, J.H. (2009) Stress-induced dendritic remodeling in the medial prefrontal cortex: effects of circuit, hormones and rest. *Brain Res.* 1293, 108–113

26 Liston, C. *et al.* (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J. Neurosci.* 26, 7870–7874

27 Liu, R.J. and Aghajanian, G.K. (2008) Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. *Proc. Natl. Acad. Sci. U. S. A.* 105, 359–364

28 McEwen, B.S. *et al.* (2012) Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology* 62, 3–12

29 Ota, K.T. *et al.* (2014) REDD1 is essential for stress-induced synaptic loss and depressive behavior. *Nat. Med.* 20, 531–535

30 Lucassen, P.J. *et al.* (2010) Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. *Eur. Neuropsychopharmacol.* 20, 1–17

31 Gronli, J. *et al.* (2006) Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. *Pharmacol. Biochem. Behav.* 85, 842–849

32 Nair, A. *et al.* (2007) Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus. *Neuropsychopharmacology* 32, 1504–1519

33 Schmidt, H.D. and Duman, R.S. (2010) Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology* 35, 2378–2391

34 Taliaz, D. *et al.* (2011) Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *J. Neurosci.* 31, 4475–4483

35 Egan, M.F. *et al.* (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112, 257–269

36 Bueller, J.A. *et al.* (2006) BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biol. Psychiatry* 59, 812–815

37 Soliman, F. *et al.* (2010) A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science* 327, 863–866

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

38 Colzato, L.S. *et al.* (2011) BDNF Val66Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults. *Psychoneuroendocrinology* 36, 1562–1569
39 Chen, Z.Y. *et al.* (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314, 140–143
40 Liu, R.J. *et al.* (2012) Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol. Psychiatry* 71, 996–1005
41 Pattwell, S.S. *et al.* (2012) The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. *J. Neurosci.* 32, 2410–2421
42 Bath, K.G. *et al.* (2012) BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity. *Neuropsychopharmacology* 37, 1297–1304
43 Duman, R.S. and Voleti, B. (2012) Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. *Trends Neurosci.* 35, 47–56
44 Berman, R.M. *et al.* (2000) Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* 47, 351–354
45 Duman, R.S. (2014) Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. *Dialogues Clin. Neurosci.* 16, 11–27
46 Iadarola, N.D. *et al.* (2015) Ketamine and other N-methyl-D-aspartate receptor antagonists in the treatment of depression: a perspective review. *Ther. Adv. Chronic Dis.* 6, 97–114
47 Curran, H.V. and Morgan, C. (2000) Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. *Addiction* 95, 575–590
48 Moghaddam, B. *et al.* (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. *J. Neurosci.* 17, 2921–2927
49 Lorrain, D.S. *et al.* (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, 697–706
50 Chowdhury, G.M. *et al.* (2012) (1)H-[(1)(3)C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biol. Psychiatry* 71, 1022–1025
51 Lepack, A.E. *et al.* (2015) BDNF release is required for the behavioral actions of ketamine. *Int. J. Neuropsychopharmacol.* 18
52 Autry, A.E. *et al.* (2011) NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 475, 91–95
53 Hoeffer, C.A. and Klann, E. (2010) mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci.* 33, 67–75
54 Voleti, B. *et al.* (2013) Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. *Biol. Psychiatry* 742–749
55 Koike, H. *et al.* (2011) Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behav. Brain Res.* 224, 107–111
56 Dwyer, J.M. *et al.* (2015) Ribosomal protein S6 kinase 1 signaling in prefrontal cortex controls depressive behavior. *Proc. Natl. Acad. Sci. U. S. A.* 112, 6188–6193
57 Furey, M.L. and Drevets, W.C. (2006) Antidepressant efficacy of the antimuscarinic drug scopolamine: a randomized, placebo-controlled clinical trial. *Arch. Gen. Psychiatry* 63, 1121–1129
58 Traynelis, S.F. *et al.* (2010) Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62, 405–496
59 Wyllie, D.J. *et al.* (2013) Influence of GluN2 subunit identity on NMDA receptor function. *Neuropharmacology* 4–17
60 Monyer, H. *et al.* (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12, 529–540
61 Zarate, C.A., Jr *et al.* (2013) A randomized trial of a low-trapping nonselective N-methyl-D-aspartate channel blocker in major depression. *Biol. Psychiatry* 257–264
62 Sanacora, G. *et al.* (2014) Lanicemine: a low-trapping NMDA channel blocker produces sustained antidepressant efficacy with minimal psychotomimetic adverse effects. *Mol. Psychiatry* 19, 978–985
63 Zarate, C.A., Jr *et al.* (2006) A double-blind, placebo-controlled study of memantine in the treatment of major depression. *Am. J. Psychiatry* 153–155
64 Gideons, E.S. *et al.* (2014) Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8649–8654
65 Hellweg, R. *et al.* (2012) Efficacy of memantine in delaying clinical worsening in Alzheimer's disease (AD): responder analyses of nine clinical trials with patients with moderate to severe AD. *Int. J. Geriatr. Psychiatry* 27, 651–656
66 Mealing, G.A. *et al.* (1999) Differences in degree of trapping of low-affinity uncompetitive N-methyl-D-aspartic acid receptor antagonists with similar kinetics of block. *J. Pharmacol. Exp. Ther.* 288, 204–210
67 Martin, H.G. and Wang, Y.T. (2010) Blocking the deadly effects of the NMDA receptor in stroke. *Cell* 140, 174–176
68 Preskorn, S.H. *et al.* (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. *J. Clin. Psychopharmacol.* 28, 631–637
69 Ibrahim, L. *et al.* (2012) A randomized, placebo-controlled, crossover pilot trial of the oral selective NR2B antagonist MK-0657 in patients with treatment-resistant major depressive disorder. *J. Clin. Psychopharmacol.* 32, 551–557
70 Zhang, X.L. *et al.* (2008) A NMDA receptor glycine site partial agonist, GLYX-13, simultaneously enhances LTP and reduces LTD at Schaffer collateral-CA1 synapses in hippocampus. *Neuropharmacology* 1238–1250
71 Burgdorf, J. *et al.* (2011) The N-methyl-D-aspartate receptor modulator GLYX-13 enhances learning and memory, in young adult and learning impaired aging rats. *Neurobiol. Aging* 32, 698–706
72 Burgdorf, J. *et al.* (2013) GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. *Neuropsychopharmacology* 38, 729–742
73 Duman, C. *et al.* (2015) *GLYX13 increases mTORC1 signaling and synaptogenesis in the prefrontal cortex.*
74 Preskorn, S. *et al.* (2015) Randomized proof of concept trial of GLYX-13, an N-methyl-D-aspartate receptor glycine site partial agonist, in major depressive disorder nonresponsive to a previous antidepressant agent. *J. Psychiatr. Pract.* 21, 140–149
75 Crane, G.E. (1959) Cyloserine as an antidepressant agent. *Am. J. Psychiatry* 115, 1025–1026
76 Heresco-Levy, U. *et al.* (2006) Controlled trial of D-cycloserine adjuvant therapy for treatment-resistant major depressive disorder. *J. Affect. Disord.* 93, 239–243
77 Heresco-Levy, U. *et al.* (2013) A randomized add-on trial of high-dose D-cycloserine for treatment-resistant depression. *Int. J. Neuropsychopharmacol.* 16, 501–506
78 Zanos, P. *et al.* (2015) The prodrug 4-chlorokynurenine causes ketamine-like antidepressant effects, but not side effects, by NMDA/Glycine$_B$-site inhibition. *J. Pharmacol. Exp. Ther.* 355, 76–85
79 Chaki, S. *et al.* (2013) mGlu2/3 and mGlu5 receptors: potential targets for novel antidepressants. *Neuropharmacology* 66, 40–52
80 Chaki, S. *et al.* (2004) MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* 46, 457–467
81 Palucha-Poniewiera, A. and Pilc, A. (2012) Involvement of mGlu5 and NMDA receptors in the antidepressant-like effect of acamprosate in the tail suspension test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 39, 102–106
82 Hascup, E.R. *et al.* (2010) Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. *J. Neurochem.* 115, 1608–1620
83 Dwyer, J.M. *et al.* (2012) mTOR activation is required for the antidepressant effects of mGluR(2)/(3) blockade. *Int. J. Neuropsychopharmacol.* 15, 429–434
84 Dwyer, J.M. *et al.* (2013) mGluR2/3 blockade produces rapid and long-lasting reversal of anhedonia caused by chronic stress exposure. *J. Mol. Psychiatry* 1, 15
85 Palucha-Poniewiera, A. *et al.* (2014) Activation of the mTOR signaling pathway in the antidepressant-like activity of the mGlu5 antagonist MTEP and the mGlu7 agonist AMN082 in the FST in rats. *Neuropharmacology* 82, 59–68
86 Drevets, W.C. and Furey, M.L. (2010) Replication of scopolamine's antidepressant efficacy in major depressive disorder: a randomized, placebo-controlled clinical trial. *Biol. Psychiatry* 67, 432–438
87 Furey, M.L. *et al.* (2010) Scopolamine produces larger antidepressant and antianxiety effects in women than in men. *Neuropsychopharmacology* 35, 2479–2488
88 Ellis, J.S. *et al.* (2014) Antidepressant treatment history as a predictor of response to scopolamine: clinical implications. *J. Affect. Disord.* 162, 39–42
89 Gillin, J.C. *et al.* (1991) The effects of scopolamine on sleep and mood in depressed patients with a history of alcoholism and a normal comparison group. *Biol. Psychiatry* 30, 157–169
90 Amar, M. *et al.* (2010) Blockade of different muscarinic receptor subtypes changes the equilibrium between excitation and inhibition in rat visual cortex. *Neuroscience* 169, 1610–1620
91 Navarria, A. *et al.* (2015) Rapid antidepressant actions of scopolamine: role of medial prefrontal cortex and M1-subtype muscarinic acetylcholine receptors. *Neurobiol. Dis.* 82, 254–261

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016

92 Witkin, J.M. *et al.* (2014) M1 and m2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly acting antidepressant scopolamine. *J. Pharmacol. Exp. Ther.* 448–456
93 Kramer, A.D. and Feiguine, R.J. (1981) Clinical effects of amitriptyline in adolescent depression. A pilot study. *J. Am. Acad. Child Psychiatry* 20, 636–644
94 Fischell, J. *et al.* (2015) Rapid antidepressant action and restoration of excitatory synaptic strength after chronic stress by negative modulators of alpha5-containing $GABA_A$ receptors. *Neuropsychopharmacology* (Epub ahead of print)
95 Atack, J. *et al.* (2005) In vivo labelling of 5 subunit containing GABAA receptors using the selective radioligand [3H]L655,708. *Neuropharmacology* 49, 220–229
96 Atack, J. *et al.* (2006) L655,708 enhances cognition in rats, but is not proconvulsant at a dose selective for 5 containing GABAA receptors. *Neuropharmacology* 51, 1023–1029
97 Wang, C. *et al.* (2013) Brain damages in ketamine addicts as revealed by magnetic resonance imaging. *Front. Neuroanat.* 23
98 Lapidus, K.A. *et al.* (2014) A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biol. Psychiatry* 76, 970–976
99 aan het Rot, M. *et al.* (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol. Psychiatry* 67, 139–145
100 Smiley, J.F. and Goldman-Rakic, P.S. (1993) Heterogeneous targets of dopamine synapses in monkey prefrontal cortex demonstrated by serial section electron microscopy: a laminar analysis using the silver-enhanced diaminobenzidine sulfide (SEDS) immunolabeling technique. *Cereb. Cortex* 3, 223–238
101 Wang, H.X. and Gao, W.J. (2009) Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. *Neuropsychopharmacology* 2028–2040

Please cite this article in press as: Gerhard, D.M. et al. Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.01.016