



## Review

## Signaling pathways underlying the rapid antidepressant actions of ketamine

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## ABSTRACT

Currently available medications have significant limitations, most notably low response rate and time lag for treatment response. Recent clinical studies have demonstrated that ketamine, an NMDA receptor antagonist produces a rapid antidepressant response (within hours) and is effective in treatment resistant depressed patients. Molecular and cellular studies in rodent models demonstrate that ketamine rapidly increases synaptogenesis, including increased density and function of spine synapses, in the prefrontal cortex (PFC). Ketamine also produces rapid antidepressant actions in behavioral models of depression, and reverses the deficits in synapse number and behavior resulting from chronic stress exposure. These effects of ketamine are accompanied by stimulation of the mammalian target of rapamycin (mTOR), and increased levels of synaptic proteins. Together these studies indicate that ketamine rapidly reverses the atrophy of spines in the PFC and thereby causes a functional reconnection of neurons that underlies the rapid behavioral responses. These findings identify new targets for rapid acting antidepressants that are safer than ketamine.

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## 1. Introduction

Depression is a widespread and heterogeneous disorder with severe health and socioeconomic consequences (Kessler et al., 2003). Despite years of research and efforts to develop effective treatments, available antidepressant medications have serious limitations. This includes low rates of treatment response (~one in three respond to the first medication prescribed, and up to two in three after testing multiple medications) (Trivedi et al., 2006). Moreover, there is a time lag of several weeks to months before a therapeutic effect is observed, a serious problem given the high rate of suicide in depressed patients.

However, recent clinical studies provide evidence of novel experimental medications that address the limitations of current antidepressant drugs. In particular these studies demonstrate that a low dose of NMDA receptor antagonist, ketamine, produces a rapid antidepressant response within hours (Berman et al., 2000; Zarate et al., 2006). Moreover, these rapid actions of ketamine are observed in patients who are resistant to two or more typical antidepressants (i.e., considered treatment resistant). Identification of a rapid acting, efficacious agent with a completely different mechanism of action represents a significant advance for the treatment of depression.

The neurobiological mechanisms underlying the antidepressant actions of ketamine are more complex than simple blockade of NMDA receptors. This hypothesis is based on the time course of the therapeutic response and sustained actions of ketamine. The very low dose of ketamine used for these studies first produces mild psychotomimetic and dissociative effects 30–40 min after administration, effects that are transient and completely dissipate by 80 min (Zarate et al., 2006). This is presumably because of the rapid metabolism of ketamine (half-life is 180 min in humans; Clements et al., 1982). After this initial psychotomimetic phase, the antidepressant effects are observed at 110 min and are sustained for approximately 7 days after a single dose of ketamine (Zarate et al., 2006). These findings indicate that ketamine initiates a cascade of events that results in a rapid response that is sustained even after the drug has been metabolized.

We have investigated the possibility that ketamine activates a change in synaptogenesis that is delayed but long-lasting. The results demonstrate that ketamine stimulates a signaling cascade that leads to increased dendritic protein synthesis and increased density and function of spine synapses. These findings are discussed in the context of the deleterious effects of stress and depression on synaptogenesis, and the implications for designing novel, rapid acting antidepressants.

## 2. Rapid antidepressant actions of ketamine

The regulation of synapse formation or synaptogenesis is a subcellular neuronal alteration that contributes to synaptic

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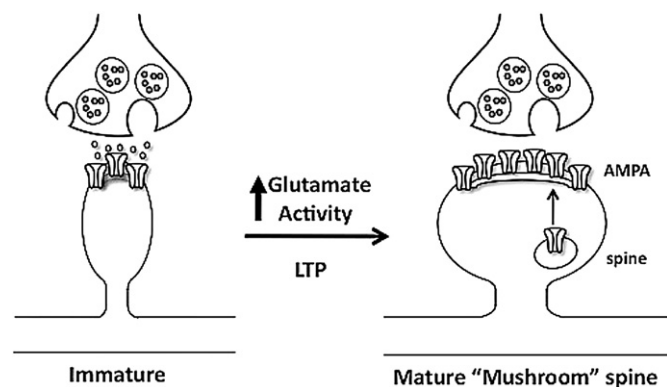
plasticity, a fundamental function of the brain. Synaptic plasticity is the ability to process information from other neuronal inputs, store that information, and make the appropriate future adaptive responses. Synaptic plasticity and synaptogenesis have been studied primarily in models of learning and memory in the hippocampus, but this critical process also plays an important role in multiple functions in other brain regions (Fig. 1). An increase in functional synaptogenesis is typically accompanied by an increase in the number of dendritic spines, the physical site of synaptic connections (Holtmaat and Svoboda, 2009; Kessels and Malinow, 2009; Yoshihara et al., 2009). Dendritic spines can be visualized by Golgi staining or by filling individual neurons with a dye that diffuses throughout the dendritic arbor, allowing for analysis of the density of spines. The shape of spines also provides information about synaptic function: shapes range from narrow and spindly to round or mushroom shaped, correlating with low to high levels of synaptic maturity, stability, and functional neurotransmitter activity (Holtmaat and Svoboda, 2009; Kessels and Malinow, 2009; Yoshihara et al., 2009).

### 2.1. Ketamine increases synaptogenesis

The potential role of dendrites and spines in stress-related illnesses such as depression is supported by basic studies demonstrating that exposure to stress causes atrophy of neurons in limbic brain regions implicated in depression, including the prefrontal cortex (PFC) and hippocampus (McEwen, 2008; Shansky and Morrison, 2009). This includes a decrease in the density of spines, as well as a decrease in the number and length of dendrite branches. These effects could contribute to the reduction in volume of PFC and hippocampus determined by imaging the brains of depressed patients (Drevets and Furey, 2010; Macqueen et al., 2008).

In contrast to the effects of stress, we have recently reported that administration of a low dose of ketamine results in the rapid induction of spine number in layer V pyramidal neurons of the PFC (Li et al., 2010). Spine analysis was conducted 24 h after ketamine administration, and it is possible that the induction of spine formation occurs sooner, given the rapid induction of synaptic proteins (i.e., as early as 2 h after ketamine administration, see below). Additional studies will be required to determine the time course for induction, as well as maintenance of spine formation.

At the 24 h time point, there is an increase in the number of mushroom or mature spines, indicating that ketamine increases spine stability and function. This possibility was directly tested by



**Fig. 1.** Model for activity-dependent induction of mature spines. Diagram shows a presynaptic terminal with synaptic vesicle adjacent to an immature spine. Synaptic activity leads to spine maturation and insertion of glutamate-AMPA receptors into the postsynaptic membrane. The signaling mechanisms underlying synaptogenesis are studied in a cellular model of learning and memory, long-term potentiation (LTP). See text for details.

analysis of neurotransmitter-induced excitatory postsynaptic currents (EPSCs) in the same PFC layer V pyramidal neurons that were analyzed for spine density. The results demonstrate that ketamine administration significantly increases the frequency and amplitude of both 5-HT- and hypocretin-induced EPSCs. The increase in EPSP amplitude is consistent with the increase in the density of mushroom spines by ketamine. The increase in 5-HT- and hypocretin-induced EPSCs indicates that there is an increase in corticocortical and thalamocortical connections, respectively. It will be important in future studies to determine the full time course for the induction of spine density by ketamine, including how long these new spines last.

Consistent with the rapid induction of synaptogenesis, we also found that ketamine produced rapid antidepressant effects in several different behavioral models. This included a significant decrease in immobility in the forced swim test (FST), decreased escape failure and latency to escape in the learned helplessness (LH) paradigm, and decreased latency to feed in the novelty suppressed feeding test (NSFT) (Li et al., 2010). Although the FST is responsive to acute administration of typical antidepressant agents, LH is only responsive to subchronic (7 days) treatment, and the NSFT, although a model of anxiety, is responsive to chronic (21 days) administration of a typical antidepressant. Together, these findings provide evidence of the fast antidepressant behavioral actions of ketamine in these rodent models.

### 2.2. Ketamine rapidly reverses the effects of chronic stress

In addition to studies in normal animals, we have examined the influence of ketamine in animals exposed to chronic unpredictable stress (CUS), considered one of the better rodent models of depression. In the CUS model, repeated exposure to stress over the course of several weeks results in the development of anhedonia, a core symptom of depression, and this effect is reversed by chronic administration of a typical antidepressant (Willner, 2005; Banasr et al., 2007). In addition, chronic stress exposure decreases the density of spines in the PFC (Liu and Aghajanian, 2008; Shansky and Morrison, 2009), providing a morphological endpoint and that is relevant to the atrophy of PFC in depression (Drevets and Furey, 2010).

We found that exposure to CUS for 3 weeks decreased the density of spines in layer V pyramidal neurons, as expected, and decreased 5-HT and hypocretin-induced EPSCs in the same neurons (Li et al., 2011). A single dose of ketamine completely reversed the deficit in spine density, and this was accompanied by a reversal of the deficit in 5-HT and hypocretin-induced EPSC. Together these studies demonstrate that ketamine reverses the spine loss caused by chronic stress exposure and normalizes PFC connectivity.

To examine the possibility that reversal of the synapse loss caused by CUS influences behavior, we also examined sucrose preference, which provides a measure of anhedonia in rodents. CUS exposure for 3 weeks significantly decreased the preference for a sweetened solution, and this effect was completely reversed by a single dose of ketamine (Li et al., 2011). In addition, CUS increased the latency to feed in the NSFT, and this effect was also reversed by a single dose of ketamine. These effects of ketamine were also sustained for approximately 7 days, similar to the time course for the antidepressant actions of ketamine in treatment resistant depressed patients (Zarate et al., 2006).

## 3. Signaling pathways underlying the rapid antidepressant actions of ketamine

The signaling pathways and mechanisms that control synapse formation and neuroplasticity have been studied primarily in models

of learning and memory (Fig. 1). In particular, cellular and behavioral forms of long-term memory are dependent on new protein synthesis and are accompanied by an increase in the number of mature synapses (Holtmaat and Svoboda, 2009; Kessels and Malinow, 2009; Yoshihara et al., 2009). These studies demonstrate that protein synthesis dependent long-term memory is dependent on stimulation of the mammalian target of rapamycin (mTOR) (Hoeffler and Klann, 2010; Livingston et al., 2010).

mTOR is a large serine/threonine kinase that regulates the initiation of protein translation. It is ubiquitously expressed, including localization in dendritic processes where it can control new protein synthesis when required for synaptogenesis (Fig. 2). Stimulation of mTOR, or more precisely the mTORC1 complex occurs via phosphorylation, particularly of the mTOR kinase domain by Akt or protein kinase B. Akt also contributes to activation of mTORC1 via repression of the tuberous sclerosis complex (TSC) 1 and 2. Akt can be activated by neurotrophic factor signaling cascades, including the phosphoinositide-3 kinase (PI3K)-phosphoinositide-dependent kinase 1 (PDK1) and by extracellular signal regulated protein kinase (ERK) pathways (Hoeffler and Klann, 2010). Another important regulatory domain is the binding site for FK506 binding protein 12 (FKBP12), which is involved in activation of mTOR signaling (Hoeffler and Klann, 2010).

Activation of mTORC1 leads to phosphorylation and activation of p70S6 kinase and repression of the inhibitory 4E binding proteins (4E-BPs). Stimulation of p70S6 kinase, in turn controls translation at a number of levels, including the synthesis of S6 ribosomal subunit, phosphorylation of the RNA helicase cofactor eIF4A, and inhibition of eukaryotic elongation factor 2 (eEF2) kinase (Hoeffler and Klann, 2010; Livingston et al., 2010). The activity of p70S6 kinase is also stimulated by ERK. Phosphorylation of 4E-BPs by mTOR results in derepression of the cap-binding activity of eIF4E thereby enhancing translation initiation.

### 3.1. Ketamine stimulates mTOR signaling and increases synaptic protein synthesis

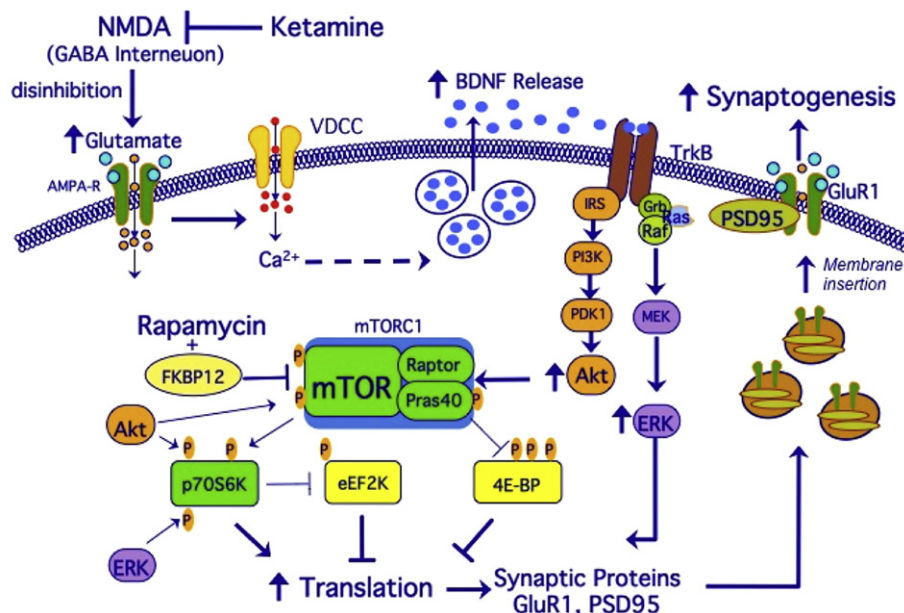
Based on these studies, we tested the hypothesis that the synaptogenic actions of ketamine occur via stimulation of mTOR

signaling and synaptic protein synthesis (Fig. 2). Levels of phosphorylated and activated forms of mTOR and p70S6 kinase were analyzed in synaptosome enriched fractions of the PFC. The results demonstrate that ketamine administration causes a very rapid induction of phospho-mTOR and phospho-p70S6 kinase, as well as increased levels of phospho-4E-BP1, which together would lead to activation of mTOR dependent translation and protein synthesis (Li et al., 2010). The induction of mTOR signaling occurs within 30 min of ketamine administration and is transient, with the mTOR phospho-proteins returning to basal, non-stimulated levels by 2 h. Further studies demonstrate an inverted U dose response for ketamine, with low doses (5 and 10 mg/kg) stimulating and a higher, anesthetic dose (80 mg/kg) having no effect on mTOR signaling.

We also found that levels of phospho-Akt, as well as phospho-ERK, possible upstream activators of mTOR, were rapidly increased by ketamine (Fig. 2). The possibility that ketamine-induction of mTOR signaling is mediated by Akt and ERK signaling was directly tested by pretreatment with inhibitors of each of these two kinases. Infusions (intracerebroventricular, ICV) of selective inhibitors of either PI3K-Akt (LY294002) or of MEK-ERK (U0126) completely blocked the ability of ketamine to stimulate the phosphorylation of mTOR, p70S6 kinase and 4E-BP1 (Li et al., 2010).

A recent paper has demonstrated that ketamine increases the phosphorylation of glycogen synthase kinase-3 (GSK-3), and that mice with a knock in mutation that blocks the phosphorylation of GSK-3 do not respond to ketamine in a behavioral model of depression (Beurel et al., in press). Phosphorylation of GSK-3, a target of mood stabilizing agents, inhibits the activity of this kinase (Li and Jope, 2010). Further studies are required to determine the mechanisms underlying the induction of GSK-3 phosphorylation by ketamine, but it is interesting to speculate that this occurs via Akt, which is activated by ketamine and is known to be a major regulator of GSK-3 (Li et al., 2010).

To determine if there was an increase in synaptic protein synthesis, levels of activity regulated cytoskeletal protein (Arc), glutamate-AMPA receptor-1 (GluR1), postsynaptic density protein-95 (PSD95), and synapsin I were examined in the synaptosome enriched PFC preparations. A single dose of ketamine significantly



**Fig. 2.** Ketamine stimulates mTOR and synaptogenesis: neurotransmitter and intracellular signaling mechanisms. Ketamine stimulates glutamate transmission, resulting in BDNF release and activation of Akt and ERK signaling, which in turn stimulate mTOR and synaptic protein synthesis. This leads to insertion of GluR1 and increased synaptogenesis, which contributes to the rapid antidepressant effects of ketamine. See text for details.

increased levels of these synaptic proteins 1 h (Arc) to 2 h (GluR1, PSD95, and synapsin I) after administration (Li et al., 2010). Surprisingly, the induction of GluR1, PSD95, and synapsin I was relatively stable, as levels remained elevated 72 h after ketamine treatment (Li et al., 2010), consistent with the formation and sustained induction of mature spine synapses (Holtmaat and Svoboda, 2009; Kessels and Malinow, 2009; Yoshihara et al., 2009). In contrast, the induction of Arc was transient, with levels returning to vehicle controls by 6 h after ketamine. Arc has been implicated in both early and late phase LTP and maturation of synapses, and the functional consequences of its transient induction by ketamine are unknown (Bramham et al., 2008).

### 3.2. Ketamine-induction of synaptogenesis is dependent on mTOR

To directly examine the role of mTOR signaling in the actions of ketamine, animals were pretreated with rapamycin, a selective mTOR inhibitor (Fig. 2). Rapamycin binds to FKBP12, and disrupts its interaction with mTOR, which is necessary for activation of mTOR (Hoeffler and Klann, 2010). For these studies, rats were infused with rapamycin (ICV) 30 min before administration of ketamine, and spine density and EPSCs were determined 24 h later. The results demonstrate that rapamycin pretreatment completely blocks the induction of spine formation by ketamine, as well as the induction of 5-HT and hypocretin-induced EPSCs (Li et al., 2010). Rapamycin pretreatment also completely blocked the induction of synaptic proteins (GluR1, PSD95, and synapsin I) by ketamine. Similarly, the ability of ketamine to reverse the deficit in synaptic proteins resulting from exposure to CUS was blocked by rapamycin infusion (Li et al., 2010).

We also examined the requirement for mTOR signaling in the behavioral actions of ketamine. Infusion of rapamycin (ICV) completely blocked the antidepressant actions of ketamine in the FST, LH, and NSFT (Li et al., 2010). Moreover, the ability of ketamine to reverse the behavioral deficits in the CUS model were also blocked by rapamycin, including the decrease in sucrose preference and increase in latency in NSFT caused by CUS exposure (Li et al., 2010).

A recent study has also reported that the behavioral actions of ketamine are dependent on protein synthesis (Autry et al., 2011). However, this paper reports that ketamine-induction of protein synthesis occurs via inhibition of eEF2 kinase and was not able to detect ketamine-induction of mTOR signaling or rapamycin blockade of the behavioral actions of ketamine (Autry et al., 2011). There are several possible technical reasons that could explain these differences. First, in the study by Autry and colleagues mTOR signaling was measured in crude homogenates of hippocampus, not synaptosome enriched fractions of PFC as reported by Li et al. (2010). Since mTOR is expressed throughout neuronal and glial cell bodies, analysis of crude homogenates could mask changes in the smaller dendritic cellular compartment. Second, analysis of behavior was conducted at an early time point (30 min) after ketamine administration, a time point when patients experience mild psychotomimetic and dissociative effects (Berman et al., 2000; Zarate et al., 2006). This also corresponds to the time when levels of extracellular glutamate are increased by ketamine (Moghaddam et al., 1997). Increased glutamate transmission could underlie the increased activity observed in the FST, and as pointed out by the authors (Autry et al., 2011), rapamycin would not be expected to block the effects of ketamine at this time point since the induction of synaptic proteins and synaptogenesis is delayed by approximately 2 h. Extracellular glutamate returns to basal levels after 2 h (Moghaddam et al., 1997), and this point corresponds very closely to the earliest time point at which an antidepressant response is observed in depressed patients (Berman et al., 2000; Zarate et al.,

2006). Thus, the increase in glutamate transmission and induction of mTOR precede and are required for the induction of synaptic protein synthesis, synaptogenesis, and antidepressant behavioral responses, which are then sustained. Another difference is that the Autry study (2011) administered rapamycin systemically, which could result in peripheral side effects, whereas it was infused ICV in the Li et al. study (2010).

The Autry study (2011) reports that a key action of ketamine is the rapid (30 min) induction of BDNF, demonstrating that blockade of protein synthesis by pretreatment with anisomycin blocks both the induction of BDNF and the behavioral actions of ketamine in the FST (Autry et al., 2011). It is also possible that anisomycin pretreatment decreases basal levels of BDNF protein synthesis in dendrites and spines, which would reduce activity-dependent release of BDNF.

### 3.3. Ro 25-6981, a selective NR2B antagonist, stimulates mTOR and synaptic protein synthesis

Ketamine is a nonselective NMDA receptor antagonist, and there have been reports that selective antagonists of the NR2B subtype produce antidepressant actions in rodent models (Maeng et al., 2008) and in humans (Preskorn et al., 2007). Therefore, we examined the influence of a selective NR2B antagonist, Ro 25-6981 on mTOR signaling and synaptic protein synthesis. The results demonstrate that the selective NR2B antagonist produces effects similar to ketamine. Ro 25-6981 administration rapidly (1 h) stimulated mTOR signaling (increased levels of phospho-mTOR, phospho-p70S6 kinase, and phospho-4E-BP1), and increased levels of GluR1, PSD95, and synapsin I, determined at a 6 h time point (Li et al., 2010).

The behavioral actions of the NR2B selective antagonist were also similar to ketamine. Ro 25-6981 administration produced an antidepressant response in the FST and NSFT, and these effects were blocked by infusion of rapamycin. In the CUS paradigm, the NR2B selective antagonist completely blocked the deficits in sucrose consumption and novelty suppressed feeding resulting from CUS exposure (Li et al., 2011). These findings suggest that the actions of ketamine are mediated by blockade of NR2B receptors, and provide further evidence of a functional connection between induction of synaptogenesis and antidepressant behavioral responses.

## 4. Role of glutamate in the rapid antidepressant actions of NMDA receptor antagonists

The neurotransmitter mechanisms underlying the molecular and cellular actions of ketamine have not been fully elucidated, although there is evidence that the effects are mediated by changes in glutamate transmission. There is a previous report that the behavioral actions of ketamine are blocked by pretreatment with a glutamate receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) (Maeng et al., 2008). NBQX is a selective antagonist of a glutamate ionotropic receptor subtype,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). In addition, ketamine is reported to increase extracellular glutamate in the PFC, determined by extracellular dialysis studies (Moghaddam et al., 1997). The latter study also demonstrated that the time course for ketamine-induction of extracellular glutamate is similar to the rapid induction of mTOR signaling (i.e., increased at 30–60 min, return to baseline by 2 h). Moreover, the dose response for the induction of extracellular glutamate is similar to that for mTOR (i.e., inverted U, with low doses increasing but no effect or slight decrease with higher anesthetic doses). In addition, cellular and behavioral studies of synaptic plasticity demonstrate that stimulation of mTOR signaling and synaptic protein synthesis is

dependent on activation of AMPA receptors (Hoeffer and Klann, 2010; Livingston et al., 2010).

Based on these reports, the requirement for glutamate-AMPA receptor activation in the ketamine-induction of mTOR signaling was determined. Pretreatment with NBQX completely blocked ketamine stimulation of phospho-mTOR, phospho-p70S6 kinase, and phospho-4E-BP1 (Li et al., 2010). NBQX pretreatment also blocked ketamine-induction of phospho-Akt and phospho-ERK, the putative upstream activators of mTOR signaling. Together, these studies are consistent with the hypothesis that ketamine-induction of mTOR signaling and synaptogenesis occurs via stimulation of glutamate transmission and AMPA receptor activation.

The cellular mechanisms underlying the ability of an NMDA receptor antagonist to increase glutamate-AMPA receptor activation has been examined using electrophysiological approaches. Administration of ketamine decreases the spontaneous activity of GABAergic interneurons in the PFC of awake rats (Homayoun and Moghaddam, 2007). In the same study, but with a delayed onset, ketamine administration was found to increase the firing rate of glutamatergic pyramidal neurons. These findings indicate that NMDA receptor antagonists block spontaneous GABAergic activity, and thereby result in disinhibition of glutamate transmission, a hypothesis that requires further testing. In addition, it is possible that ketamine also has direct effects on pyramidal neurons that further enhance synaptogenesis and the maturation of spines.

#### 4.1. Potential role of BDNF in the actions of NMDA receptor antagonists

The mechanisms by which stimulation of glutamate-AMPA receptor activation could lead to induction of mTOR signaling have been examined in models of learning and memory. These studies demonstrate that AMPA receptor regulation of synaptic function involves the activation of L-type voltage-dependent calcium channels (VDCCs) and activity-dependent release of BDNF (Hoeffer and Klann, 2010; Jourdi et al., 2009) (Fig. 2). BDNF is a key regulator of mTOR signaling and synaptic plasticity, stimulating dendritic protein synthesis, spine maturation, and synaptic transmission (Jourdi et al., 2009; Takei et al., 2004). Stimulation of AMPA receptors leads to activity-dependent release of BDNF, which in turn leads to activation of Akt and ERK, and stimulation of mTOR and synaptic protein synthesis (Hoeffer and Klann, 2010; Jourdi et al., 2009; Slipczuk et al., 2009; Takei et al., 2004).

Together, these reports are consistent with the hypothesis that the actions of ketamine are dependent on glutamate-AMPA receptor stimulation of BDNF release in the PFC, which then stimulates mTOR and spine formation. This hypothesis is supported by a report that the behavioral actions of ketamine are blocked in BDNF conditional deletion mutants (Autry et al., 2011). Together these studies suggest that increased synthesis and activity-dependent release could contribute to the actions of ketamine. Release of BDNF could stimulate TrkB receptors and PI3K-Akt and ERK signaling, which is required for ketamine activation of mTOR (Li et al., 2010). Previous studies demonstrate that typical antidepressant treatments increase the expression of BDNF in limbic brain regions (Duman and Monteggia, 2006; Krishnan and Nestler, 2008). However, there is no evidence that typical antidepressants increase BDNF release, as demonstrated for activity-dependent release of BDNF via activation of AMPA receptors and VDCC (Jourdi et al., 2009). Activity dependent release of BDNF may be a key step in the rapid actions of ketamine. Studies are currently being conducted in BDNF mutant mice to examine this possibility.

There are several BDNF mutant lines, including constitutive (heterozygous) and conditional deletion mutants. Another line of interest is a knock-in of a single nucleotide polymorphism (SNP),

Val66Met, found in humans. The Val66Met SNP is located in the BDNF prodomain and blocks trafficking into the regulated secretion pathway and activity-dependent release of BDNF (Casey et al., 2009). The Met allele is found in ~20–30% of humans, and is associated with several functional deficits, including impairments in episodic memory and executive function (Egan et al., 2003; Frodl et al., 2006), decreased hippocampal volume, both in normal and depressed patients (Bueller et al., 2006; Frodl et al., 2007; Pezawas et al., 2004), and increased susceptibility to depression in patients previously experiencing trauma or stress (Gatt et al., 2009; Kaufman et al., 2006; Kim et al., 2007). The BDNF Met knock-in mice mimic the human polymorphism, with reduced hippocampal volume, atrophy of hippocampal neurons, and increased anxiety (Chen et al., 2006). We have recently reported that the synaptogenic and behavioral actions of ketamine are blocked in BDNF Met knock-in mice, providing evidence for the role of BDNF release (Liu et al., 2011).

#### 4.2. MKP-1, a negative regulator of BDNF-ERK-mTOR signaling

In our whole genome microarray studies to elucidate the pathophysiology of depression, we have identified a negative regulator of the BDNF-ERK cascade, mitogen activated protein (MAP) kinase phosphatase-1 (MKP-1) (Duric et al., 2010). MKP-1 is a dual specificity phosphatase that dephosphorylates both threonine and tyrosine residues and is a key negative regulator of the ERK signaling cascade (Jeffrey et al., 2007). In our microarray studies of postmortem tissue, we found that levels of MKP-1 were significantly increased in the hippocampus of depressed subjects relative to matched controls (Duric et al., 2010). There was also a significant decrease in the levels of ERK, as previously reported (Dwivedi et al., 2006). In rodent studies, we found that levels of MKP-1 were increased in the hippocampus by exposure to CUS, and this effect was reversed by chronic antidepressant treatment. Increased expression of MKP-1 in depressed subjects and in response to CUS is consistent with reports that MKP-1 is an immediate early gene that is induced by cellular stress and adrenal glucocorticoids (Keyse and Emslie, 1992; Seta et al., 2001).

The functional consequences of altered MKP-1 levels were examined using viral vector and mutant mouse approaches. Over expression of MKP-1 in the hippocampus using a recombinant adeno-associated virus was sufficient to produce a depressive phenotype in rats, including a decrease in sucrose preference, an increase in escape failures in the LH model, and an increase in latency to feed in the NSFT (Duric et al., 2010). Conversely, MKP-1 deletion mutant mice displayed a resilient behavioral phenotype upon exposure to CUS (i.e., no decrease in sucrose consumption). MKP-1 null mice exposed to CUS also displayed higher levels of phospho-ERK compared to wild type controls exposed to the same CUS paradigm.

Abnormal and sustained elevation of MKP-1, combined with decreased expression of ERK, could lead to decreased BDNF-ERK signaling, and thereby contribute to decreased basal, as well as activity dependent activation of mTOR signaling. Both the PFC and hippocampus and related circuitry have been implicated in depression and antidepressant response (Drevets and Furey, 2010; Macqueen et al., 2008), and in the actions of ketamine (Li et al., 2010; Autry et al., 2011). Additional studies are required to determine if mTOR signaling and synaptogenesis are regulated by MKP-1 in both regions, as well as other limbic structures.

### 5. Rapid acting antidepressant targets

The discovery of ketamine as a rapid acting antidepressant that is effective in treatment resistant depressed patients is a major

breakthrough for mood disorders therapeutics. Ketamine is also effective for bipolar depression (Diazgranados et al., 2010a,b) and suicide ideation (Diazgranados et al., 2010b; Larkin and Beautrais, 2011). However, there are limitations: ketamine is also a street drug with abuse potential, and is reported to cause neurotoxicity with repeated use (Behrens et al., 2007).

Characterization of the cellular signaling mechanisms underlying the effects of ketamine has provided potential targets for new medications that might share the therapeutic actions of ketamine, but without the side effects. Another possibility would be to identify agents that sustain the actions of ketamine. We have discussed evidence that a selective NR2B receptor antagonist produces ketamine like effects in rodent models (Li et al., 2010) and in human subjects (Preskorn et al., 2007). However, the NR2B agent used in the latter study, CP-101,606, did cause some dissociative effects at the initial dose tested (Preskorn et al., 2007). Additional studies will be required to further demonstrate the efficacy and safety of compounds selective for the NR2B receptor.

Other possible targets that could produce ketamine like effects also influence glutamate transmission. As discussed, ketamine-stimulation of mTOR signaling and antidepressant behavioral actions are dependent on glutamate-AMPA receptor activation. Drug targets that enhance glutamate transmission or that activate AMPA receptors could also produce rapid and efficacious antidepressant actions. Regulation of glutamate transmission has been an area of interest for drug development for several years, and includes cognitive enhancing agents for neurodegenerative disorders and medications for schizophrenia, as well as depression. Two major targets are presynaptic metabotropic glutamate type 2/3 receptors (mGlu2/3) that regulate glutamate release and postsynaptic AMPA receptors (Fig. 3).

The mGlu2/3 receptors are located on presynaptic terminals and negatively regulate glutamate release. Antagonists of mGlu2/3 receptors are reported to have antidepressant actions in acute behavioral models (Pałucha-Poniewiera et al., 2010; Pilc et al., 2007). Positive AMPA receptor modulating agents have the ability to enhance AMPA receptor function without direct receptor activation (Arai and Kessler, 2007). AMPA receptor potentiating drugs are reported to increase LTP, learning and memory, and BDNF (Arai and Kessler, 2007; Arai et al., 2000), providing further evidence that these drugs could lead to activation of mTOR. There is also evidence that AMPA receptor potentiating drugs have antidepressant actions (Bai et al., 2003). Studies are currently underway to determine if mGlu2/3 antagonists or AMPA receptor potentiating drugs stimulate mTOR signaling,

increase synaptogenesis, and produce rapid antidepressant behavioral actions in the CUS model of depression.

When developing these drugs it is important to recognize that increased glutamate neurotransmission could also have deleterious effects. For example stress acutely increases glutamate (Moghaddam, 2002), and sustained elevation of glutamate transmission could produce neurotoxic effects. Studies to determine optimal levels of glutamate transmission for a therapeutic response, the relevant brain circuits to target, and safety of these treatments must be determined before developing such agents for the widespread treatment of depression and other psychiatric illnesses.

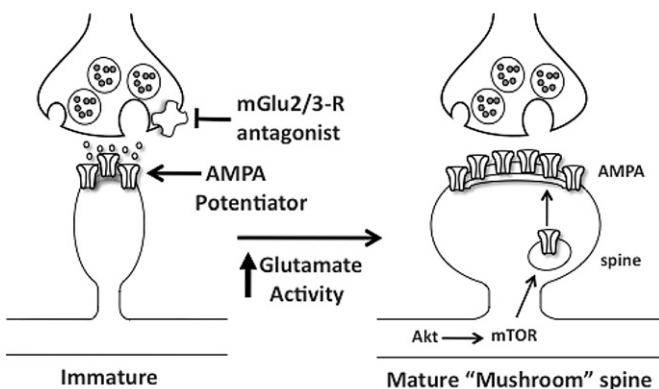
## 6. Summary and future directions

The actions of NMDA receptor antagonists on mTOR signaling and on the density and function of spine synapses represents a fundamental shift in our understanding of the mechanisms underlying rapid acting, efficacious antidepressant treatments. The ability of ketamine to increase synaptogenesis could thereby rapidly reverse the structural deficits resulting from chronic stress exposure that are thought to contribute to depressive symptoms. Sustained induction of negative regulators of BDNF-ERK signaling, such as MKP-1, could also contribute to the atrophy of neurons and decreased volume of limbic brain regions in depression.

The mTOR translational system can be influenced, both positively and negatively by a variety of neurotransmitter, endocrine, and metabolic signaling pathways, and could thereby serve as a nexus for control of synaptogenesis. This complex regulatory system raises the possibility that there are additional negative regulators of mTOR signaling and synaptogenesis that play a role in neuronal atrophy caused by stress exposure, an area that is under active investigation. Conversely, pathways that enhance mTOR signaling could also be targeted for novel therapeutic approaches for the treatment of depression, although the ubiquitous expression and function of mTOR raises the possibility of side effects. Nevertheless, novel targets that influence glutamate transmission, BDNF-mTOR signaling, and synaptogenesis offer promise for the development of safer, rapid acting and efficacious antidepressant agents for the treatment of depression.

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**Fig. 3.** Targets for induction of synaptogenesis and ketamine-like effects. Potential targets that could produce ketamine like effects include presynaptic mGlu2/3 receptor antagonists and postsynaptic positive AMPA receptor potentiating agents. Both would lead to increased glutamate transmission and AMPA receptor activation, and thereby stimulate mTOR signaling and increase synaptogenesis. See text for details.

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