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# GSK-3: a key regulatory target for ketamine's rapid antidepressant effects mediated by enhanced AMPA to NMDA throughput

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**Keywords**

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA); glycogen synthase kinase 3 (GSK-3); ketamine

In the past decade, studies that provide insights into the mechanisms of action underlying ketamine's rapid antidepressant effects have been key to identifying relevant targets for developing novel antidepressants with rapid and sustained effects[1]. In this context, glycogen synthase kinase 3 (GSK-3) appears to be a top candidate. GSK-3 has been extensively associated with mood disorders, particularly with regard to lithium's effects on this target and the potential association with clinical improvement[2–4]. GSK-3 also plays a key role in relevant biological processes such as oxidative stress, neurogenesis, and inflammation[3].

In an elegant preclinical study, Beurel and colleagues[5] recently demonstrated an integrated functional effect between GSK-3 and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in ketamine's rapid antidepressant effects. Specifically, the study found that ketamine-induced GSK-3 inhibition upregulated AMPA GluA1 subunits and stabilized AMPA receptors at the cell surface. Ketamine also decreased post-synaptic density 95 (PSD-95) phosphorylation; PSD-95 is a known substrate for GSK-3 that directly regulates the number of AMPA receptors at the cell surface and, consequently, regulates synaptic strength[6]. Notably, the limiting effects at PSD-95 led to lower internalization of AMPA GluA1, which also had a central regulatory effect on AMPA receptor trafficking[5]. It is important to note that GSK-3 was previously shown to regulate AMPA receptor trafficking[7] and is also associated with PSD-95 phosphorylation[8]. These recent findings by Beurel and colleagues underscore the relevance of GSK-3 and postsynaptic density proteins in ketamine's rapid antidepressant effects.

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Ketamine is a classic high-trapping N-methyl-D-aspartate (NMDA) receptor antagonist that displays slow 'off-rate' NMDA receptor dissociation[9]. This high-trapping ability was initially proposed as a key mechanism underlying its rapid antidepressant properties. Hypothetically, while trapped in the ion channel, ketamine would induce a prolonged receptor blockade, thereby dissociating glutamate from its binding site on the NMDA receptor. This, in turn, would exert rapid antidepressant effects in conjunction with its well-known adverse 'dissociative state' effects, even at lower concentrations[10]. However, recent studies have shown that other NMDA receptor antagonists—for instance, MK-0657 and AZD6765—do not appear to possess ketamine's robust, rapid, and sustained antidepressant effects[11, 12]. As a result, the potential roles of alternative (non-NMDA) mechanisms underlying ketamine's rapid antidepressant effects are being further explored.

The most compelling alternative model to explain ketamine's rapid antidepressant effects beyond simple NMDA antagonism is based on ketamine's ability to augment AMPA receptor activity in critical neuronal circuits, the so-called enhanced AMPA to NMDA throughput model[13, 14] (see Fig. 1). Briefly, the AMPA throughput model posits that AMPA receptor activation is a key mediator of ketamine's antidepressant effects. It builds on the observation that pre-treatment with the AMPA receptor antagonist NBQX attenuated ketamine-induced antidepressant-like effects and also upregulated hippocampal phosphorylated GluA1 AMPA receptors[15]. This model also suggests that NMDA receptor blockade increases synaptic glutamate release, thus preferentially favoring AMPA receptor activity in critical limbic circuits. Additional experiments have supported the notion that ketamine's ability to induce rapid antidepressant effects is due to a rapid and transient increase in glutamate release in the prefrontal cortex associated with acute activation of AMPA receptors[16].

Notably, the recent findings described by Beurel and colleagues[5] suggest that a direct interaction between GSK-3 and AMPA receptors plays a central role in ketamine's antidepressant effects. This association has been demonstrated in previous studies with ketamine. In addition to the recent findings showing that ketamine activates AMPA receptor signaling by inhibiting GSK-3 via a decrease in hippocampal PSD-95 phosphorylation, Beurel and colleagues had previously used GSK-3 *knockin* mice to demonstrate that cortical and hippocampal GSK-3 inhibition (based on its increased phosphorylation at serine 21 and 9) was required for ketamine's rapid antidepressant-like effects[17]. In GSK-3 *knockin* mice, serine phosphorylation did not inhibit GSK-3; the latter was also associated with arousing depressive-like behaviors in rodents following diverse stressors[18]. In humans, ketamine was shown to inactivate GSK-3 activity (by lowering its phosphorylation) after a single bolus ketamine infusion in depressed patients[19]. In addition, lithium—which is a potent GSK-3 inhibitor—potentiated the antidepressant-like effects of ketamine in mice[20]. Furthermore, a polymorphism in the GSK-3 promoter gene was found to underlie the antidepressant response to total sleep deprivation in individuals with bipolar disorder[21]; sleep deprivation is an important model for studying the neurobiological basis of rapid antidepressant efficacy.

Additional evidence supports a role for AMPA receptor activity, particularly GluA1, in ketamine's molecular actions. Specifically, low-dose ketamine significantly enhanced hippocampal AMPA—but not NMDA—receptor density in Wistar Kyoto rats[22], a finding

that may be associated with the potential role of AMPA in long-term antidepressant efficacy. Likewise, ketamine significantly upregulates AMPA receptor subunits[23]. In addition, subanesthetic doses of ketamine activate AMPA receptor-mediated prefrontal cortex synaptic transmission (AMPA-induced currents)[24]. Interestingly, potent AMPA positive modulators have recently been developed and tested in initial Phase 1 and 2 studies to overcome the lower bioavailability associated with previous studies; these newer agents have shown preliminary antidepressant efficacy in small samples[25, 26], though larger studies are needed to definitively assess the antidepressant effects of these compounds. It should be noted that the antidepressant effects of the mood stabilizer lithium, a central GSK-3 inhibitor, have also been shown to be mediated via AMPA receptor signaling potentiation[7], supporting a convergent mechanism.

Another promising area of study is the investigation of ketamine metabolites and enantiomers as tools to provide insight into potential central mechanistic models, with a convergent focus on the AMPA throughput model. Indeed, AMPA receptor throughput seems to be involved not only in ketamine's rapid antidepressant effects but also in the acute and sustained effects of its metabolites[27]. Changes in the efficiency of the AMPA receptor are commonly used to evaluate synaptic plasticity, and low-frequency synaptic stimulation capable of increasing synaptic efficiency is mostly mediated by activation of AMPA receptors[28]. Reinforcing the concept of enhanced AMPA to NMDA throughput, a recent study demonstrated that the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine (HNK) does not bind to or inhibit NMDA receptors; nevertheless, the metabolite independently and rapidly increased AMPA receptor-mediated hippocampal synaptic transmission and upregulation of GluA1 and GluA2 AMPA receptor subunits in synaptosomes[27]. (2*R*,6*R*)-HNK also induced antidepressant effects independently of NMDA receptor antagonism. Furthermore, the AMPA receptor antagonist NBQX also blocked the rapid *and* sustained antidepressant effects of (2*R*,6*R*)-HNK[27].

Finally, further evidence supports enhanced AMPA to NMDA throughput related to ketamine's effects. Several downstream targets of AMPA activate synaptic plasticity pathways, and these are known to be involved in ketamine's rapid antidepressant effects. For instance, AMPA receptor activation targeting brain-derived neurotrophic factor (BDNF), eukaryotic elongation factor 2 (eEF2), and the mammalian target of rapamycin (mTOR) signaling pathways appear relevant to the rapid antidepressant effects of ketamine and related molecules in preclinical models. Ketamine enhanced BDNF signaling by activating both post-synaptic AMPA receptor and L-type voltage-dependent calcium channels, thus inducing calcium influx and activity-dependent BDNF exocytosis[29]. These effects also seem to be related to the stimulation of eEF2- and BDNF-dependent potentiation[30]. Activation of the mTOR signaling pathway—a central regulator of cell metabolism, growth, proliferation, and survival—through AMPA receptors has also been associated with ketamine's rapid antidepressant-like effects[31]. In addition, this pathway has also been linked to reversal of stress- and/or depression-related deficits[31]. mTOR also regulates brain morphogenesis by mediating GSK-3 signaling[32]. However, it should be noted that even though mTOR has been implicated in ketamine's rapid antidepressant effects, it was not involved in the early and sustained antidepressant effects of its (2*R*,6*R*)-HNK metabolite[27].

In conclusion, the recent study by Beurel and colleagues[5] supports the notion that GSK-3 plays a key role in the enhanced AMPA throughput model underlying ketamine's rapid antidepressant efficacy. This convergent mechanism seems to involve AMPA receptor activation potentially mediated by GSK-3, with consequent glutamate release and resultant activation of downstream pathways related to ketamine's rapid and sustained antidepressant effects. Furthermore, when taken in conjunction with the recent findings of Zanos and colleagues[27], it appears that these late-phase effects may involve ketamine's metabolites targeting at the eEF2 and BDNF pathways. Antidepressant effects would thus synergistically result from elevated glutamate levels, increased AMPA receptor insertion, and increased monoamines. Indeed, an evaluation of the potential role of GSK-3 in the sustained antidepressant effects of the (2*R*,6*R*)-HNK metabolite is expected to be performed soon. At present, the extant evidence definitively confirms that a single mechanism cannot be responsible for ketamine's rapid antidepressant effects. Additional preclinical studies on enhanced AMPA throughput mechanisms that evaluate different downstream targets are needed, as are initial clinical studies exploring the therapeutic potential of ketamine metabolites.

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## Abbreviations

| | |
|---|---|
| **AKT3** | protein kinase B3 |
| **AMPA** | α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid |
| **BDNF** | brain-derived neurotrophic factor |
| **CREB** | cyclic adenosine monophosphate response element binding protein |
| **GSK-3** | glycogen synthase kinase-3 |
| **HNK** | hydroxynorketamine |
| **IRS** | insulin receptor substrate |
| **mTOR** | mammalian target of rapamycin |
| **NMDA** | N-methyl-D-aspartate |
| **PI3K** | phosphoinositide-3 kinase |

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|---|---|
| **PSD-95** | post-synaptic density-95 |
| **TrkB** | tropomyosin receptor kinase B |

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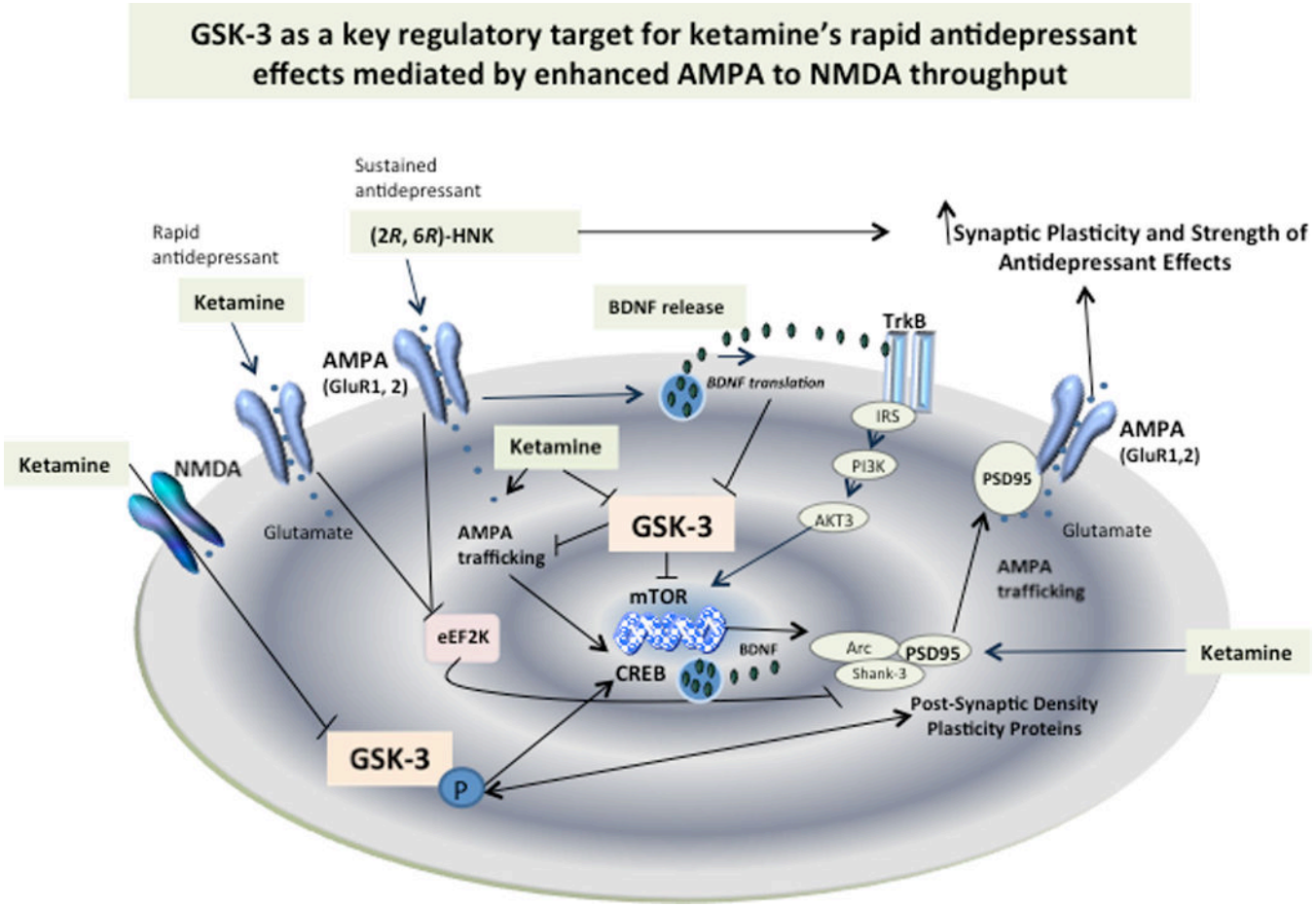


**Fig. 1.**

Enhanced AMPA to NMDA throughput is a key convergent model suggesting that augmentation of AMPA receptor signaling mediates the activation of synaptic plasticity and, consequently, the rapid antidepressant effects of the glutamatergic modulator ketamine. GSK-3 has been shown to critically regulate AMPA receptor activation and intracellular trafficking by limiting its activity and associated antidepressant efficacy. Increased GSK-3 phosphorylation by ketamine inactivates the protein and favors increases in mTOR, CREB, and PSD-95 expression. AMPA receptor trafficking is regulated by PSD-95, which also regulates GSK-3. Ketamine lowers phosphorylated PSD-95 on Thr-19, the GSK-3 target that promotes AMPA receptor internalization. Similar activation of synaptic strength and plasticity involving direct regulation at GSK-3 are expected to take place with the ketamine metabolite (2*R*, 6*R*)-HNK based on its direct ability to activate AMPA receptors and synaptogenesis in preclinical models.