The Ketamine Story: Introduction

Depression is a neurological disease


Synaptic-function–related genes, the number of spine synapses are decreased in the dlPFC of subjects with MDD.

a–d representative images of autoradiographs from in situ hybridization of matched control subjects (top) and subjects with MDD.

h Representative high-power electron micrograph. The arrowheads point to examples of spine synapses (scale bar, 500 nm). Synapses were quantified in layers II and III of the dlPFC (PFC II/III), and the results represent the mean ± s.d. (n = 5 per group, controls and subjects with MDD)
Chronic stress causes atrophy of neuronal processes and decreases synapse number. The influence of repeated-restraint stress on pyramidal neurons (layer V) in the mPFC of rat (24). Pyramidal neurons in sections of mPFC are visualized after filling the cells with neurobiotin and using two-photon laser-scanning microscopy. The left-hand set of images shows that stress induces a reduction in the number and length of apical dendrites. The right-hand set of images shows a magnified segment of dendrite, with its spines at the point of synaptic contacts with neuronal inputs to the mPFC; repeated stress significantly decreases the number and function of spine synapses

(24) Liu, R.J. & Aghajanian, G.K. Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-

Under normal conditions stimulation of the presynaptic neuron releases glutamate, resulting in the activation of postsynaptic glutamate AMPA receptors and depolarization; this causes activation of multiple intracellular pathways, including the BDNF-TrkB signaling pathway (and the downstream kinases Akt and ERK) and the mTORC1 pathway. These pathways are essential for regulation of synaptic plasticity, a fundamental adaptive learning mechanism that includes maturation (increased spine-head diameter) and an increase in the number of synapses. This process requires mTORC1-mediated de novo protein synthesis of synaptic proteins, including glutamate GluA1 AMPA receptors and PSD95. Repeated stress decreases BDNF and mTORC1 signaling in part via upregulation of the negative regulator REDD1 (regulated in DNA damage and repair), which decreases the synthesis of synaptic proteins and thereby contributes to a decreased number of spine synapses. Other proteins that are involved in the regulation of synaptic plasticity include GSK3 and protein phosphatase 1 (PP1).

The multiple heterogeneous signaling pathways that influence synapse formation and stability and that could contribute to loss of synapses in depression.

Pathways that affect synapse formation include Neurotransmitters (e.g., glutamate), growth factors and neurotrophic factors (GFs/NTFs), cytokines (e.g., TNF-alpha), energy and metabolic factors (e.g., ATP and amino acids), sex steroids (e.g., estrogen) and the HPA axis (cortisol).

One of the key pathways of interest is the mTORC1 signaling cascade, which is a sensor for synaptic activity and systems that can influence synaptic protein synthesis as shown.

Activation of mTORC1 signaling can occur via regulation of PI3K and stimulation of Akt. PI3K can be directly or indirectly (via multiple steps) stimulated by the different factors indicated, notably glutamate (via AMPA or mGlu receptors (mGluRs)), estrogen (via estrogen receptors (ER)), BDNF, and other neurotrophins and growth factors. By using the glucocorticoid receptor (GR), stress and glucocorticoids can inhibit mTORC1 signaling by inducing factors that inhibit mTORC1 stability. Metabolic factors (including ATP and amino acids) that are required for protein synthesis can also regulate mTORC1.

Mechanism of action of the fast-acting antidepressant ketamine in the mPFC.

Ketamine causes a burst of glutamate that is thought to occur via disinhibition of GABA interneurons; the tonic firing of these GABA interneurons is driven by NMDA receptors, and the active, open-channel state allows ketamine to enter and block channel activity. The resulting glutamate burst stimulates AMPA receptors, which causes depolarization and activation of voltage-dependent Ca2+ channels (VDCC), leading to release of BDNF and stimulation of TrkB and Akt, which then activates mTORC1 signaling, leading to the increased synthesis of proteins that are required for synapse maturation and formation (i.e., GluA1 and PSD95). Under conditions in which BDNF release is blocked (such as in knockin mice with the BDNF Val66Met allele) or neutralized (using a neutralizing antibody), or in which mTORC1 signaling is blocked (such as rapamycin infusion into the mPFC), the synaptic and behavioral actions of ketamine are blocked. Scopolamine also causes a glutamate burst via blockade of acetylcholine muscarinic M1 (ACh-M1) receptors on GABA interneurons. Antagonists of mGluR2/3 also produce rapid antidepressant actions via blockade of presynaptic autoreceptors that inhibit the release of glutamate. Relapse to a depressive state is associated with a decrease of synapses on mPFC neurons, which could occur via stress and imbalance of endocrine hormones (cortisol), estrogen, inflammatory cytokines, and metabolic and cardiovascular illnesses.
The Ketamine story: part 1

The non-competitive, glutamatergic NMDAR antagonist (R,S)-ketamine (ketamine) has demonstrated rapid and robust efficacy as an antidepressant by improving core depressive symptoms including depressed mood, anhedonia, and suicidal thoughts in treatment-refractory unipolar and bipolar depressed patients when administered at sub- anesthetic doses (4–8)


Figure 1. Course of Suicidal Ideation Within 230 Minutes of Ketamine Infusion in Patients With Treatment-Resistant MDD With and Without High Baseline Suicidal Ideation (N = 33)ab

A. SSIS

B. BDI Suicide

C. HDRS Suicide

D. MADRS Suicide

*High SSI score was defined as > 3; low SSI score was defined as < 4.

The values presented are mean scores, with bars representing 1 standard error.

Abbreviations: BDI = Beck Depression Inventory, HDRS = Hamilton Depression Rating Scale, MADRS = Montgomery-Åsberg Depression Rating Scale, MDD = major depressive disorder, SSI = Scale for Suicide Ideation.

Figure 1. Change in depression severity after repeated ketamine infusions in treatment-resistant major depression. Figure depicts change in depression severity as measured by the Montgomery-Åsberg Depression Rating Scale (MADRS) (mean ± SD) over a 12-day period during which ketamine (0.5 mg/kg) was administered IV on a Monday-Wednesday-Friday schedule, corresponding to study days 0, 3, 5, 8, 10, and 12. Trajectories of depression severity are plotted for phase I responder and nonresponder subgroups, defined with the final observed MADRS score. Depression severity was initially measured at baseline before the first ketamine infusion and then at 2, 4, and 24 hours while participants were inpatients. Subsequent infusions occurred on an outpatient basis, and depression severity was measured in the morning before each infusion and then at 4 hours. *MADRS score significantly decreased at given time point compared with baseline, p < .05. #MADRS score significantly different at given time point between responder and nonresponder subgroups. aThree participants in the nonresponder group did not receive all six ketamine infusions.

The rapid-acting antidepressant effects of ketamine and the potential role of synaptogenic and neurotrophic mechanisms in depression

Left, low-dose ketamine has been shown to exert a rapid antidepressant effect, and it may be effective at reducing acute suicidal ideation, even in people with TRD. Right, in parallel, studies in rats are shedding new light on the molecular effects of ketamine and other glutamate NMDA receptor (NMDAR) antagonists on depression. The antidepressant effect of NMDAR antagonists may result from enhancing the activity of the glutamate (AMPA) receptor and activating the mammalian target of rapamycin (mTOR) intracellular signaling pathway, which ultimately leads to increased synapse-related proteins and neural trophic support.

The Ketamine Story, Part 3: There are problems with the NMDA Receptor Theory

Ketamine belongs to a class of drugs that block cellular receptors for glutamate, the brain’s chief excitatory chemical messenger. Until now, the prevailing view was that ketamine produced its antidepressant effects by blocking N-methyl-D-aspartic acid (NMDA) glutamate receptors. However, human trials of other NMDA-receptor blockers failed to produce ketamine’s robust and sustained antidepressant effects.

Ketamine harbors two chemical forms that are mirror images of each other, denoted (S)- and (R)-ketamine. The investigators found that while (S)-ketamine is more potent at blocking NMDA receptors, it is less effective in reducing depression-like behaviors than the (R) form. The body breaks down (S)- and (R)-ketamine. It was known that ketamine’s antidepressant effects are greater in female mice. NIA researchers Irving Wainer, Ph.D., and Ruin Moaddel, Ph.D. identified a key metabolite (2S,6S;2R,6R)-HNK (hydroxynorketamine) and showed that it is pharmacologically active. The team then discovered that levels of this metabolite were three times higher in female mice, hinting that it might be responsible for the sex difference in the antidepressant-like effect. To find out, the researchers chemically blocked the metabolism of ketamine. This prevented formation of the metabolite, which blocked the drug’s antidepressant-like effects.
1. The authors compared the effects of two different structural forms, or enantiomers, of ketamine, called (S)- and (R)-ketamine, which are normally administered together. (S)-Ketamine is three to four times better at blocking NMDAR than (R)-ketamine, and so is predicted to be the better antidepressant under the NMDAR-inhibition model. However, the authors found that (R)-ketamine was several times more efficient at reducing depression-like behaviours in mouse models of depression. Furthermore, they confirmed that an even more potent NMDAR inhibitor, which binds to the same site as ketamine, fails to produce sustained antidepressant-like effects.

2. However, the authors found that levels of the ketamine metabolite hydroxynorketamine (HNK) were several-fold higher in the brains of females than males after the animals were given the same dose of the drug. Reducing the metabolism of ketamine to HNK reduced the effectiveness of ketamine towards depression-related behaviours in mice. Moreover, treating animals with HNK produced the same rapid and sustained antidepressant-like effects seen after treatment with ketamine. As with ketamine, the (R)-enantiomer of HNK had more-potent antidepressant-like effects than the (S)-form. And, importantly, the researchers showed that HNK neither binds to nor inhibits NMDAR.
NMDAR inhibition–independent antidepressant actions of ketamine metabolites

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The Ketamine Story, Part 3: The NMDA Receptor Theory can’t be right

**Metabolite mediator of ketamine.** How the drug ketamine exerts its antidepressant effects is unknown, although a common hypothesis states that it acts by binding to the receptor protein NMDAR on postsynaptic neurons, preventing neurotransmitter molecules released by presynaptic neurons from activating NMDAR and so inhibiting signalling processes triggered by the receptor. By contrast, Zanos *et al.* (7) report that it is a metabolite called hydroxynorketamine (HNK) that has antidepressant activity. They provide evidence that HNK, through unknown intermediates, increases the levels of another neuronal receptor protein, AMPAR, at synapses (dashed arrow), enhancing neural activity. But how this produces an antidepressant effect remains unclear.
While equivalent levels of ketamine and norketamine were found, (2S,6S;2R,6R)-HNK was approximately three-fold higher in the brains of female mice compared to males (Fig. 2c–e), suggesting a role of (2S,6S;2R,6R)- HNK in the antidepressant effects of ketamine.

Like ketamine, this metabolite includes two forms that mirror each other. By testing both forms, they found that one – (2R,6R)-HNK – had antidepressant-like effects similar to ketamine, lasting for at least three days in mice. Notably, unlike ketamine, the compound does not inhibit NMDA receptors. It instead activates, possibly indirectly, another type of glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). Blocking AMPA receptors prevented the antidepressant-like effects of (2R,6R)-HNK in mice. The experiments confirmed that the rapid antidepressant-like effects require activation of AMPA receptors, not inhibition of NMDA receptors.

Ketamine also has effects in mice that mimic its dissociative, euphoric effects in humans and underlie its abuse and addictive potential; however, these effects were not observed with (2R,6R)-HNK. (2R,6R)-HNK did not cause the changes in physical activity, sensory processing, and coordination in mice that occur with ketamine. In an experimental situation where mice were able to self-administer medication, they did so with ketamine but not the (2R,6R)-HNK metabolite, indicating that (2R, 6R)-HNK is not addictive.

The Ketamine Story, Part 4

A brief tutorial on the glutaminergic synapse
When glutamate binds to the NMDA receptor at slightly depolarized or resting membrane voltages, very few ions flow through the channel. This low conductance occurs because the pore of the channel is blocked by Mg$^{2+}$ ions, which prevents other ions from passing freely through the channel.

For example, some calcium binds to calmodulin, and this complex in turn activates several protein kinases, including calcium/calmodulin-dependent protein kinase, or CAM kinase. CAM kinase affects AMPA receptors in two ways. First, it phosphorylates AMPA receptors already present in the dendritic spine membrane, thereby increasing their conductance to sodium ions.
CaMKII also promotes movement of AMPA receptors from intracellular stores into the membrane, making more receptors available to stimulate the spine. In addition to these postsynaptic effects, Ca^{2+} may also facilitate the release of transmitter from the presynaptic axon terminal via retrograde signals, such as nitric oxide (NO).

As a result of the increase in the number of AMPA receptors, the response to a stimulus of a given strength will be stronger than it was before the NMDA receptors were activated. In this regard, the synapse is said to be "enhanced," and this physiological change is thought to be one of the mechanisms underlying the expression of long-term potentiation, or LTP.
Under normal conditions stimulation of the presynaptic neuron releases glutamate, resulting in the activation of postsynaptic glutamate AMPA receptors and depolarization; this causes activation of multiple intracellular pathways, including the BDNF-TrkB signaling pathway (and the downstream kinases Akt and ERK) and the mTORC1 pathway. These pathways are essential for regulation of synaptic plasticity, a fundamental adaptive learning mechanism that includes maturation (increased spine-head diameter) and an increase in the number of synapses. This process requires mTORC1-mediated de novo protein synthesis of synaptic proteins, including glutamate GluA1 AMPA receptors and PSD95. Repeated stress decreases BDNF and mTORC1 signaling in part via upregulation of the negative regulator REDD1 (regulated in DNA damage and repair), which decreases the synthesis of synaptic proteins and thereby contributes to a decreased number of spine synapses. Other proteins that are involved in the regulation of synaptic plasticity include GSK3 and protein phosphatase 1 (PP1).

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