NMDA Antagonism in the treatment of Major Depressive Disorder

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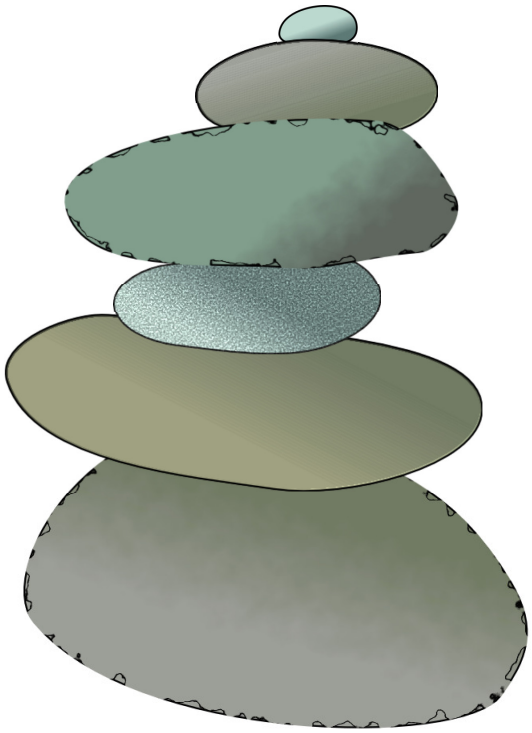

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Abstract

With a lifetime prevalence of over 16% and spread over all social classes, major depressive disorder (MDD) is one of the leading causes for psychosocial disability. Although there is no established mechanism for depression most drugs used in pharmacotherapy focus on the monoamine-deficiency hypothesis and inhibit the reuptake or metabolism of serotonin or norepinephrine in order to raise their plasma-levels. With over 20% of patients being resistant to treatment, and very low remission and high relapse rates, new strategies and methods for this treatment-resistant form of depression are warranted. In recent years the use of *N*-methyl-D-aspartate (NMDA) antagonists, and in particular the well known dissociative drug ketamine, in the treatment of clinical depression has come under increasing interest. This administration is less rigorous than more invasive treatments like deep brain stimulation. Recently the mechanism of action of this acute response, at sub-psychotomimetic doses, has been linked to eukaryotic elongation factor 2 (eEF2) kinase inhibition and desupression of brain-derived neurotrophic factor (BDNF) translation. This supports other research implying synaptic plasticity as an important factor in treating MDD. Chronic administration of ketamine is however linked to impairments of verbal fluency, cognitive processing speed, and verbal learning and its other cognitive effects are associated with potential abuse. In this report we will outline the molecular mechanisms involved in NMDA antagonism, the effects on other neurotransmitter-systems and glutamate signaling and how these relate to the positive effects on MDD. The major transduction pathways will be outlined and potential related targets highlighted.

Contents

Acronyms

4E-BP 4E binding protein.

ACC anterior cingulate cortex.
AD antidepressant.
Akt protein kinase B.
ALS amyotrophic lateral sclerosis.
AMPA α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.
AMPAR AMPA receptor.
Arc activity-regulated cytoskeleton-associated protein.

BBB blood-brain barrier.
BDNF brain-derived neurotrophic factor.
BPD bipolar disorder.

CaMKII Ca^{2+}/calmodulin-dependent protein kinase.
CBT cognitive behavioral therapy.
CNS central nervous system.
CUS chronic unpredictable stress.

E-LTP early-LTP.
eEF2 eukaryotic elongation factor 2.
EPSC excitatory postsynaptic current.
EPSP excitatory postsynaptic potential.
ERK extracellular signal-regulated kinase.

FST forced swim test.

GNDF glial-derived neurotrophic factor.
GPCR G-protein coupled receptor.
GSK-3 glycogen synthase kinase 3.

ICV intracerebralventricular.
IPT interpersonal psychotherapy.

KA kainate.
KO knockout.

L-LTP late-LTP.
LH learned helplessness.
LTD long-term depression.
LTP long-term potentiation.

MAOI monoamine oxidase inhibitor.
MDD major depressive disorder.
mGluR metabotropic glutamate receptor.
mTOR mammalian target of rapamycin.

NBQX 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione.
NMDA *N*-methyl-D-aspartate.
NMDAR NMDA receptor.
NSFT novelty suppressed feeding test.

p70S6K p70S6 kinase.
PFC prefrontal fortex.
PP1 protein phosphatase 1.
PSD-95 postsynaptic density protein 95.

SNRI serotonin-norepinephrine reuptake inhibitor.
SSRI selective serotonin reuptake inhibitor.

TCA tricyclic antidepressants.
TRD treatment-resistant depression.
TREK-1 TWIK-1-related K+ channel.
TrkB Tropomyosin receptor kinase B.
TST tail suspension test.

UCMS unpredictable chronic mild stress.

VDCC voltage-dependent Ca^{2+} channel.
VEGF vascular endothelial growth factor.

WKY Wistar-Kyoto.
WT wild-type.

1 Major Depressive Dissorder

Clinical depression, or MDD, is a major cause of disability worldwide. The lifetime prevalence of this disorder varies widely, but in most countries the percentage of people that experience depression during their lives falls within 8-12%[1]. In western countries, particularly in North American, this number can rise up to nearly 17% and is spread throughout all demographics, with slightly elevated numbers for low-income backgrounds[2]. This indicates that MDD is relatively common and widely distributed among the population with a significant impact on the medical system.

1.1 Symptoms and Treatment

MDD is characterized by a distinct change of mood, distinguished by sadness or irritability and associated with at least several psychophysiological changes such as disturbances in sleep, appetite, or reduced sex drive. Other symptoms may include constipation, general anhedonia (inability to experience pleasure in work or other activities), and suicidal thoughts[3]. These changes have to persist for several weeks and interfere substantially with work and social relations. Sometimes other related disorders are misdiagnosed such as dysthymia, which has milder symptoms, but is of a chronic variety. Some patients with MDD may encounter manic episodes that consist of additional symptoms such as hyperactivity, euphoria and an increase in pleasure-seeking behavior. Patients experiencing these episodes are diagnosed with a distinct illness termed bipolar disorder (BPD)[4].

The response to MDD treatment is generally inconsistent and there is no established mechanism[3]. The typical treatments for MDD are psychotherapy and pharmacotherapy. Both psychotherapy and medication have been shown to be viable treatments for MDD[5]. However where psychotherapy can be used more successfully in preventive follow-up care, pharmacotherapy is somewhat more successful in the treatment of dysthymia[6]. The combination of psychotherapy and pharmacotherapy is more effective than either of these therapies alone[7;8].

Psychotherapeutic treatments usually employed are cognitive behavioral therapy (CBT), interpersonal psychotherapy (IPT), psychodynamic therapy, and upportive counselling, but will depend on the background and judgment of the treating psychotherapist. For mild depression psychotherapy is the first line of treatment[9]. Pharmacotherapeutic treatments typically focus on the monoamine-deficiency hypothesis where supposed deficiencies of endogenous mood-regulatory neurotransmitters such as serotonin, dopamine or norepinephrine are enhanced using selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs) or monoamine oxidase inhibitors (MAOIs)[3].

In some acute depressive treatments the classical tricyclic antidepressantss (TCAs) are used, although their current application is limited because of their general side-effects and limited specificity[10;11]. Some melatonergic agonists, such as agomelatine, are reported to have antidepressant (AD) effects or may treat sleep disorders associated with MDD and are marketed as such[12]. MDD may occasionally be treated using lithium, although this is more common in BPD alongside an anticonvulsant or atypical antipsychotic. One of the major shortcomings of current antidepressant drugs is that

several weeks or months of treatment are needed before improvements are observed.

1.2 Animal-models and behavioral tests

In order to study the behavioral effects of treatments under controlled circumstances, a variation of translational animal-models are used to simulate the physical and psychological states associated with MDD. Most of these models focus on manipulating induced stress and behavioral despair and may use either mice or rats in their methodology[13;14]. These stress-based models can be categorized as either acute or chronic stress. Sucrose consumption is sometimes used as an inverse behavioral read-out for anhedonia, although its reproducibility has been criticized because it often depends on whether treatment with AD-medication is chronic or acute[14].

Acute stress-based tests - There are several acute tests employing behavioral despair that are used to indicate the efficacy of anti-depressant drugs and treatments. The most commonly used are the forced swim test (FST) and tail suspension test (TST). The performance in these tests has been used as a quantification of AD-effects for many decades and still counts as the 'gold standard' in AD research[15;16]. Other related tests are the learned helplessness (LH) and novelty suppressed feeding test (NSFT) in which the behavior of the animal is observed in either a conditioned environment where the animal has been taught to be helpless, or a completely new environment where the animal shows a delayed feeding response, respectively.

Chronic stress-based models - A wide variety of models have been developed using chronic stress to induce a semi-permanent state of depression in animal subjects. These stressors are typically randomly selected from a variety of methods such as water or food deprivation, 45 degrees cage tilt, exposure to rat faeces, cage overcrowding, wet bedding, overnight illumination, dark exposure during normal light cycle, cold bedding, acoustic disturbance (120 dB), strobe lights, or cagemate rotation and may last for an arbitrary length of 1 up to 12 hours[17]. These methods are also referred to as unpredictable chronic mild stress (UCMS) or chronic unpredictable stress (CUS). Some models for dysfunctional parenting, such as maternal deprivation during early postnatal life, may also be used as a chronic stress model.

Genetic models for depression - A number of genetic animal models have been developed that show endophenotypes related to depression. Wistar-Kyoto (WKY) rats were developed using selective inbreeding based on poor FST performance and show enhanced susceptibility to depressed states and resistance to a number of antidepressants, thus possibly providing a genetic base for treatment-resistent forms of depression[18;19]. The TWIK-1-related K+ channel (TREK-1) knockout mouse-model on the other hand shows increased resilience to depression in FST and TST, implicating an ion channel in depression-like animal behavior[20].

2 Synaptic Plasticity in Depression

Moving beyond the monoamine hypothesis of depression, the past 10 years we have seen many molecular and cellular studies of stress, depression and antidepressants demonstrating opposing actions on the expression of particular neurotrophic factors in limbic brain regions that regulate mood and cognition. These neurotrophic proteins are growth factors that are neurodevelopmentally expressed and regulate neuroplasticity and cellular resistance in the brain[21]. During homeostasis in the differentiated adult brain, the contacts between neurons are continuously being replaced and renewed. The presynaptic site may undergo formation of new synapses due to enhanced axonal growth, likewise terminal degeneration may eliminate existing ones. The size on the dendritic tree, or its spine density, and changes in the organization of glial cells, which maintain homeostasis in the brain, may cause the number of postsynaptic sites to increase or decrease. Depression has been associated with hypocampal neuronal atrophy and synaptic loss[22]. After chronic treatment with AD drugs however, an increase in neurotrophic factor expression and enhanced synaptic plasticity is observed[23;24].

Two forms of synaptic plasticity that are well understood are long-term potentiation (LTP) and long-term depression (LTD), which are enhancement and reduction of signal transmission between neurons respectively, thus LTP and LTD are an indication of synaptic strength and dendritic spine growth and retraction respectively[25]. Changes in synaptic strength are established through both pre- and postsynaptic mechanisms such as synthesis and movement of synaptic receptors and other proteins. Rapid changes, during early-LTP (E-LTP) for instance, depend on the movement of pre-existing proteins[26], while RNA translation and changes in gene expression are a factor in late-LTP (L-LTP)[27]. LTD similarly involves, likely oppositional, adaptations to gene expression and protein metabolism.

During synaptic plasticity many signaling systems are involved in unison between neurons. Some intracellular signal transduction pathways are crucial in this regard and central to synaptic plasticity initiation is calcium influx through several ion channels. This is particularly mediated by a number of glutamate related receptors and release mechanisms that are thus deeply involved in brain plasticity.

2.1 Glutamate and Neural Plasticity

Glutamate is the most abundant and important excitatory neurotransmitter in the brain. It is released by exocytosis and is present at very high concentrations in synaptic vesicles[28]. It is a non-essential amino acid that can be synthesized via a number of routes and as such plays an important role in many metabolic pathways[29]. Abnormalities in its metabolism have long been understood to play an important role in degenerative neurological disorders[30;31].

Glutamatergic receptors in the central nervous system (CNS) consist of two major subtypes, ionotropic and metabotropic. The G-protein coupled receptors (GPCRs) of the metabotropic glutamate receptor (mGluR) subtype consist of eight identified proteins that can be divided in to three subgroups, which are based on their signaling pathways. The ionotropic subtype of glutamatergic receptors consists of three ligand-gated ion channels that each are formed from multiple, and varying, subunits. These are

the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate (KA) and NMDA receptors respectively, which may be found pre-, post-and extra-synaptically. Post-synaptically these receptors are anchored in a scaffolding complex with postsynaptic density protein 95 (PSD-95), which is a potent regulator of synaptic strength through its control over AMPA receptor (AMPAR) concentrations[32].

These receptors are known to play an important role in synaptic plasticity. Expression of AMPARs is one of the best studied molecular correlates of synaptic strength at excitatory synapses and increases in this expression under LTP is related to dendritic spine growth and spine-head size[33]. During LTP, AMPARs containing the long-tailed subunits GluR1, GluR4 and GluR2L are added, while during the constitutive cycling of LTD the short-tailed GluR2, GluR3 and GluR4c subunits participate in AMPAR removal[34]. Similarly, following LTP and LTD, NMDA receptors (NMDARs) undergo trafficking and changes to GluN2 subunit ratios and it has been found that NMDAR synaptic response consistently decreases during LTD[35;36].

There is increasing evidence that alterations in the glutamatergic ion-channels AMPAR and NMDAR are involved in mood disorders such as MDD and BPD as their expression levels seem to be decreased in such patients[37]. Most AMPA receptors are heterotetrameric and primarily consist of the GluR2 subunit coupled as a 'dimer of dimers' with GluR1, Glur3 or Glur4[38]. Sodium and potassium are the principal ions for which AMPARs are permeable. This in contrast to NMDARs which primarily gate sodium and calcium ions, although potassium may be transferred out of the cell in a voltage dependent manner[39]. NMDARs form heterotetramers consisting of two GluN1 and two GluN2 subunits. Where AMPARs are activated at four glutamate binding sites, one at each subunit, the NMDARs have a glutumate and a glycine binding site and require co-activation.

After glutamate binds to postsynaptic AMPARs and NMDARs, Na^+ flows in to the postsynaptic cell, which results in excitatory postsynaptic potential (EPSP), or depolarization of the membrane. At resting membrane potential however, NMDAR channels are blocked by Mg^{2+} and can only open when AMPAR activation has led to depolarization, causing repulsion of Mg^{2+} cations. This then allows the NMDARs to permeate Ca^{2+} in to the cell which subsequently triggers AMPAR upregulation and a further increase in EPSP size, an indicator of LTP. The Ca^{2+} influx also results in phosphorylated Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) which phosphorylates AMPARs and enhances their conductance[40]. CaMKII is known to be an important requirement for LTP initiation[41].

2.2 Glutamatergic Drugs

A significant number of patients do not respond to the normal treatments for MDD and develop treatment-resistant depression (TRD). It is estimated that nearly 50% of patients will not reach full remission with their first AD treatment[42] while nearly half of that (20-30%) will be completely non-responsive, even after subsequent adaptations to their treatment[43;44]. The medium and long term outcomes for patients that develop TRD are very serious as they are associated with a high rate of relapse, significant disability and mortality and residual symptoms leading to this high relapse and long term disability[45;46]. TRD patients may be more likely to suffer from comorbid physical

and mental disorders[47;48]. In general, patients with TRD have a significantly higher disease burden and cost of burden than other patients[43;48]. Therefore new treatments and medications for this treatment-resistant form of depression are needed. Ever since NMDA-receptors and the glutamatergic system were implied in the mechanics of depression and anti-depressants in the 1990s their potential role as a target in the treatment of depression has increased[49–51]. This has resulted in a number of compounds that modulate the glutamate system, particularly ketamine which is a well-known dissociative anesthetic.

Ketamine - After many animal studies the first placebo-controlled, double-blinded trial with single dose ketamine, an NMDA antagonist, was published in 2000[52]. This showed the feasibility of NMDA-receptor modulation as a possible treatment for patients with MDD. Since then a number of randomized and open label trials have been conducted that support the claims of feasibility and show that the effects may last for days if not weeks in patients with TRD[53–57]. Treatment with ketamine also seems to reduce suicidality in this patient population[58]. Due to its long history of medicinal use and efficacy in TRD treatment, ketamine currently has a high interest as a potent medication for MDD.

Other glutamate modulators - Among the variety of glutamatergic compounds the glutamate uptake enhancer and amyotrophic lateral sclerosis (ALS) medication Riluzole was explored in an open-label trial for TRD[59]. GLYX-13, a NMDAR glycine-site partial agonist, has been described to induce AD-like effects without the psychoactive side effects of ketamine[60]. Likewise the low-trapping and non-selective NMDA channel blocker AZD6765 (Lanicemine) has shown rapid, albeit short-lived, AD effects in patients with TRD[61]. The NR2B subunit selective NMDA antagonist CP-101,606 (Traxoprodil) has also shown efficacy in MDD treatment, without producing any of the dissociative reactions associated with most NMDA antagonists[62].

Proposed - A number of NMDA antagonists and functional analogs of ketamine have been hypothesized to elicit similar AD effects and may be explored in future clinical research. These include the well known antitussive dextromethorphan[63] and the relatively new structural analogue of ketamine, methoxetamine[64]. It may or may not be warranted that these compounds are explored in future clinical studies.

3 Ketamine and NMDA Antagonism

Because NMDARs need agonism on both the glutamate and glycine binding site to open the ion channel, there are several methods by which they can be prevented from activation. There are competitive antagonists that inhibit glutamate binding; glycine antagonists which act at the glycine binding-site; uncompetitive channel blockers that prevent ions from passing the receptor; and finally there are non-competitive antagonists that bind to an allosteric site. Ketamine belongs to this final group of antagonists and although NMDARs may have several subunit compositions, ketamine does not appear to have a particular selectivity for these in its binding[65].

Although post-synaptic NMDARs are primarily involved in gating sodium and calcium ions, presynaptic NMDARs have been shown to modulate glutamate release[66]. Low dose NMDA antagonism by ketamine in particular increases glutamate outflow

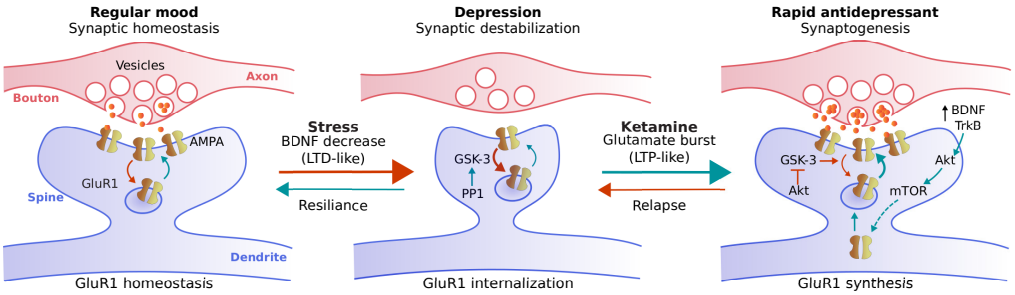

Figure 1: *Schematic representation of the synaptogenic model of depression and subsequent ketamine induced recovery. Stress induces LTD-like synapse morphology to neuronal spines with decreased BDNF activity and AMPAR internalization. This results in neuronal atrophy in its depressed state. Ketamine then induces a glutamate burst that stimulates BDNF exocytosis and subsequent mammalian target of rapamycin (mTOR) activation which restores AMPAR signaling functionality reminiscent of LTP.*

in the prefrontal fortex (PFC), which suggests that ketamine may thus increase glutamatergic neurotransmission at non-NMDA glutamate receptors[67]. While normally NMDAR activation directly leads to an influx of Ca^{2+} ions and subsequent CaMKII phosphorylation, this increase of glutamatergic neurotransmission may cause Ca^{2+} influx through hyperpolarization caused by AMPAR activation and resulting voltage-dependent Ca^{2+} channel (VDCC) opening.

Extra-synaptic NMDARs which, like the pre-synaptic receptors[68], contain NR2B subunits[69], are involved in a number of processes. ExNMDARs activation is particularly implicated in excitotoxicity and neuroprotection as part of LTD[70;71]. When extra-synaptic NR2B-containing NMDARs are excited under normal conditions they activate protein phosphatase 1 (PP1) and induce a feedback loop between glycogen synthase kinase 3 (GSK-3) and PP1[72]. Inhibition of these receptors conversely results in lower PP1 and GSK-3 deactivation, which in turn increases NR2B subunit levels[71]. This subunit increase has been observed in the antagonism by GLYX-13 and ketamine, which both led to increases in NR2B and GluR1 protein levels and a persistent enhancement of LTP[60].

3.1 Signaling Pathways

A number of signaling pathways have emerged that are modulated by NMDA antagonism and support the synaptogenic hypothesis of its fast acting and long lasting antidepressant effects. The AD effects of ketamine at least seem to be dependent on AMPAR throughput, as these effects are attenuated by the AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX)[73;74]. Likewise, the long-lasting AD-effects of both ketamine and GLYX-13 treatment are partially abolished by NBQX pretreatment in either LH, TST or FST[60;74]. Further more, chronic treatment of WKY rats with low dose ketamine induced marked changes in the hippocampal AMPA/NMDA receptor ratio in favor of AMPAR, showing the importance of AMPAR in the long-lasting AD-effects of ketamine[75].

The two major pathways currently under investigation are mediated by BDNF and

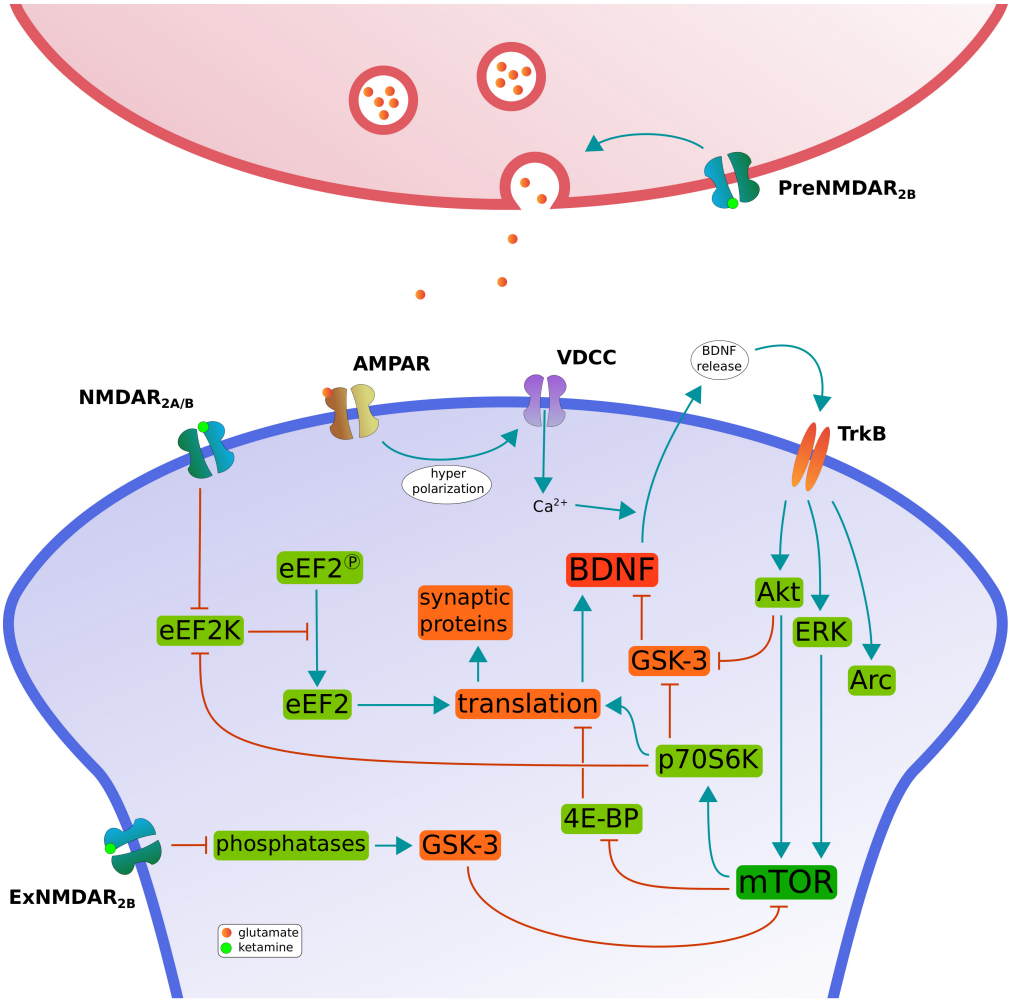

Figure 2: *Schematic representation of the pathways involved in ketamine mediated synaptic plasticity. Antagonism of pre-synaptic NMDARs leads to glutamate release while post-synaptic suppression of NMDARs results in inhibition of eEF2 kinase and dephosphorylation of eEF2. This augments BDNF translation while the aforementioned glutamate release activates AMPARs which hyperpolarize the membrane and activate VDCCs. Calcium subsequently promotes BDNF release which may then activate Tropomyosin receptor kinase B (TrkB) receptors resulting in a signaling cascade that in turn activates protein kinase B (Akt) and extracellular signal-regulated kinase (ERK). Akt and ERK then activate mTOR which enables further translation of synaptic proteins, including BDNF, GluR1 (AMPA) and PSD-95, by activating p70S6 kinase (p70S6K) and inhibiting 4E binding proteins (4E-BPs). GSK-3, which inhibits BDNF expression, may be suppressed through phosphorylation by Akt or p70S6K. Likewise, stimulation of GSK-3 is suppressed by deregulation of phosphatases from extra-synaptic NMDAR antagonism, which may also result in mTOR mediation. Finally, eEF2 kinase is also suppressed by p70S6K, feeding in to the previously mentioned BDNF translation.*

mTOR. In the following parts the role of these proteins and their relation in the overall pathway of synaptogenesis in NMDA antagonism will be highlighted. In fig. 1 a general overview of synaptic homeostasis, degerenation and genesis is presented culminating in the signaling pathway presented in fig. 2.

3.1.1 mammalian target of rapamycin

mTOR, a serine/threonine protein kinase, is a regulatory protein involved in cell growth, proliferation, motility, survival, and protein synthesis[76]. The regulation of translation initiation by mTOR is exerted through a number of downstream proteins: p70S6K, eIF4E and eIF4B. Administration of rapamycin, the principal inhibitor of mTOR, disrupts the mTOR signaling pathway leading to reduced L-LTP[26]. It has been shown that patients with MDD have a marked deficit of mTOR and its downstream components which could contribute to the observed molecular pathology of this disorder[77].

Rats exposed to CUS show these same deficits in synaptic proteins, as shown by immunoblotting of their phosphorylated activation states[77–80]. Exposure to CUS for a period of 3 weeks decreased spine density in layer V pyramidal neurons and decreased excitatory postsynaptic currents (EPSCs) and thus EPSPs[79]. Subsequently, administration of NMDA-antagonists (ketamine and Ro 25-6981) in these rats rapidly decreased the observed anhedonic and anxiogenic behavior and resulted in increased levels of these signaling proteins and increased numbers of new spine synapses in the PFC[78;79]. Other proteins influenced by mTOR are PSD-95, GluR1 and synapsin I, underlining its role in synaptogenic translation. These effects were completely blocked by pretreatment of the mTOR-inhibitor rapamycin, further supporting the role of mTOR in the synaptogenic pathway of ketamine administration.

Interestingly pretreatment with the selective AMPA inhibitor NBQX, 10 minutes before ketamine administration, resulted in complete blockade of induction of phosphorylated 4E-BP1, p70S6K and mTOR in the PFC[78]. These results underline the important role of AMPAR transmission in synaptogenesis, which is emphasized by the translation of the AMPA subunit GluR1 via the mTOR pathway.

3.1.2 ketamine mediated BDNF release

BDNF, which is critical for axonal growth and neuronal survival, has been shown to be a significant biomarker in stress models and depressed patients[81]. Hippocampal levels of BDNF are decreased in animal models using immobilization[82–84], social isolation[85], maternal deprivation[86], and swim stress[87]. Postmortem hippocampal levels of depressed suicide victims have also been found to be decreased[88–90]. And where serum levels of BDNF in depressed patients are lowered[90–92] these same levels increase during AD treatment[88;92–94], showing that ADs influence this same neurotrophic mechanism.

Although no changes in BDNF could be detected in plasma levels of patients during the initial and rapid AD response of ketamine administration[95], BDNF serum levels were in fact increased in chronic ketamine users[96]. Furthermore, mice with inducible BDNF knockout (KO) do not show the fast-acting, 30 minutes, AD-response of ketamine as seen in wild-types (WTs)[17]. This suggests that this AD-response relies on

fast BDNF expression. Finally there are clear signs that BDNF-release is involved both pre-and post-LTP[97;98]

BDNF primarily acts by binding to TrkB which is its main receptor. This results in phosphorylation, and thus activation, of Akt and ERK which are both involved in the regulation of synaptic protein synthesis[99]. It has also been found that TrkB KOs result in ketamine-insensitive fenotypes in both FST and NSFT[17]. One striking discovery is that ketamine NMDAR antagonism can lead to inhibition of eEF2 kinase, which results in dephosphorylation of eEF2 and desupression of BDNF translation[17]. The aforementioned AMPAR and VDCC regulated calcium influx may finally result in BDNF exocytosis where it can activate TrkB[100]. The glutamate modulator riluzole likewise enhances BDNF, together with other signaling proteins like vascular endothelial growth factor (VEGF) and glial-derived neurotrophic factor (GNDF)[101].

3.1.3 related targets

GSK-3 - As previously mentioned, GSK-3 has an inhibitory effect on NR2B expression, however it also mediates synaptic stimulation of mTOR[102] and is required in the phosphorylation of PSD-95 and its mobilization in LTD[103]. Inhibition of GSK-3 also seems to be necessary for the rapid antidepressant effect of ketamine in the LH-paradigm in mice[104], although this regulation of its activity was not detected in subsequent efforts[105]. Thus, GSK-3 inhibition may be a viable target for future anti-depressant research.

Arc - This activity-regulated cytoskeleton-associated protein (Arc) has involvement in both LTP and LTD[106] and is an important regulator of AMPAR endocytosis and trafficking[107]. Ketamine induced the expression of Arc in the early stages of its downstream effects, with a peak after 1 to 2 hours, in a transient time course[78]. Potentially this identifies it as a component of E-LTP in ketamine's mechanism of action, mediating its initial and fast-acting effects.

ERK and Akt - As discussed earlier, these proteins are activated by TrkB stimulation and are involved with synaptic protein synthesis. Rats exposed to CUS show reduced phosphorylation of these proteins[80]. They are consequently observed to increase in concentration after ketamine administration, but their levels are normalized with pre-administration of NBQX emphasizing the role of AMPA activation[78]. Further more, intracerebralventricular (ICV) administration of selective ERK (U0126) or Akt (LY294002) inhibitors blocked the AD actions of ketamine in FST and NSFT[78]. Both are also identified in mTOR activation, providing another key route in the overall pathway[108;109].

4 Discussion

We have seen that there are several pathways to consider in the short and long-term anti-depressant effects of ketamine and NMDA-antagonists. There is clear evidence that glutamatergic dysfunction plays an important role in the pathophysiology of depression and a number of signaling pathways can be considered as major targets.

Within the observed pathways we can see an interdependence emerging between mTOR and BDNF. Although BDNF clearly activates mTOR and its GluR1 expression[110] and the initial findings that rapamycin pre-treatment blocks the acute effects of ketamine could not be replicated[17], which may have come from particular technical differences in the study[111], there may still be some viable angles to approach mTOR as a key target.

Considering that the NR2B selective NMDA antagonist Traxoprodil is still effective in MDD treatment we may conclude that these routes in the pathway, mediated by this receptor-subtype, are most relevant to the observed outcomes. The equally rapid response to Traxoprodil, as compared to Ketamine, suggests that the post-synaptic NR2A pathway, which has been identified to disable eEF2 kinase, is not required for this result. However, mTOR activated p70S6K regulates eEF2 kinase as well, providing a different route for its inhibition[112;113]. Likewise we have seen that GSK-3 is a potential requisite in the observed activities, which may also be attenuated through NR2B receptors alone. The robust and long-term effects of both ketamine an GLYX-13 may also be a strong indication that they promote enhancement of 'metaplasticity'[60], showing changes in plasticity that last much longer than their initial triggering mechanism, where again we see that NR2B containing NMDARs play a critical role[114].

However, observing some of the potential long-term effects of mTOR modulation, we may consider its potential role in Alzheimers progression[115–117]. Although repeated ketamine use may cause impairment to cognitive processing speed and verbal fluency, no such specific effects have become apparent as of yet[118]. Concerns have previously been raised about ketamine's well known cognitive side-effects which occur during typical IV administration[119]. These concerns may be mediated by the fact that ketamine's robust AD-effects are equally effective at sub-psychotomimetic doses. Similarly the route of administration may be augmented to include oral delivery, which still has moderate bioavailability[120]. Making the drug more suitable for outpatient and nonprocedural psychiatric care.

Although many other molecules which act at some of the proposed targets show AD-like properties, the antagonist action on the NMDA receptor is currently the only clinically validated target[51]. However the current developments around this subject are continuously evolving with many clinical studies being conducted with related compounds. Primarily the elaboration of these mechanisms may result in novel directions for the development of fast-acting antidepressants and potential involvement of other neuropsychiatric disorders.

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