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Review article

# Molecular and cellular dissection of NMDA receptor subtypes as antidepressant targets

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ABSTRACT

A growing body of evidence supports the idea that drugs targeting the glutamate system may represent a valuable therapeutic alternative in major depressive disorders (MDD). The rapid and prolonged mood elevating effect of the NMDA receptor (NMDAR) antagonist ketamine has been studied intensely. However, its clinical use is hampered by deleterious side-effects, such as psychosis. Therefore, a better understanding of the mechanisms of the psychotropic effects after NMDAR blockade is necessary to develop glutamatergic antidepressants with improved therapeutic profile. Here we review recent experimental data that addressed molecular/cellular determinants of the antidepressant effect mediated by inactivating NMDAR subtypes. We refer to results obtained both in pharmacological and genetic animal models, ranging from global to conditional NMDAR manipulation. Our main focus is on the contribution of different NMDAR subtypes to the psychoactive effects induced by NMDAR ablation/blockade. We review data analyzing the effect of NMDAR subtype deletions limited to specific neuronal populations/brain areas in the regulation of mood. Altogether, these studies suggest effective and putative specific NMDAR drug targets for MDD treatment.

## 1. Introduction

Classical monoaminergic antidepressants still represent the main therapeutic option in MDD. However, two major drawbacks of these drugs are the delayed onset of action − taking in many cases several weeks − as well as the frequently observed incomplete therapeutic response. Alternative therapeutic strategies targeting other (e.g. dopaminergic, melatonergic or multiple) neurotransmitter systems have been developed but could not solve the two mentioned drawbacks either (Kasper and Hamon, 2009; Englisch et al., 2010; Orsolini et al., 2017). Due to the increasing global burden of MDD (Lépine and Briley, 2011), there is an urgent need for more effective and fast-acting antidepressants.

Over the last decades, the rapid and sustained antidepressant effect of ketamine in MDD has been labelled by some authors as 'arguably the most important discovery in depression research in half a century' (Duman and Aghajanian, 2012). Ketamine is a non-selective NMDAR antagonist intensively studied as an alternative antidepressant with fast onset and long-lasting therapeutic action (Zarate et al., 2006). Three main intracellular signaling pathways that may be responsible for the rapid antidepressive effect of ketamine have been identified: the mammalian target of rapamycin (mTOR), the eukaryotic elongation factor 2 (eEF2), and the glycogen synthase kinase-3 (GSK-3) (Niciu et al., 2014). Additionally, the enhancement of GluA1 AMPA receptor (AMPAR) activity appears critical for the antidepressant effect of ketamine (Maeng et al., 2008). However, it is less clear which NMDAR subtypes and which neurons/brain regions expressing NMDARs are mediating the antidepressant effect in the presence of ketamine. This appears of great importance, considering that NMDARs are ubiquitously expressed in the central nervous system (CNS) and are implicated in numerous brain functions in addition to the modulation of mood circuits.

The majority of NMDAR are heterotetrameric ion channels composed of two obligatory GluN1 (formerly NR1) and two GluN2 (GluN2A-D) (formerly NR2A-D) subunits (Wong and Kemp, 1991; Schorge and Colquhoun, 2003; Vyklicky et al., 2014; Regan et al.,

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2015). The predominant forebrain NMDAR subunits are GluN2A and GluN2B; in contrast, the expression of GluN2C and GluN2D subunits occurs mainly in specific extra-cortical brain regions (cerebellum and thalamus *versus* various diencephalic nuclei and olfactory bulb, respectively) and only at low level in the forebrain (Buller et al., 1994). GluN2 subunit expression shows a dynamic evolution during brain development, with GluN2 B subunits predominantly expressed pre- and early postnatally and a slow increase of GluN2A subunit containing NMDARs during adolescence ("GluN2B-to-GluN2A switch") (Monyer et al., 1994). In addition, there are important differences not only in the regional, but also in the sub-cellular localization of GluN2 subunits: in the adult brain GluN2A-containing NMDARs are present mainly at synapses, whereas GluN2Bs are located predominantly extrasynaptically, a difference with important consequences, leading, for example, to distinct, even opposite roles in cell survival (Hardingham and Bading, 2010). Thus, the remarkable functional heterogeneity of NMDARs mainly results from differences in the composition of GluN2 subunits. However, it should also be mentioned that the functional importance of the eight different GluN1 isoforms, which are selectively expressed in different brain regions (Hollmann et al., 1993; Laurie et al., 1995) has not yet been addressed. The complexity of NMDARs is even further enhanced by two other minor subunits, GluN3A and GluN3 B that can lead to the formation of heterotrimeric GluN1/GluN2/GluN3 NMDARs which compete against conventional GluN1/GluN2 NMDARs (Rozeboom et al., 2015).

Due to this very high complexity, a highly selective drug-targeting of specific NMDAR subtypes by pharmacological tools seems nearly impossible. But it might be achievable to exclude some NMDAR subtypes that mediate drug specific site effects. In the last decades, numerous modulators of NMDARs have been developed and characterized. Due to their mode of interaction with NMDARs, these substances were classified in: a) positive modulators, like polyamines, b) channel blockers, like ketamine, phencyclidine and MK-801, c) competitive antagonists, like the GluN2A-prefering antagonist NVP-AAM077 and d) negative allosteric modulators, like the GluN2B-prefering antagonists ifenprodil and Ro 25–6981 (Ogden and Traynelis, 2011). Further, very promising are novel compounds with antidepressant effect like rapastinel (formerly GLYX-13) that acts as partial agonist at the glycine site of the NMDAR and induces similar neurochemical changes as ketamine (Lepack et al., 2016), but with an improved side-effect profile (Rajagopal et al., 2016; Vasilescu et al., 2017). Of relevance for the present review are mainly substances with antagonistic effect on NMDARs.

Treatment with NMDAR channel blockers causes a persistent increase in glutamate release that may help to sustain the antidepressant action following ketamine treatment (Miller et al., 2016). Two cellular hypotheses have been proposed to explain this phenomenon: Firstly, the "indirect" hypothesis suggests preferential effects on NMDARs of GABA-ergic interneurons. This concept is consistent with studies demonstrating that ketamine has disinhibitory effects in the neocortex resulting in enhanced activity of excitatory pyramidal neurons and increases in extracellular glutamate levels (Behrens et al., 2007; Homayoun and Moghaddam, 2007; Schobel et al., 2013). Secondly, the "direct" hypothesis proposes an inhibition of NMDAR signaling on principal neurons and a reduced experience-dependent plasticity of them. Recently, several studies using new mouse models with conditional genetic ablation restricted to specific NMDAR subtypes or NMDA receptors in distinct neuronal populations or brain regions improved our understanding of molecular and cellular substrates of ketamine's antidepressant effect.

Here we review recent data analyzing the antidepressant effect of ketamine from the perspective of different NMDAR subtypes. This allows to dissect antidepressant, anxiolytic, motor, or psychotomimetic effects mediated by distinct NMDAR subtypes. Of note, the potential clinical use of ketamine is seriously restricted by psychosis-like site-effects (Krystal et al., 1994) which are accompanied by cortical neurotoxicity in animal models (Olney et al., 1989). Therefore, ketamine is not in common clinical use and more specific glutamatergic antidepressants with fewerside-effects are needed.

## 2. The role of gluN2A-mediated mechanisms

The GluN2A containing NMDARs are recognized as NMDAR subtypes that mediate NMDAR-induced neuronal plasticity in the mature brain. GluN2A-type NMDARs are expressed postnatally and reach their final high expression level after puberty (Monyer et al., 1994). Therefore, a major role of GluN2A in mechanisms underlying various psychotropic effects of NMDAR antagonists is expected. Indeed, several pharmacological and genetic models of GluN2A-specific blockade/ablation revealed the role of GluN2A in mechanisms underlying the treatment of depression and in the emergence of abnormalities associated with psychosis.

### 2.1. Pharmacological paradigms

The competitive NMDAR antagonist NVP-AAM077 has about a10-fold higher selectivity for GluN2A-containing NMDARs compared to GluN2B-containing receptors (Auberson et al., 2002). NVP-AAM077 was proposed recently as possible alternative to ketamine, since acute treatment with this compound elicited antidepressant effects, without inducing − like uncompetitive NMDAR antagonists − stereotypy as correlate of psychosis (Jimenez-Sanchez et al., 2014). However, only this study reported antidepressant effects of NVP-AAM077, whereas the evidence for similar actions of GluN2B-prefering NMDAR antagonists is much more consistent (see 3.1). Interestingly, both NVP-AAM077 and the GluN2B-prefering NMDAR antagonist Ro 25–6981 triggered antidepressant-like effects in the forced swim test (FST), but only the former compound increased, similar to MK-801, the efflux of serotonin and glutamate in the prefrontal cortex (PFC) (Jimenez-Sanchez et al., 2014). This suggests different and yet unknown molecular mechanism underlying the antidepressant-like effect of GluN2A *versus* GluN2Btype-specific antagonists.

However, other data revealed serious other effects of NVP-AAM077 that may hamper its future clinical use. Similar to ketamine, but unlike Ro 25–6981 or GluN2C/D NMDAR antagonists, NVP-AAM077 induced important deleterious effects accompanying psychosis, like aberrant cortical gamma oscillations, similar to ketamine (Kocsis, 2012). In addition, NVP-AAM077 was shown to generatealterations of working memory, another important feature of schizophrenia (Smith et al., 2011). This result is not surprising considering the recently described main role of GluN2A in the regulation of working memory (Bannerman et al., 2008; McQuail et al., 2016). Finally, NVP-AAM077 was reported to disrupt spatial memory by inhibiting adult hippocampal neurogenesis (Hu et al., 2009). This is of relevance considering the postulated role of adult neurogenesis in the antidepressant effect of classical antidepressants, like the selective serotonin reuptake inhibitors (SSRI) (Santarelli et al., 2003). Such opposite effects of NVP-AAM077, i.e. the antidepressant-like action in the FST *versus* the inhibition of adult hippocampal neurogenesis may appear at a first glance counter-intuitive. However, one should distinguish between the effects triggered by acute compared to chronic treatments. For example, also the SSRI fluoxetine stimulated adult hippocampal neurogenesis following a 28-days administration, whereas 5-days treatment was without effect (Santarelli et al., 2003). Nevertheless, the antineurogenic action of NVP-AAM077 seriously questions its therapeutic use and up to date no data are available regarding the effect of this compound in chronic pharmacological models.

### 2.2. Genetic models of GluN2A deletion

Genetic models appear necessary to validate data obtained in pharmacological experiments, also due to limitations of