Author: kdevos12

NR2D and NR2C

Useless vestiges or key targets for cognitive enhancement ?

INTRO

The NMDA receptor subunits NR2C and NR2D are relatively little known and understood, and their role in the pathogenesis of psychiatric disorders and cognitive enhancement remains unclear and somewhat obscure. In the field of nootropic research, these targets are sometimes even considered inferior to other subunits deemed more important, such as the NR2A subunit. Here, I compile extensive information in the form of an enumeration of facts (and hypotheses) about these subunits, addressing their role in synaptic plasticity, the regulation of interneuron and astrocyte activity, their distinct functions, and the harmonious and complementary pair they form — a pair that proves to be crucial for maintaining the Excitation-Inhibition balance.

NR2C Itself

NMDA receptors containing the GluN2C subunit (NR2C) play a crucial role in cognition by influencing the cortical excitation/inhibition balance as well as synaptic plasticity.

The GluN2C subunit, like the GluN2A subunit, is relatively under-expressed during the embryonic period in the CNS, and its expression becomes notable during the first two postnatal weeks (Watabe et al., 1993).

GluN2C knockout (KO) mice show a reduction in the frequency of Miniature Excitatory Postsynaptic Currents (mEPSCs). Their amplitude decreases with age, but not their frequency [2]. They also exhibit a decrease in dendritic spine density in the mPFC [1]. Paradoxically, this leads to an increase in inhibition: indeed, the frequency of mIPSCs increases, along with the density of GABAergic synapses (observed via the vGAT marker), suggesting an excitation/inhibition (E/I) imbalance in favor of inhibition [1]. It has been revealed that an E/I imbalance oriented towards inhibition is associated with issues of vigilance [3]. Even more paradoxically, this results in a reduction of parvalbumin-positive interneurons but an increase in GAD67 perisomatic terminals, further emphasizing the trend towards an aberrant yet dysfunctional modulation of the inhibitory network in the GluN2C KO phenotype [1].

This reduction in parvalbumin-positive interneurons is linked to the observed role of GluN2C/GluN2D subunits in the maturation of parvalbumin-positive GABAergic interneurons. Indeed, when the activation of NMDA receptors containing these subunits is blocked, interneuron maturation is impaired, and cortical networks exhibit abnormal hyperexcitability. [22]

It also appears that the GluN2C subunit may play a privileged role in schizophrenia and other psychoses. Indeed, spontaneous alternation in the Y-maze is more impaired in GluN2C KO mice after PCP administration than in wild-type mice, reflecting a role in the reduction of working memory in the schizophrenic phenotype [1]. This remains consistent with dysfunctions observed in the excitation/inhibition balance and earlier documentation [4], which also mentions a deficit in conditioned fear acquisition, suggesting a role for GluN2C subunits in associative memory. However, no effect of GluN2C knockout on spatial memory has been observed.

But that’s not all, as GluN2C KO mice also show a reduction in Prepulse Inhibition (PPI) [1], a clear marker of sensory gating disorders, which is of course linked to schizophrenia. This is consistent with the decrease in PV interneurons, inhibitory interneurons identified as key elements in PPI, whose dysfunctions are, of course, associated with schizophrenia.

NR2D Itself:

Penchantbio has written a comprehensive article on NR2D subunits, which will certainly overlap with this one. Credit is therefore given to them for much of the information in this section, and you are encouraged to read their article as well [5]. The next two paragraphs will be paraphrases of Penchantbio's article, and you may skip them if you have already read the original article.

Receptors containing the NR2D subunit (also called GluN2D) exhibit increased sensitivity to agonists and a much slower deactivation than those containing NR2A, and even slower than those containing NR2B. Additionally, NR2D is exclusively located on extrasynaptic membranes [6]. When the density of active synapses increases, the accumulation of released glutamate significantly prolongs the decay of the excitatory postsynaptic current (EPSC) by activating a greater number of extrasynaptic receptors containing NR2B and NR2D. Induction of long-term potentiation (LTP) through repeated burst stimulation is correlated with a prolonged increase in the contribution of extrasynaptic receptors to the EPSC following the burst [6].

NMDARs containing GluN2D are present at synapses of hippocampal interneurons and can modulate their activity. The GluN2D subunit is also selectively expressed in fast-spiking parvalbumin-positive (PV+) interneurons (FSINs) of the cortex [7], where it plays a role in the dorsolateral prefrontal cortex (dlPFC). Among the NMDAR subunits, NR2B is already known to exhibit very slow deactivation, but NR2D is even slower, with durations that could potentially last several hours. The deactivation times of diheteromeric NMDARs vary by a factor of 50, in the following order: NR2A < 2C = 2B << 2D [8].

Concerning the predominant presence of GluN2C/D subunits in interneurons, the study highlighting this feature (Shiokawa et al., 2010) was conducted on young mice at a developmental stage that may not be representative of the NMDA receptor subunit composition.

This slowness in EPSC decay may indicate a particularly important role of the GluN2D subunit in the formation of neural circuits by allowing an extended time window for synaptic plasticity. These mechanisms, beyond being crucial in consolidation, may also be extrapolated as playing an essential role in the transfer effect [9] during learning.

Furthermore, during embryonic development, the NR2D subunit is expressed before NR2B and NR2C, while NR2A would only begin to be expressed around birth. This would tend to validate, through an evolutionary reasoning, the primary and superior role of NR2D subunits in development. While in adulthood, the expression of GluN2D subunits strongly decreases, it remains present in the diencephalon and mesencephalon [11].

It is also found that GluN2D subunits are highly expressed in inhibitory GABAergic interneurons of the cortex and hippocampus. Their modulation via (+)-EU1180-453 increases inhibitory activity. It should be noted that (+)-EU1180-453 is also a PAM of NMDA GluN2B. In animal models, this leads to anxiolytic and antidepressant effects, while Grind2D$^{-/-}$ mice exhibit alterations in inhibitory transmission, suggesting a link between GluN2D and excitation-inhibition imbalances associated with anxiety disorders [9]. In perspective with the fact that an excitation-inhibition balance with predominant inhibition is associated with a decrease in vigilance [3], this may lead to the hypothesis that dysfunctions of NR2C/NR2D subunits could play a role in the pathogenesis of ADHD.

NR2C and NR2D – Complementary Roles and Effects of Their Modulation

NR2C and NR2D and the Bidirectional Feedback Mechanism in Brain Oscillation Regulation

Although individually, NR2C and NR2D receptors appear to perform distinct functions, particularly with respect to the excitation-inhibition balance, they are nonetheless complementary in terms of E-I, as evidenced by the effects of knocking out one of these two subunits. A 2020 study entitled "NMDA receptors containing GluN2C and GluN2D subunits have opposing roles in modulating neuronal oscillations; potential mechanism for bidirectional feedback" proposes the following model [17]:

Firstly, GluN2C NMDA receptors, which are primarily present in cortical astrocytes, would detect local excitatory synaptic activity in the cortex and provide excitatory feedback to surrounding postsynaptic neurons.

Secondly, GluN2D NMDA receptors in parvalbumin-positive GABAergic inhibitory interneurons in the cortex and mesencephalic structures also detect local excitatory synaptic activity (just like the GluN2C NMDA receptors in cortical astrocytes). However, in this case, the feedback provided to surrounding excitatory postsynaptic neurons is inhibitory.

In this model, GluN2C and GluN2D subunits become central elements in maintaining the cortical excitation-inhibition balance, highlighting a finely tuned regulatory mechanism of oscillatory power. It is conceivable that a disruption of this bidirectional feedback mechanism could be the cause of many psychiatric and neurodegenerative disorders, and conversely, enhancing this mechanism could be a solution to improve cognitive abilities.

The Current State of GluN2C/D PAMs

(±) CIQ, a positive allosteric modulator of GluN2C/D, has demonstrated the ability to rescue the detrimental effects of MK-801 (NMDA receptor pore blocker) on working memory and prepulse inhibition, indicating pro-cognitive and anti-psychotic effects [11]. Additionally, the CIQ isomers (±, +) improved memory recognition in the novel object recognition test and reduced hyperlocomotion as well as stereotyped behaviors [12].

From a functional perspective, the administration of CIQ has been shown to potentiate short-term potentiation (STP) without affecting long-term potentiation (LTP) [18]. Moreover, Hebbian STP has been proposed as a key mechanism in working memory in a 2017 model [19].

In the Parkinson's disease animal model, CIQ restores LTP in dopamine-depleted striatum [13]. CIQ has also shown another intriguing property: in control mice, its administration inhibits tonic dopamine release and induces an initial inhibition followed by a prolonged increase in phasic release (which is consistent with the observed role of NR2C/NR2D subunits in the E-I balance). However, a muscarinic receptor antagonist, prenzepine, blocked the CIQ-induced decrease in release but not the prolonged potentiation [14].

(+)-EU1180-453, another positive allosteric modulator of GluN2C/D, increases the response of NMDA receptors containing GluN2C or GluN2D, including triheteromeric receptors (composed of two different GluN2 subunits). It also prolongs deactivation time and increases the amplitude of the glutamate response. Notably, there is an increase in gamma oscillation power induced by carbachol in hippocampal slices, suggesting an enhancement in neuronal synchronization and the function of inhibitory networks [15].

PTC-174, another PAM of NMDA receptors containing GluN2C/GluN2D subunits, has demonstrated the ability to improve markers of inhibitory control in the animal model [5] [15] (see Penchantbio article for more details). Furthermore, it is highly likely that the improvement in behaviors associated with inhibitory control is more closely linked to the GluN2D subunits than to the GluN2C subunits, due to the higher presence of GluN2D subunits in inhibitory interneurons.

Interesting Points on pathogenesis

This section, which is more of an additional section, will cover points that I find interesting to highlight regarding this pair of subunits and their roles in pathogenesis. It will mostly present a few observed curiosities.

Depression & Pain

A first curiosity is that GluN2C- and GluN2D-containing NMDA receptors in the nucleus accumbens seem to play an important role in nociception. Indeed, GluN2D KO mice exhibited a reduced mechanical nociceptive response [20].

Validating the role of GluN2C subunits in excitatory activity, it has been reported that the GluN2C subunit is present at higher levels in the locus coeruleus during depression [22]. The locus coeruleus has been associated with panic disorders. It has been proven that stimulation of this area induces an anxious state in animals. Therefore, negative modulators of NMDA receptors containing the GluN2C subunit, but also potentially positive

modulators of NMDA GluN2D, in line with their joint role in controlling brain oscillations, could become novel targets for depression and anxiety disorders.

Additionally, GluN2D KO mice exhibit depressive traits (potentially linked to schizoid personality disorder, though this remains speculative), including deficits in social recognition, social stress, dysfunction of 5-HT2C receptors (the mechanism behind this dysfunction remains unclear), and anhedonia [23].

Epilepsy

A particularly important point is the potential central role of the GluN2C/GluN2D balance in epilepsy. Rare cases of dysfunction in the gene encoding GluN2D (GRIN2D) have been reported in the literature in association with epilepsy, and all of these dysfunctions have been shown to impair this subunit's function [24].

A specific disorder, GRIN2D-Related Developmental and Epileptic Encephalopathy, has been identified in 22 individuals to date. This disorder is caused by dysfunctions in the GluN2D subunit and manifests as epilepsy, intellectual disability, autism spectrum disorders, and abnormal movements [25].

While the role of GluN2D subunit impairment in epilepsy is marginal, it is still worth mentioning. However, the role of the GluN2C subunit appears to be crucial. As a reminder, it has been proposed that the GluN2C subunit, present in cortical astrocytes, detects local excitatory synaptic activity in the cortex and provides excitatory feedback to surrounding postsynaptic neurons. This mechanism aligns perfectly with the excitatory cascade observed in epilepsy. The inhibition of GluN2C is currently considered a target for developing new antiepileptic drugs [27].

This +GluN2C/-GluN2D relationship also explains why the antipsychotic Neboglamine does not seem to increase the risk of epilepsy. Since Neboglamine is a PAM of the glycine site, which is present in all NMDA receptors, its administration should not disrupt the GluN2C/GluN2D balance. However, given the available data, it is very likely that a PAM selectively targeting the GluN2C receptor could increase the risk of epilepsy. It is even possible that a selective GluN2C PAM could be used to develop animal models of the disease in the future.

Final words

Despite recent discoveries about this pair of NMDA receptor subunits, many uncertainties remain, particularly regarding their exact role in the pathogenesis of psychiatric disorders and higher cognitive functions, such as working memory and inhibitory control. Unfortunately, clinical trials on PAMs targeting NMDA receptors containing GluN2C/GluN2D subunits remain scarce, and concerns persist regarding their safety. Further investigation into their potential role in ADHD would be valuable, as increased inhibitory control and the regulation of brain oscillations are undoubtedly key factors in addressing attention disorders. GluN2D could therefore play an important role in neuropathic pain despite its potential as a target for cognitive enhancement. [20]

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