Neurosci Bull December 1, 2013, 29(6): 761–772. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1336-9


·Review·

# GluN2A *versus* GluN2B: twins, but quite different

Xiao-Min Zhang, Jian-Hong Luo

*Department of Neurobiology, Key Laboratory of Medical Neurobiology of the Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, China*

Corresponding author: Jian-Hong Luo. E-mail: luojianhong@zju.edu.cn



N-Methyl-*D*-aspartate receptors (NMDARs) play vital roles in the central nervous system, as they are primary mediators of $Ca^{2+}$ influx during synaptic activity. The subunits that compose NMDARs share similar topological structures but are distinct in distribution and pharmacological properties, as well as physiological and pathological functions, which make the NMDAR one of the most complex and elusive ionotropic glutamate receptors. In this review, we focus on GluN2A and GluN2B, the primary NMDAR subunits in the cortex and hippocampus, and discuss their differences in developmental expression, brain distribution, trafficking, and functional properties during neuronal activity.

**Keywords:** N-methyl-*D*-aspartate receptors; GluN2A; GluN2B; developmental expression; brain distribution; function; trafficking

## Introduction

N-Methyl-*D*-aspartate receptors (NMDARs) mediate excitatory signal transmission in the central nervous system (CNS). The combinations of their subunits, each with distinct pharmacological and electrophysiological characteristics, result in NMDARs with multiple functions. GluN2, the main regulatory subunit, is important in determining NMDAR function. Invertebrates have only one GluN2 gene. The first round of duplication gave rise to GluN2AB and GluN2CD, and the second round separated GluN2AB into GluN2A and GluN2B, while GluN2CD generated GluN2C and GluN2D[1]. Thus far, these four GluN2 subunit genes, especially GluN2A and GluN2B, with their distinct temporal and spatial expression patterns and channel properties, enrich and complicate the functions of the brain. With highly similar amino-acid sequences (70% identity), both GluN2A and GluN2B are expressed in the cortex and hippocampus, and are involved in important physiological and pathological processes such as neuronal signal transduction, long-term potentiation (LTP), long-term depression (LTD), and excitotoxicity, as well as in neurodegenerative diseases. This review focuses on two protein homologs, GluN2A and GluN2B, with emphasis on comparison of their behavior in physiological processes in the CNS.

## Developmental Expression and CNS Distribution

NMDARs play important roles in excitatory signal transduction in the CNS. Like other ionotropic glutamate receptors, they are tetramers usually composed of two obligatory GluN1 subunits and two modulatory GluN2 or GluN3 subunits[2,3]. All of these subunits share a classical topological structure: a large extracellular N-terminus, three and a half transmembrane segments, and an intracellular C-terminus. In 1991, Moriyoshi and collaborators first cloned the GluN1 gene from rat brain; it encodes a 938 amino-acid peptide and has eight splice variants[4]. Later, researchers found that the GluN1 subunit is required for a functional NMDAR, and GluN1 gene knockout leads to neonatal death in mice[5]. The assembly of GluN1 subunits with different GluN2 or GluN3 subunits confers distinct characteristics of channel properties, expression pattern

and function on the receptor.

As the structural subunit, GluN1 is expressed ubiquitously in the brain, while the modulatory GluN2 subunits are under strict regional and age-dependent regulation. GluN2B and GluN2D are expressed early in the prenatal brain. GluN2B is widely distributed in most brain regions, and GluN2D is restricted to the diencephalon and brain stem. After birth, the predominance of GluN2B is soon overtaken by GluN2A during development. Specifically, the GluN2B expression level is high prenatally, reaches 1.5-fold of the adult level 14 days after birth, and then continuously decreases, while GluN2A expression begins 7 days after birth and surpasses GluN2B in the cortex and hippocampus at day 14[6]. This developmental switch is critical for brain maturation and is under extensive study[7,8].

## Trafficking

### *Early Trafficking: from Endoplasmic Reticulum to Golgi*

The translation and assembly of multi-subunit transmembrane receptors begin in the endoplasmic reticulum (ER). Correct folding and assembly are required for the normal expression and functioning of NMDARs on the neuron membrane. Various ER quality control mechanisms guarantee that the immature subunits are not exported from the ER until completely assembled[9]. The GluN1 splice variants have distinct properties in the early trafficking stage because of the different C-terminal cassette combinations they contain. The C1 cassette has an RRR ER retention signal, while the C2' cassette has a PDZ-interacting domain that suppresses the ER retention of the C1 cassette[10,11]. As a result, GluN1-1a/b are retained in the ER before assembly because of the C1 cassette, and GluN1-4a/b are automatically expressed on the cell surface because they contain both C1 and C2' cassettes.

For GluN2, an HLFY motif in the C-terminus immediately following the transmembrane domain is required for the exit of the assembled receptor from the ER[12,13]. An ER retention signal has been found in the third transmembrane domain (M3) of GluN2B, which can be masked by the same topological region of the GluN1 subunit. However, a recent study suggests that this M3 retention signal also exists in the GluN1 subunit[14,15]. Despite the fact that most NMDAR subunits carry an identical or overlapping retention signal on the intracellular C-terminus, GluN2A has an ER retention signal in its extracellular amino terminal domain, and this retention signal can also be masked through assembly[16]. This specific ER retention signal is worth noting for two reasons: (1) during translation, the extracellular N-terminus of GluN2A is inside the ER lumen, so it is able to interact with ER chaperone proteins, which commonly participate in ER quality control, and (2) no similar retention signal so far has been found in the homogenous amino terminal domain of GluN2B. This raises the possibility that the ratio of functional GluN2A-containing to GluN2B-containing NMDARs can be regulated at an early trafficking stage, from ER to Golgi.

### *Canonical Trafficking*

Neurons are morphologically specialized polarized cells with long protrusions, the axon and dendrites. In some large animals, the length of an axon can reach one meter. Neurons, therefore, require highly-developed transport mechanisms to ensure the appropriate delivery and function of newly-synthesized proteins that are essential for the maintenance of morphology and survival[17,18]. The conventional hypothesis is that the soma is responsible for the synthesis and assembly of proteins, then nascent proteins are sorted out from the Golgi to distal dendrites and axons. Long-distance transport along an axon or dendrite depends on microtubules and motor proteins, while actin is responsible for short-distance transport inside spines. This is called canonical trafficking, and has been studied over decades.

NMDARs are mainly expressed on dendrites, where they participate in synaptic transmission. According to the canonical trafficking hypothesis, NMDARs are first sorted into vesicles after their synthesis in the soma, and then the motor protein family member kinesin or dynein carries these vesicles along microtubules towards the distal dendrites. Such a pathway has been confirmed for the trafficking of NMDARs and is believed to be important for the development and survival of neurons and even for learning and memory[17-20].

GluN2B trafficking through the canonical pathway has been well studied. It is generally believed that vesicles carrying GluN2B are linked to microtubules and conveyed by the kinesin superfamily motor protein 17 (KIF17) at a speed of ~0.76 μm/s[21,22]. These vesicles are 50 nm

in diameter and contain both KIF17 and GluN2B. The interaction of GluN2B and KIF17 is mediated by mLin10 (Mint1), which directly binds the KIF17 tail region through its C-terminal PDZ domain[22]. The Ser1029 phosphorylation of KIF17 by CaMKII disrupts its binding to mLin10, and in turn triggers the release of cargo vesicles from KIF17[23]. In cultured hippocampal cells from the *kif17*$^{-/-}$ mouse, the mobility of GluN2B is significantly decreased[24]. By introducing a mutated KIF17 to mimic a phosphorylated or non-phosphorylated Ser1029, Yin *et al.* discovered that interrupting the binding of cargo vesicles with KIF17 reduces the velocity of GluN2B, and impairs the spatial memory of mice[25]. KIF17-knockout mice also display a decreased transcription of GluN2B and a loss of GluN2B in the synapse. This is consistent with recent studies showing that KIF17 and GluN2B are both regulated by the same transcription factor, NRF-1[26, 27].

However, there is not much research on the canonical trafficking of GluN2A. So far, no kinesin or dynein protein has been found to specifically interact with GluN2A, or participate in GluN2A trafficking. It is reported that in KIF17-knockout mice, the expression of GluN2A is reduced while its trafficking is unaffected[24]. Consistently, Yin *et al.* detected a decreased number of GluN2A clusters along dendrites without any alteration of GluN2A mobility[25].

### *Non-Canonical Trafficking*

ER, Golgi and mRNA are not restricted to the soma, but are widely distributed along dendrites and axons. The identification of dendritic ribophorin I, alpha-mannosidase II and galactosyltransferase demonstrates that local ER and Golgi outposts may be functional[28]. Using live-cell imaging, researchers found that the membrane protein VSVG accumulates in dendritic ER or Golgi outposts when the temperature is low[29,30]. So a new pathway, non-canonical trafficking, has been identified. In this pathway, proteins that are destined to function in distal dendrites and axons can be translated and assembled in dendritic or axonal local ER and Golgi outposts[31,32].

This local apparatus raises the possibility that several neuronal receptors, including α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) and NMDARs, might be transported by non-canonical means. In fact, evidence shows that SAP97 and CASK mediate the sorting of NMDARs by dendritic Golgi outposts. It is suggested that NMDARs bypass the Golgi apparatus in the soma, and are transported within the ER until they reach the Golgi outpost in the distal dendrite. The ER-NMDAR vesicles exhibit a much slower velocity than KIF17-GluN2B vesicles, at 0.2–0.3 μm/s[33]. It is known that SAP97 mainly interacts with the GluN2A subunit, and studies show that GluN2A co-localizes with SAP97 in the ER soon after its synthesis. Phosphorylation of SAP97 at Ser39 promotes the release of SAP97/GluN2A clusters from the ER, without changes of GluN2B sub-cellular localization[34]. These results, combined with the fact that no kinesin or dynein has been identified to carry Golgi sorting vesicles containing GluN2A, support the theory that GluN2A subunits undergo non-canonical trafficking. However, GluN2B cannot be ruled out from non-canonical trafficking, since ER vesicles can be carried by KIF17 and GluN2B also accumulates in dendritic Golgi outposts when Golgi trafficking is blocked by ARF1-Q71I[33]. So far, no research has been conducted to distinguish or define in detail the post-Golgi sorting vesicles and ER vesicles. Although the functional importance of non-canonical trafficking is not clear, this alternative secretory pathway might provide a platform for rapid synaptic insertion of NMDARs in response to synaptic activity.

### *Lateral Diffusion*

Thousands of neurons make up delicate networks to fulfill various kinds of neuronal activity and plasticity. The interactions between neurons depend on synapses, the minimal functional unit in the CNS. Therefore, correct functioning of the CNS requires strict regulation of specific proteins such as ionotropic glutamate NMDARs and AMPARs in specific quantities and locations. A considerable number of NMDARs is located in the postsynaptic density (PSD). The localization of NMDARs in the PSD is mediated by scaffolding proteins of the MAGUK family, including PSD-95, PSD-93, SAP102 and SAP97. All MAGUK proteins contain three PDZ domains at the N-terminus, one SH3 domain, and a C-terminal GK domain. MAGUK proteins are located in the PSD, where they anchor NMDARs by interacting with the C-terminal PDZ binding domain of NMDARs[35]. However, the localization of NMDARs is not unchanging. The dynamic regulation of these receptors has been studied extensively.

Using electrophysiological methods, Tovar and Westbrook demonstrated that the extra-synaptic NMDARs are mobile. They used the open-channel antagonist MK-801 to block the synaptic NMDARs and detected a

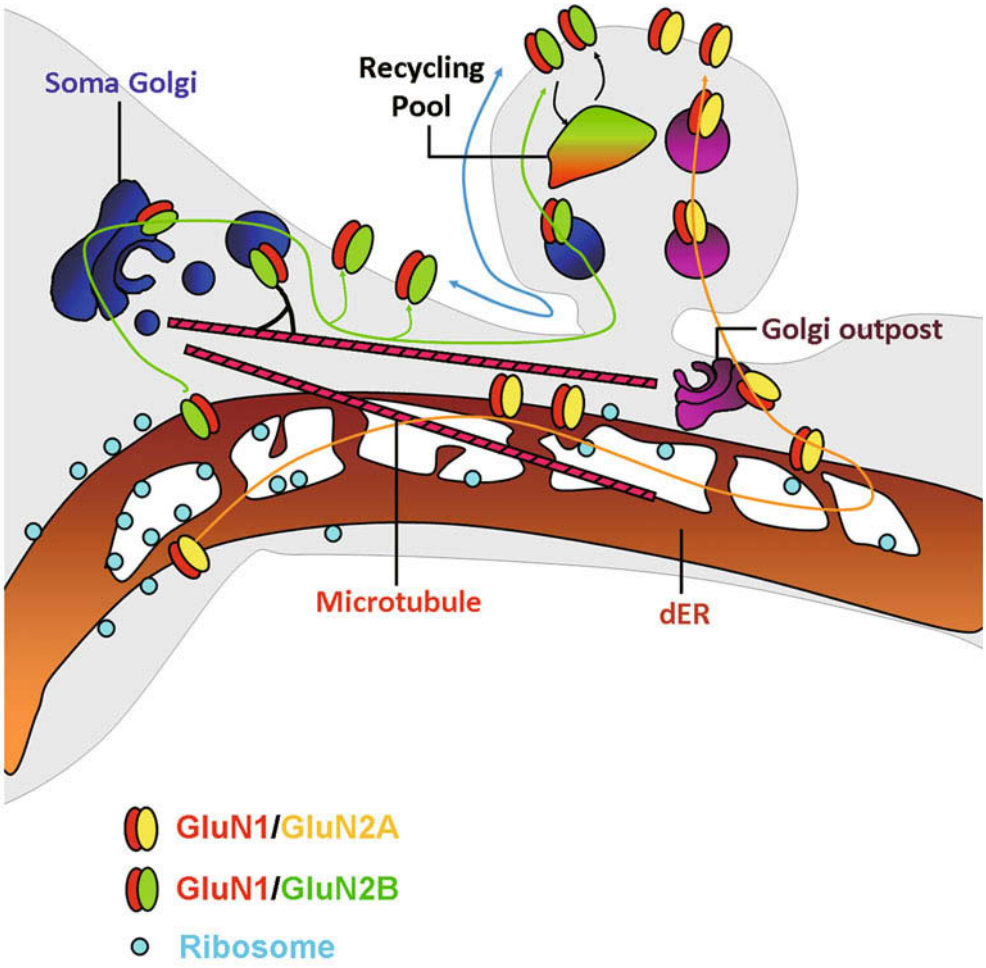


**Fig. 1. Canonical and non-canonical trafficking of NMDARs. GluN2B-containing NMDARs traffic in the canonical pathway. They are synthesized in the somatic endoplasmic reticulum (ER) and Golgi, and are sorted as post-Golgi vesicles along microtubules to distal dendrites. GluN2B-containing NMDARs localize in extra-synaptic regions, and move laterally into the synapse (green line and arrows). Also, in the synapse, the GluN2B-containing NMDARs go through continuous recycling (black line and arrows). GluN2A-containing NMDARs may traffic in the non-canonical pathway in which they bypass the somatic Golgi and are transported inside the ER to distal dendrites. They can be delivered from the dendritic ER directly into the synapse (yellow line and arrow). Modified from Ramirez *et al*., 2011, Trends in Cell Biology[32].**

recovery of synaptic miniature excitatory synaptic currents (mEPSCs), suggesting that extrasynaptic receptors move laterally into synapses to replace the previously-occupied synaptic NMDARs blocked by MK-801. This was the first study to indicate that NMDARs can move laterally between extrasynaptic and synaptic regions[36]. A few years later, the Groc lab recorded the mobility of GluN2A and GluN2B using quantum dots conjoined with antibodies specifically binding to GluN2A or GluN2B. They found that both synaptic GluN2A and GluN2B subunits are very stable, while the mobility of extrasynaptic GluN2B subunits is much higher than that of the extrasynaptic GluN2A subunits[37]. Later, they discovered that the mobility of GluN2B is regulated by the extracellular matrix protein reelin. The increase of reelin during brain development accelerates the mobility of extrasynaptic GluN2B. As a result, the dwell-time of GluN2B in the synapse is reduced, and this provides one explanation for the developmental switch of GluN2A and GluN2B subunits[38]. Another extracellular matrix protein, matrix metalloproteinase-9 (MMP-9), is able

to increase the mobility of both synaptic and extrasynaptic NMDARs[39]. Interestingly, the transmembrane protein intergrin1-β mediates the function of these two extracellular matrix proteins. It seems that the binding of different extracellular proteins to intergrin1-β triggers different signal cascades to modulate NMDAR mobility. Nevertheless, it is currently believed that GluN2A subunits are more stable than GluN2B subunits. This is consistent with the notion that GluN2A subunits are mainly located inside the synapse while GluN2B subunits are expressed in both synaptic and extrasynaptic regions, and GluN2B subunits can quickly move into synapses in response to synaptic activity[40-42].

## Function

### *Neuronal Fate Governed by Differentiated NMDAR Signaling*

**Localization of NMDARs links differential signaling of neuronal survival or death** Activation of synaptic NMDARs is thought to trigger signal cascades for the protection and survival of neurons, and the activation of extrasynaptic NMDARs leads to neuronal death. $Ca^{2+}$ influx through the synaptic NMDARs activates the CAM kinase kinase and Ras–ERK1/2 signaling cascade as well as Akt and RSk2 in the Ras–MAPK pathway to phosphorylate Ser136 or Ser112 of the pro-apoptosis protein BAD, which causes the inactivation of BAD and protects neurons from apoptosis[43, 44]. In addition, the $Ca^{2+}$ influx evoked by synaptic NMDAR activation is also a trigger for genomic processes that increase the transcription of neuroprotective genes like BDNF and BCL2 by the transcription factor cAMP-response-element-binding-protein (CREB)[45-47]. However, the extrasynaptic NMDARs play a contrasting role, because they are coupled to a CREB shut-off pathway by phosphorylating Ser133 of CREB to inactivate this transcription factor[48]. Besides, the $Ca^{2+}$ overload through extrasynaptic NMDARs strongly impairs mitochondria and leads to cell death[49]. But interestingly, the same level of $Ca^{2+}$ influx through synaptic NMDARs has no effect on mitochondria and is well-tolerated by neurons[47].

**Synaptic and extrasynaptic localization of GluN2A- and GluN2B-NMDARs** Whether GluN2A and GluN2B behave differently in deciding neuronal fate remains a question. One popular theory states that GluN2A is coupled with protection while GluN2B is linked with neurotoxicity, because most synaptic NMDARs are GluN2A-containing and the extrasynaptic receptors are GluN2B-containing. A series of studies has shown that early in the neonatal brain, when GluN2A is not expressed, both synaptic and extrasynaptic regions are occupied by GluN2B-containing NMDARs, then synaptic GluN2B is gradually substituted by GluN2A during development[50]. Some evidence supports this location preference of different subunits, since in GluN2A-knockout mice, only synaptic mEPSCs are impaired, but not the evoked NMDAR current[51].

Based on recent findings, however, GluN2A and GluN2B are not distributed solely in the synaptic or extrasynaptic region. Combining electrophysiology, local photolysis, and confocal imaging, Harri and Pettit defined synaptic receptors as those responding to the glutamate release of a 0.1 Hz synaptic response, and found that GluN2B subunits are present in both compartments[52]. This was supported by an electrophysiological study which confirmed the existence of both GluN2A and GluN2B in synapses by whole-cell recording from cultured hippocampal neurons[53]. It seems that GluN2A and GluN2B are distributed asymmetrically, and the synaptic content of NMDARs is able to respond quickly to synaptic activity[41,42].

Although the distribution of GluN2A and GluN2B is still controversial, one agreement that has been reached is that GluN2A subunits are located mainly in synapses, and GluN2B subunits mainly in the extrasynaptic region. Therefore, the hypothesis that GluN2As mediate neuronal survival and GluN2Bs mediate neurotoxicity sounds reasonable and is widely accepted. Pharmacological experiments on mice under ischemia or primary cultured cortical neurons with subunit-specific antagonists have revealed that the different NMDAR subtypes, GluN2B and GluN2A, play different roles in cellular damage and stress tolerance. Furthermore, the receptor subtype but not its location is believed to be the determining factor for neuronal fate, since blockade of synaptic NMDARs by MK-801 cannot block the neuroprotective or death pathway entirely[54].

**GluN2A and GluN2B in mediating neuronal survival and death** It is conventionally believed that GluN2A-containing receptors activate neuroprotection against ischemia by regulating CREB phosphorylation[55]. However, recent studies suggest that other signaling pathways may also be involved. The activation of GluN2A-containing

receptors down-regulates PTEN, which has an inhibitory effect on the expression of TDP-43 (TAR DNA-binding protein), conferring protection against cortical neuronal death at the early stage of several neurodegenerative diseases[56]. Besides, the ERK1/2 pathway is activated to suppress apoptosis when neurons are exposed to glucocorticoids. In contrast, GluN2B is coupled to several signaling pathways that lead to neurotoxicity. For example, the over-expression and over-phosphorylation of tau in Alzheimer's disease induces neuronal death mediated by GluN2B-containing receptors, which can dephosphorylate CREB to inhibit protection and activate calpain by which tau is degraded into highly toxic N-terminal peptides[57]. Like GluN2A-containing receptors, GluN2B-containing receptors can also regulate PTEN but in a contrary way. Activation of GluN2B-containing receptors directly phosphorylates PTEN which subsequently dephosphorylates Akt and BAD, resulting in deactivation of the Akt survival pathway and promoting neuronal apoptosis[58]. Moreover, recent studies have revealed several previously unknown apoptosis signaling pathways. DAPK1 has been shown to be recruited to the GluN2B complex during cerebral ischemia, and activated DAPK1 is able to phosphorylate the Ser1303 of the extrasynaptic GluN2B subunit to increase its channel conductance and strengthen excitotoxicity[59]. Another study points to a transcriptional factor, SREBP-1, which can be activated by GluN2B-containing NMDARs and mediate excitotoxicity[60].

In addition to the signaling pathway, the extent and location of $Ca^{2+}$ influx by GluN2A- or GluN2B-containing NMDARs are also considered to be key factors in deciding neuronal fate. Synaptic $Ca^{2+}$ is well-tolerated by neurons, but extrasynaptic $Ca^{2+}$ leads to strong depolarization of the mitochondrial membrane and causes cell death[47]. One explanation is that GluN2B subunits, located mainly in the extrasynaptic region, have a longer decay time which allows for long-lasting $Ca^{2+}$ influx to reach a high intracellular concentration and subsequently triggers mitochondria-mediated apoptosis. On the other hand, the synaptic GluN2A subunits mediate $Ca^{2+}$ inflow to a lesser extent, which is incapable of triggering apoptosis but sufficient to activate $Ca^{2+}$/calmodulin and promote neuroprotection[61,62].

However, many studies have reported contradictory findings: in immature hippocampal neurons, specific blockade of GluN2B-containing NMDARs by ifenprodil or Ro-25-6981 has a potent neuroprotective effect, reducing the cell death rate to 20% during glutamate- and NMDA-induced apoptosis, because extrasynaptic GluN2B subunits mediate extensive $Ca^{2+}$ inflow which causes $Ca^{2+}$ overload in mitochondria. In mature hippocampal neurons however, $Ca^{2+}$ inflow is mediated by both GluN2A- and GluN2B-containing NMDARs. Moreover, a series of studies showed that Cdk5 may phosphorylate Ser1232 in the GluN2A C-terminal, resulting in increased expression of GluN2A in the retina and the hippocampal CA1 region. This glutamate-induced neurotoxicity may increase calpain and calpain-specific alpha-spectrin breakdown products, and aggravate neuronal injury.

### *GluN2A- and GluN2B-NMDARs and the Direction of Synaptic Plasticity*

LTP was first discovered in 1973 and, being considered a possible molecular model for learning and memory, has attracted massive attention over the decades[63]. Classical LTP requires the participation of NMDARs and AMPARs. NMDAR activation following high-frequency stimulation allows extracellular $Ca^{2+}$ to flow into the cell and phosphorylate AMPARs through a signal cascade that upregulates AMPAR surface expression, increases cationic conductance, and eventually strengthens synapses. However, in recent years, the specific role of NMDARs, with particular interest in GluN2A and GluN2B subunits, in the formation of LTP/LTD has been controversial.

**Evidence from pharmacological procedures** The primary debate is whether LTP is potentially facilitated by NMDARs. Many studies claim that during LTP induction, the AMPAR/NMDAR ratio increases, and this change may last for 2–4 h. But the fact that these studies were carried out only in neonatal neurons has been overlooked. On the other hand, in animal behavior experiments, natural stimulation that occurs during development, such as light, is more often used. Recent studies using adult rat neurons revealed that LTP-induced stimulation increases the surface expression of NMDARs but not AMPARs. This increased surface expression depends on the phosphorylation sites of GluN2A (Tyr842) and GluN2B (Tyr1472)[64,65]. Other studies have reported that even in neonatal neurons, NMDAR surface expression may be up-regulated 2 h after LTP induction and maintain a balanced AMPAR/NMDAR ratio in the face of ongoing plasticity[66].

In 2004, using pharmacological methods, Yu-Tian Wang and collaborators discovered that specifically blocking GluN2B suppresses LTD, while blocking GluN2A suppresses LTP, therefore drawing the conclusion that GluN2A subunits are involved in LTP while GluN2B subunits participate in LTD[67,68]. Though many subsequent studies are in favor of this model[38,69,70], others have found opposing results[71]. Notably, the GluN2A and GluN2B subunit knockout mouse models do not fully support this.

Studies have reported that GluN2A-containing NMDARs play a pivotal role during chemically-triggered LTP and LTD in brain slices. Furthermore, suppression of the protein kinase CK2 inhibits NMDAR-mediated LTP, while LTD as well as non-NMDAR-dependent LTP are not affected[72]. CK2 suppression also leads to up-regulation of the surface expression of GluN2B-containing receptors and down-regulation of that of GluN2A-containing receptors, which suggests that increased GluN2A surface expression is essential for hippocampal LTP or that increased GluN2B expression suppresses LTP. These experiments also suggest that increased GluN2B surface expression is of no relevance to LTD[73].

However, in other experiments, GluN2B appears to be critical for LTP. Recent studies have reported that the D4 dopamine receptor may regulate LTP through modification of GluN2B[74]. Knockdown by siRNA and over-expression assays indicate that the C-terminal signaling sequence but not the channel properties of GluN2B contributes to LTP regulation, while the C-terminus of GluN2A has little relevance to hippocampal LTP[75,76]. It is also suggested that during early development, hippocampal LTP is chiefly affected by the dominant GluN2B subunit, and gradually switches to a GluN2A-dominant phase as the GluN2A/GluN2B ratio increases. Notably, GluN2B may exert versatile effects on LTD, as studies have shown in mature hippocampal regions that neither ifenprodil nor Ro25-6981 affects LTD[77].

**Evidence from genetic procedures** Studies concerning LTP and behavior have also yielded contradictory results. Newborn mice maintained in an enriched environment for 1 month have increased *egr-1* in the anterior cingulate cortex, enhanced LTP and decreased LTD, together with an increased GluN2B/GluN2A ratio[78]. In the mood and social activity-related lateral nucleus of the amygdala, GluN2A and GluN2B are both essential for LTP and LTD. Remarkably, IQGAP1 and c-Fos knockout leads to inhibition of LTP that depends on GluN2A. This impairs spatial learning, while general behaviors such as anxiety or depression-related behaviors, are not affected[79,80].

Despite the myriad of results *in vitro*, studies on genetically-modified animal models support the idea that GluN2B facilitates learning and memory. Overexpression of GluN2B potentiates learning and memory[81] while genetic knockout of GluN2B in the frontal lobe or hippocampal region impairs behavior in many aspects, both spatially or non-spatially, or induces selective short-term spatial working memory loss[82]. In addition, treatment with the selective GluN2B antagonist Ro25-6981 abolishes fear-associated memory in 3-month-old mice, and the effect weakens as the mouse ages.

However, it seems that GluN2A knockout also partially affects learning and memory. GluN2A-knockout or GluN2A C-terminus-deficient mice exhibit impaired spatial working memory while spatial reference memory remains unaffected[83]. Another study also showed that GluN2A-knockout mice have normal spatial reference memory, but the rapidly-acquired spatial working memory is dysfunctional. Subcutaneous injection of deoxy-ephedrine may upregulate GluN2A and cause spatial working memory impairment in rats[84]. These results invariably point to a consensus that GluN2B is essential for learning and memory, while the association between GluN2A and learning and memory appears to be more complex.

**Controversy and weakness in methodology** It is conceivable that differences in species, strains, ages, brain structures and protocols might account for these discrepancies. However, other reasons have been indicated. First, no GluN2A-specific antagonist has been found. So far, various GluN2B-specific antagonists such as ifenprodil and Ro 25-6981 have been identified, with significantly higher affinity for GluN2B than GluN2A (ifenprodil >400-fold, Ro25-6981 >1000-fold). Therefore, it is now possible to distinguish GluN2B-mediated activities from overall NMDAR activities. Although GluN2A-specific antagonists are currently absent, NVP-AAM077 exhibits a 130-fold affinity for GluN2A compared with GluN2B in oocytes. In rodents, however, the affinity rate is only 13-fold. The concentration-dependent characteristics of NVP-AAM077 specificity for NMDAR subunits remain unknown. NVP-AAM077 can even inhibit NMDAR-mediated LTP

in GluN2A-knockout mice and block GluN2B-containing NMDAR current. Furthermore, the interactions between specific antagonists and triheteromeric NMDARs are undetermined[85,86]. It is also recognized that LTP and LTD can be generated *via* different mechanisms, therefore different forms of LTP and LTD may be associated with different subunits[87].

The presence of a considerable number of NMDAR triheteromers is another obstacle hindering the discrimination of GluN2A from GluN2B functions. Studies have found that in the CNS, particularly in cortex, the proportion of NMDAR triheteromers may be more than half of the overall NMDARs[88,89]. These triheteromers may function differently from GluN1/GluN2B and GluN1/GluN2A receptors, as their ionic channel properties and signaling may be basically different from those of the heterodimers. To study triheteromers, an effective method is required to distinguish them from the NMDAR pool. Recent studies have extracted triheteromers by mutation, and their affinity for ifenprodil and $Zn^{2+}$ has been investigated. The results show that triheteromers respond poorly to ifenprodil or $Zn^{2+}$ with no significant changes in affinity. A double antagonist may be required to achieve a satisfactory blockade of the triheteromers[90]. Clearly, all the pharmacological studies on the function of NMDAR subtypes described above should take the triheteromers into account, but most studies have not considered them, which inevitably causes more ambiguity over GluN2A and GluN2B subunits.

Recently, some studies have attempted to explain these discrepancies in other ways. For example, it is reported that inhibition of CaMKII for 2 h may specifically decrease synaptic GluN2B-containing NMDARs, with no influence on GluN2A distribution. Abolishing the GluN2B and PSD-95 interaction yields the same result, and LTP but not LTD is affected[91]. And LTD induced in rodent cortex does not require synaptic NMDAR activation[68]. These results all suggest that the location, synaptic or extrasynaptic, but not the type of subunit may be responsible for LTP and LTD.

Even though conflicting results remain between animal models and *in vitro* experiments, it is easy to see that both LTP and LTD are somehow related to learning and memory behaviors. Learning and memory correspond in the microscopic view to synaptic plasticity, including LTP and LTD. In other words, failure to establish learning and memory behavior may be ascribed to disorders in inducing both LTP and LTD. Therefore, the notion that learning and memory improve with LTP and weaken with LTD is erroneous. It is important to establish a concept that can clear up the discrepancy between genetic manipulation in mice and *in vitro* experiments.

## Conclusion and Perspectives

NMDARs have been extensively studied for over 20 years since their first cloning. It is now well-known that NMDARs are more complex than AMPARs or kainate receptors in many ways, particularly in the composition of subunits, of which each has unique features and plays major roles in deciding NMDAR function. The two main GluN2 subunits in the frontal cortex and hippocampus, GluN2A and GluN2B, are involved in many neuronal processes. These two subunits are highly similar in their amino-acid sequences, but possess distinct pharmacological and electrophysiological characteristics. And they may traffic in different ways and finally be located in different subregions in dendrites. As a result, they play contradictory or overlapping roles in neuronal plasticity and mediate different signaling cascades in neuronal survival or death. However, the absence of a specific GluN2A antagonist and the existence of triheteromeric NMDARs with GluN1/GluN2A/GluN2B composition make it very difficult to clarify the functional distinctions between GluN2A- and GluN2B-containing NMDARs. Thus, a specific GluN2A antagonist and an effective method to identify triheteromeric NMDARs are required in future studies.

### ACKNOWLEDGEMENTS

This review was supported by grants from the National Basic Research Development Program of China (2010CB912002) and the National Natural Science Foundation of China (30730038 and 81171164).

Received date: 2012-10-09; Accepted date: 2012-11-15

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