NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease

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Abstract | NMDA receptors (NMDARs) are glutamate-gated ion channels and are crucial for neuronal communication. NMDARs form tetrameric complexes that consist of several homologous subunits. The subunit composition of NMDARs is plastic, resulting in a large number of receptor subtypes. As each receptor subtype has distinct biophysical, pharmacological and signalling properties, there is great interest in determining whether individual subtypes carry out specific functions in the CNS in both normal and pathological conditions. Here, we review the effects of subunit composition on NMDAR properties, synaptic plasticity and cellular mechanisms implicated in neuropsychiatric disorders. Understanding the rules and roles of NMDAR diversity could provide new therapeutic strategies against dysfunctions of glutamatergic transmission.

Throughout the brain and spinal cord, the amino acid glutamate mediates the vast majority of excitatory neurotransmission[1]. Glutamate acts on various membrane receptors, including ionotropic glutamate receptors (iGluRs), which form cation-permeable ion channel receptors and can be subdivided into three large families[1]: AMPA receptors (AMPARs), kainate receptors and NMDA receptors (NMDARs). Since their discovery three decades ago, NMDARs have kept fascinating neuroscientists because of their central roles in CNS function. These glutamate-gated ion channels are essential mediators of brain plasticity and are capable of converting specific patterns of neuronal activity into long-term changes in synapse structure and function that are thought to underlie higher cognitive functions[1]. NMDAR dysfunctions are also involved in various neurological and psychiatric disorders[1–3], including stroke, pathological pain, neurodegenerative diseases and schizophrenia, and there is growing interest in developing new drugs that target these receptors. Recent studies have highlighted the functional diversity of NMDARs[1,4,5]. NMDARs are diverse in their molecular (subunit) composition, their biophysical and pharmacological properties, their interacting partners and their subcellular localization. Subunit composition varies across CNS regions during development and in disease states[1–3]. There is also evidence that even at fully mature synapses, the NMDAR subunit content changes depending on neuronal activity. Further comprehension of the distinct roles of the various NMDAR subtypes should help to define new strategies to counteract the deleterious effects of deregulated NMDAR function.

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Diversity in subunit composition and expression

NMDAR subunits and genes. The notion that NMDARs exist as multiple subtypes endowed with distinctive properties emerged almost 30 years ago from early patch-clamp and binding studies on neuronal preparations[1,5]. Subsequent cloning studies revealed that NMDARs are assembled as heteromers that differ in subunit composition. To date, seven different subunits, falling into three subfamilies according to sequence homology, have been identified[1,4,5] (FIG. 1a): the GluN1 subunit, four distinct GluN2 subunits (GluN2A, GluN2B, GluN2C and GluN2D), which are encoded by four different genes, and a pair of GluN3 subunits (GluN3A and GluN3B), arising from two separate genes. The total number of amino acids per subunit ranges from 900 to over 1,480. The difference in subunit size is almost entirely accounted for by differences in the length of the intracellular carboxyl (C)-terminal domain (CTD), a region that is involved in receptor trafficking and couples receptors to signalling cascades[1]. NMDARs function as heterotetrameric assemblies that typically associate GluN1 subunits with GluN2 subunits or a mixture of GluN2 and

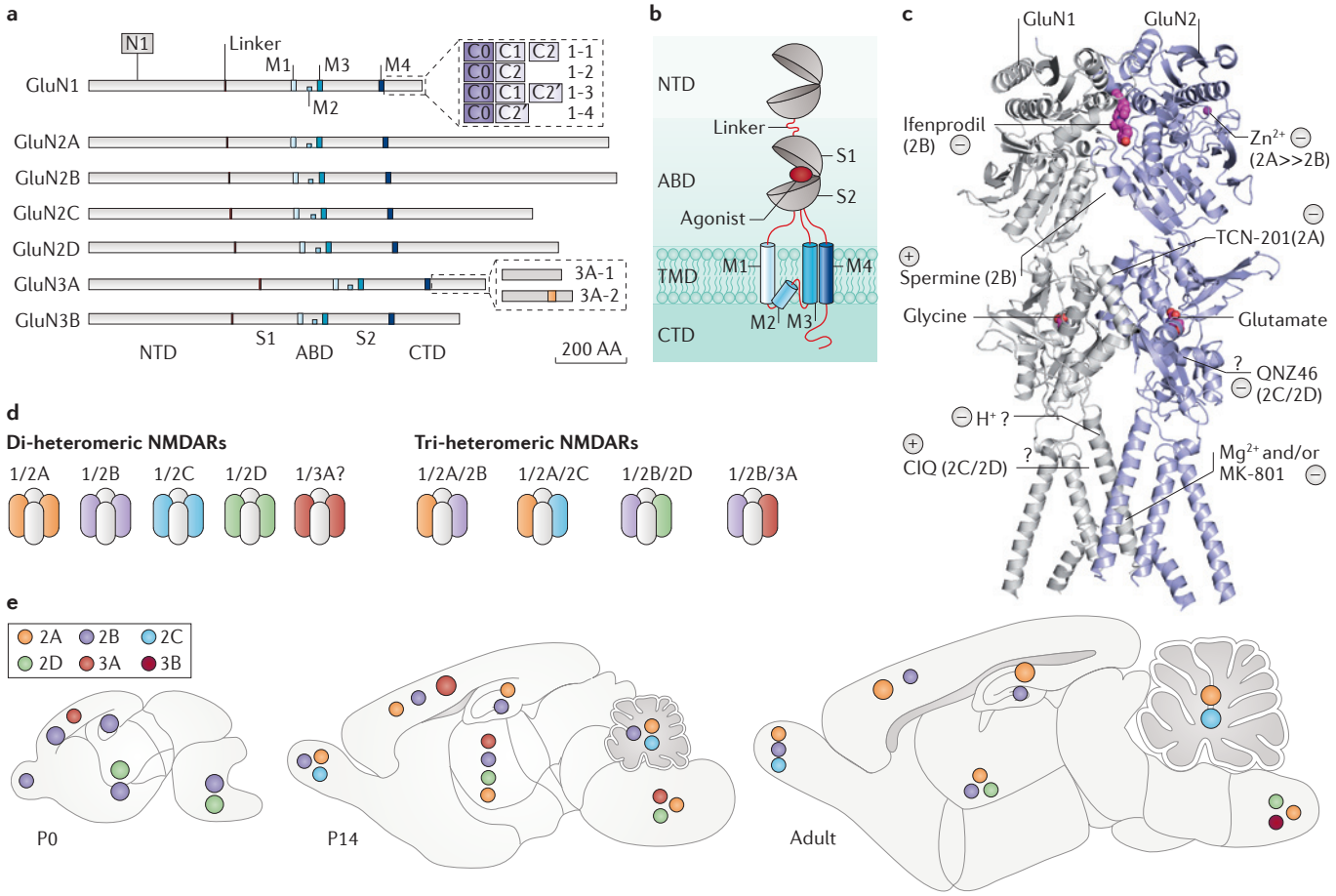


Figure 1 | **NMDAR subunit diversity, structure and expression.** **a** | Seven NMDA receptor (NMDAR) subunits have been identified: GluN1, GluN2A– GluN2D and GluN3A and GluN3B. Subunit heterogeneity is further enhanced by alternative splicing of GluN1 and GluN3A subunits. M1–M4 indicate membrane segments. **b** | All GluN subunits share a modular architecture that is made of four distinct domains: the N-terminal domain (NTD), the agonist-binding domain (ABD) that binds to glycine or D-serine in GluN1 and GluN3 and glutamate in GluN2, the transmembrane domain (TMD) containing the ion channel, and an intracellular C-terminal domain (CTD). The NTD and CTD are the most divergent regions. **c** | NMDARs harbour multiple binding sites for extracellular small-molecule ligands acting as subunit-selective allosteric modulators. A model of a GluN1/GluN2 heterodimer based on the X-ray crystal structures of GluN1/GluN2B NTDs[39], GluN1/GluN2A ABDs[40] and the AMPA receptor GluA2 pore region[43] is shown. The + and – signs indicate positive and negative allosteric modulators, respectively. Question marks (?) indicate uncertainty concerning the exact location of the binding site. **d** | A sample of the various populations of di-heteromeric and tri-heteromeric NMDARs that are thought to exist in the CNS is shown. **e** | The developmental profile of GluN subunit expression in the mouse brain at day of birth (postnatal day 0 (P0)), 2 weeks following birth (P14) and at the adult stage.

GluN3 subunits[1,4,5]. The existence of a large repertoire of homologous NMDAR subunits allows for various combinations of subunit assembly, which gives rise to a multiplicity of receptor subtypes in the CNS (FIG. 1d).

The GluN1 subunit is encoded by a single gene but has eight distinct isoforms (GluN1-1a–GluN1-4a and GluN1-1b–GluN1-4b) owing to alternative splicing (FIG. 1a). The GluN1-b isoforms (or exon 5-containing isoforms) possess an additional extracellular 21-amino-acid stretch (known as the N1 cassette) that affects the receptor's gating and pharmacological properties[6,7]. The four other splice variants arise from alternative splicing of exon 21 and exon 22, giving rise to CTDs of variable length and differential subunit trafficking properties[8]. In accordance with the widespread CNS distribution of NMDARs, the GluN1 subunit is ubiquitously expressed from embryonic stage E14 to adulthood[9–11]. There are specific differences in GluN1 isoform expression however[5]. Whereas GluN1-2 is widely distributed, GluN1-1 and GluN1-4 have a complementary distribution: the former is concentrated in more rostral regions (including the cortex and hippocampus). The GluN1-a and GluN1-b isoforms have largely overlapping expression patterns but their relative abundance varies from one region to another. Notably, in the hippocampus, GluN1-a isoforms are found in all principal cells, whereas the GluN1-b isoforms are largely restricted to the CA3 layer[12]. However, the functional significance of the differential expression of GluN1 isoforms remains unclear.

The four GluN2 subunits, which are major determinants of the receptor's functional heterogeneity, show strikingly different spatiotemporal expression

Isoforms
Different versions of a given receptor subunit. The term usually refers to different splice forms.

profiles[10,11,13] (FIG. 1e). In the embryonic brain, only GluN2B and GluN2D subunits are expressed, and the latter is mostly found in caudal regions. Major changes in the expression patterns of the GluN2 subunits occur during the first 2 postnatal weeks. GluN2A expression starts shortly after birth and rises steadily to become widely and abundantly expressed in virtually every CNS area in the adult. Concomitant to this progressive rise in GluN2A expression, GluN2D expression drops markedly, and in the adult, it is expressed at low levels mostly in the diencephalon and mesencephalon. In sharp contrast to GluN2D expression, GluN2B expression is maintained at high levels following birth, peaks around the first postnatal week and becomes progressively restricted to the forebrain. Lastly, expression of GluN2C appears late in development (postnatal day 10 (P10)), and its expression is mainly confined to the cerebellum and the olfactory bulb. The GluN3A and GluN3B subunits also display differential ontogenetic profiles[14,15] (FIG. 1e). GluN3A expression peaks in early postnatal life and then declines progressively. Conversely, GluN3B expression slowly increases throughout development, and in the adult, it is expressed at high levels in motor neurons and possibly other regions. The specific expression of GluN2B, GluN2D and GluN3A subunits early in development strongly suggests that these subunits are important for synaptogenesis and synaptic maturation[14,15]. In the adult CNS, particularly in higher brain structures (such as the hippocampus and cortex), GluN2A and GluN2B are the predominant subunits[9–11], indicating that they have central roles in synaptic function and plasticity.

Multiple receptor subtypes, multiple locations. In line with the large number of subunits and their overlapping expression in several brain regions, many different NMDAR subtypes coexist in the CNS. Taking into account the various GluN1 splice variants, at least a dozen functionally distinct NMDAR subtypes have been described[4,5], but the exact number may be significantly larger. All NMDAR subtypes are thought to combine two copies of the obligatory GluN1 subunit plus two copies of GluN2 and/or GluN3 subunits. Examples of a receptor with two GluN1 isoforms within the same receptor complex have been reported[13] (although GluN1-a and GluN1-b isoforms seem mutually exclusive). The two non-GluN1 subunits can also be identical or different, giving rise to so-called di-heteromeric and tri-heteromeric receptors[13], respectively (FIG. 1d). Di-heteromeric GluN1/GluN2B and GluN1/GluN2A receptors represent an important fraction of juvenile and adult NMDARs. Tri-heteromeric GluN1/GluN2A/GluN2B receptors also populate many regions in the adult brain, particularly in the hippocampus and cortex, with estimates of abundance ranging from 15% to >50% of the total receptor population[16–18]. Tri-heteromeric GluN1/GluN2A/GluN2C receptors and GluN1/GluN2B/GluN2D receptors have also been described[4,5]. Di-heteromeric GluN1/GluN3 receptors can generate glycine-activated excitatory currents[19,20], but *in vivo*, GluN3 subunits are generally believed to participate in tri-heteromeric GluN1/GluN2/GluN3 assemblies[14,15]. Hence, the differential incorporation of GluN2 and GluN3 subunits is a major source of functional diversity[1,4,5]. However, although di-heteromeric receptors have been extensively studied in recombinant expression systems, much less is known about the functional properties of tri-heteromeric receptors.

Different neuronal types usually express distinct complements of NMDAR subunits. For instance, *GRIN2C* and *GRIN2D* mRNAs (which encode GluN2C and GluN2D, respectively) are expressed in hippocampal and cortical interneurons but are barely expressed in principal cells[11]. In addition to this cell-specific expression, within individual neurons, several NMDAR subtypes can coexist and may even segregate in an input-specific manner[4]. Thus, in adult hippocampal CA3 neurons, GluN2B is detected at connections from the perforant path and neighbouring CA3 cells but is barely detected at mossy fibre synapses[21]. At CA3–CA1 synapses, the GluN2B content also differs between the left and right hemispheres[22]. On ganglion retinal cells, OFF synapses preferentially accumulate GluN2A, whereas ON synapses are enriched in GluN2B[23]. Layer 5 pyramidal cells in the neocortex provide another example of pathway-specific subunit localization, with intracortical inputs mainly containing GluN2B and callosal inputs mainly containing GluN2A[24]. The differential expression of these two subunits directly affects synaptic timing and summation properties[24,25].

NMDAR subtypes also vary according to subcellular localization. Typically, NMDARs are found at postsynaptic sites. In the adult forebrain, synaptic NMDARs are predominantly di-heteromeric GluN1/GluN2A and tri-heteromeric GluN1/GluN2A/GluN2B receptors, although their ratios may vary considerably between inputs. By contrast, peri- and-extrasynaptic sites are enriched in GluN2B-containing receptors[26,27]. However, the idea that GluN2B subunits segregate outside synapses, whereas GluN2A subunits are confined to synaptic sites is an over-simplification[28–30]. Similarly, GluN2C and GluN2D subunits can participate in synaptic transmission in specific brain regions[31–34]. NMDARs are mobile[35] (at least in cultured neurons), particularly the GluN2B-containing ones[36], and probably exchange through lateral diffusion between synaptic and extrasynaptic sites, thus allowing for fine regulation of receptor number and subunit composition. Presynaptic NMDARs with differing subunit composition can also populate axon terminals and modulate synaptic strength (BOX 1). The heterogeneity of NMDAR subtypes in the CNS may be further increased by the existence of non-neuronal pools of NMDARs, both in astrocytes and oligodendrocytes, with atypical (GluN2C- and/or GluN3-containing) subunit compositions[14,37].

Subunit architecture and operation

Similar to all other iGluR subunits, NMDAR subunits consist of four discrete modules[1,5,38] (FIG. 1b): in the extracellular region there are a tandem of large globular bilobate (or clamshell-like) domains comprising the amino (N)-terminal domain (NTD), which is involved in subunit assembly and allosteric regulation, and the agonist-binding domain (ABD) that is formed by two

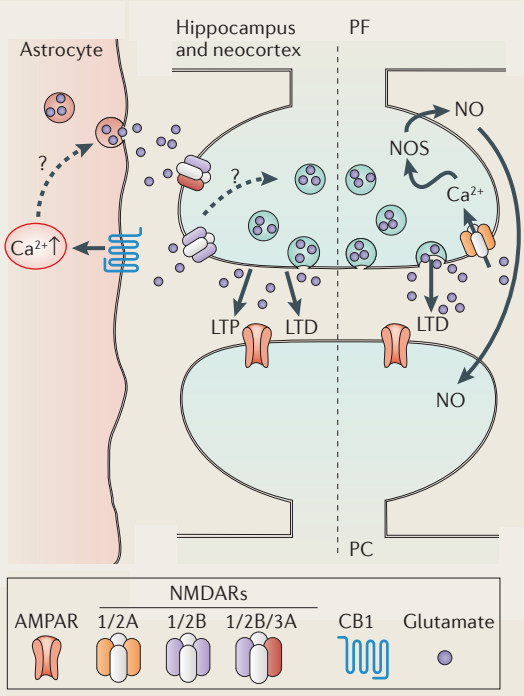
Tri-heteromeric receptors
A class of NMDA receptors that contains three distinct subunits in the tetrameric receptor complex (for example, GluN1/GluN2A/GluN2B receptors).

CA3–CA1 synapses
Excitatory synapses in the hippocampus formed between axons (Schaffer collaterals) of CA3 pyramidal cells and dendrites of CA1 pyramidal cells. NMDA receptor-mediated plasticity (long-term potentiation and long-term depression) has been extensively studied at these synapses.

Allosteric regulation
A form of receptor modulation that involves domains or ligand-binding sites that are distinct from those to which the agonist binds.

Box 1 | **Presynaptic NMDARs**

In addition to being expressed at extra-, peri- and postsynaptic sites, NMDA receptors (NMDARs) are found in the presynaptic compartment (preNMDARs; see the figure). PreNMDARs have been identified at several synapses throughout the CNS, but their exact roles are still debated[180]. They may facilitate glutamatergic release by increasing both spontaneous and evoked excitatory postsynaptic currents (EPSCs). Their activation is also necessary for the induction of long-term depression (LTD) at both cerebellar parallel fibre (PF)–Purkinje cell (PC) synapses and neocortical synapses[180]. PreNMDARs can be activated by glutamate release from afferent fibres[181–183] or from neighbouring astrocytes[184]. At PF–PC synapses, it has been suggested that Ca^{2+} entry through preNMDARs activates nitric oxide synthase (NOS) and triggers the production of the anterograde messenger NO[183], but whether this cascade occurs at other synapses remains unclear. The subunit composition of preNMDARs varies according to brain regions and developmental stages. At PF–PC synapses, preNMDARs are mostly composed of di-heteromeric GluN1/GluN2A receptors[183]. At mature cortical and hippocampal synapses, preNMDARs contain mostly GluN1/GluN2B receptors[181,182,184,185], whereas in the juvenile mouse visual cortex, preNMDARs form predominantly GluN3A-containing tri-heteromeric assemblies[185]. However, the presence of preNMDARs has been challenged [186]. Although some of the discrepancies could be explained by the large heterogeneity of preNMDAR expression[187], future studies are required to clarify the existence and roles of this pool of receptors. LTP, long-term potentiation; CB1, cannabinoid receptor type 1.



discontinuous segments (S1 and S2), which binds glycine (or D-serine) in GluN1 and GluN3 subunits and glutamate in GluN2 subunits; the transmembrane domain (TMD) made of three transmembrane helices plus a pore loop (M2) that lines the ion selectivity filter; and an intracellular CTD, which is involved in receptor trafficking, anchoring and coupling to signalling molecules. Although the structure of a full NMDAR is still lacking, several high-resolution crystal structures of isolated NTDs[39] and ABDs[40] captured in different conformational states are available. Within a tetrameric receptor complex, the NTDs and ABDs assemble as dimers, with the full receptor operating as a dimer-of-dimers. In 'classical' GluN1/GluN2 receptors, the two constitutive GluN1/GluN2 dimers adopt an alternating GluN1/GluN2/GluN1/GluN2 subunit arrangement around the pore[41,42].

NMDARs probably have a comparable 'layer' organization to that of AMPA receptors (AMPARs)[43] in which the ABDs are sandwiched between the TMD at the 'bottom' and the NTDs at the 'top' (FIG. 1c). The basic gating principles that involve agonist-induced closure of the ABDs are also conserved between iGluR families[5,38]. By contrast, NMDAR NTDs have a unique twisted clamshell conformation[39], resulting in looser NTD dimer assemblies than the tightly-packed AMPA and kainate NTD dimers. In agreement, structural rearrangements occurring distally at the NTD level can be sensed by the downstream gating machinery[39,44,45]. The dynamic nature of NMDAR NTDs, together with their ability to recognize a host of small ligands acting as subunit-specific allosteric modulators, confers a central role of the N-terminal region in generating functional and pharmacological diversity in the NMDAR family.

Subunit content determines receptor properties

NMDARs exhibit remarkable properties that distinguish them from other types of ligand-gated ionotropic receptors[1,4,5]. First, their ion channel is subject to a voltage-dependent block by Mg^{2+}; second, NMDAR channels are highly Ca^{2+}-permeable; third, they display unusually slow kinetics owing to slow glutamate unbinding; fourth, their activation requires the presence not only of glutamate but also of a co-agonist (glycine or D-serine); and fifth, they are equipped with an array of modulatory sites conferring an exquisite sensitivity to the extracellular microenvironment. Moreover, NMDARs have particularly long CTDs that allow for a multiplicity of intracellular interactions. However, there are marked variations in properties between receptor subtypes, with each subunit affecting the receptor's biophysical, pharmacological and signalling attributes.

Permeation and gating properties. Single-channel conductance, Mg^{2+} blockade and Ca^{2+} permeability are all influenced by subunit composition[1,4,5] (FIG. 2). For example, di-heteromeric GluN2A- or GluN2B-containing receptors generate 'high-conductance' channel openings (main conductance of ~50 pS) with high sensitivity to Mg^{2+} blockade (half-maximal inhibitory concentration (IC_{50}) of ~15 μM at –70 mV) and Ca^{2+} permeability (p_{Ca}/p_{Cs} of ~7.5). By contrast, GluN2C- or GluN2D-containing di-heteromeric receptors have lower conductances (37 pS), sensitivity to Mg^{2+} (IC_{50} of 80 μM) and Ca^{2+} permeability (p_{Ca}/p_{Cs} of 4.5). These marked differences, which are all controlled by a single GluN2 residue in the M3 segment[46], significantly affect the relative contribution of NMDAR subtypes to synaptic integration and plasticity. At cerebellar granule cells, for instance, an incomplete Mg^{2+} blockade conferred by GluN2C subunits enables signalling during low-frequency stimulation even at hyperpolarized potentials[34]. Incorporation of a GluN3 subunit results in an even more dramatic decrease in Mg^{2+} blockade, although the exact drop in Mg^{2+} sensitivity (and Ca^{2+} permeability) of tri-heteromeric GluN1/GluN2/GluN3 receptors remains unclear[14,15]. Insensitivity to Mg^{2+} may explain why GluN3- and GluN2C-containing NMDARs expressed on oligodendrocytes[20,37] can be active while the membrane (myelin sheath) on which they reside experiences little depolarization.

Single-channel conductance
The single-channel current divided by the electrical driving force. It refers to the number of charges flowing through a single open channel under a given transmembrane potential and is usually expressed in picoSiemens (10^{-12} S).

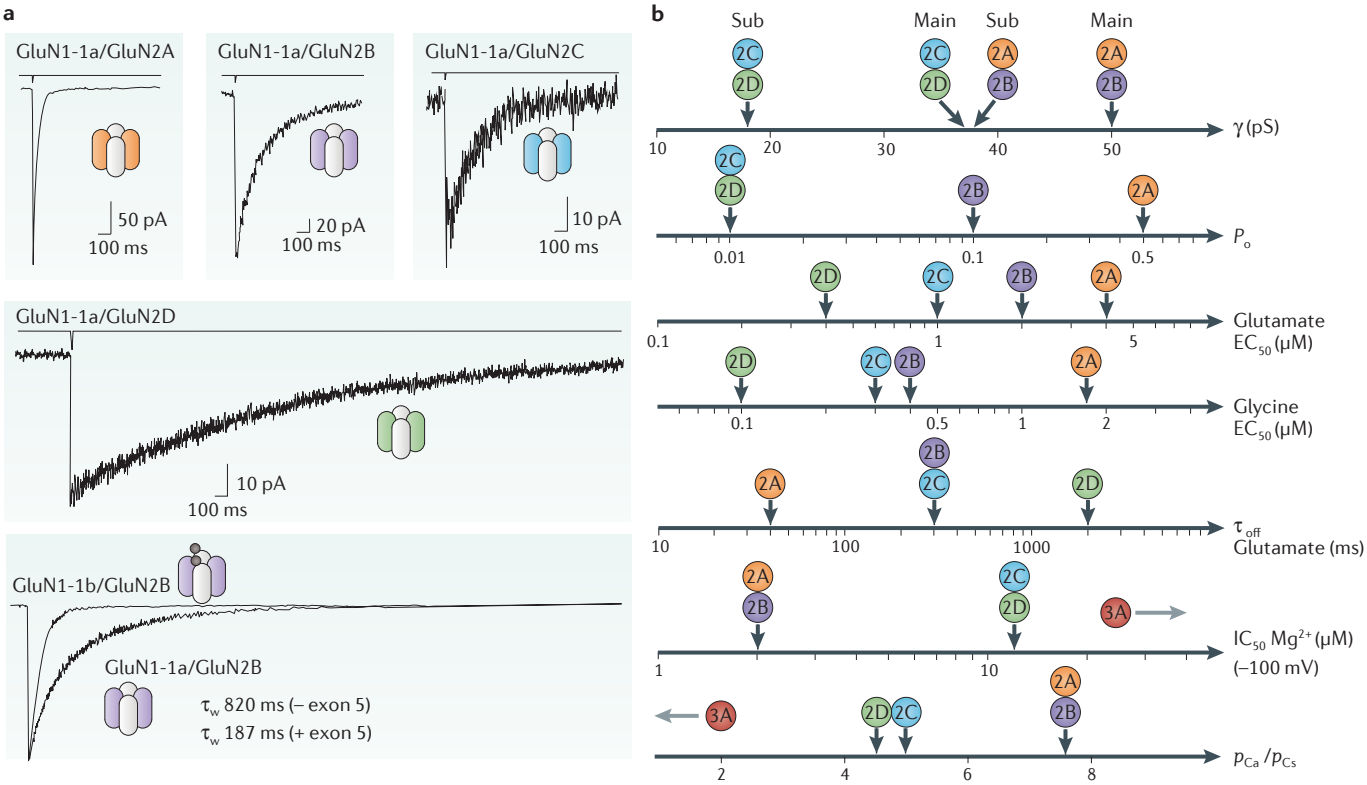


Figure 2 | **Subunit composition determines receptor properties.** **a** | Influence of GluN1 and GluN2 subunit composition on glutamate deactivation kinetics. NMDA receptor (NMDAR)-mediated currents recorded from transfected human embryonic kidney cells were induced by a brief (<5 ms) application of saturating glutamate (1 mM). GluN2A-containing receptors deactivate much faster than all other receptor subtypes. The presence in GluN1 of the extracellular N1 cassette (GluN1-1b splice variant) accelerates glutamate deactivation kinetics. **b** | The various NMDAR subtypes display distinct gating and permeation properties. Single-channel conductance (γ, main and sub-levels (sub)), channel maximal open probability (P_o), sensitivity to the agonists glutamate and glycine (effector concentration for half-maximum response (EC_{50})), glutamate deactivation time constant (τ_{off}), sensitivity to Mg^{2+} blockade (half-maximal inhibitory concentration (IC_{50}) at –100 mV) and Ca^{2+} permeation (p_{Ca}/p_{Cs}) are shown. Values are shown for di-heteromeric GluN1-1a/GluN2 receptors. The influence on the permeation properties of the presence of a GluN3 subunit in the receptor complex is also indicated (decreased Mg^{2+} blockade and Ca^{2+} permeability). Part **a** is modified, with permission, from REF. 47 © (1998) American Physiological Society and from REF. 6 © (2000) American Physiological Society.

The identity of the GluN2 subunit is also crucial for determining several gating properties, including maximal channel open probability, agonist sensitivity and deactivation kinetics[1,4,5] (FIG. 2). GluN1/GluN2A receptors have a higher open probability than GluN1/GluN2B or GluN1/GluN2C and GluN1/GluN2D receptors; these two latter subtypes having a surprisingly low open probability. However, GluN1- and GluN2A-containing receptors have the lowest sensitivity to both glutamate and glycine. Glutamate deactivation kinetics govern the excitatory postsynaptic current (EPSC) decay, which spans a 50-fold range depending on the type of GluN2 subunit[47], with GluN1/GluN2A having the fastest decay (40 ms) and GluN1/GluN2D the slowest (2 s). The distal GluN2 NTDs and the short ABD–NTD connecting linkers are major determinants of this subunit-specific gating behaviour[45,48]. Glutamate deactivation kinetics are also influenced by GluN1 isoforms, with GluN1-b isoforms decaying faster than GluN1-a isoforms[6,7] (FIG. 2a), an effect that complicates inference of GluN2 subunit composition based on the EPSC decay time course. These distinct gating properties confer unique charge transfer capacities and temporal signalling profiles to each receptor subtype. Simulations of synaptic responses show that under a low-frequency stimulation regime typically used to trigger long-term depression (LTD), GluN1/GluN2B receptors make a larger contribution to the total charge transfer than GluN1/GluN2A receptors. By contrast, under high-frequency tetanic stimulation, as used to induce long-term potentiation (LTP), the charge transfer mediated by GluN1/GluN2A receptors considerably exceeds that of GluN1/GluN2B receptors[49]. Unfortunately, little is known about the gating properties of tri-heteromeric receptors containing more than one type of GluN2 subunit or a GluN2 subunit and a GluN3 subunit.

Pharmacological properties. NMDARs are studded with regulatory sites binding small molecule ligands that act as positive or negative allosteric modulators and that allow for subunit-specific modulation[1,5] (FIG. 1c). Several allosteric modulators can distinguish between receptor subtypes and hold strong therapeutic potential.

Deactivation kinetics
The time course of the current decrease following agonist removal.

Excitatory postsynaptic cuurent (EPSC) decay
The decay time course of the EPSC. EPSC decay is a key parameter in the control of synaptic integration.

Long-term potentiation
A long-lasting (> 1 h) and activity-dependent strengthening of synaptic transmission. It is widely considered to be a major cellular substrate for several forms of learning and memory.

Numerous substances that are endogenously found in the CNS, such as protons, polyamines and Zn^{2+}, act as potent modulators of NMDARs[5]. Sensitivity to these cations is strongly influenced by subunit composition. Protons preferentially inhibit GluN2B- or GluN2D-containing receptors[50], whereas extracellular polyamines specifically enhance GluN2B-containing receptors by stabilizing the heterodimer NTD interface[51]. Interestingly, this potentiation is lost in receptors incorporating GluN1-b isoforms[1]. By contrast, Zn^{2+} ions act as highly specific antagonists of GluN1/GluN2A receptors. Both GluN2A and GluN2B NTDs harbour Zn^{2+}-binding sites[52], but the difference in affinity is such that when low Zn^{2+} concentrations (<1 μM) are applied, only GluN2A-containing receptors are affected[53,54]. An increasing number of pharmacological agents can also discriminate between NMDAR subtypes. The best characterized subunit-specific drugs are ifenprodil and its derivatives (such as Ro 25-6981), which are synthetic compounds that selectively inhibit GluN2B-containing receptors by acting at the GluN1–GluN2B NTD interface[39]. The opposite sensitivity of GluN2A- and GluN2B-containing receptors to Zn^{2+} and ifenprodil is classically used to distinguish between GluN2A- and GluN2B-containing receptors[55,56]. However, the presence of tri-heteromeric receptors complicates the interpretation of pharmacological results. Indeed, GluN1/GluN2A/GluN2B receptors bind both Zn^{2+} and ifenprodil with high-affinity, but maximal inhibition is weak[57] (~20%). Thus, low ifenprodil sensitivity does not necessarily mean that GluN2B subunits are absent but instead might be indicative of preferential co-assembly with other subunits. Recent screening efforts have identified several compounds with novel subunit-selectivity and sites of action (FIG. 1c): a GluN2A-specific antagonist that acts by reducing glycine potency (TCN-201 (REFS 58,59)) and GluN2C- and GluN2D-specific allosteric modulators, both negative (QNZ46 (REF. 60)) and positive (CIQ[61]). These compounds could prove to be particularly useful for probing NMDAR subunit composition[61,62]. No GluN3-specific drug has been identified yet.

Trafficking properties and coupling to downstream cascades. The cytoplasmic CTDs are the least conserved regions among NMDAR subunits, and thus they are the site of several subunit-specific regulations that have implications for receptor trafficking, localization and signalling[63–65]. Export from the endoplasmic reticulum (ER) and synaptic delivery of NMDARs varies according to GluN1 C-terminal splicing[8], a process that is regulated by neuronal activity[66]. Similarly, GluN2A, GluN2B[2] and GluN3A[67] CTDs contain distinct motifs that control their intracellular and surface trafficking.

Retention of GluN2B at synapses requires binding of the C terminus to membrane-associated guanylate kinases[68] (MAGUKs; also known as PDZ domain-containing scaffold proteins). By contrast, GluN2A localizes to postsynaptic sites even in the absence of PDZ binding[68], although the differential interaction of GluN2A and GluN2B subunits with MAGUKs is controversial[69]. The idea that GluN2B subunits preferentially interact with synapse-associated protein 102 (SAP102; also known as DLG3) and GluN2A subunits interact with postsynaptic density 95 (PSD95)[70], thus controlling the differential postsynaptic localization of GluN2A and GluN2B subunits, has been questioned[16]. Surface mobility also differs between receptor subtypes. In cultured neurons, GluN2B-containing receptors can move in and out of synapses much more readily than GluN2A-containing receptors[36]. Phosphorylation of the PDZ-binding motif of GluN2B, but not of GluN2A, by casein kinase 2 (CK2), which disrupts association with scaffolding proteins, may account for this difference[71,72]. Several other kinases, including cyclin-dependent kinase 5 (CDK5), protein kinase A (PKA), protein kinase C (PKC) and SRC tyrosine kinases phosphorylate specific sites of GluN2 subunits and are therefore likely to participate in subunit-specific trafficking and signalling[65,73]. There is also strong evidence that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) interacts more strongly with the CTD of GluN2B than with that of GluN2A[74]. Because of the crucial role of CaMKII in LTP induction[75], the GluN2B–CaMKII interaction has major implications for synaptic plasticity. This interaction is also critical for proper synapse maturation and regulation of AMPAR content during brain development[76,77]. The RAS-guanine-nucleotide-releasing factor 1 (RASGRF1 (REF. 78)) and the PSD95–neuronal nitric oxide synthase complex[64] are other preferential binding partners of GluN2B's CTD. Their physical association with GluN2B couples NMDAR activation to the extracellular signal-regulated kinase (ERK; also known as MAPK) pathway, and to cyclic AMP-responsive element-binding protein (CREB)-mediated signalling pathways, respectively, thus affecting neuronal survival and plasticity[26].

NMDAR subunit composition is plastic

The subunit composition of NMDARs is not static but changes during development in response to neuronal activity or sensory experiences. This plasticity, which was long thought to occur exclusively during development, can also occur at adult synapses. Changes in subunit composition can be rapid (timescale of minutes) and can have profound influences on the functioning of synapses and networks.

Developmental switch in subunit composition. During early postnatal development, NMDARs switch their subunit composition from primarily containing GluN2B subunits to predominantly containing GluN2A subunits (FIG. 3). This subunit exchange occurs throughout the CNS, is evolutionary conserved from amphibians to mammals and occurs during a time window that coincides with synapse maturation, circuit refinement and acquisition of learning abilities[79]. The replacement of GluN2B with GluN2A subunits is not absolute, as GluN2B subunits still populate many regions of the adult CNS. The increased contribution of GluN2A subunits is accompanied by several distinctive changes in NMDAR-mediated synaptic currents, including a marked acceleration in decay time kinetics

Postsynaptic density
A protein-dense specialization that is attached to the postsynaptic membrane of excitatory synapses. It contains hundreds of proteins, including glutamate receptors, scaffold proteins and signalling molecules.

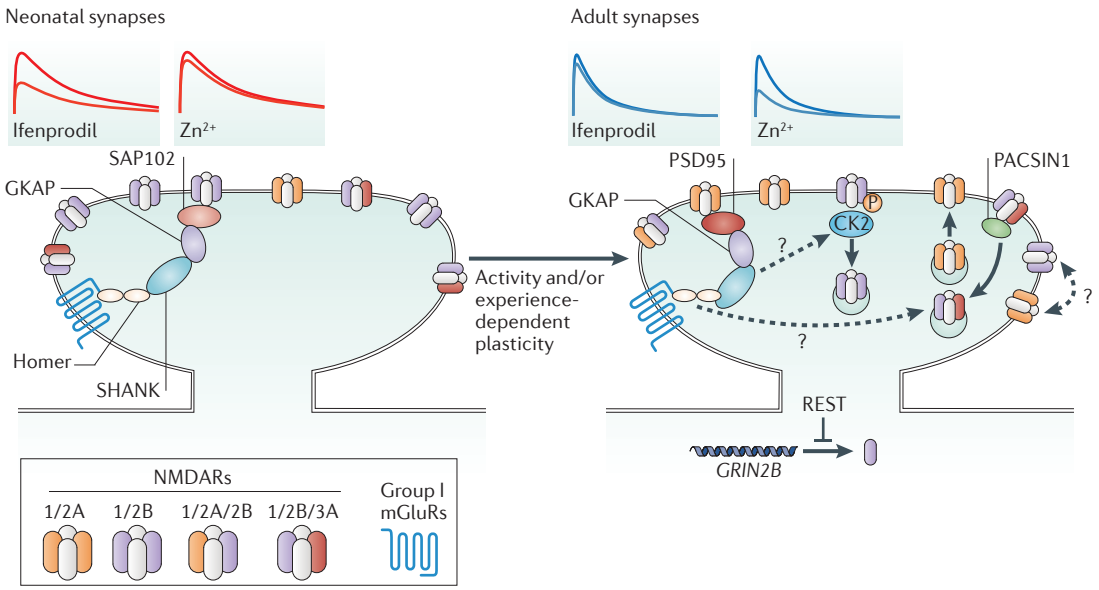


Figure 3 | **Activity and experience-dependent switch in NMDAR subunit composition.** Neonatal synapses throughout the CNS are mainly composed of di-heteromeric GluN1/GluN2B (which is encoded by *GRIN2B*) and tri-heteromeric GluN1/GluN2B/GluN3A receptors. These receptors are replaced by di-heteromeric GluN1/GluN2A and tri-heteromeric GluN1/GluN2A/GluN2B receptors in adults. Differential sensitivity to the allosteric inhibitors Zn^{2+} and ifenprodil as well as the kinetic properties of synaptic currents allow discrimination between NMDA receptor (NMDAR) subtypes at neonatal and adult synapses. The activation of group I metabotropic glutamate receptors (mGluRs) has been shown to play an important part in the induction mechanism of the subunit switch during postnatal development. Less is known about the expression mechanisms of this form of plasticity, although the activity of casein kinase 2 (CK2) has been implicated in the removal of GluN2B-containing receptors. Future studies will need to investigate whether group I mGluR activation can promote the CK2 signalling pathways and the exact mechanisms underlying NMDARs trafficking at neonatal synapses (internalization versus lateral surface diffusion). GluN3A-containing NMDARs play an important part during the postnatal development, and the endocytic adaptor PACSIN1 is responsible for the removal of GluN3A-containing receptors. Repressor element 1-silencing transcription factor (REST) is critically involved in the developmental switch of synaptic NMDARs; it is responsible for epigenetic remodelling and long-lasting transcriptional repression of *GRIN2B* early in postnatal development. GKAP, guanylate kinase-associated protein (also known as DLGAP1); PSD95, postsynaptic density 95; SAP102, synapse-associated protein 102; SHANK, SH3 and multiple ankyrin repeat domains protein.

and a consequent change in the temporal integration properties. The GluN2B-to-GluN2A subunit switch is also accompanied by a change in synaptic current pharmacology, with a progressive increase in Zn^{2+} sensitivity concomitant to a decrease in the sensitivity to GluN2B-specific antagonists (ifenprodil and its derivatives[3]) (FIG. 3). The changes in kinetics and pharmacology may not strictly correlate in time, as changes in pharmacology can occur first[80] (which is probably due to the presence and unique properties of tri-heteromeric GluN1/GluN2A/GluN2B receptors). The molecular and cellular mechanisms responsible for the GluN2B-to-GluN2A subunit switch have yet to be fully defined. It is likely that novel receptors are inserted through forward trafficking from the ER and that existing synaptic receptors are removed through regulated endocytosis. Internalization of GluN2B subunits occurs via an adaptor protein 2-binding YEKL motif on the distal C-terminal that is unique to GluN2B[81]. Clearance of fast-moving GluN2B-containing receptors from synaptic sites through lateral diffusion may also be involved[36].

The postnatal developmental GluN2B-to-GluN2A switch is likely to be driven by neuronal activity. In cultured hippocampal slices, GluN2A trafficking at the synapse requires synaptic activity, whereas GluN2B insertion does not[82]. An LTP protocol at neonatal hippocampal synapses (P2–P9) speeds up the decay kinetics of NMDA-mediated EPSCs and results in decreased ifenprodil sensitivity, which is indicative of newly inserted GluN2A subunits[83]. In the hippocampus, as well as in the visual cortex and the ventral tegmental area (VTA), induction of this type of plasticity depends on group I metabotropic glutamate receptor (mGluR), phospholipase C and/or PKC activation and Ca^{2+} release from internal stores[84,85]. Activity-dependent phosphorylation of the GluN2B PDZ-binding domain by CK2 has also been implicated in synaptic clearance of GluN2B-containing receptors[72]. How mGluRs are activated during development and whether group I mGluRs activate the CK2 pathway remain to be established (FIG. 3). At the gene level, the transcriptional repressor repressor element 1-silencing transcription factor (REST) participates in the postnatal switch in synaptic NMDAR subunit content by downregulating GluN2B expression through epigenetic remodelling of *Grin2b*[55]. *In vivo*, the postnatal GluN2B-to-GluN2A switch in subunit composition appears to be driven by sensory experience and appears to coincide with the critical period. The importance of

Critical period
A finite temporal window following birth during which neuronal circuits are shaped; it is characterized by heightened plasticity and experience-dependent remodelling.

experience in regulating synaptic NMDAR subunit content has been particularly well studied in the visual and somatosensory cortex[86]. Rearing animals in the dark extends the critical period for ocular dominance plasticity and prolongs the expression of GluN2B subunits. Strikingly, however, in these animals, subunit switching can be rapidly induced with only a 2-h exposure to visual stimuli[87]. Interestingly, stressful or pathological conditions during the critical period, such as maternal deprivation[55] or *in utero* cocaine exposure[84], delay the postnatal switch in NMDAR subunit composition, further strengthening the importance of sensory experience in this process.

Involvement of GluN3A in the developmental maturation of synaptic NMDARs. The bell-shaped expression profile of the GluN3A subunit — peaking at early postnatal stages — suggests a role for GluN3A-containing NMDARs in synapse maturation[14,15] despite the fact that it only contributes to a small fraction of the total NMDAR subunit pool[88]. Deletion of GluN3A increases dendritic spine growth and density[89], and accelerates the expression of markers of synaptic maturation, including GluN2A in P8 mice[90]. By contrast, overexpression of GluN3A limits structural plasticity and inhibits the developmental sculpting of glutamatergic synapses[91]. During normal postnatal developmental, the upregulation of the endocytic adaptor PACSIN1, which directly interacts with GluN3A, promotes the removal of GluN3A-containing NMDARs (FIG. 3), allowing for the insertion of mature NMDARs that in turn trigger AMPAR insertion[67]. Therefore, GluN3A appears to limit premature synaptic maturation by acting as a molecular break during neuronal development. Unique properties imparted by the GluN3A subunit, such as weak association with PSD scaffolding proteins (GluN3 subunits lack PDZ-binding sequences), reduced Mg^{2+} sensitivity and Ca^{2+} permeability, are likely to contribute to the developmental role of GluN3A-containing receptors. Whether GluN3A subunits operate as di-heteromeric GluN1/GluN3 or tri-heteromeric GluN1/GluN2/GluN3 complexes (or both) is still an open question.

Plasticity of NMDARs at mature synapses

NMDARs have long been thought to be less dynamic than AMPARs, especially at mature synapses in which long-term plasticity is usually mediated by changes in AMPAR-mediated transmission. However, accumulating evidence indicates that adult NMDARs are also dynamically regulated and subject to activity-dependent long-term plasticity. The number of receptors can be modulated by neuronal activity, but little is known about the regulation of subunit composition at mature synapses. As the NMDAR-mediated transmission contributes to fundamental aspects of neurons and neural circuit function — namely, temporal summation, integrative properties and plasticity — long-term changes of the NMDAR-mediated component ought to have important functional implications for information processing and brain function[92].

LTP_{NMDA}. Since the discovery of AMPAR-mediated LTP (LTP_{AMPA}), the question of whether NMDAR-mediated transmission undergoes changes after activity-dependent stimulation or whether the receptors are only necessary for triggering plasticity of AMPARs has raised much debate. Early studies, mostly on hippocampal slices, reported that the induction protocols that trigger LTP_{AMPA} could similarly induce NMDAR-mediated LTP (LTP_{NMDA})[93,94] but others did not[95]. Despite these diverging results, it was generally admitted that LTP_{NMDA} required a stronger induction protocol and developed slower than the LTP_{AMPA}. Moreover, at CA1 synapses, the prevailing view was that LTP_{AMPA} can occur independently of changes in NMDAR-mediated transmission, which is in line with the presumed weaker mobility of NMDARs. However, subsequent studies have established that AMPAR and NMDAR intracellular and surface mobility follow distinct activity-dependent rules[2]. Accordingly, the trafficking and expression mechanisms that underlie both forms of plasticity (LTP_{NMDA} and LTP_{AMPA}) may differ completely[96], or the plasticity of one component may subsequently influence the plasticity of the other[97].

Recently, both at mossy fibre–CA3 synapses[98,99] and at glutamatergic synapses onto midbrain dopaminergic neurons[100] (in the substantia nigra and VTA), brief bursts of synaptic activity have been shown to elicit prolonged increases in NMDAR-mediated transmission without changes in AMPAR-mediated transmission. These forms of LTP_{NMDA} share a common induction mechanism (FIG. 4): a rise in postsynaptic Ca^{2+} as a consequence of the activation of group I mGluRs or adenosine 2A receptors and NMDARs themselves. The expression mechanism is also postsynaptic, but the precise underlying events remain poorly defined. At mossy fibres synapses, PKC[98] and SRC[99] gate LTP_{NMDA}, whereas in midbrain neurons PKA is important[100]. Although exocytosis of NMDARs has been implicated at mossy fibre–CA3 synapses, which is in agreement with LTP_{NMDA} at other synapses[101], lateral movement or long-lasting changes in the properties of the existing receptors cannot be excluded[92]. Intriguingly, activity-dependent plasticity of the Ca^{2+} permeability of NMDARs has been described[102–104] but is not necessarily associated with a change in NMDAR-response amplitudes[102,104]. At perforant path–dentate gyrus synapses, LTP_{NMDA} involves a change in receptor subunit composition through the diffusion of extrasynaptic GluN2D-containing receptors to synaptic sites[33]. At CA1 synapses, LTP_{NMDA} is accompanied by an increase in synaptic GluN2A content[96]. However, a shift in subunit composition may not be a general mechanism[100].

LTD_{NMDA}. NMDAR-mediated LTD (LTD_{NMDA}) has been reported at several synapses[100,105–109]. Synaptic activity[106], as well as pharmacological activation of muscarinic receptors[109] or group I mGluRs[110] can induce LTD_{NMDA}. At many synapses, including hippocampal CA1 synapses, induction protocols used to trigger AMPAR-mediated LTD (LTD_{AMPA}; for example, low-frequency stimulation) can also induce LTD_{NMDA}. However, under certain stimulation patterns, LTD_{NMDA} occurs without

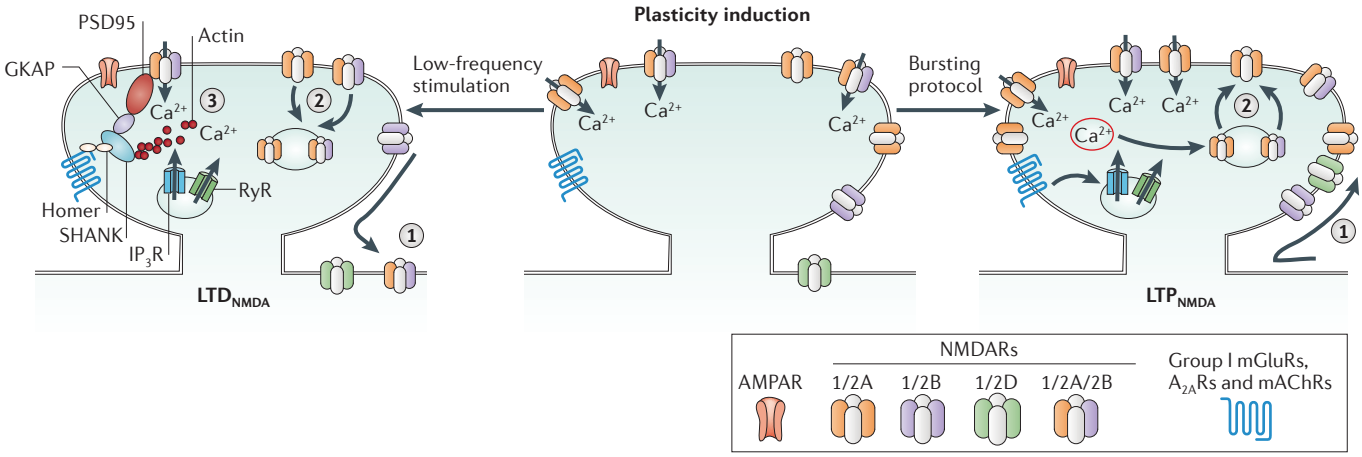


Figure 4 | **Plasticity of the NMDAR component at mature synapses.** At adult synapses, NMDA receptors (NMDARs) can undergo plasticity independently of AMPA receptors (AMPARs). The induction of both forms of synaptic plasticity — NMDAR-mediated long-term potentiation (LTP_{NMDA}) and NMDAR-mediated long-term depression (LTD_{NMDA}) — requires a postsynaptic rise in Ca^{2+}. Ca^{2+} enters through NMDARs or is released by intracellular stores after the activation of metabotropic receptors, including group I metabotropic glutamate receptors (mGluRs), adenosine 2A receptors ($A_{2A}Rs$) and muscarinic acetylcholine receptors (mAChRs). It then activates intracellular signalling pathways (involving inositol triphosphate (IP_3), guanylate kinase-associated protein (GKAP), postsynaptic density 95 (PSD95) and SH3 and multiple ankyrin repeat domains protein (SHANK)). Lateral diffusion (1), exocytosis and endocytosis (2) and actin depolymerization (3) have been identified as possible mechanisms underlying synaptic NMDAR plasticity. Ryr, ryanodine receptor.

concomitant LTD_{AMPA}[106]. The precise mechanisms that underlie LTD_{NMDA} are unclear and may differ from mechanisms underlying LTD_{AMPA}[106,108]. At CA3–CA3 (REF. 107) and CA3–CA1 (REF. 109) synapses, dynamin-dependent internalization of synaptic NMDARs has been involved. At CA3–CA1 synapses, there is also evidence for an endocytosis-independent mechanism that relies on Ca^{2+}-mediated actin depolymerization, which could promote destabilization of the postsynaptic scaffold[108] and lateral displacement of predominantly GluN2A-containing NMDARs away from synaptic sites[96] (FIG. 4).

GluN2 subunits and AMPAR plasticity

Which GluN2 subunits are involved in synaptic plasticity of AMPAR responses? NMDARs are essential mediators of many forms of activity-dependent synaptic plasticity that are thought to underlie higher cognitive functions, such as learning and memory. At prototypical CA1 synapses, both LTP_{AMPA} and LTD_{AMPA} require Ca^{2+} influx through NMDARs, and several lines of evidence suggest that the direction of plasticity primarily depends on the magnitude and time course of the postsynaptic Ca^{2+} increase[111]. As the various GluN2 subunits confer unique charge transfer capacities and signalling properties[1,4,5], there has been intense speculation that the subunit composition of NMDARs dictates whether LTP or LTD is produced. To unmask possible GluN2-specific roles in synaptic plasticity, both genetic and pharmacological approaches have been taken.

Several lines of evidence implicate GluN2A subunits in triggering LTP. GluN2A-knockout mice[112] and mice lacking the GluN2A CTD ($GluN2A^{\Delta C/\Delta C}$; REF. 63) show reduced LTP at CA3–CA1 synapses. In the superior colliculus[113] and cerebellar granule cells[114], synapses from GluN2A-knockout mice lack LTP. In the hippocampus[115], perirhinal cortex[116] and amygdala[117], ifenprodil or Ro 25-6981 (GluN2B-selective antagonists) specifically blocks LTD, whereas NVP-AAM077, a GluN2A-preferring antagonist, prevents LTP but not LTD. Similarly at CA1 synapses, loss of GluN2B abolishes LTD[118], whereas selective inhibition of GluN2A-containing receptors with low Zn^{2+} concentrations impairs LTP but not LTD[56]. According to these findings, GluN2A seems specifically important for LTP induction, whereas GluN2B would be specifically involved in LTD induction. Although appealing, this dichotomy between GluN2A and GluN2B subunits in governing the polarity of synaptic plasticity has been challenged by many studies that provide convincing evidence that GluN2B subunits are critically required for LTP but not necessarily for LTD[74,119–129]. Although some of this discrepancy might be explained by developmental and regional differences in NMDAR subunit expression, differences in LTP and LTD induction protocols[111,126] or poor subunit-specificity of NVP-AAM077 (REFS 130,131), there seems to be no simple rule that relates a single NMDAR subunit to the direction of synaptic plasticity.

For LTP at mature synapses, an attractive possibility is that actually both GluN2A and GluN2B subunits are engaged in the induction process[132,133] (FIG. 5). In such a scenario, LTP induction relies on the cooperation of di-heteromeric GluN1/GluN2A and tri-heteromeric GluN1/GluN2A/GluN2B receptors, the two receptor populations that predominate at adult forebrain synapses[16–18]. Di-heteromeric GluN1/GluN2A receptors would be essential to flux a large amount of Ca^{2+} (because of the high channel open probability

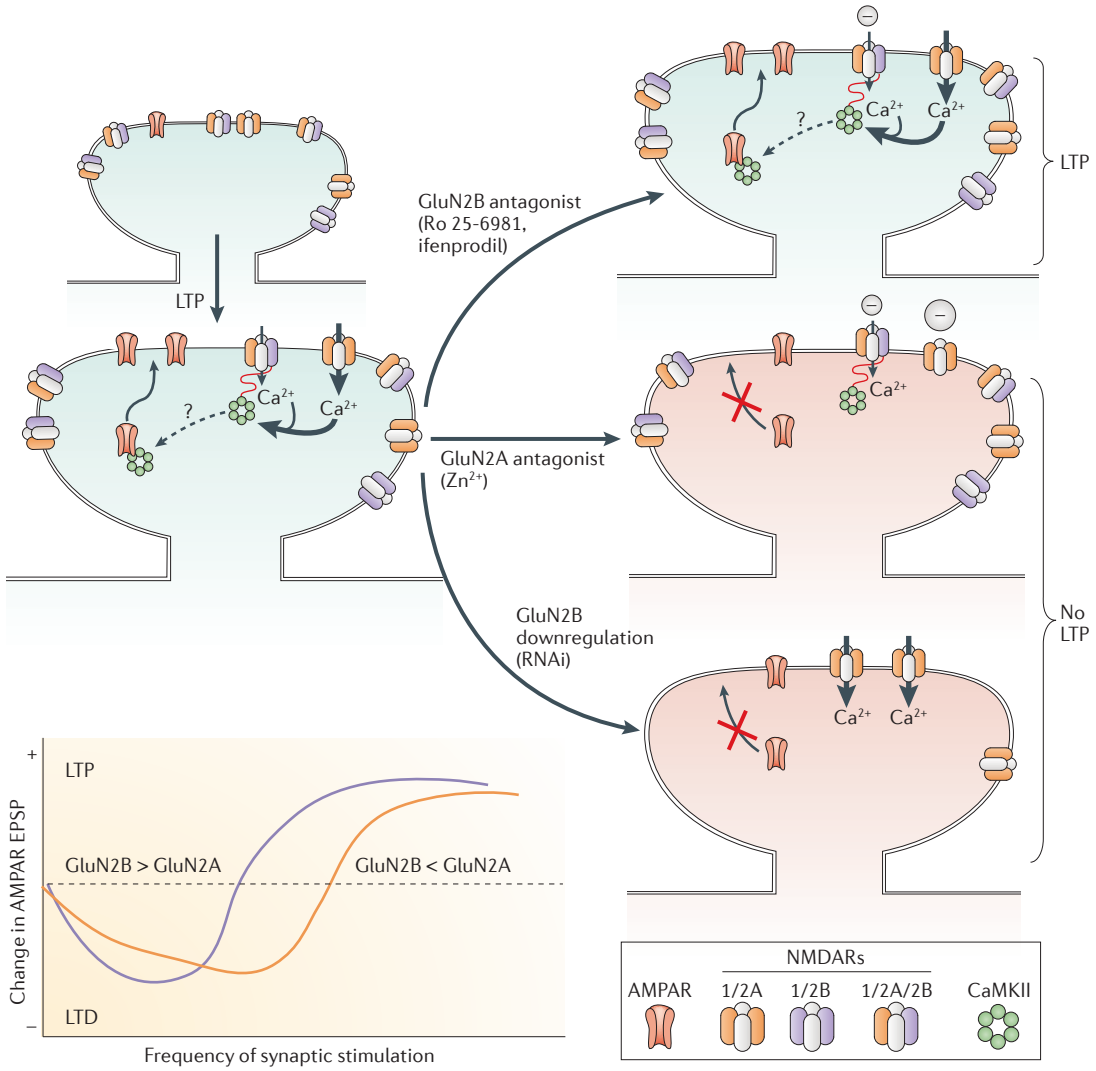


Figure 5 | **Role of tri-heteromeric GluN1/GluN2A/GluN2B receptors in AMPAR-mediated synaptic plasticity.** Induction of long-term potentation (LTP) at adult synapses is usually mediated by NMDA receptors (NMDARs). In the proposed model, both di-heteromeric GluN1/GluN2A receptors and tri-heteromeric GluN1/GluN2A/GluN2B receptors cooperate to trigger LTP. Ca^{2+} entry through both receptor subtypes is required to activate Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which in turn promotes the trafficking and stabilization of AMPA receptors (AMPARs) at synaptic sites. CaMKII is recruited by the C-terminal domain of GluN2B and is a key component for LTP expression. In the presence of a GluN2B-selective antagonist (such as ifenprodil or Ro 25-6981), tri-heteromeric GluN1/GluN2A/GluN2B receptors are only weakly inhibited[57], and LTP can be induced. By contrast, in presence of the GluN2A-selective antagonist Zn^{2+}, or when GluN2B subunits are genetically removed from the synapses, LTP is abolished. Zn^{2+} strongly inhibits GluN1/GluN2A receptors and thus markedly reduces Ca^{2+} influx. Removal of GluN2B subunits also abolishes LTP as it prevents CaMKII from accumulating in close vicinity of the synaptic receptors and thus uncouples Ca^{2+} influx and CaMKII activation. The graph shows the influence of NMDAR subunit composition on the direction of the AMPAR-mediated synaptic plasticity. When the synaptic GluN2A-to-GluN2B ratio is low following synaptic stimulation, LTP is more likely to occur rather than long-term depression (LTD). Conversely, when the GluN2A-to-GluN2B ratio is high, LTD is favoured. EPSP, excitatory postsynaptic potential.

of GluN1/GluN2A receptors[49]), whereas the GluN2B subunit in tri-heteromeric receptors would be essential for recruiting (via their CTD) molecules that are key for LTP. This interplay between an ionotropic role for GluN2A and a structural role for GluN2B[132,133], together with the unique pharmacology of tri-heteromeric GluN1/GluN2A/GluN2B receptors[57], offers a potential reconciliation between pharmacological experiments that suggest that GluN2B subunits are not important for LTP and genetic manipulations that suggest the opposite (FIG. 5). The specific contribution of the GluN2B subunit in tri-heteromeric complexes as a scaffolding element also offers a molecular explanation for the critical importance of the GluN2B–CaMKII interaction in LTP induction and memory formation[74,75,128,134].

Functional consequences of shifting the ratio of GluN2A to GluN2B: metaplasticity. The unique biophysical properties of NMDARs contribute several functions to neuronal excitability. Owing to their slow decay kinetics, NMDARs facilitate temporal integration. Their rectification properties can generate non-linear signals in dendrites that may be involved in bursting activity. NMDARs are also essential for metaplasticity[86,92], and accordingly, changes in NMDAR-mediated responses can profoundly affect synaptic transmission. At hippocampal mossy fibre synapses, which were long thought to lack the machinery involved in 'classical' postsynaptic LTP, an increase in the number of NMDARs renders mossy fibre synapses competent for generating NMDAR-dependent LTP of AMPAR-mediated responses[135]. Given the differential impact of subunit composition on NMDAR signalling properties, activity-dependent changes in subunit content are expected to broadly affect neuronal functions[92]. Several studies *in vitro* and *in vivo* indicate that a change in the ratio of GluN2A to GluN2B, as occurs during sensory experience, affects subsequent NMDAR-dependent synaptic modifications. Manipulations of the GluN2A-to-GluN2B ratio, either through pharmacological means[136] or activity-dependent alterations[137,138], regulate both the magnitude and sign of subsequent plasticity, leading to a shift in the LTP and LTD threshold (see the graph in FIG. 5). In agreement with a critical role of GluN2B in LTP, when the ratio of GluN2A to GluN2B increases, stronger stimulation (for example, a higher stimulation frequency) is required to induce LTP, whereas a wider range of weaker stimulations can induce LTD[86,139]. There are exceptions to this rule, however[140], as besides the GluN2A-to-GluN2B ratio, several other factors, such as signalling molecules and inhibitory inputs, are likely to contribute to metaplastic changes[86].

NMDAR subunits in CNS disorders

It is increasingly recognized that many neuropsychiatric disorders are linked to synaptic defects (synaptopathies) and NMDAR dysfunction, expressed either as altered subunit expression, trafficking, localization or activity, can contribute to numerous neurological and psychiatric conditions[1–3,141] (TABLE 1). Not only is NMDAR hyperactivity deleterious (excessive Ca^{2+} influx through NMDARs leads to neuronal death) but so is NMDAR hypofunction. Therefore, there is potential for both NMDAR antagonists and potentiators as CNS therapeutics. Because not all NMDAR subtypes contribute equally to CNS diseases, current efforts are focused on exploiting the diversity in subunit composition and allosteric sites, with the rationale that subunit-selective modulators can be more effective and better tolerated than the non-selective modulators. In this section, we focus on a few diseases in which the contribution of NMDARs to pathophysiology is relatively well understood, especially in terms of subunit specificity.

Reducing NMDAR signalling

As glutamate levels are raised following brain insults (for example, stroke and traumatic brain injury) and directly contribute to neuronal death, antagonists of NMDARs have been pursued for decades. Moreover, chronically increased glutamate levels may contribute to a loss of synapses and neurons in degenerative conditions, such as Huntington's disease, Parkinson's disease (PD) and Alzheimer's disease (AD).

In agreement with a prominent role for NMDAR-mediated excitotoxicity, NMDAR antagonists have been shown to be neuroprotective when administered before or shortly after traumatic brain injury or an ischaemic insult in animal models[142]. However, all of the clinical trials of first-generation NMDAR antagonists were disappointing because of intolerable side-effects and short therapeutic windows. Among the contributing factors are the short durations (<1 h) of glutamate increases and the need for preserved NMDAR activity for recovery[142]. The lack of subunit-selectivity of the drugs ('broad-spectrum' antagonists) was also raised as a potential limitation. Indeed, GluN2B-specific antagonists combine significant neuroprotection with a much improved side-effect profile[1,3]. GluN2B antagonists are likely to act by preferentially targeting extrasynaptic GluN1/GluN2B receptors, which constitute a major hub for signalling pathways that lead to neuronal death[26,27]. Despite an improved therapeutic index, GluN2B-selective antagonists have not been successful in the clinic yet[3]. Attempts to disrupt interactions between GluN2B subunits and downstream signalling molecules using small interfering peptides has shown promise in reducing ischaemic damage in rodents[143,144], non-human primates[145] and humans[146]. Besides GluN2B, there is also evidence that extrasynaptic GluN2D subunits may contribute to excitotoxicity[147]. Conversely, activation of GluN2A-containing receptors may convey pro-survival effects[148,149] through CREB signalling[150,151], although the protective role of GluN2A remains debated[64].

Excessive activation of NMDARs has also been implicated in AD, and the NMDAR channel blocker memantine is currently used to treat patients with moderate to severe AD. There is no consensus on how memantine alleviates cognitive deficits in patients with AD[152]. Various mechanisms, from selective inhibition of extrasynaptic NMDARs[153] to more effective inhibition of GluN2C (or GluN 2D)-containing NMDARs[154] have been proposed. Both amyloid-β (Aβ)[155] and tau[156] have been shown to perturb synaptic functions by removing synaptic NMDARs. Recent studies indicate that GluN2B-containing NMDARs have an essential role in mediating the harmful effects of Aβ (FIG. 6a). GluN2B antagonists can rescue Aβ-induced impairment of LTP[157–159], Aβ-induced synaptic loss[157] and Aβ-induced facilitation of LTD[160]. Some of these effects seem to be mediated by activation of extrasynaptic GluN2B-containing receptors owing to altered glutamate uptake[160]. The production of Aβ also requires activation of extrasynaptic NMDARs (probably GluN2B-enriched NMDARs)[161]. Tau transports the tyrosine protein kinase FYN to dendritic spines in order to phosphorylate GluN2B subunits, which enhances their association with PSD95 and leads to downstream excitotoxic effects[162]. Disrupting the interaction between GluN2B and PSD95 with a synthetic peptide improved memory functions

Metaplasticity
A term that refers to the phenomenon whereby previous synaptic activity influences the occurrence of subsequent synaptic plasticity. It is commonly regarded as a mechanism to adjust synaptic plasticity according to the history of the synapse.

Synaptopathies
A term used to define disorders caused by disruption in synaptic structure and function. Synaptopathy is increasingly seen as a key feature of neurodegenerative and psychiatric diseases.

Excitotoxicity
Cell death induced by excessive extracellular glutamate concentrations.

Table 1 | **Contribution of NMDAR subunit alterations to neurological and psychiatric disorders**

Disease indications	Key NMDAR-subunit related alterations	Therapeutic treatments	Refs
Cerebral ischaemia and traumatic brain injury	Excessive activation of NMDA receptors (NMDARs), especially extrasynaptic GluN2B-containing receptors, owing to increased extracellular glutamate levels. Hyperactivation of NMDARs leads to neuronal death	• GluN2B-selective antagonists • Peptides disrupting GluN2B-interacting partners	See text
Pain	GluN2B-containing receptors are majorly implicated, and alterations include overexpression and altered phosphorylation state of GluN2B. GluN2A-containing receptors can also be involved	GluN2B-selective antagonists	54,188–190
Alzheimer's disease	Activation of GluN2B-containing NMDARs mediates amyloid-β (Aβ)-induced alterations in synaptic plasticity and synapse loss, enhanced production of Aβ- and tau-induced excitotoxicity	NMDAR antagonist (memantine) or GluN2B-selective antagonists	See text
Huntington's disease	Activation of extrasynaptic GluN2B-containing NMDARs increases mutant huntingtin protein-induced excitotoxicity	NMDAR antagonists or GluN2B-selective antagonists	27,191–192
Parkinsons's disease (dyskinesia)	Enhanced synaptic GluN2A expression and redistribution of GluN2B-containing receptors from synaptic to extrasynaptic locations	• GluN2B-selective antagonists • Potential interest for interventions targeting GluN2A subunits	See Text
Depression	Inhibitors of NMDARs, in particular GluN2B-containing receptors, induce rapid (hours) and sustained (days) reduction in depressive symptoms	NMDAR antagonists (ketamine) or GluN2B-selective antagonists	193–195
White matter injury	NMDAR activation leads to oligodendrocyte damage and loss of the myelin sheath. Specific involvement of GluN2C and/or GluN3A subunits	Potential interest for GluN2C- or GluN3A-selective antagonists	14,15,20,37
Autism spectrum disorders	Either reduced or enhanced NMDAR function is implicated. Mechanisms are unclear and there is no clear subunit specificity	Potential interest for NMDAR antagonists or NMDAR potentiators	196,197
Cognitive impairments	Reduced expression of NMDARs, especially GluN2B-containing receptors, correlates with decline in cognitive functions	Potential interest for NMDAR potentiators	See text
Schizophrenia	Reduced NMDAR presence and/or activity, probably in GABAergic inhibitory neurons, which leads to an imbalance in neural network activity. Suggestions for a preferential loss of GluN2A-containing receptors	• NMDAR potentiators • D-serine, glycine and glycine transporter 1 inhibitors • Potential interest for GluN2A-selective potentiators	See text
Anti-NMDAR encephalitis	Anti-NMDAR antibodies lead to reduced NMDAR density and impaired synaptic plasticity. Specific dispersal of GluN2A subunits from synaptic sites	Potential interest for NMDAR potentiators	198–200

and reduced premature death in mouse models of AD[162]. Interestingly, this peptide does not affect synaptic NMDAR-mediated currents[143,162] but reduces ischaemic cell death in stroke models[143]. One intriguing suggestion is that conformational changes in GluN2B-containing receptors, rather than Ca^{2+} influx through these receptors, mediate the above effects[163,164]. Indeed, in young neurons, Aβ-induced synaptic depression is abolished in the presence of antagonists that inhibit NMDAR activation (such as APV and ifenprodil) but not by channel blockers (such as ketamine and MK-801)[164]. Whether such a mechanism operates in ageing neurons remains to be tested. Moreover, one should emphasize that the evidence supporting GluN2B's role in mediating Aβ-induced excitotoxicity has largely been obtained using *in vitro* preparations and synthetic Aβ. Thus, it remains to be seen whether long-term dosing of GluN2B antagonists in adult or even aged mice can reduce or prevent synapse loss and ameliorate cognitive deficits in AD models.

In patients with PD, the degeneration of nigral dopaminergic neurons results in overactivation of glutamatergic projections to the striatum and basal ganglia output nuclei[165]. Moreover, dyskinesia induced by deep brain stimulation or dopamine replacement therapy is linked to dysregulation of glutamate transmission.

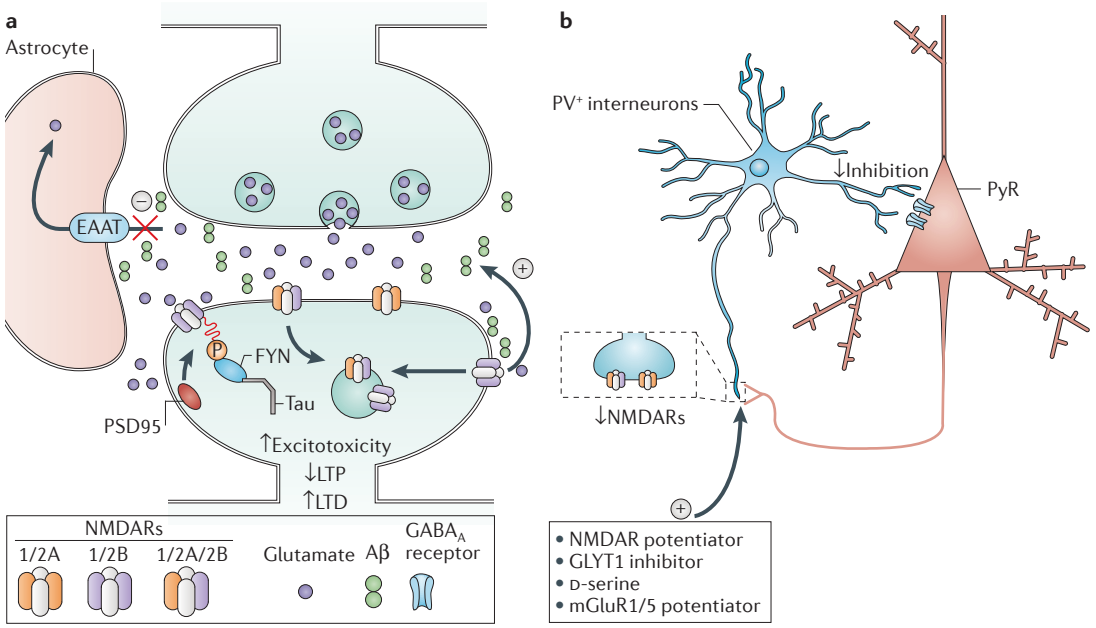


Figure 6 | **Contribution of NMDAR subunits to Alzheimer's disease and schizophrenia. a** | The contribution of NMDA receptors (NMDARs) to amyloid-β (Aβ)-induced effects on synaptic function and plasticity. NMDARs are present at both synaptic and extrasynaptic regions. Aβ can induce the endocytosis of GluN2B-containing NMDARs. Activation of extrasynaptic NMDARs enhances the production of Aβ. Aberrant activation of extrasynaptic GluN2B-containing NMDARs, which is probably mediated by an Aβ-induced deficit in glutamate uptake by excitatory amino acid transporters (EAATs), also leads to impaired long-term potentiation (LTP) and facilitated induction of long-term depression (LTD). Hyperphosphorylated tau targets the receptor protein kinase FYN to phosphorylate GluN2B-containing NMDARs to enhance their association with postsynaptic density 95 (PSD95) and downstream excitotoxic pathways. **b** | Enhancing NMDAR function may be beneficial in treating schizophrenia. Reduced NMDAR activation leads to reduced output from inhibitory GABAergic interneurons, especially those containing parvalbumin (PV$^+$ interneurons), and the ultimate result is reduced inhibition (also called disinhibition) of excitatory pyramidal neurons (PyR). Normal (non-pathological) PV$^+$ interneurons are enriched in GluN2A subunits. Also shown are strategies to enhance NMDAR function on the interneurons: NMDAR potentiators that can directly enhance NMDAR activity; glycine transporter 1 (GLYT1) inhibitors, which by reducing the uptake of glycine, enhance the synaptic concentration of glycine; D-serine, another co-agonist of NMDARs; and positive allosteric modulators of group I metabotropic glutamate receptors (mGluR1 and mGluR5), which couple to NMDARs and enhance their function.

Accordingly, the therapeutic potential of NMDAR antagonists as anti-parkinsonians or antidyskinetics has been investigated. However, low-affinity, broad-spectrum NMDAR antagonists, such as amantadine and memantine, only showed modest clinical benefits[165]. Selectively targeting specific NMDAR subtypes might be more promising based on the observation of increased synaptic abundance of GluN2A and redistribution of GluN2B subunits from synaptic to extrasynaptic regions in the striatal membrane of L-3,4-dihydroxyphenylalanine (L-DOPA)-treated dyskinesic animals[166,167]. Interestingly, the expression of MAGUKs is also reduced in animal models of PD, and disrupted GluN2B and MAGUK binding with a synthetic peptide results in dyskinesia in animal models of PD[167]. However, GluN2B-selective antagonists produced mixed results in PD models[3] and have not shown any consistent antidyskinesia effects in human patients with PD[165]. Encouragingly, in PD rat models, interfering specifically with GluN2A synaptic localization led to a reduction of the percentage of parkinsonian rats developing dyskinetic movements[168].

Potentiating NMDAR signalling

Recent studies have strongly suggested that enhancing NMDAR functions may be beneficial for certain neuropsychiatric conditions. The pathology of these diseases may be caused by reduced NMDAR signalling, and there are indications that enhancing NMDAR function could be neuroprotective if conveyed in a subunit-selective manner. Below, we examine the evidence in cognitive impairment and schizophrenia.

A decline in memory function with ageing is generally linked to impaired synaptic plasticity, which could be caused by altered function and/or expression of NMDARs. Selective vulnerability of NMDARs among glutamate receptors is observed in aged animals, and correcting NMDAR decline (either through drug intervention or caloric restriction) led to improvement in LTP[169]. This altered NMDAR functioning involves reduced expression of particular NMDAR subunits[169]. Aged rats have deficits in GluN2B protein expression, which correlate with impaired LTP and learning[120]. Consistent with a crucial role of GluN2B subunits in cognition, transgenic mice overexpressing GluN2B

show enhanced LTP and superior learning and memory functions even at senile age[170]. As there is no gross alteration in the expression of GluN2A in aged animals, the remaining NMDARs at synaptic sites are probably pure GluN1/GluN2A di-heteromeric receptors rather than GluN1/GluN2A/GluN2B tri-heteromeric receptors, and thus have fast kinetics and reduced coupling to CaMKII signalling (due to a loss of GluN2B subunits). In such a scenario, enhancing GluN1/GluN2A receptors could be effective in restoring functions and could compensate for the loss of tri-heteromeric receptors. Alternatively, GluN2B could primarily be lost from extrasynaptic regions, and hence the critical intervention might be to prevent this loss. Treatments aimed at preventing or reversing the effects of ageing on NMDARs may thus aid in ameliorating the memory decline that is associated with ageing and age-related diseases.

Traditionally, excess dopamine has been considered as the major underlying cause of schizophrenia. However, this classic hypothesis does not readily explain the negative symptoms and cognitive deficits that are often observed in patients with schizophrenia[171]. Altered glutamate signalling may provide a better pathological basis for the genesis of schizophrenia[171]. This hypothesis states that reduced functioning of glutamatergic synapses, especially NMDARs in GABAergic interneurons, leads to an imbalance between excitation and inhibition (disinhibition; see FIG. 6b) and, ultimately, to persistent alterations in the neural circuitry that drives psychosis and impairment in cognitive and executive functions. NMDAR antagonists cause schizophrenia-like symptoms in healthy humans, and they exacerbate the symptoms in patients with schizophrenia[171]. Mice with reduced NMDAR expression show schizophrenia-like phenotypes[172]. Strikingly, most of these phenotypes could be reproduced in mice when NMDARs are selectively deleted from a subpopulation of corticolimbic GABAergic interneurons[173]. Interestingly, GABAergic interneurons express specific NMDAR populations: hippocampal interneurons are enriched in GluN2A and GluN2D[11], whereas cortical parvalbumin-positive (PV^+) interneurons display a fivefold higher GluN2A-to-GluN2B ratio than pyramidal neurons[174]. The activity of GluN2A-containing receptors seems to be essential for the maintenance of the GABAergic function of PV^+ interneurons[174], and the density of GluN2A-positive GABAergic interneurons is decreased in post-mortem brain samples of patients with schizophrenia[175]. Another possible mechanism that may account for NMDAR hypofunction is the enhanced expression of GluN3A[176], which is due to this subunit's unique ability to act as a suppressor of NMDAR function[14,15]. Enhancing NMDAR activity by increasing the concentration of glycine or D-serine has shown mixed results in improving the negative symptoms and cognitive deficits in patients with schizophrenia[171]. One strategy currently being pursued to increase co-agonist site occupancy is to inhibit glycine uptake using glycine transporter 1 inhibitors[171] (FIG. 6b). It remains to be tested whether the observed benefit is mediated by the enhanced function of inhibitory neurons due to NMDAR potentiation, and whether subunit-selective NMDAR enhancers will be more effective than boosting the concentration of glycine and/or D-serine.

Given that reduced NMDAR presence or function is likely to occur under various neurological and psychiatric conditions and during ageing, key questions arise: how much potentiating is required to convey functional rescue? Are remaining NMDARs sufficient in number to support the required enhancement? Is it better to potentiate NMDARs containing GluN2A or GluN2B? Regarding subunit specificity, targeting GluN2B could be supported by the findings that animals overexpressing GluN2B exhibited superior memory functions throughout their lifespan[119,129,177], GluN2B levels might be reduced with ageing[169], and GluN2B is critical for LTP induction (see above). However, an obvious concern about enhancing GluN2B-containing receptors relates to excitotoxicity[26,27,64,142]; enhancing GluN2A could be less excitotoxic[26]. Moreover, certain cognitive functions may rely more on GluN2A- than GluN2B-containing receptors[126,177–179]. Thus, there is a rationale for targeting either GluN2A or GluN2B subunits, but only future studies will tell whether the benefits of targeting one surpasses the other. A promising strategy may reside in developing compounds selective for tri-heteromeric GluN1/GluN2A/GluN2B receptors, but targeting this receptor subtype without interfering with others may be particularly challenging.

Concluding remarks

The heteromeric nature of NMDARs provides a wide variety of receptor subtypes, allowing for a rich diversity in receptor signalling properties. There is thus little doubt that this large diversity allows specific NMDAR subtypes to engage in various distinct functions. Moreover, the recent recognition that NMDAR subunit composition can be remodelled according to activity even in adults provides further credence that the molecular composition of the receptor is tailored to match the strict requirements of specific neural functions. Assigning specific roles to individual subunits remains a difficult task, however, and the enticing view that each NMDAR subtype has a unique and exclusive role may turn out to be an oversimplification. There is a great need for novel pharmacological compounds and genetic approaches that permit discrimination between receptor subtypes and the various associated signalling pathways. Efforts in this direction should not only provide invaluable tools to dissect the contribution of the different GluN subunits but should also foster new therapeutic developments for addressing unmet clinical needs in neurology and psychiatry. One should keep in mind, however, that the effects of NMDAR activation are dual in nature, with both neurotoxic and pro-survival effects. The therapeutic window for NMDAR-based therapeutics may thus be a narrow one. Continuous and sustained efforts are still needed before NMDAR-targeted interventions can turn into valuable treatments.

Negative symptoms
A set of symptoms seen in patients with schizophrenia, including social withdrawal, loss of motivation and reduced affect.

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