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Shuffling the Deck Anew: How NR3 Tweaks NMDA Receptor Function

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Abstract The *N*-methyl-D-aspartate (NMDA) receptors are the most complex members in the family of ionotropic glutamate receptors. They are involved in long-term potentiation and underlie higher cognitive functions like memory formation and learning. On the other hand, overstimulation of NMDA receptors (NMDARs), leading to a massive influx of Ca^{2+} ions into the cell, is linked to neurodegenerative disorders such as for example Huntington's disease and epilepsy. NMDARs are generally considered to be heteromeric tetramers and are conventionally thought to assemble from NR1 splice variants and NR2 subunits, which determine crucial channel properties. With the recent discovery of the functionally different NR3 subunits, many of the known features of NMDARs are being reassessed: The presence of NR3 in NMDARs decreases Mg^{2+} sensitivity and Ca^{2+} permeability and reduces agonist-induced current responses. Between altering those essential key characteristics of conventional NMDARs and forming a new class of excitatory glycine receptors when coassembling with NR1, the NR3 subunits give rise to a functionally entirely new array of "NMDA" receptors. Understanding the multifaceted influence of NR3 is imperative to further the understanding of the complex role of NMDARs in neurotransmission and higher brain functions.

Keywords Glutamate receptors · NMDA receptors · NR3A · NR3B · Ca^{2+} permeability · Glycine receptor

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Introduction

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels mediating the vast majority of all excitatory neurotransmission in the vertebrate central nervous system. Additionally, they are involved in higher cognitive functions such as memory formation and learning. By contrast, abnormal stimulation of these receptors can have deleterious effects: On the one hand, NMDAR overstimulation is implicated in neurodegenerative diseases such as Morbus Parkinson, Alzheimer's disease, Huntington's disease, and epilepsy. On the other hand, reduced NMDAR activity—due to antagonistic blockade or hypofunction—is equally damaging: The block of NMDAR-mediated signaling during synaptogenesis triggers neuronal apoptosis [1] and is associated with the fetal alcohol syndrome [1]. Furthermore, hypoactivity of NMDARs appears to be linked to schizophrenia, as suggested by pharmacological studies [2] and animal models [3].

The subfamily of NMDA receptors (NMDARs) comprises NR1, NR2, and NR3 subunits, which are generally believed to assemble into heteromeric tetramers. The classical NMDARs (in the following called "conventional" NMDARs) were thought to form out of two dimers of NR1 and NR2 subunits, the composition of which determines receptor properties. Generally, NMDARs need glycine in addition to glutamate for activation [4]. Concur-

rently, depolarization of the membrane is required to release Mg^{2+} ions binding inside the NMDAR pore and inhibiting ion flux. Upon activation, conventional NMDARs comprised of NR1/NR2 subunits become highly permeable for Ca^{2+}. Via the coincidence detection of depolarization and the presence of agonists, NMDARs are thought to provide the molecular basis for long-term potentiation (LTP).

Apart from their subunit-dependent heterogeneity, NMDAR function can also be influenced via multiple modulatory sites. Inside the ion pore, binding sites exist for the channel blockers MK801 and 1-(1-phenylcyclohexyl)-piperidine (phencyclidine, PCP) [5]. Zn^{2+} ions, antagonists for NMDARs, bind to the N-terminal domain [6–8]. Furthermore, NMDARs possess extracellular binding sites for polyamines and protons and can be modified via phosphorylation (intracellularly) and glycosylation (extracellularly) [5].

NR1 as the compulsory subunit is expressed ubiquitously in the brain. Alternative N- and C-terminal splicing generates nine isoforms from the single gene transcript, eight of which form functional ion channels [8]. While the NR1 subunit contains the glycine-binding site [9, 10], NR2 binds glutamate [11].

The four NR2 subunits (NR2A-D) are independent gene transcripts that are expressed in a regionally and developmentally regulated manner [12]. NR2B and NR2D are expressed prenatally. NR2B is localized not only in cortex, thalamus, and the spinal cord but also in the hippocampus, colliculus, and hypothalamus, while NR2D is found mainly in midbrain structures. Perinatally, a shift in expression occurs in favor of NR2A and NR2C. While NR2A is expressed in cortex and hippocampus, NR2C is restricted mainly to the cerebellum [12]. The main functional differences between the NR2 subunits concern comparatively minor changes regarding the Mg^{2+} block, the gating, and receptor kinetics.

As most studies did not account for the existence of the NR3 subunits, little if anything was known about their contribution to the receptor complex. Of late, those members of the subfamily have become a focus of NMDAR research, and recent leaps in the understanding of their remarkable influence on receptor function might necessitate a reevaluation of NMDAR key properties.

Molecular Biology of the NR3 Subunits

First identified in 1995 and independently described by Ciabarra et al. and Sucher et al. [13, 14], this new member of the iGluR family was originally termed χ-1 (chi-1, GenBank accession number L34938) and NMDAR-L (NMDAR-like, GenBank accession number U29873), respectively.

The complementary DNA identified from rat brain has an open reading frame of 3,345 bp, encoding a 1,115-amino-acid protein with a calculated molecular mass of 124.5 kDa [14]. The human homolog (GenBank accession number AJ416950, RefSeq accession number NM_133445) has the same length [15, 16], with the mature protein comprising 1,089 amino acids [15].

At the amino acid level, this subunit showed highest homology to NMDAR subunits [13, 14]. Based on sequence comparison and its influence on heterologously expressed NMDARs (see "Electrophysiological Properties of NR3-Containing NMDARs"), the clone was established as a member of a new group of NMDAR subunits and later renamed NR3A [17].

At least one alternatively spliced variant of the gene transcript exists in rodents. Termed NR3A-l for NR3A-*long* [18] or NR3A-2 [19], this variant comprises a 60-bp insertion within the C terminus. The additional 20 amino acids feature two potential sites for phosphorylation by protein kinase C, cyclic AMP-dependent protein kinase A and calmodulin-dependent protein kinase II [18]. This is reminiscent of the C1 cassette of the NR1 subunit, where splicing determines the presence or absence of phosphorylation sites. In the human CNS, no NR3A variant has been identified [16].

The other member of the NR3 group of NMDAR subunits (RefSeq accession number NM_138690) was first identified in the rodent CNS [20]. The 1,003-amino-acid protein was termed NR3B (GenBank accession number AF396649, RefSeq accession number NM_13055) due to its 51% sequence similarity to NR3A [21]. The rat homolog is a 1,002-amino-acid protein of 109 kDa [22] (RefSeq accession number NM_133308). NR3B is encoded by nine exons [21], with exon nine being considerably longer in the human than in the rodent subunit [23] (RefSeq accession number NM_138690). Alternatively, a 900-amino-acid protein encoded by eight exons has been reported for human and mouse NR3B [15]. Although the existence of the short variant could not be corroborated for the human subunit [23], it is still conceivable that for NR3B, as for its sibling NR3A, several variants exist due to alternative splicing.

Hydropathy analyses and sequence comparisons suggest that all major topological features of iGluRs are present in the NR3 subunits (Fig. 1A): One hydrophobic region at the N terminus encodes a putative signal peptide, while four additional predicted hydrophobic segments correspond to the three transmembrane domains (TMD) and one membrane-associated region of other iGluR subunits [13, 14, 21].

Like NR1 and NR2, both NR3 subunits probably undergo posttranslational modifications. Nine (human NR3A), 11 (rat NR3A), three (human NR3B), and four (mouse NR3B) putative glycosylation sites have been identified in their N-terminal domains, plus one site in the extracellular region between TMDs B and C (Fig. 1C) [13, 15, 21]. Potential sites for phosphorylation by several

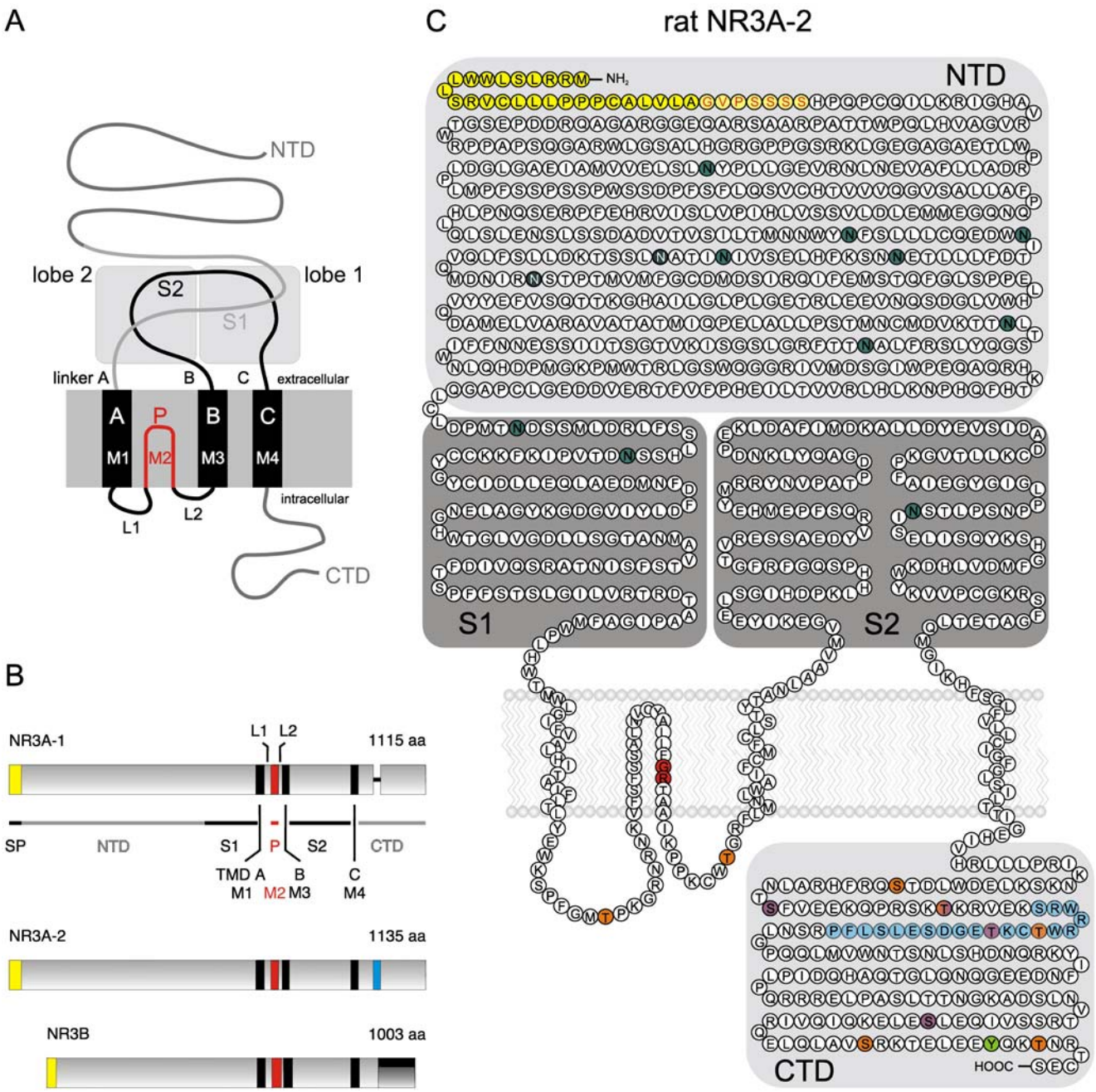

Fig. 1 **A** Topology of an iGluR subunit. *NTD* N-terminal domain; *S1* and *S2* sequences interacting to form lobes 1 and 2 of the LBD; *A*, *B*, and *C* TMDs (also termed *M1*, *M3*, and *M4*, respectively); *linkers A, B and C* linkers connecting the TMDs to the S1 and S2 regions; *L1* and *L2* loops connecting the pore to the adjacent TMDs; *CTD* C-terminal domain. **B** Schematic representation of the NR3 subunits. Indicated are the signal peptide (*SP*, *yellow*), N-terminal domain (*NTD*), ligand-binding sequences S1 and S2, TMDs A, B and C (*black bars*), the pore loop (*red bar*), linker regions L1 and L2, and the C-terminal domain (*CTD*). The *blue bar* represents the insert in the long NR3A splice variant. For NR3B, a 900 aa protein—a potential splice variant—has also been described [12], lacking the C-terminal region encoded by exon nine (represented in *dark gray*). **C** Amino acid sequence of rat NR3A and approximate representation of the transmembrane topology [10, 11]. The length of the signal peptide is estimated as either 26 aa [10, 12] (*yellow balls*) or 33 aa [11, 13] (*light yellow balls, red lettering*). *Cyan balls* mark the 20-aa C-terminal insert of NR3A-2 [15]. The N-site and N+1 position in the pore loop are shown in *red*. *Green balls* denote putative glycosylation sites [10], two dark green positions (*white lettering*) are glycosylation sites found in the rodent, but not in the human NR3A protein [13]. Also indicated are putative sites for phosphorylation via Ca^{2+}/calmodulin-dependent protein kinase [10, 12, 15] (*lilac balls*), protein kinase C [12] (*orange balls*) and tyrosine kinase [11, 12] (*light green ball*)

protein kinases as well as *N*-myristoylation have been reported [13–15, 21].

Strikingly, one major functional determinant of electrophysiological properties in iGluRs has a unique trait in NR3: At the Q/R/N (glutamine/arginine/asparagine) site (hereafter: N site), also known as the Q/R editing site in α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors, both NR3 subunits possess glycine (G) followed by arginine [13, 14, 21]. One exception is the human NR3B subunit, which features two arginines at the N− and N+1 positions [15, 21]. The amino acid at the N site critically determines the selectivity of the channel and the block by Mg^{2+} ions. In "conventional" NMDARs an asparagine occupies this position, conferring a high Ca^{2+} permeability to NR1/NR2 heteromers. In AMPA receptors, RNA editing of the genomically encoded glutamine into arginine dramatically reduces Ca^{2+} permeability. The unprecedented glycine/arginine combination found at the N and N+1 positions in NR3 might relate to the distinctive electrophysiological properties conferred to NMDARs by this subunit (see "Electrophysiological Properties of NR3-Containing NMDARs").

Expression Profile of the NR3 Subunits

In the rodent CNS, both NR3 subunits are expressed in specific regional and temporal patterns. NR3A expression is high in the developing CNS [13, 14, 24–26], suggestive of a critical role of this subunit during that period. NR3A messenger RNA (mRNA) is present in the spinal cord, thalamus, hypothalamus and structures of the rodent brainstem as early as embryonal age E15. Transcript levels for this subunit are increased at E19, and NR3A mRNA is found additionally in the hippocampus and cortical neuroepithelium [13]. While transcript levels remain elevated during the first postnatal week, with NR3A mRNA increasingly detectable in cortical layers II–VI, a rapid decrease occurs after postnatal age P14 to levels hardly detectable in the adult brain [14]. NR3A protein levels change correspondingly: Peak levels between P7 and P10 subsequently decline to low adult levels [25, 26]. In the cerebellum and olfactory bulb, peak NR3A protein levels are reached as early as P2 and P5, respectively [25]. Pronounced mRNA expression in the adult is retained only in the thalamus, amygdala, nucleus of the lateral olfactory tract and laminae 2 and 3 of the dorsal horn of the spinal cord [13, 14]. NR3A has also been found in the rodent retina from an early postnatal age and persisting into adulthood [27]. Evidence exists for a region-specific mRNA distribution for the two splice variants of NR3A. For the longer variant, NR3A-2, a slightly different mRNA expression pattern was reported by Sun and colleagues [18]. During the developmental stages, mRNA for the longer variant is expressed at much lower relative levels. Notably though, this variant is the predominant form in the cerebellum, where RNA for the shorter form, NR3A-1, is barely detectable. As reported for the short variant, NR3A-2 transcript levels also decrease postnatally to low adult levels. The olfactory bulb constitutes an exception: Here, NR3A-2 mRNA levels remain almost unchanged from birth to adulthood [18].

The extensive expression of the NR3A subunit during development has been interpreted in terms of a regulatory role of this subunit during the early stages of the CNS. In the human brain, a similar course of NR3A levels is observed: In the dorsolateral prefrontal cortex, NR3A transcript and protein levels peak postnatally and decline during adolescence [28].

However, recent reports paint a different picture of NR3A distribution in primates: Widespread in the human fetal brain [16] and widely distributed even in the adult, mRNA for this subunit is present in the cerebral cortex and subcortical areas but hardly detectable in the spinal cord [29]. Accordingly, NR3A mRNA and protein are found in many regions of the adult macaque brain, including hippocampus, amygdala, thalamus and hypothalamus, as well as neocortex and cerebellum [30].

The expression pattern of NR3B differs from that of its sibling subunit: In the rodent brain and spinal cord, NR3B mRNA appears in motoneurons during the second postnatal week and reaches its maximal expression level by P21 [31]. Expression of NR3B is pronounced during adulthood but is restricted to motoneurons in limited regions of the brainstem and the ventral horn of the spinal cord [21, 22]. Its strictly localized expression has raised suggestions about a neuroprotective role of NR3B in somatic motoneurons, which upon a functional loss of this subunit might become selectively susceptible to glutamate-induced excitotoxicity [21].

However, recent investigations indicate a more widespread localization of NR3B, including its expression in cortex, cerebellum and hippocampus in the adult rat CNS [15, 32]. In the human forebrain, NR3B mRNA is expressed in the CA1 to CA4 regions and dentate gyrus of the hippocampus [23].

Trafficking and Assembly of NR3 Subunits

Until recently, studies of NMDAR function have not accounted for the NR3 subunits. Consequently, as soon as they had been identified as members of the subfamily, their interaction with so-called conventional NR1/NR2 heteromers and their influence on the receptors' cellular localization has become a focus of interest.

In heterologous expression systems, both NR3 subunits assemble readily at least with the NR1 subunit or when

both NR1 and NR2 are present [33–36]. In transfected hippocampal neurons of adult mice, exogenous NR3B is incorporated readily into endogenous conventional NMDARs [34]. In vivo however, only a fraction of NMDARs in the rodent brain might actually contain NR3A [25].

Whether NR3 and NR2 assemble in the absence of NR1 is controversial: Independent NR2/NR3A assembly in the ER has been reported in a heterologous expression system [33], but recent investigations argue against the formation of NR2/NR3 dimers in the absence of NR1 [35, 36].

For both NR3 subunits, however, transport to the plasma membrane appears to require the presence of NR1 in the complex [33, 34]. This prerequisite for surface localization of NMDAR heteromers suggests that the NR3 subunits—like NR2 and other interacting proteins [37–39]—can mask retention signals in the C terminus of NR1. The region responsible for this was narrowed down to the C-terminal amino acids 952–985 of mouse NR3B [34]. For mere assembly with NR1, on the other hand, the C terminus of NR3 is not essential [34]. Neither is the N-terminal domain of NR1, as subunits lacking this region still assemble and form functional receptors with NR3A [36].

Both NR3 subunits contain C-terminal ER retention motif-like signals themselves [33, 34]. However, mutants without any retention motifs can still not exit the ER, so NR3B does probably not contain forward trafficking signals [34].

Homomerically expressed NR3A—as well as NR2, for that matter—does not simply become retained in the ER [33, 34]; rather, it is only partially folded and actively degraded [35, 36]. Homo-oligomerization of NR3A is a controversial matter that awaits further clarification: While it has been reported that homo-dimers of NR3A subunits (human and rat) do not exist [35, 36] except in the presence of NR1 [36], it has also been suggested that NR3A—much like NR1—dimerizes in the adult human CNS [29].

Assembly, export and function of NR3-containing NMDARs have all been proven possible without any NR2 subunit present in the complex. The stoichiometry of the NR1/NR3 tetramer is suggested to adhere invariantly to a 1:1 ratio, with two NR1 and two NR3 subunits in the complex [36, 40]. Much less is known about the exact composition of a triheteromeric (NR1/NR2/NR3) receptor, but a number of accounts report not only the assembly of all three subunit types but also functional peculiarities of such triheteromeric NMDARs [33, 34, 36, 41, 42] (cf. "Electrophysiological Properties of NR3-Containing NMDARs").

NR3A also impacts the synaptic localization and stabilization of receptor complexes. Via C-terminal interaction, NR3A specifically links to PACSIN1, an accessory protein involved in clathrin-mediated endocytosis and actin arrangement [43]. NR3A-containing receptors are localized mainly peri- and extrasynaptically in dendritic spines; they are thought to be actively and selectively removed from the postsynaptic density and subjected to PACSIN1-mediated endocytosis [43]. This process, which is dependent on synaptic activity, could be one of the factors of postnatal NR3A downregulation [43].

Electrophysiological Properties of NR3-Containing NMDARs

The functional ramifications of NMDA receptors stem from their key properties: blockade by Mg^{2+} that necessitates predepolarization, requirement of a coagonist, and high permeability for Ca^{2+} ions.

Like the conventional NMDAR subunits NR1 and NR2, NR3 does not function as a homomer [13, 14, 21, 22, 33, 44]. Neither does its ion pore domain support function when transplanted into NR1 [45]. This is consistent with evidence that the transplanted pore regions of NR3 subunits might be differently involved in ion fluxing (see below).

Both NR3 subunits have been suggested to modulate NR1/NR2 heteromers by reducing current responses (Fig. 2B), both in non-mammalian [13, 14, 17, 19, 22] and mammalian [19, 21, 24, 44] expression systems, as well as in cortical and hippocampal neurons from wild-type [19] and NR3A-overexpressing transgenic [42] mice, respectively (for an overview, see Table 1). As reduced amplitudes also apply to glycine-induced currents (Fig. 2B), it may be concluded that—at least in a heterogeneous expression system—all three types of subunits assemble into a triheteromeric NMDAR instead of forming a heterogeneous population consisting of NR1/NR2 and NR1/NR3 receptors.

Even though a number of studies reported otherwise [14, 21, 46], the presence of NR3 might also lower the sensitivity to Mg^{2+} ions, as shown in heterologous expression systems [19] and by comparing NMDARs in NR3A-transgenic [42] and NR3A knockout mice [19] to NMDAR wild-type animals.

Most strikingly, though, coexpressed NR3 appears to decrease the Ca^{2+} permeability of conventional NMDARs [14, 17, 33, 34, 42, 44]. Most surveys, however, describe the additional presence of NR1/NR2 heteromers. Their contribution to current responses observed complicates analysis and affects quantification; reported Ca^{2+} permeabilities range from twofold lower values in NR3A-transgenic mice [42] and a fivefold reduction in NR1/NR2A/NR3A heteromers in HEK cells [33] to a ninefold decrease in *Xenopus* oocytes for the same subunit combination [19]. The number of NMDARs actually containing NR3 might vary in the different expression systems, resulting in different ratios of conventional to triheteromeric receptors and therefore leading to divergent values. In

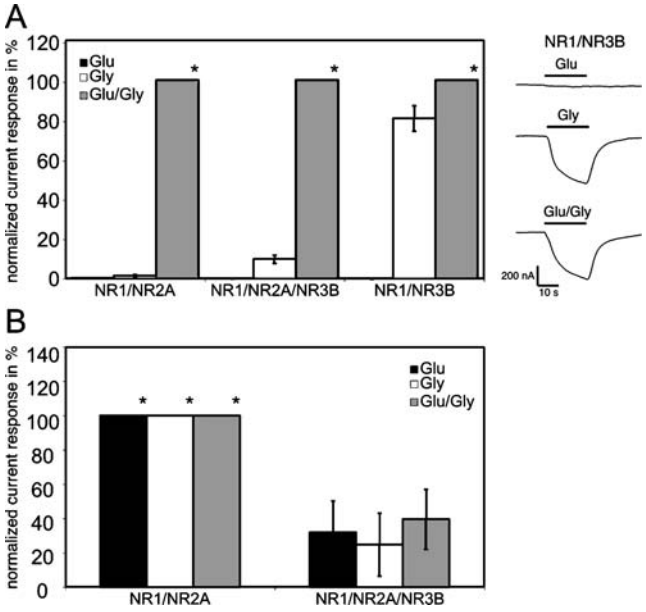

Fig. 2 Current responses of NMDARs expressed in *Xenopus* oocytes and measured via two-electrode voltage clamp. Data averaged from three to six oocytes per subunit combination. Applied agonists: 300 μM glutamate (*Glu*), 10 μM glycine (*Gly*), or coapplication of both (*Glu/Gly*). **A** Mean amplitudes of different NMDAR subunit combinations (±SEM). Responses to co-applied Glu/Gly were set independently to 100% for each combination (*columns marked with asterisk*); responses to separately applied agonists were normalized to these values respectively. To the *right*, exemplary current responses are shown for NR1/NR3B receptors. The *black bar* denotes the duration of agonist application. Note that these receptors are fully activated by glycine and insensitive to glutamate. **B** Mean current responses of di- and triheteromeric NMDARs (±SEM). Responses for NR1/NR2A receptors were independently set to 100% for each agonist (*columns marked with asterisk*); responses of triheteromeric receptors were normalized to the respective values of diheteromeric receptors. Mean values for NR1/NR2A were 29.79±9.98 nA (Glu), 142.97±62.78 nA (Gly), and 7,732.50±671,97 nA (Glu/Gly). Note that the presence of NR3 in the complex results in reduced agonist-induced NMDAR current amplitudes

retinal cells of NR3A-deficient mice, Ca^{2+} influx is significantly larger than in wild-type mice [27].

In heterologous expression systems, NR3 subunits can also assemble with NR1 to form receptors gated by glycine alone [22] (Fig. 2A). These diheteromers are only weakly sensitive to channel blockers like Mg^{2+}, MK801, and memantine [22, 47] and display a strongly decreased Ca^{2+} permeability [22]. D-serine, a glycine site agonist of conventional NMDARs, inhibits glycine-induced currents mediated by NR1/NR3 receptors but is able to induce small currents when applied alone [22, 47].

Though characterized in mammalian [47, 48] and non-mammalian expression systems [22], it remains to be elucidated whether these excitatory glycine receptors are present *in vivo*, co-existing with conventional NMDARs. Alternatively, it is also conceivable that NR1/NR3 dimers *in vivo* occur exclusively in functional assembly with NR1/NR2 dimers.

Lowered current responses, reduced Mg^{2+} block and reduced Ca^{2+} fluxing: How does NR3 confer these properties to NMDARs? Contrary to the SYTANLAAF motif in other subunits [49], the TYTANLAAF motif of NR3A does not display state-dependent changes and might form a rigid structure that affects gating [46] and thus current amplitudes. For binding of Mg^{2+} ions and for Ca^{2+} permeability, the amino acids at the N site and N+1 position are essential. The unusual glycine–arginine combination of the NR3 subunits, which replaces asparagine–serine in NR1 and asparagine–asparagine in NR2 subunits, might mediate the atypical functional traits found in NR3-containing NMDARs. However, NR3A subunits in which the arginine residue has been substituted by either glutamine [13] or asparagine [14] do not differ in their functional interaction with NR1 or both NR1 and NR2. Similarly, NR3B mutants featuring two asparagines instead of the glycine–arginine combination do not alter the sensitivity of NR1/NR2/NR3 complexes to Mg^{2+} ions, anesthetics, or ethanol [50]. In line with that, a recent study identified the narrow constriction in NR1/NR3A dimers at the external channel vestibule [46], as opposed to the pore loop. Consequently, and in contrast to the N-site of NR1, the N-site of NR3A might not contribute to the selectivity filter of the channel. Instead, homologous residues of the second transmembrane domains (TMD B) of NR1 and NR3A align at the vestibular level in the NR1/NR3A channel to form this newly identified constriction, which possibly disrupts Ca^{2+} hopping relay stations and thus impairs Ca^{2+} permeability [46].

NR3 Pharmacology and the Ligand-Binding Domain: Properties and Peculiarities

Even though NR1 and NR3 both bind glycine, they display a very different electrophysiological behavior, which is in part due to differences in ligand-binding properties.

Experiments with a soluble NR3A ligand-binding domain (LBD) suggest NR3 to bind glycine with approximately 650-fold higher affinity than does NR1 (NR3A: K_d ~40.4 nM, [51]). Similarly, human NR3A has been shown to bind glycine with high affinity in radioligand-binding assays (K_d ~535 nM, [29]). D-serine also binds to NR3A S1S2 with high affinity (K_d ~643 nM), indicating that *in vivo*, the NR3A LBD might be saturated by either of those agonists [51].

This is especially noteworthy in the context of two recent studies unveiling an asymmetric effect of ligand binding on the NR1/NR3 diheteromers in the absence of NR2: Mutations inhibiting glycine binding to NR1 led to a potentiation of steady-state NR1/NR3-mediated currents in *Xenopus* oocytes. Functionally homologous mutants of NR3A and NR3B displayed the opposite effect, allowing

Table 1 Summary of some of the electrophysiological properties of the NR3 subunits

NR3A coexpressed with	*expression system*	*agonists / antagonists*	*response*	*ref.*
NR1	*Xenopus* oocytes	glycine	inward current that cannot be potentiated by glutamate or NMDA, EC_{50} ~ 1 µM, desensitization above 3 µM glycine	[22]
NR1/NR2A		NMDA + glycine	with NR3: appearance of a small conductance state (35 pS) apparent mean open time of 4.3 ms, mean closed time of 185 ms reduced Ca^{2+} permeability compared to NR1/NR2; P_{Ca2+}/P_{mono} ~0,8	[19]
		+ Mg^{2+}	receptors containing NR3A hardly sensitive to Mg^{2+}	
NR1/NR2B NR1/NR2D		NMDA + glycine	reduced current response compared to NR1/NR2	[13]
		+ Mg^{2+}	full block of current response	[14,17]
NR1/NR2A	HEK293 cells	NMDA + glycine	reduced current response compared to NR1/NR2 reduced Ca^{2+} permeability compared to NR1/NR2; P_{Ca2+}/P_{mono} ~0,6	[33]
		+ Mg^{2+}	receptors containing NR3A hardly sensitive to Mg^{2+}	[19]
		glutamate + glycine + ethanol	ethanol inhibition of NMDARs unaltered in the presence of NR3A	[54]
		glutamate + glycine + ifenprodil	ifenprodil inhibition of NMDARs unaltered in the presence of NR3A	
NR1/NR2C NR1/NR2D		glutamate + glycine + ethanol	ethanol inhibition of NMDARs unaltered in the presence of NR3A	
NR1/NR3B		glycine	inward current, desensitization above 10 mM glycine, EC_{50} values for peak current: 44.3 µM, for steady-state current: 14.0 µM	[47]
		+ cyclothiazide	no effect on desensitization	
		NFPS, ALX-1339, picrotoxin, strychnine, acamprosate, APV, MK801, memantine, ifenprodil, Mg^{2+}	no effect on glycine-evoked currents	
		L-alanine, β-alanine, taurine	no or very small current responses	
		D-serine	induction of small current responses and reduction of glycine-evoked current	
		ethanol	concentration-dependent inhibition of glycine-evoked current responses	
NR1/NR2	primary neurons from NR3A-KO-mice	NMDA + glycine	reduced current response compared to WT neurons with NR3: appearance of a small conductance state (20 pS), mean open time of 3.6 ms	[17,19]
		+ APV	inhibition of current response	[19]
NR1/NR2	cultured hippocampal neurons	NMDA + glycine	with NR3: appearance of a small conductance state (40 pS), mean open time of 6.5 ms	[42]
		+ Mg^{2+}	synaptic NMDAR EPSCs in slices from NR3A-overexpressing mice are less sensitive to Mg^{2+} than WT or KO-controls	
NR3B coexpressed with	***expression system***	***agonists / antagonists***	***response***	***ref.***
NR1	*Xenopus* oocytes	glycine	inward current that cannot be potentiated by glutamate or NMDA, EC_{50} ~ 1 µM, desensitization above 3 µM glycine	[22]
		D-serine, D-alanine, D-cycloserine, ACPC	inhibition of gly-evoked currents	
		APV, memantine, Mg^{2+}	hardly inhibition of gly-evoked currents	
NR1/NR2A		NMDA + glycine	reduced current response compared to NR1/NR2	
NR1/NR2A	HEK293 cells	glutamate + glycine	reduced current response compared to NR1/NR2 reduced Ca^{2+} permeability compared to NR1/NR2; P_{Ca2+}/P_{mono} ~1.7	[21,44]
		glutamate + glycine + Mg^{2+}	full block of current response	[21]
NR1/NR2	transfected hippocampal neurons	NMDA + glycine	reduced Ca^{2+} permeability compared to untransfected neurons; P_{Ca2+}/P_{mono} ~2.2	[34]

For homomeric expression of NR3 or coexpression with non-NMDAR subunits, no function has been demonstrated; these combinations are not shown in this table.

only very small current responses upon application of glycine [52, 53]. These effects are reflected in pharmacological studies: NR1-specific glycine site antagonists like MDL and 5,7-DCKA potentiate glycine-induced current responses dose-dependently in NR1/NR3 heteromers [47, 52, 53]. Conversely, antagonists binding also to NR3 (like CNQX) attenuate receptor currents [52].

With opposing effects mediated by the different subunits' LBDs, the NR1/NR3 receptor has been suggested to constitute a glycine biosensor that is regulated in a concentration-dependent manner via NR1. In this model, the NR1 *apo* state (=unoccupied glycine-binding site) is permissive for receptor activation. Consequently, agonist binding to NR3 alone activates the heteromers, while binding to NR1 induces current decay [53]. Accordingly, the sequential dissociation of glycine from first NR1, then NR3, leads to a short relief of current inhibition, as evidenced by a distinct peaking of the current response of heterologously expressed receptors [52]. In a diheteromeric NR1/NR3 receptor, current responses decay rapidly at saturating glycine concentrations. Consequently, in a triheteromeric receptor composed of NR1/NR2 and NR1/NR3 dimers, the NR1/NR2 and NR1/NR3 dimer's discrepant contribution might explain the NR3-mediated attenuation of current responses compared to conventional NMDARs [52].

But why is the affinity for glycine different in NR1 and NR3 at all? The key residues for glycine binding are conserved among the subunits [51], so the explanation has to lie elsewhere. For example, Val689 and Trp731 of NR1 are replaced by the smaller alanine and methionine residues in NR3A, which effectively increases the volume of the ligand-binding cavity. This allows glutamate to bind to the NR3A LBD—but with a very low affinity, much too low to be of physiological importance (K_d ~9.55 mM [51]). Concerning glycine binding, solvent-mediated hydrogen bond networks might differ in the expanded cleft of NR3A and influence ligand binding. Alternatively, the glycine-bound conformation might be stabilized more effectively via interdomain contacts in NR3 [51].

D-serine, a high-affinity agonist of both NR1 and NR3 [51], has been shown to have a greater efficacy at the NR1 subunit [53]. Owing to NR1-mediated current decay [52, 53], D-serine has been shown to elicit only small current responses at NR1/NR3 receptors and to inhibit glycine-mediated responses [22]. The partial NR1 agonist ACPC behaves rather similarly: High-affinity binding to NR1 mediates current decay, resulting in the inhibition of glycine-induced responses of NR1/NR3B receptors. A similar effect can be expected for NR3A, as ACPC has been shown to bind with greater affinity to the NR1 LBD than to that of NR3A [51].

Antagonist affinities also differ dramatically between NR1 and NR3. As demonstrated for a soluble NR3A LBD, only CNQX binds to NR3 and NR1 with similar affinities, while more potent NR1 antagonists (CNQX and quinoline derivatives) strongly favor NR1 over NR3 [51]. Inclusion of NR3A to form triheteromeric NMDARs does not alter the sensitivity to ethanol or ifenprodil found for NR1/NR2 diheteromers [54]. By contrast, glycine-activated NR1/NR3 diheteromers are slightly blocked by ethanol [47].

Physiological Relevance of NR3-Containing NMDARs

NMDA receptors in vivo constitute the molecular basis for complex processes like LTP, which in turn influence synaptic plasticity and underlie higher cognitive functions. Consequently, dysfunction or overstimulation of NMDARs are implicated in various pathological conditions. Most of the NMDARs' critical functional traits can be attributed to their unique coincidence detection and resulting Ca^{2+} flux through receptors relieved from Mg^{2+} block. With an NR3 subunit present that potentially alters each of these essential qualities, the question about the physiological consequences is rather obvious.

Due to its dominant-negative influence on NMDAR current amplitudes and Ca^{2+} permeability, NR3 was ascribed a modulatory role, especially when considering its highly restricted expression. The developmentally regulated NR3A subunit might attenuate NMDAR currents in maturing neurons and prevent overexcitation of newly established synapses. The NR3B subunit might influence cell death in the spinal cord [34] or specifically protect somatic motoneurons [21]—cells that selectively succumb to glutamatergic excitotoxicity in neurodegenerative disorders like amyotrophic lateral sclerosis (ALS). However, a recent study describes high genetic diversity of the NR3B gene in humans. In approximately 10% of the worldwide population, a null allele exists that does not correlate with an ALS phenotype [55].

A primarily modulatory role of NR3 in NMDAR-mediated transmission is also consistent with the finding that NR3A knockout mice are viable and do not display an aberrant behavioral phenotype or altered expression of the other NMDAR subunits [17]. NMDAR-mediated current responses, however, are larger in cerebrocortical neurons from NR3A knockout mutants than wild-type controls. And while dendritic branches are unaltered, the number and density of dendritic spines increase significantly in the absence of endogenous NR3A [17]. NR3B-deficient mice display slightly impaired motor learning, but increased social interaction in familiar environments [56].

Considering its suppressive effects on NMDARs, NR3A has been suggested to act as a neuroprotective modulator. In NR3A-overexpressing transgenic mice, NR3A is incorporated into at least a subset of NMDARs, mitigating their

responses in hippocampal neurons [42]. In the same cells, however, no glycine-activated NR1/NR3A diheteromers could be detected [42]. It remains questionable whether NR3 *in vivo* participates in an excitatory glycine receptor or whether its physiological effects are confined to triheteromeric channels.

In vivo, triheteromers containing NR3A selectively undergo rapid endocytosis [43] (cf. "Trafficking and Assembly of NR3 Subunits"). Eliminating NR3A-containing NMDARs from sites of synaptic input might serve to stabilize signaling in developing neurons and play a role in experience-dependent plasticity [43].

In fact, NR3A might be involved in LTP and long-term depression by governing the phosphorylation states of NMDAR subunits and initiating signaling pathways dependent on recent receptor activity [57]: Specific interaction with NR3A recruits protein phosphatase 2A (PP2A) to the receptor complex, where it dephosphorylates serine 897 of NR1. Dephosphorylation of this residue is apparently dependent on receptor excitation; stimulation of NMDARs interferes with PP2A activity [57]. The specific NR3A residues for interaction with PP2A are conserved in the human sequence and in rodent NR3B, suggesting a common mechanism of PP2A recruitment [58]. Considering the decreased dephosphorylation of NR1 Ser897 in the schizophrenic brain [59], a putative role of NR3 in this disorder presents itself.

Schizophrenic disorders go along with deficits in the prepulse inhibition (PPI) of a startle response. Interestingly, male NR3A knockout rats display age-dependent abnormalities in PPI [60]. Studies on patients with schizophrenia reveal increased NR3A mRNA expression in specific regions of the dorsal lateral prefrontal cortex in these subjects [61]. In the same area, strikingly, NR3A expression is decreased in subjects with bipolar disorder, suggesting region-specific, NR3-dependent neurochemical mechanisms involved in these disorders [61]. This is a matter awaiting further research, however, as conflicting evidence argues against a change in NR3A protein levels in the prefrontal cortex in schizophrenics when compared to control individuals [28].

New fields of action for NR3 are currently discovered in glial cells, where all three subtypes of NMDAR subunits are expressed [41, 62–64], and even in central myelin [63]. NMDA-evoked currents in oligodendrocytes are only feebly blocked by Mg^{2+} [41, 62, 64], which suggests the functional involvement of NR3. Oligodendrocytic NMDARs, possibly composed of NR1, NR2C, and NR3 subunits [41], have been implicated in damaging glial cells under ischemic conditions [41]. Along this line, induced ischemia in the optic nerves of adult rats causes Ca^{2+} accumulation in central myelin [63]. This influx has been suggested to be mediated by myelinic NMDARs, likely composed of NR1, NR2C or NR2D and NR3 [63], and might constitute a form of axo-glial signaling [63] or influence myelination itself [41].

Conclusion

NMDA receptors have been well recognized for their involvement in higher cognitive functions and their unique electrophysiological characteristics. But after decades of NMDAR research, the NR3 subunits have now shuffled the deck anew. From modulating just about every single key property of "the NMDA receptor" to recruiting NR1 subunits for the formation of excitatory glycine receptors, the NR3 subunits unveil a whole new world of possible implications for the vertebrate CNS. Whether concerning their unique pharmacological traits or their role in major neurodegenerative diseases, exciting new discoveries are bound to follow—and eagerly awaited.

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