SYMPOSIUM REVIEW

GluN3A NMDA receptor subunits: more enigmatic than ever?

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Abstract Non-conventional N-methyl-d-aspartate receptors (NMDARs) containing GluN3A subunits have unique biophysical, signalling and localization properties within the NMDAR family, and are typically thought to counterbalance functions of classical NMDARs made up of GluN1/2 subunits. Beyond their recognized roles in synapse refinement during postnatal development, recent evidence is building a wider perspective for GluN3A functions. Here we draw particular attention to the latest developments for this multifaceted and unusual subunit:

Oliver Crawley undertook his PhD with Dr Enrique Martinez-Perez at Imperial College London where he studied how chromosome cohesion is regulated during meiosis. He continued using *C. elegans* imaging during his postdoctoral work with Professor Brock Grill at Scripps Florida to identify genetic pathways determining axonal and presynaptic development. His work helped uncover how autophagy is controlled in the distal axon via ubiquitination of UNC-51/ULK1. His current research in Professor Isabel Pérez-Otaño’s group at the Instituto de Neurociencias de Alicante, funded by Severo-Ochoa and Marie-Curie fellowships, is revealing how NMDA receptors influence axonal morphology during circuit refinement in the mouse brain. Isabel Pérez-Otaño is Full Professor at the Instituto de Neurociencias de Alicante. Her team explores mechanisms that control the construction and refinement (or ‘rewiring’) of complex neural circuits, and what goes wrong in disorders of brain development, mood, cognition or memory. Her work has contributed to our current understanding of GluN3-containing brain receptors from cell biological mechanisms that control their assembly, forward secretory and local synaptic trafficking, to discovering their roles in driving synapse destabilization and pruning during postnatal critical periods, and linking their adult reactivation to neurodegeneration.

Oliver Crawley and Maria J. Conde-Dusman contributed equally to this study.

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Introduction

N-Methyl-d-aspartate receptors (NMDARs) are a major class of ionotropic glutamate receptors that mediate a slow component of excitatory neurotransmission in the central nervous system (CNS), acting as critical mediators of experience-dependent synaptic plasticity, learning and memory (Paoletti et al. 2013). NMDARs are large tetrameric complexes composed of an obligatory GluN1 subunit and combinations of GluN2 (A-D) and GluN3 (A-B) subunits. Each subunit confers distinct properties to NMDARs, such as ion permeability, subcellular localization and trafficking patterns, or signalling interactions, and displays unique spatiotemporal expression profiles across the nervous system (Lau & Zukin, 2007; Paoletti et al. 2013; Hansen et al. 2018). Complexes composed of GluN1 and GluN2 subunits have been more extensively studied and are referred to as classical or conventional NMDARs whereas non-conventional NMDARs denote incorporation of GluN3 subunits.

One of the most enigmatic of the NMDAR subunits is GluN3A (encoded by the human gene GRIN3A, Grin3a in rodents), the more widely expressed of the ‘non-conventional’ GluN3 subfamily. Work to date has established GluN3A as an important regulator of neural circuit refinements by preventing the maturation of synapses until the arrival of sensory experience and later determining which synapses will be maintained or eliminated (Pérez-Otaño et al. 2016). In line with this role, in many brain regions GluN3A shows a characteristic peak of expression during narrow windows of postnatal development that precedes or overlaps with critical periods of experience-dependent plasticity. However, here we highlight how growing evidence is expanding this view and places GluN3A as a broader regulator of brain functions at later ages, in multiple areas and cell types.

This article briefly encompasses some of the established knowledge around GluN3A that has been previously discussed in several thorough reviews (Henson et al. 2010; Low & Wee, 2010; Pachernegg et al. 2012; Pérez-Otaño et al. 2016) but is primarily focused on more recent findings. In doing so, we hope to draw attention to the strides forward that are being made, especially in building a more complete picture of the spatiotemporal distribution of GluN3A throughout the CNS within the context of brain-wide functional implications, and the underappreciated roles of this subunit in excitatory glycine receptors and at presynaptic locations, as well as the ever-expanding links between brain disorders and GluN3A.

GluN3A expression patterns in the CNS

Temporal and regional patterns

GluN3A expression begins at low levels in the embryonic CNS and rises after birth, peaking at the end of the first postnatal week in rodents (early years in humans) (Henson et al. 2010; Jantzie et al. 2015; Pérez-Otaño et al. 2016; Wee et al. 2016) (Fig. 1A). During this zenith high GluN3A levels are found in many brain regions including the cortex, hippocampus CA1, thalamus, amygdala, hypothalamus, olfactory nuclei and others (Wong et al. 2002; Henson et al. 2010; Pachernegg et al. 2012; Pérez-Otaño et al. 2016), demonstrating the broad roles of this non-canonical subunit. Expression drops during the second and third postnatal weeks in rodents (childhood and adolescence in humans) (Fig. 1A). Yet the time courses of GluN3A emergence and down-regulation vary across brain regions, correlating with differences in the timing of circuit maturation, sensory modality, and degrees of functional specialization (Murillo et al. 2021). For instance, a detailed time series across postnatal days revealed that in primary somatosensory cortex GluN3A expression is initially constrained to layer 5 and later extends to layers 2–4 (Murillo et al. 2021) (Fig. 1B). This layer profile sequence evokes the inside-outside
patterning model of cortical maturation and was found to be conserved in motor, visual and auditory cortices. However, both GluN3A expression and down-regulation are delayed in primary visual cortex, which matures later following eye-opening (around P12–P14 in rodents) (Murillo et al. 2021); and visual deprivation further delays the developmental loss of GluN3A, demonstrating a remarkable coupling of GluN3A expression with sensory experience (Larsen et al. 2014) (Fig. 1A). Studies where the calcium-regulated transcription factor CaRF promotes GluN3A expression (Lyons et al. 2016) further supported a link between neuronal activity and GluN3A levels and offered mechanistic insight into how regional and temporal patterns might be produced.

This distinctive profile of postnatal expression coinciding with windows of experience-driven synaptic plasticity and refinements is a unique hallmark of GluN3A among glutamate receptor subunits. However, GluN3A levels are retained into adulthood to some extent (Wong et al. 2002; Allen Brain Atlas) and a recent paper systematically mapped regions where Grin3a mRNA expression persists in the adult mouse brain (Murillo et al. 2021), most notably in nuclei of the amygdala, medial habenula (MHb), association cortices and high-order thalamic nuclei. The work elegantly complements a different study which identified Grin3a as one of the genes most strongly correlated with hierarchy gradients of functional integration across the neocortex – from primary sensorimotor to higher-order association areas – established using the MRI T1w:T2w ratio (Fig. 1C). Low T1w:T2w ratios and high GluN3A expression were found to be typical of less differentiated association and transmodal cortical areas with strong needs for plasticity and functional integration throughout life such as the claustrum, rhinal, insular or prefrontal cortex (Fulcher et al. 2019; Murillo et al. 2021). By contrast, low adult

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**Figure 1. Temporal and regional GluN3A expression patterns in the rodent CNS**

A, regional postnatal expression profiles of GluN3A protein (shaded area = predominant expression window; adapted from Pérez-Otaño et al. 2016); visual deprivation (dashed green line) delays the developmental expression drop (Larsen et al. 2014). B, time course of Grin3a mRNA emergence and down-regulation varies across layers in primary somatosensory cortex (SS1). *In situ* hybridization images and quantification of Grin3a mRNA levels across layers at indicated postnatal ages are shown; dashed lines mark layer 5 (L5) boundaries. Data are means ± S.E.M. ****P < 0.0001; ###P < 0.001. C, correlation between mouse Grin3a transcription and MRI T1w:T2w ratio (for abbreviations, see Fulcher et al. 2019). Colours represent connectivity-based area groupings; light green = somatomotor, turquoise = medial, yellow = temporal, pink = visual, red = anterolateral, dark green = prefrontal. D, RNAscope analysis of Grin3a mRNA localization in SS1 interneurons in a P6 Nkx2–1-cre:Aig tdTomato mouse brain: Grin3a mRNA (red); tdTomato mRNA (green); somatostatin (Sst) mRNA (blue). Filled arrows = Grin3a+ MGE-derived SST interneurons. open arrowheads = Grin3a+ excitatory neurons. E, molecularly distinct interneuron categories defined by scRT-PCR (Pfeffer et al. 2013). Note high levels of Grin3a expression in SST interneurons (boxed).
Grin3a expression was observed in primary sensorimotor unimodal cortices with highly consolidated circuitry and lower plasticity requirements. Correlations between functional hierarchy and Grin3a expression also apply to organization of the primate and human brain (Burt et al. 2018; Fulcher et al. 2019).

**Cell-type specificity**

Expression of Grin3A has been documented in excitatory neurons and also within inhibitory GABAergic or cholinergic interneurons (Pérez-Otaño et al. 2016). Using RNAscope hybridization techniques, the Murillo et al. study dissected the proportion of Grin3A-expressing cells belonging to particular neuron types and demonstrated particularly strong Grin3a expression in somatostatin (SST) interneurons of the neocortex (Fig. 1D) and hippocampus. This is in line with single-cell transcriptional analyses of mouse somatosensory and visual cortex interneurons (Pfeffer et al. 2013; Paul et al. 2017) (Fig. 1E) that identified Grin3a as a secondary molecular marker for SST interneurons, most prominently Martinotti cells. Further work found the Grin3a locus in SST interneurons to be a site of prominently open chromatin and low DNA methylation (Yao et al.), two marks of actively transcribed genes that promote/maintain cell identity. Of note, SST cell densities, like Grin3a expression, are negatively correlated with T1w:T2w ratios (Fulcher et al. 2019). SST interneurons innervate distal dendrites of pyramidal neurons and other interneurons to control the gating of dendritic inputs, consistent with integrative transmodal areas having a preference towards greater input control. Beyond neurons, morphological and RNAseq methods detected GluN3A/Grin3a expression in oligodendrocytes (Káradóttir et al. 2005; Salter & Fern, 2005; Spitzer et al. 2019), microglia (Murugan et al. 2011) and brain endothelial cells (Mehra et al. 2020).

**Subcellular localization**

A defining characteristic of classical NMDARs is their concentration at postsynaptic densities (PSDs) of excitatory synapses. By contrast, high-resolution electron microscopy (EM) and biochemical fractionation studies have shown that GluN3A, while present at PSDs, predominates at perisynaptic and extrasynaptic locations (Fig. 2A–C). Within the PSD itself, GluN3A particle density increases towards the lateral edge, differing from GluN1, which concentrates at the centre (Fig. 2C). This localization has been attributed to decreased physical association with PSDs relative to classical GluN1/2 NMDARs due to the absence in GluN3A of PDZ-binding motifs for synaptic scaffolds (Pérez-Otaño et al. 2016), and could reflect a specialized function such as sensing specific patterns of glutamate release or activating signalling pathways in dendrites. Alternatively, it might reflect a higher mobility of GluN3A-NMDARs and out of the plasma membrane via endo-exocytosis or lateral diffusion that might contribute to keep synapses in a labile state. The latter is supported by the observation that the density of GluN3A particles peaks at the PSD edge, indicating the presence of a rate-limiting step at this level which is in line with models of the PSD acting as a ‘diffusion trap’ (Fig. 2C). Of note, the postsynaptic enrichment of GluN3A is higher at early postnatal stages and declines into adulthood in contrast to the progressive synaptic stabilization of GluN1 or GluN2 subunits (Pérez-Otaño & Ehlers, 2004, Pérez-Otaño et al. 2006; Henson et al. 2012). These observations broadly correlate with proposed roles of GluN3A in postsynaptic signalling and refinement of dendritic spines (see below) (Das et al. 1998; Roberts et al. 2009; Fiuzzi et al. 2013; Kehoe et al. 2014).

On the other hand, immunogold EM studies have also observed GluN3A at presynaptic locations (Fig. 2B). Presynaptic GluN3A-NMDARs are rarer than their postsynaptic counterparts and exhibit remarkable synapse, temporal and circuit selectivity. To date, they have been identified in layer 4 to layer 2/3 synapses of the juvenile visual cortex (Larsen et al. 2011) and perforant path (PP) synapses in juvenile and adult hippocampus (Savtchouk et al. 2019). Interestingly, presynaptic GluN3A immunolabelling was almost exclusively found in medial perforant path (MPP) axon terminals but not in lateral perforant path (LPP) terminals, away from the synaptic cleft and often facing astrocytic membranes (Fig. 2B). At both synapses, presynaptic labelling decreased with age, but the decline was sharp at visual cortex (between P16–P23) while significant expression (~50%) persisted into adulthood at MPP axons. These topographical data are supported by electrophysiology experiments where presynaptic GluN3A function was isolated by blocking postsynaptic NMDAR activity (Larsen et al. 2011, 2014; Savtchouk et al. 2019).

**Non-conventional GluN3A-NMDARs and excitatory glycine receptors**

GluN3A can assemble with other NMDAR subunits to form two types of functional complexes: (1) GluN1/2/3A triheteromers that display non-conventional biophysical and signalling properties, and (2) GluN1/3A diheteromers that behave as excitatory glycine receptors (Fig. 3A).

**GluN1/2/3A triheteromeric NMDARs (or ‘non-conventional’ GluN3A-NMDARs)**

Composed of GluN1, GluN2 and GluN3A subunits, GluN3A-NMDARs receptors are activated by glutamate
or NMDA and require glycine as co-agonist; as such they are considered bona fide NMDARs (Henson et al. 2010; Low & Wee, 2010; Pachernegg et al. 2012; Pérez-Otaño et al. 2016). Compared to classical GluN1/2 NMDARs, they exhibit atypical biophysical properties such as smaller single-channel conductance (Perez-Otano et al. 2001); 10-fold lower Ca\(^{2+}\) permeability as estimated from single-channel recordings (Sasaki et al. 2002); and diminished sensitivity to Mg\(^{2+}\) block at hyperpolarized potentials (Sasaki et al. 2002; Roberts et al. 2009) (Fig. 3A and B). FRET-based assembly studies support a GluN1:GluN2:GluN3 stoichiometry of 2:1:1 (Schüler et al. 2008; but see Ulbrich & Isacoff, 2008). The identity of the GluN2 subunit varies: using biochemical or electrophysiological approaches GluN3A has been shown to complex with GluN2A or 2B in neurons (Das et al. 1998; Al-Hallaq et al. 2002; Nilsson et al. 2007; Larsen et al. 2011; Martínez-Turrillas et al. 2012; Pilli & Kumar, 2012; Savtchouk et al. 2019) and with GluN2C in oligodendrocytes (Káradóttir et al. 2005; Burzomato et al. 2010).

Because of their non-conventional properties GluN3A subunits have been hypothesized to act as negative regulators of classical NMDAR activity (Pachernegg et al. 2012; Kehoe et al. 2013; Pérez-Otaño et al. 2016). In a traditional view, postsynaptically located NMDARs detect coincident pre- and postsynaptic activity and couple calcium entry to intracellular signalling pathways that trigger long-lasting synaptic structural and functional plasticity (Hardingham, 2019). By contrast, GluN3A-NMDARs inhibit synapse plasticity and stabilization and have been shown to delay synapse maturation until the arrival of sensory experience and later target non-used synapses for pruning (Roberts et al. 2009; as summarized in Pérez-Otaño et al. 2016). This model is supported by: (1) restriction of GluN3A expression to immature synapses shown by EM; (2) the match of GluN3A expression and down-regulation with the timing of plasticity and refinements across different brain regions; (3) the control of synaptic GluN3A levels by activity and sensory experience. How this works at a mechanistic level is unknown and might involve impaired

**Figure 2. Subcellular distribution of GluN3A-NMDARs**

A, membrane distribution of GluN3A-containing (synaptic, peri- and extra-synaptic) and GluN3A-lacking NMDARs (mostly anchored to the PSDs via their C-terminal PDZ-binding motif). Diagram also illustrates the presence of GluN3A-NMDARs in presynaptic membranes next to astrocytes and the high rates of endocytosis driven by the adaptor PACSIN1 in mature neurons. B, post-embedding immunogold electron microscopy demonstrated that GluN3A is expressed at synaptic sites in CA3-CA1 hippocampus (arrows), but preferentially localizes at perisynaptic (100–120 nm from the edge of PSD) and extrasynaptic sites (>120 nm away from PSDs) (double arrows). Presynaptic GluN3A immunolabelling is also detected at MPP-GC synapses. C, quantification of GluN3A gold particle density as a function of distance to the PSD. Inset shows differential tangential distribution of GluN3A and GluN1 immunoparticles along the PSD of CA1 synapses. Note that labelling for GluN1 is highest at the centre of the PSD while GluN3A increases towards the PSD periphery. Panels B and C are adapted from Pérez-Otaño et al. (2006) and Savtchouk et al. (2019).
coincident detection due to the lesser Mg\(^{2+}\) block of GluN3A-NMDARs, inhibition of calcium-activated signalling cascades or coupling to distinct signalling adaptors (see below). Furthermore, a series of recent studies have brought to light other unconventional modes of NMDAR signalling that do not rely on ion flux or postsynaptic localization. As such, NMDARs can be dynamically regulated by synaptic activity even in the absence of Ca\(^{2+}\)-dependent functions or via presynaptic localization (summarized in Dore et al. 2017).

**Presynaptic GluN3A**

Despite the canonical view of NMDARs as postsynaptic coincidence detectors, presynaptic NMDARs have been reported in many locations of the nervous system (Bouvier et al. 2015; Banerjee et al. 2016; Wong et al. 2021). The first reports of a presynaptic GluN3A function (Larsen et al. 2011, 2014) demonstrated a role of (probably GluN1/2B/3A) in promoting spontaneous glutamate release and mediating spike-timing-dependent long-term depression (tLTD) at young visual cortex synapses (L4–L2/3). Loss of presynaptic GluN3A expression was associated to the developmental loss of tLTD, a form of plasticity thought to be involved in the refinement of visual maps (Larsen et al. 2011).

Presynaptic GluN3A-NMDARs have been more recently identified in juvenile mouse hippocampus (P17–P22) at PP synapses onto dentate gyrus granule cells (GCs) (Savtchouk et al. 2019). As in visual cortex, presynaptic GluN3A-NMDARs contained GluN2B subunits and increased glutamate release probability in a circuit-specific manner due to their selective localization at MPP (but not LPP) axons. LTP is strongly influenced by presynaptic release probability and was enhanced in Grin3a-null mice, which could be explained by the lack of basal ‘prepotentiation’ dependent on pre-NMDARs at GluN3A-null MPP-GC synapses and consequently increased dynamic range for LTP induction. Blocking local astrocyte Ca\(^{2+}\) signalling in wild-type controls reproduced the enhanced LTP at MPP synapses found in Grin3a knockouts (Savtchouk et al. 2019), implying astrocyte modulation in conferring differential release and plasticity properties to LPP-GC and MPP-GC synapses. As in visual L4–L2/3 synapses, GluN3A expression decays with age but the significant levels retained into

**Figure 3. Unique properties of GluN3A-containing receptors**

**A**, cation permeabilities of different types of GluN3A-containing complexes; the calcium permeability of both GluN1/2/3A triheteromers and GluN1/3A diheteromers is lower than conventional GluN1/2 receptors but the exact value for relative permeability of GluN1/3A receptors remains less well established. **B**, Mg\(^{2+}\) sensitivity is an electrophysiological signature of GluN1/2/3 NMDARs: at early developmental stages (P6-P8), genetic deletion of GluN3A increases NMDAR current rectification at CA1 hippocampal synapses due to loss of GluN3A-NMDARs, which exhibit reduced voltage-dependent Mg\(^{2+}\) block (left panel); at later stages when synaptic GluN3A is typically down-regulated, transgenically prolonging GluN3A expression (tgGluN3A) decreases NMDAR current rectification (right panel; adapted from Roberts et al. 2009). **C**, CGP-78608, an antagonist of the GluN1 glycine binding site, unmasks glycine currents mediated by GluN1/3A receptors (from Grand et al. 2018).
adulthood support a life-long role in setting different modes of information processing between LPP and MPP circuits. The presence of GluN3A, which confers low calcium permeability, has been suggested as an explanation for why many studies failed to detect presynaptic NMDAR-mediated calcium signals (Wong et al. 2021). It would also explain why presynaptic NMDARs can be activated tonically in the absence of axon firing or previous depolarization.

**GluN1/3A diheteromeric NMDARs**

Initial work in recombinant systems showed that GluN3A co-assembles with GluN1 in the absence of GluN2 subunits to form functional excitatory glycine receptors (Chatterton et al. 2002). These complexes are: (1) not activated by NMDA or glutamate, (2) insensitive to APV, a competitive antagonist at the glutamate binding site in GluN2 subunits, as well as to open-channel NMDAR blockers such as Mg$^{2+}$, memantine or MK-801, and (3) relatively Ca$^{2+}$-impermeable (Chatterton et al. 2002; Madry et al. 2010), but estimates vary (see Otsu et al. 2019) (Fig. 3A). Studies with selective antagonists and site-directed mutagenesis revealed that glycine binding to the GluN3A ligand binding domain (LBD) triggers channel opening and activation whereas binding to the GluN1 LBD causes rapid desensitization (Awobuluý et al. 2007; Madry et al. 2007).

Demonstrating the existence of GluN1/3A receptors in vivo proved difficult due to the rapid desensitization that derives from the GluN1 glycine binding site, combined with ambient levels of free glycine in brain slice preparations. Intriguingly, native GluN1/3A receptors were first observed in oligodendrocytes (OLs) of mice optic nerves rather than neurons, and specifically within myelin sheaths but not somas of OLs (Piña-Crespo et al. 2010). The exact role of these receptors in myelin remains a mystery but is in line with GluN3A expression in the OL lineage. Work on neuronal cultures later proposed a role in metaplasticity of excitatory hippocampal synapses by showing that, upon induction of chemical LTP, putative GluN1/3 receptors are recruited to enlarged synapses to facilitate depotentiation (Rozeboom et al. 2015).

Strong evidence has more recently emerged thanks to the use of CGP-78608, a competitive antagonist with pronounced preference for the glycine-binding site of GluN1 over GluN3A (Yao & Mayer, 2006; Grand et al. 2018). By preventing glycine binding to GluN1, CGP-78608 bypasses desensitization and unmasks large glycine-activated currents mediated by GluN1/3A receptors (Fig. 3C). Recordings of CA1 neurons in young (P8–P12) mouse hippocampal slices in the presence of CGP-78608 revealed a massive potentiation of glycine-induced inward currents in wild-type but not Grin3a knockout mice (Grand et al. 2018), clearly implicating GluN1/3A receptor function. Although expression of GluN3A is typically highest in young brains as discussed earlier, regions such as the MHb retain high levels into adulthood. Building from their initial study, the same group demonstrated the presence of GluN1/3 receptors in this region in adult mice (Otsu et al. 2019). In wild-type (but not GluN3A knockout) MHb neurons, glycine puffs increased firing rates and induced rapidly rising inward currents. GluN3A immunolabelling was detected in dendrites and somata of MHb neurons, often in juxtaposition to glial profiles. Selective expression of DREADDs in glial cells pointed towards astrocytes as the physiological source of glycine (Otsu et al. 2019), resembling the ability of this cell type to modulate specific circuit functions via presynaptic GluN3A-NMDARs (Savtchouk et al. 2019). Aptly, a specific requirement for MHb GluN1/3 receptors was found in behavioural tests for the ability to acquire conditioned place-aversion, a readout that depends on this region.

**Pharmacology**

The lack of pharmacological reagents has hampered our understanding of the physiological roles of NMDA or glycine GluN3A-containing receptors, particularly triheteromeric GluN1/2/3, given the difficulty of expressing a pure population in cell lines. Among the limited information available, GluN3A-NMDARs have been shown to be blocked by the general NMDAR antagonist APV (see Sasaki et al. 2002 and Larsen et al. 2014 for pre-GluN3A-NMDARs), and are less sensitive to open-channel blockers such as memantine or MK-801, in line with their reduced block by Mg$^{2+}$ block (McClymont et al. 2012). More extensive compound screening using glycine-activated GluN1/3A receptors has led to the identification of GluN3A- and GluN3B-competitive (TK80) and non-competitive (TK13 and TK30) antagonists with modest (5- to 10-fold) preferences for GluN1/3A or 3B vs GluN1/2 receptors (Kvist et al. 2013) and a remarkably selective negative allosteric modulator, EU1180-438 (Zhu et al. 2020) (Table 1). EU1180-438 produced robust inhibition of native GluN1/3A glycine-activated currents in hippocampal CA1 pyramidal neurons (Zhu et al. 2020). This work demonstrates that the structural differences between the glycine-binding sites of GluN1 and GluN3 can be exploited to develop GluN3A-selective ligands (Kvist et al. 2013). Information regarding these ligands’ effects on GluN1/2/3A triheteromeric receptors is lacking.

**GluN3A modulates plasticity and cognition**

Structural plasticity allows stable rewiring of synaptic networks through the formation of new connections
and the stabilization of specific contacts with pruning of others. Converging lines of evidence place GluN3A as a critical modulator of functional and structural plasticity. First, regulated GluN3A expression has been shown to control the timing of long-lasting forms of plasticity implicated in the opening and closure of critical periods of postnatal development. At CA3–CA1 hippocampal synapses, ablation of GluN3A accelerates the developmental onset of LTP while prolonging expression beyond the physiological window reduces the magnitude of LTP (Roberts et al. 2009). At L4–L2/3 visual cortex synapses, developmental down-regulation of GluN3A correlates with reductions in tonic glutamate release and ability to induce presynaptic tLTD, both processes linked to the stabilization of sensory maps (Larsen et al. 2011; Feldman, 2012). Genetic deletion or overexpression of GluN3A was sufficient to accelerate or delay the plasticity loss. Crucially, sensory input was identified as a driving force for down-regulation of GluN3A and the associated shifts in functional properties of visual cortex synapses (Larsen et al. 2014). In both preparations, loss of GluN3A allows a switch to GluN1/2 NMDARs that changes plasticity properties (GluN1/2A: Henson et al. 2012; GluN1/2B: Larsen et al. 2014). Further work will be required to establish whether this is a general mechanism that operates at other synapses to control different modes of developmental plasticity, as suggested by the conserved profile of GluN3A down-regulation in primary cortices.

Second, studies in vivo and with organotypic slices showed that GluN3A inhibits the long-lasting stabilization of excitatory synapses by plasticity-inducing stimuli and regulates the number of synapses and associated dendritic spines without modifying initial spine formation (Roberts et al. 2009; Kehoe et al. 2014). Inhibition of actin cytoskeleton remodelling by direct binding to the postsynaptic scaffold and actin regulator GIT1 has been implicated (Fiuza et al. 2013). Whether GluN3A also modulates NMDAR-dependent gene expression remains largely unexplored, but a recent study in cortical cultures suggests that GluN3A might interfere with MEF2C-mediated transcription (Chen et al. 2020).

Genetically modified mice have provided some initial insight into how GluN3A expression affects behavioural output. Young (3- to 4-week-old) Grin3a knockout mice exhibit increased pre-pulse inhibition (Brody et al. 2005), an indicator of sensorimotor gating related to schizophrenia but the phenotype fades later on. Enhanced object recognition and spatial learning have been reported in adult Grin3a knockouts (Mohamad et al. 2013), in line with human studies that correlate enhanced cognitive performance with low GluN3A levels (Sadat-Shirazi et al. 2019). Whether this is a consequence of altered developmental plasticity or reflects adult roles of GluN3A in cognition is yet to be established, but will be of paramount importance for the development of GluN3A-based therapeutics (see below). The retention of adult GluN3A expression in areas implicated in higher cognitive processing with strong requirements for plasticity and input control supports the latter, as does the observation that elevating GluN3A levels in adult brain is sufficient to impair memory consolidation (Roberts et al. 2009). Also, genetic variations in GRIN3A influence prefrontal cortex activation during attention tasks and consolidation of episodic memories (Gallinat et al. 2007; Papenberg et al. 2014). Additional studies report altered odour discrimination (Lee et al. 2016) and social deficits (Lee et al. 2018) in constitutive Grin3a knockouts, consistent with high GluN3A levels in olfactory areas and defects in oxytocin signalling in the prefrontal cortex. CRISPR-generated Grin3a knockouts are short-sleepers and display lower transitions from awake to sleep states, possibly reflecting GluN3A expression in brain centres mediating wakefulness (Sunagawa et al. 2016; Murillo et al. 2021). Finally, glycine-gated GluN1/3A receptors in the MHB have been implicated in the control of aversive behaviours (Otsu et al. 2019).

### GluN3A/Grin3a disease links

Genetic and expression data are unveiling connections between brain diseases and GluN3A which implicate this subunit not only in neurodevelopmental processes, but

| Ligand     | Functional property               | GluN1/3A | GluN1/3B | GluN1/2A | Reference          |
|------------|-----------------------------------|----------|----------|----------|--------------------|
| EU1180-438 | Negative allosteric modulator     | 1.8 μM   | 2.2 μM   | N.E.     | Zhu et al. (2020)  |
| TK13       | Non-competitive antagonist         | 67 μM    | 49 μM    | >300 μM  | Kvist et al. (2013) |
| TK30       | Non-competitive antagonist         | 14 μM    | 7.4 μM   | 270 μM   | Kvist et al. (2013) |
| TK80       | GluN3B competitive antagonist     | N.E.     | 79 μM    | >300 μM  | Kvist et al. (2013) |

Inhibition measured in Xenopus oocytes expressing the indicated recombinant receptor. The compounds were co-applied with 100 μM glutamate and 0.5 μM glycine. N.E. no significant effect. Maximal inhibition is 100%. IC50 > 300 μM indicates that the compound showed some inhibition but less than 50% at 300 μM.
also in disorders where adult reactivation of GluN3A expression reinstates juvenile modes of plasticity thought to underlie many of the synaptic and behavioural alterations (Table 2).

Schizophrenia

One of the most prominently GluN3A-associated disorders is schizophrenia, a psychiatric disease characterized by delusions, hallucinations and impaired cognition and where aberrant synapse pruning is a key neuropathological feature. Elevated Grin3a transcripts have been found in schizophrenic brains (Mueller & Meador-Woodruff, 2004) and prepulse inhibition (PPI) (Brody et al. 2005), a behavioural readout strongly related to schizophrenia, is altered in Grin3a knockout mice. Human genetic studies have reported altered prevalence of common and rare GRIN3A variants in schizophrenia patients (Table 2), as well as a linkage peak on chromosome 9 close to the GRIN3A locus in a schizophrenia family study (Greenwood et al. 2016), correlated to specific cognitive traits in patients. GRIN3A variants have also been associated with post-operative delirium (Kazmierski et al. 2014), a disorder sharing major features with schizophrenia.

Bipolar disorder

Early studies found reduced mRNA and protein levels for GluN3A in the frontal cortex of bipolar disorder (BD) patients (Mueller & Meador-Woodruff, 2004; Rao et al. 2010). Later genetic work identified a GRIN3A SNP as the top associated variant with severely affected patients (suicide attempters) versus patients with milder symptoms (Table 2). A further connection was suggested by the finding that Grin3a was among the top three down-regulated genes in neurons treated with widely prescribed BD drugs (Kidnapillai et al. 2020).

Neurodevelopmental disorders

Autism spectrum disorder (ASD) is thought to arise from incorrect configuration of circuits due to altered synapse refinement and resulting excitation/inhibition imbalances (Peça & Feng, 2012). Deficits in social behaviour tasks, a hallmark of ASD (Lee et al. 2018), have been observed in Grin3a knockout mice alongside decreases in oxytocin signalling in the prefrontal cortex, correlating with claims that oxytocin treatment improves symptoms in ASD patients (Gordon et al. 2013). Interestingly, a study that also looked at schizophrenia concurrently found some rare missense GRIN3A variants in ASD patients (Yu et al. 2018), disorders with overlap in symptoms and genetic risk factors. A recent meta-analysis combining RNA-seq and microarray data from multiple ASD mouse models with human brain transcriptional datasets identified Grin3a as a hub gene within the ‘Juvenile-Cortex’ and ‘Adult-Hippocampus’ gene networks (Duan et al. 2020). Single cell transcriptomics in cortex samples from human patients with epilepsy, another primarily neurodevelopmental disorder, showed major upregulation of GRIN3A (Pfisterer et al. 2020). Upregulation was particularly evident in certain L5/6 excitatory neurons and two SST interneuron subtypes.

Addiction and hedonistic behaviour

Substance addictions are chronic and relapsing disorders that are extremely dangerous to personal and public health. Next-generation sequencing (NGS) or genome-wide association (GWA) approaches have placed GluN3A centre-stage in the aetiology of nicotine and alcohol addiction (Chen et al. 2018). GRIN3A mRNA levels are increased in the hippocampus and orbitofrontal cortex of individuals with alcoholism (Jin et al. 2014). Risk of nicotine dependence has been linked with a number of rare non-synonymous variants in GRIN3A, as shown in a series of studies in European, African-American and Chinese Han populations (Table 2). Moreover, GRIN3A gene variants or changes in GluN3A expression have been associated with addiction to illicit drugs such as heroin and opioids (Table 2) (Roozafzoon et al. 2010; Liu et al. 2021). In mouse studies, a single cocaine injection drives the insertion of GluN3A-NMDARs at synapses in reward-related regions with subsequent recruitment of calcium-permeable AMPARs, a form of adaptive plasticity involved in relapse (Yuan et al. 2013). Chronic methamphetamine also enhances GluN3A expression, reducing cortical plasticity and impairing motor learning (Huang et al. 2017).

Behavioural addictions are disorders analogous to substance addiction, in which there is a behavioural core based on repeated performances. The analysis of mRNA levels of different NMDAR subunits in human blood lymphocytes surprisingly revealed a reduction in GRIN3A mRNA levels in computer game addicts (Sadat-Shirazi et al. 2018). Dysregulated Grin3a expression has also been observed during transgenerational transmission of hedonistic and addictive behaviours such as increased preference of palatable foods (Särker et al. 2019).

Neurodegeneration

Reactivation of GluN3A expression in adult medium-spiny neurons of the striatum has been documented in human and mouse models of Huntington’s disease (HD) (Marco et al. 2013; Wesseling
| dbSNP ID | dbSNP allele | Amino acid change | Domain | Phenotype | Disease related | Reference |
|----------|-------------|------------------|--------|-----------|----------------|-----------|
| -        | G > A       | Val132Leu        | Extracellular (NTD) | Possibly damaging | Nicotine dependence | (Yang et al. 2015) |
| rs556419599 | C > T | Asp133Asn        | Extracellular (NTD) | — | Schizophrenia (SCZ) | Shen et al. (2009) |
| rs769491656   | G > A, C, T | Arg137Ser       | Extracellular (NTD) | Disease causing | Autism spectrum disorder (ASD) | Yu et al. (2018) |
| rs773593066   | G > A       | Arg337Trp       | Extracellular (NTD) | Possibly damaging | ASD, SCZ | Yu et al. (2018) |
| rs10989591    | C > A, T    | Val362Met       | Extracellular (NTD) | Higher P300 amplitude | Prefrontal cortex-dependent-memory | Gallinat et al. (2007); Papenberg et al. (2014) |
| -           | A > C       | Val389Leu       | Extracellular (NTD) | Possibly damaging | Nicotine dependence | (SCZ) | Yang et al. (2015) |
| rs200120504  | C > T       | Val389Ile       | Extracellular (NTD) | Disease causing | SCZ | Yu et al. (2018) |
| rs34755188   | C > T       | Arg480His       | Extracellular (NTD) | Possibly damaging | Nicotine dependence | (SCZ) | Yang et al. (2015) |
| rs149729514  | G > A, C    | Arg480Gly       | Extracellular (NTD) | Probably damaging | SCZ | Shen et al. (2009); Takata et al. (2013) |
| rs189425146  | T > C       | Lys488Glu       | Extracellular (NTD) | Disease causing | ASD, SCZ | Yu et al. (2018) |
| -           | C > T       | Gln508*         | Extracellular (NTD) | Patient with catatonic SCZ, inherited from mother in SCZ spectrum | SCZ | Tarabeux et al. (2011) |
| rs75981117   | T > C       | Asn549Ser       | Extracellular - glycosylation site (LBD S1) | Possibly damaging | Nicotine dependence, Bipolar suicide attempting | Yang et al. (2015); Gaynor et al. (2016) |
| rs10989563   | C > T       | Asp835Asn       | Extracellular (LBD S2) | Susceptibility for Alzheimer’s disease (AD) pathogenesis | AD | Liu et al. (2009) |
| -           | C > A       | Gly898Trp       | Extracellular (LBD S2) | Possibly damaging | ASD | Yu et al. (2018) |
| -           | C > T       | Arg1024*        | Intracellular (CTD) | Possibly damaging | SCZ | Shen et al. (2009) |
| rs3739722    | C > T       | Arg1041Gln      | Intracellular (CTD) | AD susceptibility, increased risk of cerebrovascular disease | AD, dementia, cerebral palsy, non-substance-abuse delirium | Liu et al. (2009); Cacabelos et al. (2012); Costantine et al. (2012); Kazmierski et al. (2014) |
| -           | G > C       | Gln1091His      | Intracellular (CTD) | Possibly damaging | SCZ | Shen et al. (2009) |

(Continued)
Table 2. (Continued)

| dbSNP ID     | allele | Amino acid change | Domain          | Phenotype                      | Disease related                | Reference                  |
|--------------|--------|-------------------|-----------------|-------------------------------|-------------------------------|----------------------------|
| rs10121600   | C > T  | -                 | Intronic        | Possibly damaging             | Nicotine dependence           | Ma et al. (2010)           |
| rs11788456   | G > A  | -                 | Intronic        | Possibly damaging             | Nicotine dependence           | Ma et al. (2010); Yang et al. (2015) |
| rs1323423    | T > A, C, G | - | Intronic | Surrounding DNA region harbours an enhancer element | Nicotine dependence | Chen et al. (2019) |
| rs17189632   | T > A, G | - | Intronic | Possibly damaging             | Nicotine dependence, heroin addiction | Ma et al. (2010); Yang et al. (2015); Xie et al. (2016) |
| rs2067056    | T > C  | -                 | 5’ UTR          | Upstream transcript variant    | Nicotine dependence           | Chen et al. (2019)         |
| rs2485530    | C > G, T | - | Intronic | Possibly damaging             | Bipolar suicide attempting    | Gaynor et al. (2016)        |
| rs45537432   | C > G, T | - | Intronic | Possibly damaging             | Bipolar suicide attempting    | Gaynor et al. (2016)        |
| rs7030238    | A > C  | -                 | 3’ UTR          | Possibly damaging             | Nicotine dependence           | Ma et al. (2010)           |

*dbSNP is a NCBI public database for human single nucleotide polymorphisms. *Non-sense mutation. NTD; amino terminal domain; LBD, ligand-binding domain; CTD, carboxy-terminal intracellular domain.

& Pérez-Otaño, 2015; Mahfooz et al. 2016). The disease mechanism involves sequestration by mutant huntingtin of the GluN3A-selective adaptor PACSIN1, leading to accumulation of GluN3A-NMDARs at the cell surface of striatal neurons and age-inappropriate synapse pruning (Marco et al. 2013). Suppression of aberrant GluN3A expression by genetic deletion or AAV-mediated RNAi in HD mouse models was able to prevent and even reverse disease phenotypes, including striatal dendritic spine loss and motor performance (Marco et al. 2013, 2018). Also, GRIN3A genetic variants have been associated with increased risk of developing Alzheimer’s disease (Table 2).

**Summary and future directions**

Research into GluN3A continues to reveal new and surprising functions for this unusual NMDAR subunit. The expanding knowledge of GluN3A expression is transforming our perception, and paves the way to a better understanding of its roles in neurodevelopment and adult functions. Of note, the remarkable correlation between GluN3A expression in the mature brain and functional hierarchy adds particular weight to argument for the importance of this subunit in the adult beyond its better recognized functions in controlling developmental plasticity. An open question is how such specific spatio-temporal patterns of GluN3A are achieved between areas, cell types and cellular compartments? Also, how do these patterns influence the wider assembly and function of circuits, and how is behavioural output affected by local or collective expression across the brain?

The continued support for presynaptic GluN3A in long-term plasticity at very particular circuits and synapses (Savtchouk et al. 2019) raises questions on how exactly presynaptic GluN3A-NMDARs mediate their function. Is it via ionotropic or metabotropic mechanisms? Is there preferential trafficking regulation to axons in certain cell types? Why do astrocytes appear to be important for the function of synapses containing GluN3A-NMDARs presynaptically, as well as postsynaptically in certain situations? (Otsu et al. 2019). The evidence for functional roles of excitatory glycine-gated GluN1/3A receptors in the CNS and their effects on behaviour are especially compelling (Grand et al. 2018; Otsu et al. 2019), but there is still much to learn. Do these receptors co-exist with non-conventional GluN1/2/3A receptors in terms of temporal, regional or cell type patterns? What governs their abundance in certain cell types or subcellular compartments? What synaptic and circuit properties do they provide to neurons? Are these the same in all locations where they exist?

The increasing breadth of studies documenting the effects of GluN3A on forms of plasticity and multiple...
readouts including sensory processing, social cognition, sleep and learning place GluN3A as an important regulator of various behaviours and add insight to some of the disease links for this gene. More extensive behavioural profiling in transgenic mice in which GluN3A is removed at specific developmental times should allow us to distinguish defects caused by altered development from GluN3A functions within the adult brain. Finally, the long and diverse list of diseases from psychiatric and addiction disorders to neurodevelopmental conditions and neurodegeneration that have continued over recent years to show links to GluN3A demonstrates the remarkable brain-wide impacts of this unusual NMDAR subunit. Future studies in disease models, as well as new tools, are required to elucidate how GluN3A influences the various diseases it is linked to, which could lead to direct therapies for these disorders, as hinted at for HD (Marco et al. 2018). Such work may additionally provide avenues for improving symptoms in other conditions where affected brain regions and phenotypes overlap with the diverse roles for GluN3A.

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**Additional information**

**Competing interests**

None declared.

**Author contributions**

All authors researched and wrote up their own specific sections, after which all authors edited the full document together. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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