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Presynaptic NMDA Receptors Also Make the Switch

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In spike timing-dependent plasticity (STDP), the precise temporal association of pre- and post-synaptic spiking events bidirectionally regulates synaptic strength. STDP is important for the maturation of neuronal circuits and could be altered in disease states. Due to their unique gating properties, both pre- and post-synaptic NMDA receptors function as excellent coincidence detectors, and NMDARs are known to modulate spike-timing long-term potentiation (tLTP) and depression (tLTD) at numerous cortical synapses (1). In the primary visual cortex, there is a developmental switch at visual cortical synapses that reduces spontaneous glutamate release and the ability to induce tLTD (2), for which the molecular basis has been unknown. Utilizing an impressive combination of genetic, pharmacological and histological approaches, Philpot and colleagues (3) now report how the regulated expression of the NR3A subunit can account for a developmental switch of pre-synaptic NMDAR (preNMDAR) composition and function, and show that ablation of NR3A subunit prevents the induction of tLTD at young visual cortex layer 4 to layer 2/3 synapses (L4-L2/3). While this specific molecular mechanism to regulate preNMDA-dependent STDP may not be universal, these findings open new exiting avenues for understanding at the molecular level how pre- and post-synaptic NMDARs cooperate as coincident detectors to regulate STDP at certain synapses.

NMDARs are heterotetramers comprised of two obligatory NR1 subunits and two NR2 and/or NR3 subunits (4). They are well suited to function as coincident detectors because their gating requires the binding of glutamate, as well as membrane depolarization to release a magnesium (Mg^{2+}) ion that blocks the channel pore at negative resting potentials. Among the receptor properties conferred by NR2 and NR3 subunits are the affinities for glutamate and extent of voltage-dependent Mg^{2+} block. The NR2 subunits bind glutamate, whereas NR1 and NR3 subunits bind the co-agonist glycine. Heterodimeric receptors containing NR2A and/or NR2B have high affinity for Mg^{2+} , while those incorporating with NR2C, NR2D or NR3 subunits are less sensitive to block by Mg^{2+} . Because NR2 and NR3 subunits also affect channel open duration, NMDAR subunit composition critically determines channel gating and extent of calcium ion permeation that is important for facilitating neurotransmitter release by preNMDARs and modulating synaptic plasticity during development at both sides of the synapse (5, 6). Elucidation of NMDAR composition in vivo has been hampered by the lack of specific pharmacological reagents that selectively target distinct NMDARs heteromers, particularly those imparting low Mg^{2+} sensitivity.

After a brief temporal window that coincides with sensory experience, there is a developmental switch at visual cortical synapses that reduces spontaneous glutamate release

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Demonstrating regulation of glutamate release by NR3A subunit-containing presynaptic NMDA receptors, the study by Larsen et al. shows that a subunit switch in receptor composition underlies the developmental loss of spike timing-dependent long-term depression at cortical synapses.

dependent on preNMDAR opening and induction of tLTD, processes generally associated with stabilization of sensory maps (2). A common property of preNMDARs in slice preparations, particularly at L4-L2/3 synapses, is that they facilitate spontaneous and evoked neurotransmitter release more effectively and can exhibit reduced sensitivity to Mg^{2+} block during early postnatal development (1, 5). Taking this into account, Larsen *et al.* began by analyzing the relative expression of NMDAR subunit protein at different ages – before (< postnatal days 20) and after (PN>23) – and found that expression of NR3A protein paralleled quite closely the loss of preNMDAR-dependent spontaneous neurotransmitter release at L4-L2/3 synapses. This suggested that NR3A subunit is a likely culprit for the developmental loss of tonic preNMDAR activity. Traditionally there has been a predilection to consider a role of NR2C- or NR2D-containing NMDARs when studying receptors with reduced sensitivity to voltage-sensitive Mg^{2+} block, so the potential role for NR3A, as suggested by this subunits expression profile, was novel and unexpected.

To delineate the potential role of NR3 in preNMDARs at L2/L3 neurons during development, the authors first measured spontaneous mEPSCs in cortical slices prepared from NR2A and NR2D knockout mice of different ages in conditions that blocked postNMDARs by including the activity-dependent NMDAR blocker MK-801 in the intracellular recording solution (See Fig. 1A). The contribution of either subunit, especially NR2D, was excluded because spontaneous mEPSCs in NR2A and NR2D knockouts were unaltered as compared to their wild-type littermate controls. In stark contrast, spontaneous mEPSCs in young NR3A null mice were significantly reduced and similar to those measured in adults, strongly suggesting that early in development, L4 synaptic terminals harbor NR3A-containing preNMDARs. The possible contribution of NR2C subunits was not considered because its expression is undetectable in cortical neurons using highly sensitive techniques (7).

Three additional types of experiments strongly support the conclusion that an NR3A subunit switch in preNMDARs regulates spontaneous and evoked glutamate release during development. First, the authors show that transgenic mice that begin to over-express NR3A in excitatory neurons at a time that approximately coincides with downregulation of the NR3A gene in the visual cortex exhibit preNMDAR-driven spontaneous mEPSC in L2/3 neurons into adulthood. Second, paired-pulse ratios of evoked EPSPs during early development change from facilitating to depressing in NR3A null mice, similar to ratios in adult mice. Last, NR3A immunoreactivity is observed at presynaptic terminals of L2/3 asymmetric synapses, close to active zones, in cortical sections from young mice; an earlier study by the authors failed to detect presynaptic NR3A immunoreactivity in sections from adult mice.

Do preNMDARs at L4-L2/3 synapses include other subunits? Based on pharmacological experiments using NR2A- and NR2B-selective antagonists, preNMDARs at somatosensory, entorhinal and visual cortical synapses are thought to be NR2B-containing (reviewed in (6)). Because NMDARs comprised of NR1 and NR3A are gated by glycine, and not glutamate, L4-L2/3 preNMDARs could be expected to contain NR2B (4). Indeed, Larsen *et al.* found that the NR2B antagonists Ro-25-6981 and ifenprodil reduced preNMDAR-mediated spontaneous mEPSC frequency in slices from young mice. Intriguingly, removal of extracellular Mg^{2+} uncovered the potential existence of NR2B-containing preNMDARs in slices from older mice, suggesting that tonic glutamate release at mature L4-L2/3 synapses is regulated by extracellular Mg^{2+} levels.

Based largely on the aforementioned findings, the authors conclude that at young L4-L2/3 synapses preNMDARs are triheteromeric complexes comprised of NR1, NR2B and NR3A, but switch to NR1 and NR2B-containing diheteromeric receptors during synaptic maturation

(See Fig. 1B). Therefore, developmental changes of NMDAR subunit composition can occur at both sides of the synapse. Given the similarities in preNMDAR developmental expression and plastic properties of ascending excitatory synapses onto L2/3 pyramidal neurons at different cortical areas, in contrast to parallel within layer synapses (8), it will be interesting to determine the universality of the NR3A subunit switch and its potential to regulate the specificity of synaptic connections onto L3/3 neurons.

The developmental preNMDAR subunit switch coincides with a loss of tLTD, a mechanism that has been associated with the refinement of tectal maps in response to visual experience (9). Interestingly, Larsen *et al.* report that tLTD cannot be induced at L4-L2/L3 synapses in slices from young mice either treated with Ro-25-6981 or obtained from NR3A knockout mice. In contrast, tLTD is unaffected at these synapses in NR2D null mice, suggesting that the switch from NR3A/NR2B-containing trimeric preNMDARs to dimeric NR2B-containing receptors underlies the developmental loss of tLTD at L4-L2/L3 synapses.

Why synapses lacking NR3A fail to induce tLTD is not evident from this work. A different study recently reported that maximum “large” Ca^{2+} accumulation and facilitation of transmitter release occurs at presynaptic Schaffer collateral buttons when preNMDAR autoreceptors are selectively activated at theta frequencies (10), suggesting that matching of network frequency with preNMDAR properties may be important for synaptic integration and modulating plasticity. It remains to be tested if NR3A-containing preNMDARs are necessary for regulating the temporal integration of stimuli and/or calcium accumulation to modify the probability of neurotransmitter release. Additionally, NR3A-containing preNMDARs may synergize with other types of mechanisms that detect coincident activity between pre- and post-synaptic spiking, as previously reported for the role of retrograde cannabinoid signaling in tLTD at visual (11) and somatosensory (12) cortices.

The work by Larsen *et al.* raises a series of intriguing questions relevant to the potential role of NR3A-containing preNMDARs, not only in the developing visual cortex, but also in the adult brain. A common feature at visual, sensory and entorhinal cortical synapses is the progressive loss of preNMDAR-mediated spontaneous glutamate release during development (5); however, the reduction of preNMDAR function may not be irreversible. Expression of preNMDARs re-emerges at adult synapses following pilocarpine-induced chronic seizures (13), and some anticonvulsant drugs (i.e. felbamate) target these receptors selectively ((14)). If the developmental regulation of preNMDARs in the visual and entorhinal cortices is similar, it suggests NR3A expression is reversible and may be a potential molecular target for existing (14) or future anticonvulsant drugs.

Another interesting question raised by the study of Larsen *et al.* is if NR3A-containing preNMDARs are expressed at heterologous terminals (i.e. GABAergic)? The present study has focused on preNMDARs at asymmetric glutamatergic synapses where they function strictly as autoreceptors. However, pre-NMDARs are found in axons and terminals of GABAergic neurons in the cortex and cerebellum (6). In GABAergic neurons heterosynaptic preNMDARs function to coordinate glutamate and GABA release and consequently regulate excitatory:inhibitory balance. The role of preNMDARs on GABA terminals is of fundamental interest for understanding mechanisms that regulate experience-dependent refinement of neuronal connections and excitatory:inhibitory balance, which has important implications cognitive functions altered in psychiatric disorders. To date, most theories of the association of NMDAR hypofunction with schizophrenia have focused on postsynaptic, but not presynaptic, receptors (see (15)). Taking into account the importance of preNMDAR function for bidirectional STDP, modulation of neurotransmitter release and regulation of excitatory:inhibitory balance, and the possibility that preNMDARs can re-emerge under maladaptive conditions, it is important that future studies on the association of

NMDAR hypofunction with cognitive functions and schizophrenia consider that switches in NMDAR composition and function can occur at both sides of the synapse.

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Figure 1. Measurements of preNMDAR activity and the developmental switch of receptor subunits that coincide with loss of tLTD

NMDARs are expressed pre- and post-synaptically. **A)** In order to measure preNMDAR-mediated mEPSCs, recordings are performed from postsynaptic neurons using intracellular solutions containing MK-801 to selectively block postNMDARs (modified from (2)). Comparison of mEPSC frequency before (*middle panel*) and after (*right panel*) addition of APV to the recording bath identifies tonic glutamate release facilitated presumably by preNMDARs; additional experiments are required to unequivocally determine that changes in mEPSC frequency and plasticity originate from NMDARs located presynaptically and not from glia or axons (see (5, 6)). **B)** Reduction of spontaneous glutamate release during development (*upper*) and loss of tLTD (*bottom*) coincide with a subunit switch of NR3A-containing triheteromeric receptors (NR1, NR2B, NR3A) to diheteromeric (NR1, NR2B) preNMDARs at visual cortex L4 synaptic terminals onto L2/3 neurons (3). tLTD is induced by sequentially associating stimuli via electrodes 2 and 1 in the post- and pre-synaptic cells, respectively. Induction of tLTD is observed at young L4-L2/3 synapses (*left*), but not older synapses (>P23; *right*), following the NR3A subunit switch (3). The observation that preNMDAR activity reappears at adults synapses following chronic seizures (13), questions the potential reversibility of the developmental NR3A subunit switch at preNMDARs.