COMMENTARY

Presynaptic NMDARs and astrocytes ally to control circuit-specific information flow

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The entorhinal cortex (EC) conveys spatial, limbic, and sensory information to the hippocampus, which performs critical brain functions, including learning and memory processes and spatial information coding. Axons from superficial [layer (L)2] EC neurons make excitatory synapses onto granule cells (GCs) of the hippocampal dentate gyrus (DG), which prepare the information for further processing in other hippocampal regions (1). Afferents from the lateral and medial perforant path (LPP and MPP, respectively) convey different aspects of information to the DG, with the former related more to sensory information, and the latter to spatial location and limbic signals related to attention and motivation. They also have distinct patterns of input, contacting the outer (LPP) or middle (MPP) third of the molecular layer of the DG, and exhibit different functional properties (1, 2). The mechanistic bases of these differences are unknown and represent a challenge to understand circuit-specific biological computations as well as susceptibility to pathological insults. In PNAS, Savtchouk et al. (3) identify presynaptic *N*-methyl-D-aspartate receptors (pre-NMDARs) as a source of the differences in information processing between MPP and LPP fibers.

Previous work by the group (4) demonstrated that glutamate released by astrocytes enhances the strength of PP-GC synapses. The effect seemed to be mediated by pre-NMDARs, which increased the probability of transmitter release and contained the glutamate receptor subunit GluN2b. However, the work made no distinction between LPP and MPP contributions and left unsolved an apparent incongruence: GluN2b subunits confer high voltage-dependent Mg^{2+} block to NMDAR channels, but pre-NMDARs at PP-GC synapses could be activated without previous depolarization or in the absence of action potential firing in the axons. The current work (3) demonstrates that this is possible because of the additional presence in the receptor channel of the atypical GluN3a subunit, which largely relieves NMDAR dependence on Mg^{2+} block (5). Combining patch-clamp electrophysiology and high-resolution immunogold electron microscopy in wild-type and GluN3a knockout (KO) mice, Savtchouk et al. (3) report that the observed increase in glutamate release probability is circuit specific and occurs at MPP (but not LPP) fibers due to an anatomical difference: the selective expression of pre-NMDARs containing GluN3a subunits (GluN3a–pre-NMDARs) at MPP axons contacting GCs. Beautiful electron microscopy images show how GluN3a–pre-NMDARs are located in presynaptic terminals, away from synaptic clefts, and often face astrocytic membranes. The authors additionally demonstrate that pre-NMDARs decrease in number with age and control the dynamic range of long-term potentiation (LTP) at MPP-GC synapses (Fig. 1).

Savtchouk et al. (3) performed state-of-the-art experiments, simultaneously recording patch-clamped GCs and neighboring astrocytes in the molecular layer of hippocampal slices. Because the previously described increase in glutamate release probability (4) was not demonstrated by localized activation of NMDARs at PP-GC synapses, they applied brief local puffs of NMDA to the presynaptic compartment and recorded miniature excitatory postsynaptic currents (mEPSCs) in conditions that minimize activation of postsynaptic NMDARs in GCs; one experimental advantage of this synapse is that axonal and somatodendritic compartments are distant. They observed a clear increase in mEPSC frequency without change in amplitude, consistent with increased presynaptic release probability of glutamate resulting from activation of pre-NMDARs.

In a variety of brain areas, pre-NMDARs have been proposed to work as autoreceptors to tonically control the spontaneous release of glutamate (6, 7). To test this possibility, Savtchouk et al. (3) applied the broad-spectrum NMDAR antagonist D-2-amino-5-phosphonovalerate to the bath. However, no change in the frequency or amplitude of mEPSCs was observed, ruling out such a role for pre-NMDARs at PP-GC synapses.

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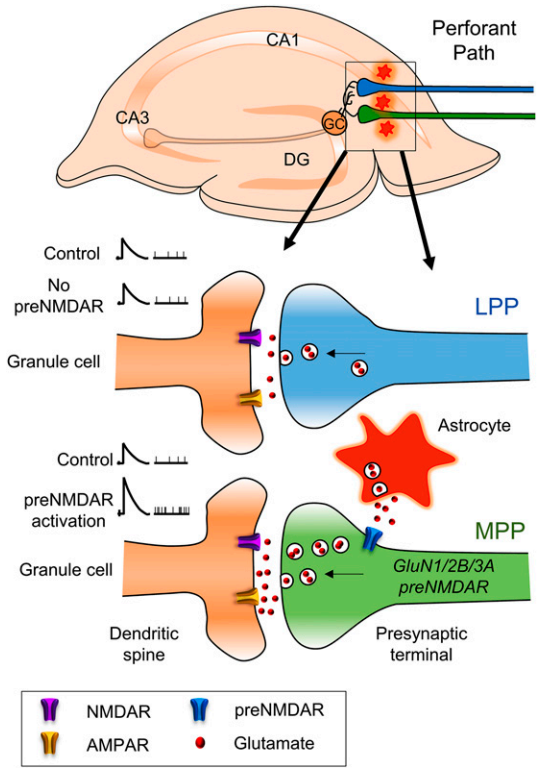

Fig. 1. Heterotrimeric pre-NMDARs containing GluN1/GluN2b/GluN3a subunits are specifically located at medial (but not lateral) entorhinal axonal inputs to the GCs of the hippocampal DG (MPP-GC synapses). These receptors are activated by a gliotransmitter (probably glutamate) released after stimulation of astrocytes situated in close apposition to pre-NMDARs. The activation of these pre-NMDARs produces an increase in glutamate release, thus strengthening synaptic transmission at this synapse. AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

It is important to note that spontaneous activity measurements do not allow distinguishing MPP-GC from LPP-GC synapses, and the specific activation of MPP and LPP axons is not trivial. To target electrical stimulation toward MPP or LPP inputs, Savtchouk et al. (3) placed the stimulating electrodes in the middle or outer third of the dentate molecular layer, respectively, and monitored evoked EPSC (eEPSC) activity. In separately stimulating these pathways, the addition of the GluN2b-selective antagonist ifenprodil decreased the amplitude of eEPSCs and increased paired-pulse facilitation ratios (PPRs) at MPP (but not LPP) synapses onto GCs, suggesting that modulation of glutamate release by GluN2b–pre-NMDARS is specific for MPP-GC synapses. While technically challenging, one approach that would directly distinguish between pre-NMDAR actions on spontaneous and evoked responses at these 2 different synapses would be to load presynaptic terminals with the open-channel NMDAR blocker MK-801 and study differences in spontaneous activity and evoked responses with paired recordings of synaptically connected neurons (8, 9).

Synapse and circuit selectivity is one of the most interesting findings of this work (3) and emerges as a general rule for pre-NMDARs. Selective expression of pre-NMDARs in medial (but not lateral) afferents from the EC to the hippocampus endows 2 bunches of axons (that have different origins but impinge on the same type of cell) with unique synaptic transmission properties and the ability to modulate information flow in response to specific stimuli. Analogous selectivity has been described in the visual cortex (10), where L5 pyramidal neurons that connect with neighboring L5 cells express pre-NMDARs, whereas L5 cells contacting basket cells do not. Another example is the somatosensory cortex, where pre-NMDARs mediate presynaptic forms of plasticity such as spike-timing dependent long-term depression (t-LTD) at subsets of synapses (11). At L4-to-L2/3 synapses, pre-NMDARs mediate presynaptic t-LTD, whereas at L2/3-to-L2/3 synapses, NMDARs are postsynaptic and mediate postsynaptic t-LTD. It might be of interest to determine whether MPP-GC synapses bearing pre-NMDARs also express a form of presynaptic t-LTD.

Because GluN2b–pre-NMDARs cannot account for the Mg^{2+} insensitivity, Savtchouk et al. (3) explored the presence of additional subunits in the receptor complex. Functional NMDARs are heterotetrameric assemblies of an obligatory GluN1 subunit with different combinations of GluN2(a–d) and GluN3(a, b) subunits. Of these, GluN2c, GluN2d, or GluN3a lower the sensitivity to Mg^{2+} block of the channel and have been reported to incorporate into pre-NMDARs in other brain areas (12, 13). Using GluN3a KO mice, Savtchouk et al. (3) found that local puffs of NMDA did not affect mEPSC frequency, as seen in control mice. The PPR was increased at MPP-GC (but not LPP-GC) KO synapses, possibly reflecting a lower probability of release due to lack of pre-NMDARs containing GluN3a, and ifenprodil effects were occluded. The contribution of GluN2c/2d subunits was not addressed.

For the moment, the data from Savtchouk et al. (3) strongly indicate that pre-NMDARs are composed of GluN1, GluN2b, and GluN3a subunits, with incorporation of GluN3a avoiding Mg^{2+} block dependence and permitting activation at resting membrane potentials. Preembedding immunogold analysis lends key experimental support. While previous work found GluN3a–NMDARs at postsynaptic and extrasynaptic plasma membranes (5), Savtchouk et al. (3) observed that on MPP axons, the majority of GluN3a immunogold particles are presynaptic. GluN3a particles were located away from the synaptic cleft, closely apposed to astrocytic membranes, and were virtually absent from LPP axons. The abundance of GluN3a in MPP axons decreases during development [from postnatal day (P)21 to P45], as described at other synapses (7, 13, 14), but some expression (~50%) persists into adulthood. At visual cortex synapses, down-regulation of GluN3a–pre-NMDARs drives the developmental loss of a presynaptic form of t-LTD linked to formative periods of circuit development. It will be of interest to explore whether GluN3a–pre-NMDARs are functional during later periods of life, because experiments were performed with P20 to P40 animals, which might not be old enough to close the window of plasticity for specific circuits (15).

The unavailability of pre-NMDARs to sense spontaneous release and their proximity to astrocytic membranes, together with convergent evidence for astrocyte control of release probability at PP-GC synapses (4, 16), supports a gliotransmitter (likely glutamate) as the source of pre-NMDAR activation. However, many questions remain unanswered—most critically, what the role of this circuit-specific astrocytic modulation is; which signals trigger astrocytic glutamate release to activate pre-NMDARs; and even whether release of coagonists such as D-serine might be involved, as demonstrated at other synapses (13, 17). Of upmost importance will be to establish the physiological or pathological contexts in which the astrocytic modulation of pre-NMDARs plays a role.

To date, no information is available on intracellular pathways that couple pre-NMDAR activation to changes in transmitter release. Candidates range from modifications in presynaptic

proteins involved that modulate release, phosphorylation/dephosphorylation events involved in exocytosis or endocytosis, or regulation of the size of the releasable pool of synaptic vesicles as well as presynaptic calcium channels and their association to the release machinery. A link worth exploring is provided by the finding that the intracellular C-terminal tail of GluN3a binds GIT1, a synaptic scaffold implicated in regulating probability of transmitter release (18, 19).

As mentioned, one commonality of pre-NMDARs is their strategic location at subsets of synapses to control the induction of time-restricted or circuit-specific forms of short- or long-term plasticity. Expanding on this literature, Savtchouk et al. (3) report that LTP is enhanced at GluN3a-null MPP-GC synapses and suggest that GluN3a–pre-NMDARs impose a basal "prepotentiation" state that narrows the dynamic range for LTP induction. Blocking Ca^{2+} elevation in astrocytes by loading the Ca^{2+}chelator 1,2-Bis(2-aminophenoxy)ethane *N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) recapitulated the enhanced LTP, suggesting a role for pre-NMDAR–astrocyte coupling in this modulation. Whether BAPTA has less of an impact in GluN3a KO mice was not assessed by the authors. Increased LTP was previously reported at GluN3a KO hippocampal CA1 synapses (20) and was deemed to be postsynaptic, thus the role of astrocytes would need to be further tested.

The new work underscores the emerging roles of gliotransmission in modulating brain function in concert with neurons, as well as the general idea that animal behavior results from simultaneous and coordinated activity of astrocytes and neurons. Undoubtedly, much more will come regarding the relationship between pre-NMDAR and astrocytes to modulate brain activity.

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