Response to Comment on “Role of NMDA Receptor Subtypes in Governing the Direction of Hippocampal Plasticity”

As a result of the differential (intrasyaptic versus extrasynaptic) localization and agonist affinity of NR2A-containing and NR2B-containing NMDA receptors (NMDARs), Rusakov et al. (1) propose that the production of long-term potentiation (LTP) versus long-term depression (LTD) in a cell might depend on the degree to which synaptic and extrasynaptic NMDARs are activated. We alluded to this idea in a previous study (2) but did not discuss it further in (3).

Although a substantial amount of NR2B subunits are localized at extrasynaptic sites (4–6), they are also expressed in hippocampal synapses of adult rats. We argue that it is the activation of these synaptic NR2B-containing NMDARs that produced the CA1 LTD in our study (3) for the following reasons. First, we demonstrated that about 30 to 40% of evoked NMDAR-mediated synaptic currents at CA1 synapses were sensitive to NR2B antagonists (3) and, more important, that a similar proportion of spontaneously occurring miniature excitatory postsynaptic currents (mEPSCs) were sensitive to the same antagonists (Fig. 1). Because mEPSCs are primarily activated by glutamate spontaneously released from presynaptic terminals (as opposed to spillover from adjacent synapses), functional NR2B-containing NMDARs must have been present within CA1 synapses in the adult rats used in our study. Second, if activation of extrasynaptic NMDA receptors by glutamate spillover is responsible for the induction of LTD, one might expect that high-frequency stimulation, rather than low-frequency stimulation, would be more likely to produce LTD, because it should cause more spillover. However, high-frequency and low-frequency stimulation produce LTP and LTD, respectively. Finally, the CA1 LTD shown in (3) is the homosynaptic type that has a high degree of input specificity. Such specificity can only occur after the activation of synaptic NMDARs because the activation of extrasynaptic NMDARs by glutamate spillover would be expected to produce a heterosynaptic LTD in nearby synapses. As noted in (1), that the majority of extrasynaptic NMDARs are NR2B-containing might explain why bath application of NMDA produces LTD in hippocampal neurons in both brain slices (8) and primary cultures (2). Together, these results are consistent with the idea that, regardless of their synaptic or extrasynaptic localization, sufficient activation of NR2B-containing receptors can lead to the induction of CA1 LTD.

We agree with Rusakov et al. (1) that the higher affinity for glutamate of NR2B receptors (9) makes extrasynaptic NR2B-containing NMDARs well suited to sense glutamate spillover from strongly activated synapses. This could be one of the mechanisms underlying homeostatic regulation of excitatory transmission (10), but there are potential pitfalls to consider. Because the induction of NMDAR-dependent LTD typically requires a temporal stimulation threshold of at least several minutes (3, 11, 12), the activation of extrasynaptic NR2B-containing receptors may not be sufficiently sensitive as a feedback mechanism for the maintenance of synaptic homeostasis. Moreover, heterosynaptic LTD in an unstimulated pathway after the induction of LTP in another pathway appears not to require the activation of NMDARs (13). Nonetheless, Rusakov et al. raise interesting ideas that should provoke more research into the physiological effects of activation of extrasynaptic NMDARs during conditions of glutamate spillover.

**Fig. 1.** Evidence for the presence of functional synaptic NR2B-containing NMDA receptors in CA1 hippocampal neurons from 3-week-old rats. Spontaneous mEPSCs were recorded in whole-cell voltage-clamp mode at a holding membrane potential of −60 mV in the presence of tetrodotoxin (0.3 μM) and bicuculline (10 μM) in artificial cerebral spinal fluid with no Mg$^{2+}$ added. (A) Examples of mEPSC traces (averaged from 100 individual events) obtained in the absence and presence of the broad spectrum NMDA receptor antagonist APV (50 μM) demonstrate that, under this recording condition, mEPSCs comprise both α-amino-3-hydroxy-5-methyl-isoazole-4-propionic acid (AMPA) and NMDA receptor–mediated components. The AMPA component (APV), isolated by recording of mEPSCs in the presence of APV, was completely blocked by non-NMDA receptor antagonist DNQX (data not shown). The NMDA component (orange) was obtained by subtracting the AMPA component (APV) from control mEPSCs (control). (B) Examples of averaged mEPSC traces illustrate pharmacological isolation of the component of mEPSCs mediated by NR2B-containing NMDA receptors (pink). The NR2B component was obtained by subtracting mEPSCs recorded in the presence of a specific NR2B-containing NMDA receptor antagonist Ro25-6981 (1 μM; Ro25-6981) from control mEPSCs (control). The inset shows the overlay of the NR2B component [pink area in (B)] with the total NMDA component [orange area in (A)]. On average, the NR2B-containing receptor-mediated component accounts for 38.9 ± 6.7% of the synaptic NMDA current (n = 3).

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References

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