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Thinking Outside the Synapse: Glycine at Extrasynaptic NMDA Receptors

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In this issue, Papouin et al. show that glycine is the endogenous coagonist for extrasynaptic NMDA receptors (NMDARs), unlike at synapses where the coagonist is D-serine. By enzymatically degrading endogenous glycine, they begin to address the enigmatic physiological and pathological roles for extrasynaptic NMDARs.

N-methyl-D-aspartate glutamate receptors (NMDARs) play key roles in synaptic transmission, synaptic plasticity, and learning and memory, and disturbances in NMDAR function have been implicated in a broad range of neuropsychiatric disorders. NMDARs are unique among neurotransmitter receptors in that they require both glutamate and a coagonist for activation. The coagonist was initially identified as glycine (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), although subsequent evidence, largely by Sol Snyder and colleagues, has revealed that D-serine is similarly potent and is found at high concentrations at synapses in which NMDARs are prevalent. In a pioneering study, Mothet et al. (2000) were the first to show that depletion of endogenous D-serine considerably reduces synaptic NMDAR currents, and it is now generally accepted that glial-derived D-serine is the endogenous coagonist for synaptic NMDARs (Wolosker, 2007).

Neuronal NMDARs are also present on nonsynaptic membranes, although identification of clear physiological roles for these extrasynaptic NMDARs has remained elusive. There is, however, significant clinical interest in studying their function and regulation given their potential involvement in the pathogenesis of Alzheimer's and Huntington's disease and in ischemic cell death (Hardingham and Bading, 2010). Nevertheless, basic essential questions regarding the activation of extrasynaptic NMDARs have remained largely unresolved, including the source of extrasynaptic glutamate, the depolarization signal required to relieve the Mg^{2+} block, as well as the identity and source of the glycine site coagonist. In this issue, Papouin et al. (2012) show that glycine and D-serine are both endogenous coagonists for NMDARs but act at distinct populations of receptors, with D-serine present at synaptic NMDARs and glycine at their extrasynaptic counterparts (Figure 1).

To assess the individual functions of glycine and D-serine, the authors treat hippocampal slices from adult rats with D-amino acid oxidase (DAAO), which specifically degrades D-amino acids, such as D-serine, and a recombinant glycine oxidase (GO) from *Bacillus subtilis* to remove endogenous glycine. They find that treatment with DAAO reduces synaptic NMDAR responses by nearly 60%, confirming previous studies that D-serine is the major coagonist at synaptic NMDARs. The remaining 40% of synaptic NMDAR responses could be due to a number of factors, including poor penetration of the recombinant enzyme into the slice preparation or insufficient kinetics of D-serine oxidation (which they favor), or due to additional coagonism by glycine. To address the latter, they added GO along with DAAO and saw no further reduction in synaptic NMDAR responses, suggesting that endogenous glycine is not contributing. However, they also show that glycine *can* act as a coagonist at synaptic NMDARs by exogenous administration of glycine and by pharmacological inhibition of perisynaptic glycine transporters. In both of these situations, GO now results in a reduction of the synaptic NMDAR response. These results strongly support the existing literature suggesting that D-serine is the predominant coagonist at synaptic NMDARs.

To look at extrasynaptic NMDARs, they apply a brief puff of NMDA onto the dendrites of patch-clamped, depolarized pyramidal cells, which results in long-lasting outward NMDAR-mediated currents. Although this method samples both synaptic and extrasynaptic receptors, they find that, in contrast to the synaptic responses, both DAAO and GO reduce these NMDA-evoked responses. They then enrich the extrasynaptic contribution to the NMDA-evoked responses by blocking a portion of synaptic NMDARs with the open-channel inhibitor MK-801 and show a greater effect of GO treatment, further supporting their conclusion that glycine acts as a coagonist at extrasynaptic NMDARs. In addition, they show GO-selective effects on the small, tonic extrasynaptic NMDAR currents first reported by Sah et al. (1989) and internally consistent effects on GluN2 subunit-selective antagonism. Taken together, Papouin et al. convincingly, though not definitively, show that glycine is a major coagonist at extrasynaptic NMDA receptors. Not fully answered is whether D-serine contributes to the extrasynaptic NMDAR responses. Indeed, it is uncertain how D-serine is removed, as DAAO is at low levels in the forebrain and no unambiguous transport mechanism has been identified.

These limitations aside, the selectivity of GO for reducing extrasynaptic, but not synaptic, NMDARs can be used as a tool to begin to probe the elusive physiological and pathological roles of the extrasynaptic population of NMDARs. Along these lines, Papouin et al. show that long-term potentiation (LTP) is blocked by D-serine depletion but is unaffected by decreasing glycine, whereas long-term depression (LTD) is blocked by either. Surprisingly, glycine depletion completely abolishes LTD, whereas depletion of D-serine only attenuates it, suggesting that extrasynaptic NMDARs are crucial for LTD induction. However, it is uncertain how extrasynaptic NMDARs would get activated during the low-frequency LTD induction protocol.

They next examined the effects of glycine or D-serine removal on NMDA-induced neurotoxicity in hippocampal slices. They again confirm previous findings (Katsuki et al., 2004) that DAAO treatment is strongly protective against NMDA-induced cell death and additionally show that GO treatment does not prevent neurotoxicity. These data support the conclusion that synaptic rather than extrasynaptic NMDARs mediate the NMDA-induced neurotoxicity in CA1 pyramidal neurons. This result is in striking contrast to a large body of literature in cultured neurons that posits that activation of extrasynaptic NMDARs promotes excitotoxicity (Hardingham and Bading, 2010). It could be argued that acute tissue slices more closely approximate intact brain, where differential roles for synaptic and extrasynaptic NMDARs have not been rigorously examined.

Finally, they provide the quite unexpected and compelling finding in cultured neurons that glycine and D-serine differentially affect the surface diffusion rate of NMDARs in a subunit-dependent manner. Specifically, exogenous application of glycine selectively impairs the surface diffusion of GluN2B-containing NMDARs, whereas D-serine application impairs the mobility of GluN2A-containing receptors. Most surprising is that this effect remains even after blocking channel. These results suggest a unique functional role for the glycine site coagonist independent of channel gating but leave the reader somewhat dangling, as they provide no clues as to the basis of this intriguing effect. An obvious experiment would be to examine surface mobility with transfected channel-dead receptors.

In summary, Papouin et al. convincingly demonstrate that glycine acts as a major coagonist for extrasynaptic NMDARs in the hippocampus and is excluded from contributing to synaptic receptor activity. Many questions remain. What advantage is provided by anatomically segregating the coagonists for NMDARs? When is this functional compartmentalization solidified during neuronal development, and what implications might this division have on synaptogenesis? Regardless, the findings will now allow proposed roles for extrasynaptic NMDARs to be tested, including their involvement in neuronal synchrony (Fellin et al., 2004), the shaping of synaptic potentials prior to somatic integration, and the de novo synthesis of dendritic spines (Kwon and Sabatini, 2011). Finally, the findings may allow the development of therapeutic approaches to enhance or inhibit extrasynaptic NMDAR activation without affecting synaptic transmission by targeting the glycine regulatory machinery.

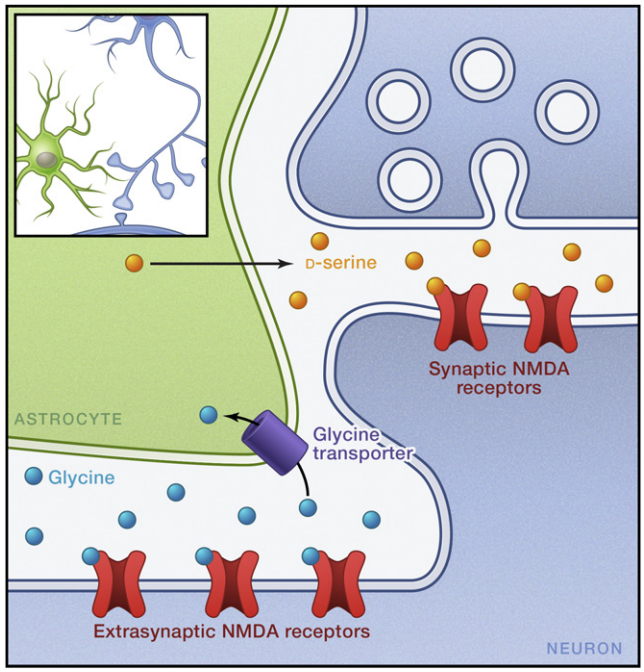

Figure 1. Synaptic and Extrasynaptic NMDA Receptors Use Different Coagonists

NMDA receptors are unique among neurotransmitter receptors in that activation requires binding of both glutamate and a coagonist. Initially, this coagonist was thought to be glycine (Johnson and Ascher, 1987), though over the past decade it has become clear that, in many brain regions, synaptic NMDA receptors utilize D-serine (Mothet et al., 2000) released by synapse-enveloping astrocytes as coagonist. NMDA receptors are also found on extrasynaptic membranes, though the physiological roles of these receptors are poorly understood. Papouin et al. (2012) now show that glycine is the major coagonist at extrasynaptic NMDA receptors and is largely excluded from synapses by perisynaptic glycine transporters. Enzymatic degradation of ambient glycine now allows a dissection of the physiological roles for this enigmatic class of NMDA receptors.

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