

NEUROTRANSMISSION

Silence of the synapses



A significant proportion of excitatory glutamatergic synapses in the CNS are functionally silent, as they contain NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) but no AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPA). It is not well understood how silent synapses are established during development. A study by Adesnik *et al.* now demonstrates that NMDARs limit rather than promote synapse maturation during hippocampal development.

The authors transfected hippocampal slice cultures from floxed NR1 (NR1^{fl/fl}) mice with a plasmid encoding Cre-recombinase, to abolish NMDAR expression, and slices from NMDAR-knockout mice with a plasmid encoding the NR1 receptor subunit, to reintroduce NMDARs (concomitantly labelling transfected

cells with green fluorescent protein (GFP) for identification purposes). To transfected only a few cells per slice, they used a gene gun to deliver biolistic particles coated with the plasmids. This allowed them to study the role of NMDARs in circuit maturation (postnatal days (P) 5–9) while avoiding potential indirect effects stemming from changes in overall network activity.

Adesnik *et al.* showed that NMDAR depletion augmented AMPA currents in the transfected neurons, owing to a significant increase in the number of AMPAR-containing synapses two weeks after transfection. Conversely, reintroduction of NMDARs into hippocampal slices obtained from NMDAR-knockout mice decreased synaptic AMPA currents; this effect was dependent on NMDAR activation. These results suggest that NMDARs inhibit synapse maturation by preventing AMPAR recruitment.

The authors further tested this hypothesis by injecting NR1^{fl/fl} mouse embryos with a CRE-GFP-expressing adenoassociated virus, to delete NMDARs in hippocampal pyramidal neurons before the onset of synaptogenesis. Electrophysiological recordings in acute hippocampal slices obtained from these animals at P12–P19, when the excitatory circuitry has formed, demonstrated that AMPA currents in transfected neurons were increased. Furthermore, the authors showed that the spine density and overall dendritic structure of the

pyramidal neurons was unaffected, suggesting that the total number of synapses in NMDAR-deficient cells was unchanged. This implies that the increased AMPA currents were the result of a reduction in the number of silent synapses, indicating that premature recruitment of AMPARs to synaptic sites is normally prevented by the presence of NMDARs.

In summary, these results imply that NMDARs are not required for the morphological development of pyramidal neurons, and that during early development NMDARs inhibit the formation of functional synapses by preventing recruitment of AMPARs to synaptic sites. Interestingly, previous studies have shown that NMDAR activation is crucial for the insertion of AMPARs in the early formation of synapses. Explanations for these apparent contradictions will need to be provided by further studies. Moreover, in other brain areas, such as the nucleus tractus solitarius (NTS), alternative mechanisms seem to account for the silencing of synapses. In this brain area, blocking NMDARs does not impair AMPAR insertion during development. In addition, a recent study used immunodetection to show that electrophysiologically silent synapses contain AMPARs. It seems that the field of silent synapses will not be quiet for a long time to come.

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ORIGINAL RESEARCH PAPER Adesnik, H. *et al.* NMDA receptors inhibit synapse unsilencing during brain development. *Proc. Natl Acad. Sci. USA* **105**, 5597–5602 (2008)

FURTHER READING: Balland, B. *et al.* Silent synapses in developing rat nucleus tractus solitarius have AMPA receptors. *J. Neurosci.* **28**, 4624–4634 (2008)