

Spike timing-dependent long-term depression requires presynaptic NMDA receptors

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NMDA receptors are necessary for both synaptic potentiation and depression, but the precise location of these receptors has not been established. By loading MK-801 into pre- or postsynaptic neurons during paired recordings of synaptically connected layer 4 and layer 2/3 neurons in mouse barrel cortex, we found that synaptic potentiation requires postsynaptic, but not presynaptic, NMDA receptors, whereas synaptic depression requires presynaptic, but not postsynaptic, NMDA receptors.

Spike timing-dependent plasticity (STDP) is a strong candidate for a synaptic mechanism that is involved in cortical development and map plasticity^{1,2}. In STDP, the temporal order and precise timing of pre- and postsynaptic action potentials (spikes) determine the direction and magnitude of synaptic change. Thus, long-term potentiation (LTP) occurs when a presynaptic spike is followed by a postsynaptic spike, whereas long-term depression (LTD) is induced when this order is reversed^{3–5}. Both timing-dependent LTP (t-LTP) and timing-dependent LTD (t-LTD) depend on NMDA receptors^{4–7}. However, the means by which the same type of receptor can be involved in opposite changes in synaptic efficacy is not well understood. It has been reported that LTP and LTD that were induced by high-frequency and low-frequency afferent stimulation, respectively, could be dissociated using subunit-selective NMDA receptor antagonists^{8,9}, but this finding has been controversial^{10,11}. Recently, it was reported that postsynaptic loading of a use-dependent, noncompetitive NMDA receptor antagonist, MK-801, blocked the induction of t-LTP, but not t-LTD^{12,13}. This finding raises the possibility that NMDA receptors at different synaptic locations are involved in t-LTP and t-LTD. Here, we investigated whether presynaptic or postsynaptic NMDA receptors are necessary for t-LTD and t-LTP in layer (L) 4-to-L2/3 excitatory synapses in mouse barrel cortex.

First, we wanted to confirm that both t-LTP and t-LTD at these synapses require NMDA receptors. We monitored excitatory postsynaptic potentials (EPSPs) that were evoked by extracellular stimulation in L4 during whole-cell recording of L2/3 pyramidal cells in slices prepared from the mouse barrel cortex (postnatal days 9–14), as described previously¹⁴ (**Supplementary Methods** online). In control experiments, t-LTP and t-LTD were induced in current clamp using 100 pairings of single EPSPs and postsynaptic spikes at 0.2 Hz.

Consistent with previous results^{6,12,13}, a pre-before-post pairing protocol (with a postsynaptic spike occurring in 10 ms of EPSP onset) elicited robust t-LTP ($150 \pm 6\%$, $n = 12$, $P < 0.01$, t -test; **Fig. 1a**), whereas an unpaired control pathway was unchanged ($98 \pm 7\%$, $n = 12$). Conversely, a post-before-pre pairing protocol (with a postsynaptic spike occurring ~ 15 ms before presynaptic stimulation) induced robust t-LTD ($73 \pm 3\%$, $n = 15$, $P < 0.01$, t -test), whereas an unpaired pathway remained unchanged ($103 \pm 6\%$, $n = 15$; **Fig. 1b,c**). In slices treated with the NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5), a pre-before-post pairing protocol failed to induce t-LTP ($94 \pm 6\%$, $n = 5$; versus interleaved controls, $154 \pm 8\%$, $n = 5$; $P < 0.01$, t -test; **Fig. 1d,e**). D-AP5 also blocked t-LTD. In D-AP5-treated slices, a post-before-pre pairing protocol did not induce t-LTD ($98 \pm 5\%$, $n = 5$; versus interleaved controls, $76 \pm 5\%$, $n = 5$; $P < 0.01$, t -test; **Fig. 1e,f**). These results indicate that both t-LTP and t-LTD require NMDA receptors.

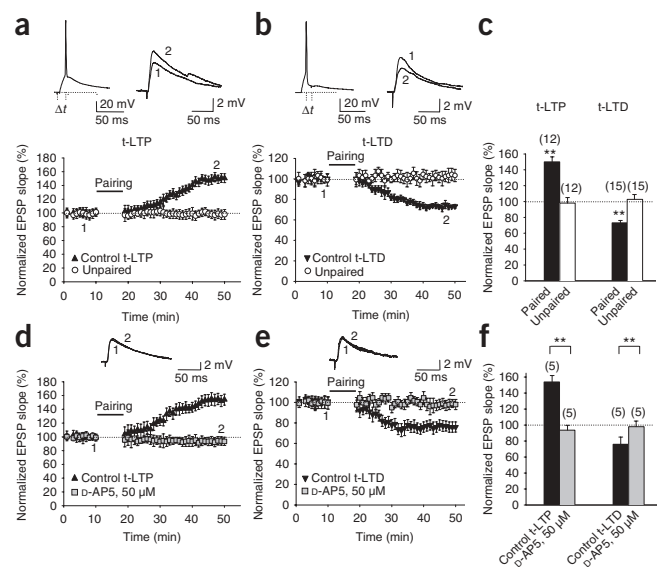


Figure 1 NMDA receptor dependence of timing-dependent plasticity in barrel cortex. **(a)** Pre-before-post pairing protocol induced t-LTP. The EPSP slopes monitored in paired (black symbols) and unpaired control pathway (open symbols) are shown. Inset, pairing protocol (Δt , time between EPSP onset and peak of spike). Traces show EPSP before (1) and 30 min after (2) pairing. **(b)** Post-before-pre pairing protocol induced t-LTD. Symbols and traces are presented as in **a**. **(c)** Summary of results. **(d,e)** t-LTP and t-LTD required NMDA receptors. In the presence of 50 μM D-AP5, t-LTP (**d**) and t-LTD (**e**) were completely blocked. Symbols and traces are presented as in **a**. **(f)** Summary of results. Error bars are s.e.m. $** P < 0.01$, Student's t -test. The numbers of slices are shown in parentheses.

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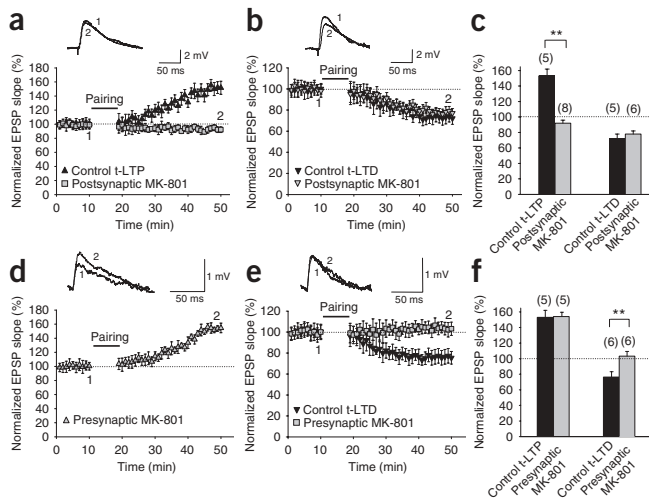


Figure 2 t-LTP and t-LTD require postsynaptic and presynaptic NMDA receptors, respectively. **(a)** Postsynaptic MK-801 completely blocked induction of t-LTP. The EPSP slopes monitored in MK-801-treated (gray symbols) and nontreated cells (black symbols) are shown. Inset, EPSP before (1) and 30 min after (2) the pairing protocol. **(b)** Inclusion of MK-801 in the postsynaptic pipette did not block t-LTD. Symbols and traces are presented as in **a**. **(c)** Summary of results. **(d,e)** During paired recordings, presynaptic MK-801 did not block the induction of t-LTP **(d)**, but completely blocked t-LTD **(e)**. Symbols and traces are presented as in **a**. **(f)** Summary of results. Control t-LTP refers to values obtained using extracellular stimulation. Error bars are s.e.m. ** $P < 0.01$, Student's t -test. The numbers of slices are shown in parentheses.

To investigate whether postsynaptic NMDA receptors are necessary for timing-dependent plasticity, we repeated the pairing experiments, including the NMDA receptor blocker MK-801 (1 mM) in the postsynaptic recording pipette. Consistent with previous reports^{12,13}, postsynaptic MK-801 blocked the induction of t-LTP ($92 \pm 4\%$, $n = 8$; versus interleaved controls, $153 \pm 9\%$, $n = 5$; $P < 0.01$, t -test; **Fig. 2a**). In contrast, t-LTD was unaffected ($77 \pm 4\%$, $n = 6$; versus interleaved controls, $72 \pm 6\%$, $n = 5$; **Fig. 2b,c**). These results show that postsynaptic NMDA receptors are necessary for induction of t-LTP but not t-LTD. To further support this conclusion, we carried out both pre-before-post and post-before-pre pairing in the same cells treated with MK-801. Potentiation was not observed after pre-before-post pairing, but subsequent post-before-pre pairing in the same pathway induced robust t-LTD (**Supplementary Fig. 1** online). Thus, t-LTD could still be successfully induced during inhibition of postsynaptic NMDA receptors sufficient to completely block the induction of t-LTP.

The fact that t-LTD was blocked by extracellular, but not by postsynaptic intracellular application of an NMDA receptor antagonist, raises the possibility that nonpostsynaptic, possibly presynaptic, NMDA receptors are necessary for t-LTD. To doubly dissociate the involvement of NMDA receptors in timing-dependent synaptic plasticity, we investigated the role of presynaptic NMDA receptors in t-LTP and t-LTD. To this end, we used paired whole-cell recordings of synaptically connected L4 and L2/3 cells. Out of 104 pairs recorded, 19 pairs showed a clear monosynaptic EPSP, of which we used 17 in plasticity experiments. In five pairs, a pre-before-post pairing protocol was applied with 1 mM MK-801 in the presynaptic pipette. Robust t-LTP was induced ($154 \pm 5\%$, $n = 5$; $P < 0.01$, t -test; **Fig. 2d**), that was of similar magnitude to that seen with extracellular stimulation ($153 \pm 9\%$, $n = 5$; **Fig. 2a**), suggesting that presynaptic NMDA receptors are not necessary for induction of t-LTP. In contrast, t-LTD was completely blocked when MK-801 was included in the presynaptic pipette ($104 \pm 6\%$, $n = 6$, **Fig. 2e,f**), whereas this protocol induced robust t-LTD in pairs of cells without MK-801 ($76 \pm 6\%$, $n = 6$; $P < 0.01$, t -test), indicating that presynaptic NMDA receptors are necessary for t-LTD. It has recently been shown that functional presynaptic NMDA receptors are present at this synapse¹⁵. To exclude the possibility that blocking these receptors could, by itself, reduce synaptic transmission and interfere with the expression of t-LTD in our experiments, we measured

the effect of D-AP5 on synaptic efficacy. Only a small, nonsignificant reduction in the slope of the EPSP was observed ($95 \pm 5\%$, $n = 9$, $P = 0.34$, t -test) and t-LTD was completely blocked (**Supplementary Fig. 2** online), which is consistent with a role for NMDA receptors during the induction of t-LTD⁷.

Other receptors, such as endocannabinoid receptors and metabotropic glutamate receptors, have also been reported to be necessary for induction of t-LTD^{12,13}. Our experiments do not exclude the possibility that presynaptic NMDA receptors are permissive or modulatory¹², rather than mediating t-LTD, or that MK-801, when applied internally, interferes with some other presynaptic target. Nevertheless, the experimental double dissociation observed here supports a model in which induction of t-LTP requires postsynaptic NMDA receptors and t-LTD requires presynaptic NMDA receptors. The different sites of NMDA receptors necessary for t-LTP and t-LTD, together with their possibly distinct loci of expression⁷ (**Supplementary Fig. 3** online), may have important consequences for the computational operation of cortical microcircuits and map plasticity^{1,2}.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

A.R.-M. conducted the experiments and analyzed the data. A.R.-M. and O.P. designed the experiments and wrote the manuscript.

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- Feldman, D.E. & Brecht, M. *Science* **310**, 810–815 (2005).
- Dan, Y. & Poo, M.M. *Physiol. Rev.* **86**, 1033–1048 (2006).
- Markram, H., Lubke, J., Frotscher, M. & Sakmann, B. *Science* **275**, 213–215 (1997).
- Bi, G.Q. & Poo, M.M. *J. Neurosci.* **18**, 10464–10472 (1998).
- Debanne, D., Gähwiler, B.H. & Thompson, S.M. *J. Physiol. (Lond.)* **507**, 237–247 (1998).
- Feldman, D.E. *Neuron* **27**, 45–56 (2000).
- Sjöström, P.J., Turrigiano, G.G. & Nelson, S.B. *Neuron* **39**, 641–654 (2003).
- Liu, L. *et al. Science* **304**, 1021–1024 (2004).
- Massey, P.V. *et al. J. Neurosci.* **24**, 7821–7828 (2004).
- Berberich, S. *et al. J. Neurosci.* **25**, 6907–6910 (2005).
- Morishita, W. *et al. Neuropharmacology* **52**, 71–76 (2007).
- Bender, V.A., Bender, K.J., Brasier, D.J. & Feldman, D.E. *J. Neurosci.* **26**, 4166–4177 (2006).
- Nevejan, T. & Sakmann, B. *J. Neurosci.* **26**, 11001–11013 (2006).
- Mierau, S.B., Meredith, R.M., Upton, A.L. & Paulsen, O. *Proc. Natl. Acad. Sci. USA* **101**, 15518–15523 (2004).
- Brasier, D.J. & Feldman, D.E. *J. Neurosci.* **28**, 2199–2211 (2008).