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Involvement of Synaptic NR2B-Containing NMDA Receptors in Long-Term Depression Induction in the Young Rat Visual Cortex *In Vitro*

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Abstract

Activation of N-methyl-D-aspartate receptors (NMDARs) has been implicated in various forms of synaptic plasticity depending on the receptor subtypes involved. However, the contribution of NR2A and NR2B subunits in the induction of long-term depression (LTD) of excitatory postsynaptic currents (EPSCs) in layer II/III pyramidal neurons of the young rat visual cortex remains unclear. The present study used whole-cell patch-clamp recordings *in vitro* to investigate the role of NR2A- and NR2B-containing NMDARs in the induction of LTD in visual cortical slices from 12- to 15-day old rats. We found that LTD was readily induced in layer II/III pyramidal neurons of the rat visual cortex with 10-min 1-Hz stimulation paired with postsynaptic depolarization. D-APV, a selective NMDAR antagonist, blocked the induction of LTD. Moreover, the selective NR2B-containing NMDAR antagonists (Ro 25-6981 and ifenprodil) also prevented the induction of LTD. However, Zn^{2+}, a voltage-independent NR2A-containing NMDAR antagonist, displayed no influence on the induction of LTD. These results suggest that the induction of LTD in layer II/III pyramidal neurons of the young rat visual cortex is NMDAR-dependent and requires NR2B-containing NMDARs, not NR2A-containing NMDARs.

Key Words: LTD, NMDA receptors, NR2A, NR2B, visual cortex, rat

Introduction

The alteration of synaptic strength by patterns of neuronal activity is thought to be a mechanism *via* which information is stored in the CNS (1). Long-term depression (LTD), defined as a persistent weakening of synaptic efficacy, is currently one of the most widely investigated models of the synaptic mechanisms underlying learning (3). LTD can reliably be induced by low-frequency stimulation (LFS) in the hippocampus and neocortex of young animals (22). Accordingly, there is support for the view that LFS may mimic physiological activity and, thus, a process like LTD may contribute to the structural refinement of cortical circuits (30, 33). Previous studies have shown that LTD can be induced by LFS of presynaptic afferents or by pairing presynaptic stimulation with modest depolarization in glutamatergic synaptic transmission in the rat visual cortex (2, 8, 32). Interestingly, the induction of LTD induced by LFS in this area is dependent on activation of NMDA-type glutamate receptors (6, 32, 34).

NMDARs, a subtype of glutamate receptors, are composed of two obligatory NR1 subunits in combination with two NR2A-D or NR3A-B subunits (25, 26). Of the four NR2 subunits, rodent cortex contains

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primarily NR2A and NR2B (11). Previous investigations have revealed that functional and pharmacological properties of NMDARs are determined by the types of NR2 subunits they contain (28). Developmentally, NR2B subunits are already expressed at birth and increase to near a plateau level within the first 2 weeks, whereas NR2A subunits are not expressed until the second week and increase gradually in subsequent weeks. Therefore, the ratio of NR2A- to NR2B-containing NMDARs increases with maturation (36). Additionally, the four NR2 subunits have distinct distributions in the CNS, with patterns of expression that change drastically during development (14). For example, only NR2B and NR2D subunits are expressed in the embryo, while NR2A is ubiquitously expressed in the adult brain. Moreover, NR2B expression is restricted to the forebrain while NR2C is highly enriched in the cerebellum (28). Because NR2A-containing NMDARs possess shorter current durations than NR2B-containing NMDARs, NMDAR-mediated current durations shorten over development (12). Synaptic plasticity, with regard to its dependence on NR2 subunits, has been investigated in some brain regions such as hippocampus, cortex and nucleus accumbens (NAc) (17, 21, 24, 35, 38). However, the types of subunits containing NMDARs involved in LTD in the young rat visual cortex have not been investigated.

In this study, we used whole-cell voltage-clamp recordings to assess the roles of NR2A- and NR2B-containing NMDARs in the induction of LTD in the young rat visual cortex. Our results demonstrate that LTD in the layer II/III pyramidal neurons of the young rat visual cortex is dependent on NMDARs, and requires NR2B, but/and not NR2A-containing, NMDARs.

Materials and Methods

Slice Preparation

Visual cortical slices were prepared from Sprague-Dawley rats aged 12-15 days. All animals were housed in a standard environment on a 12/12-h light/dark cycle with light on at 07:00, and they were allowed *ad libitum* access to water and food. The use and care of animals in this study followed the guidelines of the Xi'an Jiaotong University Animal Research Advisory Committee, and all efforts were made to minimize animal suffering and reduce the use of their number. Animals were deeply anesthetized with ether and then immersed in ice-cold water, with the nose exposed, for 3 min to reduce brain temperature. After decapitation, the brain was quickly removed and placed in cold (1-4°C) oxygenated artificial cerebrospinal fluid (ACSF). The ACSF consisted of the following: 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM glucose, and had an osmolality of 305-310 mOsm/kg H_2O and equilibrated with 95% O_2-5% CO_2 saturation to obtain a pH value of 7.4. The visual cortices were dissected, and coronal slices were cut at a thickness of 350 μm with a vibratome (Campden Instruments, London, UK). Slices were then transferred to an incubating chamber containing oxygenated ACSF and incubated for at least 1.5 h at room temperature (20°C) prior to electrophysiological recording.

Electrophysiological Recordings

For recording, slices were individually transferred to a recording chamber where they were perfused (2.5-3 ml/min) with oxygenated ACSF at 31 ± 0.5°C. The temperature of the recording chamber was continuously monitored and controlled by a custom-made temperature controller. The slices were placed on an upright infrared video microscope with differential interference contrast (DIC) optics (Olympus BX51WI, Tokyo, Japan) which was mounted on a Gibraltar X-Y table. Slices were observed through a 40× water immersion objective lens using an infrared-sensitive camera (IR 1000, DAGE-MTI, Michigan City, IN, USA). Layer II/III pyramidal neurons of the visual cortex were visually selected (20, 23). Patch clamp recordings were performed in the whole cell configuration. Unpolished and uncoated patch pipettes (1.5 mm/1.10 mm; Sutter Instruments, Novato, CA, USA) with a resistance of 4-6 MΩ were pulled using a horizontal puller (P-97, Sutter Instruments) and backfilled with internal solution containing 135 mM K-gluconate, 5 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM EGTA, 10 mM HEPES, 2 mM Na_2ATP, 0.25 mM Na_3GTP (pH adjusted to 7.3 with KOH, 280-290 mOsm/kg H_2O). Currents were recorded at a holding potential of -70 mV. Experimental recordings were initiated no later than 10 min after whole-cell configuration was obtained. A monopolar glass stimulating electrode (1-2 MΩ when filled with ACSF) was placed 50-100 μm lateral to the recording pipette. Test stimulation (0.1-ms duration) was delivered at a frequency of 0.05 Hz to elicit an excitatory postsynaptic current (EPSC). EPSC was recorded using an Axopatch 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) and digitized using a data acquisition board (Digidata 1322A) operated by pCLAMP 9.2 software (Molecular Devices). Stable responses were recorded for 10 min prior to tetanic conditioning. After baseline recording, LTD was induced using 1-Hz stimulation for 10 min paired with postsynaptic depolarization to -40 mV. The percentage change in EPSC amplitude was determined

by normalizing the mean response measured 35-40 min post LFS-stimulation to the mean response of baseline. Currents were filtered at 2 kHz and digitized at 10 kHz. Series resistance was monitored continuously using a 5 ms, 10 mV hyperpolarizing pulse and compensation was not employed. Statistical comparisons were performed only when series resistance was lower than 20 MΩ and did not change by more than 20%. All experiments were performed in the presence of picrotoxin (100 μM) to inhibit $GABA_A$R-mediated inhibitory postsynaptic currents.

D-2-amino-5-phosphonovalerate (D-APV), zinc chloride, Ro 25-6981, ifenprodil, picrotoxin and N-tris(hydroxymethyl) methylglycine (tricine) were purchased from Sigma. Stock solutions of APV (50 mM), Ro 25-6981 (1 mM), ifenprodil (3 mM) and picrotoxin (100 mM) were prepared in distilled water and stored frozen at -20°C. On the day of the experiment, antagonists were diluted to their respective final concentration in ACSF. The 30 nM free Zn^{2+} was made by adding 7.8 μM Zn^{2+} to 10 mM tricine (29). Zn^{2+} is a voltage-independent antagonist of the NR2A-containing NMDARs (IC_{50} in the nanomolar range) and displays strong selectivity for NR2A-containing receptors over all other NR1/NR2 combinations (> 100 fold) (29).

Data Analysis

The amplitude of EPSC was measured by using pCLAMP 9.2 software. Each point shown in the graphs of Figs. 1, 2 and 3 represents the average of the responses recorded in 1 min. All data were expressed as means ± SEM, with n indicating the number of slices tested. Statistical analysis was performed using paired and unpaired Student's *t* test. The significance level was established at $P < 0.05$.

Results

Induction of LTD Is Dependent on the Activation of NMDA Receptors

Whole-cell EPSCs recordings were performed on pyramidal neurons in layer II/III of the visual cortical slices. After stable responses were collected for 10 min, LTD was induced by 10-min 1-Hz afferent stimulation paired with postsynaptic depolarization to -40 mV (pairing protocol). As illustrated in Fig. 1A, the peak amplitude of EPSCs showed obvious depression lasting for 40 min after induction (when the experiment was usually terminated). The corresponding mean value of EPSC depression at 40 min was 71.8 ± 5.6% and was significantly different from the baseline ($P < 0.001$, n = 13, Fig. 1A). To confirm the role of NMDARs in the induction of LTD, the

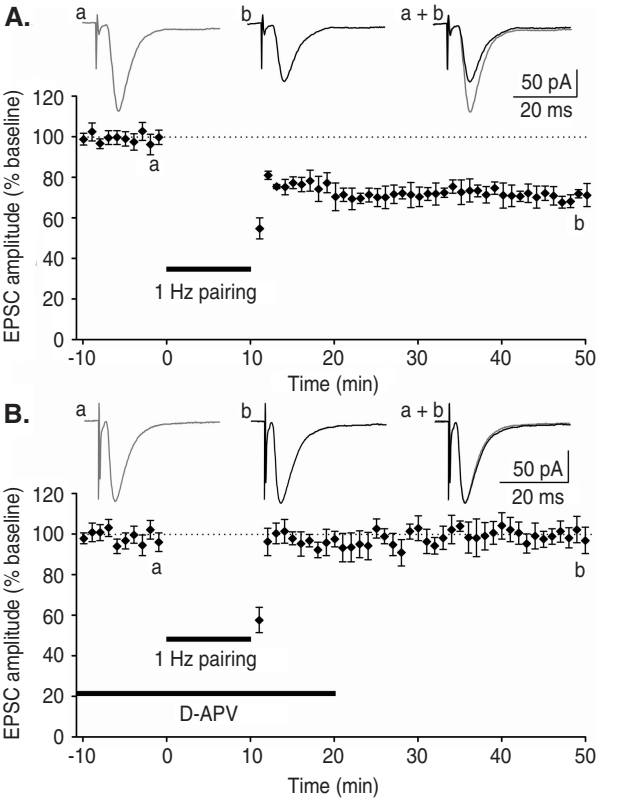

Fig. 1. LTD induced by LFS is blocked by the NMDAR antagonist, D-APV. **A.** The amplitude of EPSCs showed obvious depression lasting for 40 min after induction in layer II/III of the rat visual cortical slices. The average response, 40 min after LFS, was 71.8 ± 5.6% (n = 13) of the baseline EPSC amplitude. **B.** LFS-induced LTD is inhibited by the NMDAR antagonist D-APV (50 μM). Average EPSC amplitudes, 40 min after LFS, were 97.0 ± 6.5% (n = 8) of the baseline in the presence of D-APV. Each point represented average amplitude of three responses over one minute. EPSCs (average of 3 individual responses) during baseline (a), 40 min after LFS protocol (b), and overlay (a + b) are shown above the graphs. Short bar indicates 1-Hz pairing and long bar indicates duration of drug treatment. Error bars indicate S.E.M.

selective NMDAR antagonist, D-APV, was used. As shown in Fig. 1B, when LFS was given in the presence of D-APV (50 μM), this depression of the EPSC amplitude was blocked and no significant LTD in synaptic responses was observed (97.0 ± 6.5% of baseline EPSC amplitude 40 min after LFS, $P > 0.85$, n = 8). These results suggest that LFS-induced LTD in layer II/III of the visual cortical slices is fully dependent on NMDARs.

LTD Induction is Dependent on NR2B-Containing NMDA Receptors

As mentioned above, synaptic NMDARs may

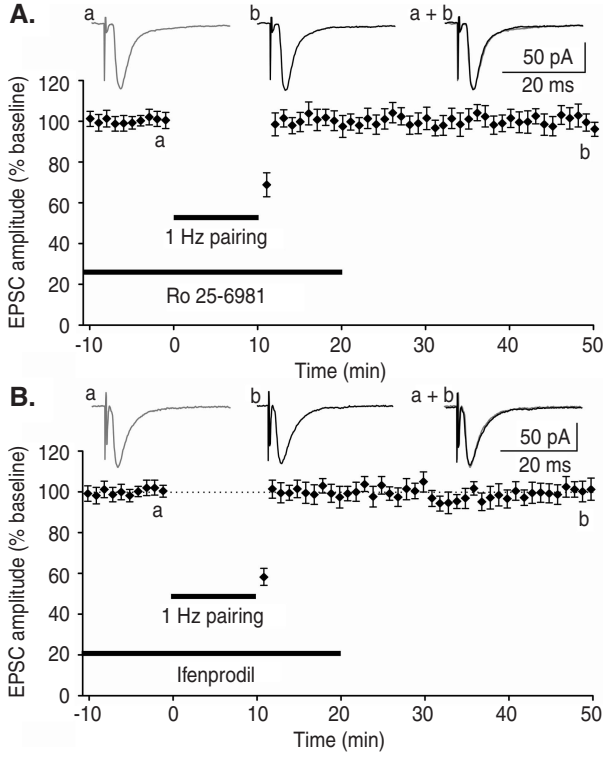

Fig. 2. NR2B-containing NMDAR inhibition blocked induction of LTD. **A.** During continuous bath application of the NR2B antagonist, Ro25-6981 (1 μM), the induction of LTD was fully prevented (n = 11). **B.** A similar result was obtained in the same type of experiment but treated with ifenprodil (3 μM, n = 6). Inserts show EPSCs (average of 3 individual responses) taken at the indicated time points (a and b), and overlay (a + b). Short bar indicates 1-Hz pairing and long bar indicates duration of drug treatment. Error bars indicate S.E.M.

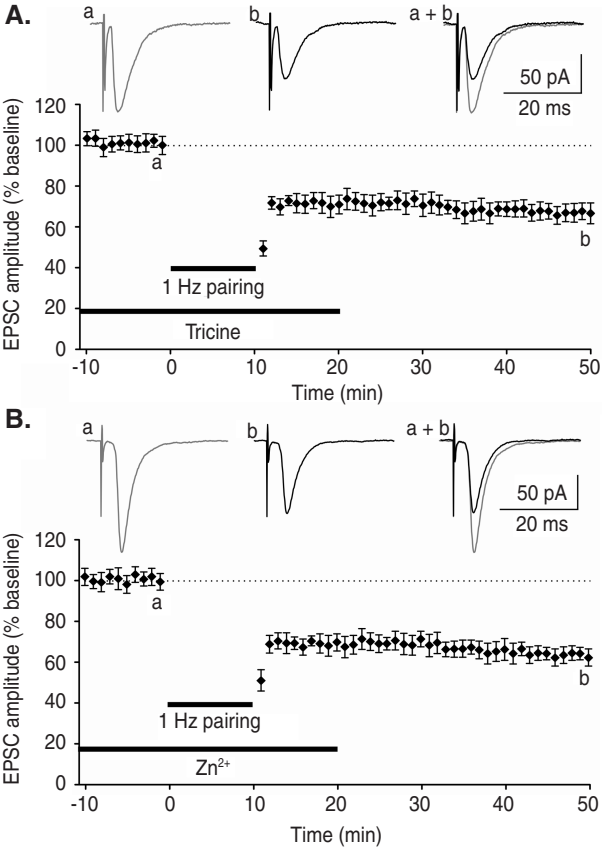

Fig. 3. NR2A-containing NMDAR inhibition did not prevent the inducton of LTD. **A.** In the tricine control (10 mM), LTD was induced by LFS (n = 5). **B.** LTD was still induced by LFS when Zn^{2+} (30 nM) is present (n = 9). Inserts show EPSCs (average of 3 individual responses) taken at the indicated time points (a and b), and overlay (a + b). Short bar indicates 1-Hz pairing and long bar indicates duration of drug treatment. Error bars indicate S.E.M.

be composed of different subunits, mainly NR2A or NR2B, in rodent cortex (11). To determine which subtype of NMDARs is involved in the induction of LTD, we blocked NR2B-containing NMDARs and examined the amplitude of EPSCs. In the presence of the highly specific NR2B antagonist, Ro25-6981 (1 μM), LFS did not induce LTD in layer II/III of the visual cortical slices (Fig. 2A). The mean EPSC normalized to the baseline was 101.3 ± 5.4% measured at 40 min post-tetanus. The average amplitudes of EPSCs in the control group and in the Ro25-6981 group recorded at 40 min post-tetanus showed no significant difference ($P > 0.58$, n = 11). Another NR2B-specific antagonist, ifenprodil (3 μM), also abolished the induction of LTD (96.1 ± 3.6% of baseline at 40 min post-tetanus, $P > 0.62$, n = 6, Fig. 2B). Thus, LTD induction was blocked by NR2B-containing NMDAR inhibition. These results indicate that LTD in layer II/III of the visual cortical slices critically relies on the activation of NR2B-containing NMDARs.

NR2A-Containing NMDAR Antagonist Does Not Affect the Induction of LTD

To investigate whether NR2A-containing NMDARs are involved in the induction of LTD, we applied Zn^{2+}, a highly potent inhibitor of NR2A-containing NMDARs (19, 20, 29). As shown in Fig. 3B, LFS continued to significantly decrease the amplitude of EPSCs compared with the baseline similarly to the control group. The amplitude of the EPSCs measured 40 min after LFS was 66.0 ± 5.1% ($P < 0.001$, n = 9) of the baseline EPSC amplitude and was not significantly different from control slices in which LTD was induced ($P > 0.75$). Moreover, we also found that the Zn buffer ticine when applied alone at 10 mM had no effect on LTD induction (n =

5, Fig. 3A). These results suggest that NR2A-containing NMDAR activation is not necessary for the LTD induced by LFS in layer II/III pyramidal neurons of the young rat visual cortical slices.

Discussion

In this study, we investigated whether LTD induced by the combination of presynaptic stimulation with postsynaptic depolarization in layer II/III of the visual cortical slices required a particular type of NMDAR subunit. All data were obtained in the presence of picrotoxin in order to eliminate $GABA_AR$-mediated inhibitory postsynaptic currents. Our studies provided direct electrophysiological evidence that the induction of LTD relied on NR2B- but not NR2A-containing NMDARs.

It was initially shown that the induction of LTD mediated by LFS in the CA1 hippocampal region was dependent on the activation of NMDARs (9). Other studies have reported NMDAR-dependent LTD in the visual cortex (15, 32), anterior cingulate cortex (37), peripheral cortex (7) and hippocampus (16, 21, 24). In this study, we used visual cortex slices that were from Sprague-Dawley rats aged 12-15 days to examine the roles of NMDARs in the induction of LTD. We found that LTD was readily induced by LFS and that it was blocked completely by D-APV, a nonsubunit selective antagonist of NMDARs. The observation that application of NMDAR antagonist can block the induction of LTD in the young rat visual cortex *in vitro* support the supposition that activation of NMDARs is not only necessary but also sufficient for the induction of NMDAR-dependent LTD in these cortical regions (15, 32, 34).

The present study showed that application of NR2B antagonist, not NR2A antagonist, prevented the induction of LTD in layer II/III pyramidal neurons of the young rat visual cortex. There is strong evidence that NR2B subunits are already expressed at birth and increase to near a plateau level within the first 2 weeks, whereas NR2A subunits are not expressed until the second week and increase gradually in subsequent weeks. Therefore, the ratio of NR2A- to NR2B-containing NMDARs increases with maturation (31, 36). Moreover, synaptic NR2A- and NR2B-containing NMDARs are known to exist in the visual cortex (4) and play key roles in excitatory synaptic transmission (21). Previous studies have shown that NR2A-containing NMDARs display a lower affinity for glutamate and a higher open probability than NR2B-containing NMDARs (5, 10, 28). In addition, studies have found that NR2A-containing NMDARs are more effective at mediating charge transfer and Ca^{2+} influx into neurons at high-frequency stimulation, which can induce LTP in many brain areas. On the contrary, NR2B-containing NMDARs are more effective at mediating charge transfer at lower stimulus frequencies (*e.g.*, 1 Hz, the frequency used to elicit LTD) than NR2A-containing NMDARs (10). Additionally, the finding that NR2B subtype-specific antagonist ifenprodil abolished the induction of LTD suggests that the triheteromeric (NR1-NR2A-NR2B) NMDARs, which have a reduced sensitivity to ifenprodil (13), are not critical for the induction of LTD.

In recent years, the special roles of the different NMDAR subunits in synaptic plasticity have been described in brain areas such as hippocampus, cerebral cortex and nucleus accumbens (NAc) (17, 21, 24, 35). In this study, we examined the roles of these two NMDAR subunits in LTD induction in layer II/III of the rat visual cortical slices. We found that selective inhibition of NR2A-containing NMDARs did not prevent the induction of LTD; however, selective inhibition of NR2B-containing NMDARs completely prevented the induction of LTD. While these findings contrast with a recent report in the mice visual cortex (8), the hippocampus (27) and the NAc (35), our results are in agreement with previous reports in the hippocampus and cortex (21, 24, 37). In 2008, De Marchena *et al*. reported that LTD was not attenuated by application of ifenprodil, an NR2B-type antagonist (8). This controversy can be explained, at least in part, by the species of the animal (rats *vs*. mice) used and by low levels of NR2A-containing NMDARs. The roles of NR2A and NR2B subunits may be better understood in the future through careful consideration of the limitations of subunit-selective antagonists, the contribution of triheteromeric NMDARs, the developmental stage and species of the experimental animal, and the passway and brain region being examined (8).

In summary, the present study demonstrates that NR2B-containing, not NR2A-containing, NMDARs are required for the induction of LTD in layer II/III pyramidal neurons of the young rat visual cortex. These results suggest that NMDAR subunits may differentially mediate synaptic plasticity and physiological function in the young rat visual cortex.

Acknowledgments

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References

1. Abraham, W.C. and Bear, M.F. Metaplasticity: The plasticity of

synaptic plasticity. *Trends Neurosci*. 19: 126-130, 1996.
2. Artola, A., Brocher, S. and Singer, W. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature* 347: 69-72, 1990.
3. Bi, G.-Q. and Poo, M.-M. Synaptic modifications in cultured hippocampal neurons: Dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci*. 18: 10464-10472, 1998.
4. Cao, Z., Lickey, M.E., Liu, L., Kirk, E. and Gordon, B. Postnatal development of NR1, NR2A and NR2B immunoreactivity in the visual cortex of the rat. *Brain Res*. 859: 26-37, 2000.
5. Chen, N., Luo, T. and Raymond, L.A. Subtype-dependence of NMDA receptor channel open probability. *J. Neurosci*. 19: 6844-6854, 1999.
6. Chistiakova, M., Balaban, P., Eysel, U.T. and Volgushev, M. NMDA receptor blockade prevents LTD, but not LTP induction by intracellular tetanization. *Neuroreport* 10: 3869-3874, 1999.
7. Cho, K., Kemp, N., Noel, J., Aggleton, J.P., Brown, M.W. and Bashir, Z.I. A new form of long-term depression in the perirhinal cortex. *Nat. Neurosci.* 3: 150-156, 2000.
8. de Marchena, J., Roberts, A.C., Middlebrooks, P.G., Valakh, V., Yashiro, K., Wilfley, L.R. and Philpot, B.D. NMDA receptor antagonists reveal age-dependent differences in the properties of visual cortical plasticity. *J. Neurophysiol*. 100: 1936-1948, 2008.
9. Dudek, S.M. and Bear, M.F. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci. USA* 89: 4363-4367, 1992.
10. Erreger, K., Dravid, S.M., Banke, T.G., Wyllie, D.J. and Traynelis, S.F. Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *J. Physiol*. 563: 345-358, 2005.
11. Flint, A.C., Maisch, U.S., Weishaupt, J.H., Kriegstein, A.R. and Monyer, H. NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J. Neurosci.* 17: 2469-2476, 1997.
12. Flint, A.C., Maisch, U.S., Weishaupt, J.H., Kriegstein, A.R. and Monyer, H. NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J. Neurosci*. 17: 2469-2476, 1997.
13. Hatton, C.J. and Paoletti, P. Modulation of triheteromeric NMDA receptors by N-terminal domain ligands. *Neuron* 46: 261-274, 2005.
14. Jiang, B., Trevino, M. and Kirkwood, A. Sequential development of long-term potentiation and depression in different layers of the mouse visual cortex. *J. Neurosci*. 27: 9648-9652, 2007.
15. Kirkwood, A. and Bear, M.F. Homosynaptic long-term depression in the visual cortex. *J Neurosci*. 14: 3404-3412, 1994.
16. Kollen, M., Dutar, P. and Jouvenceau, A. The magnitude of hippocampal long term depression depends on the synaptic location of activated NR2-containing N-methyl-D-aspartate receptors. *Neuroscience* 154: 1308-1317, 2008.
17. Li, P., Li, Y.H. and Han, T.Z. NR2A-containing NMDA receptors are required for LTP induction in rat dorsolateral striatum *in vitro*. *Brain Res*. 1274: 40-46, 2009.
18. Li, Y.-H. and Han, T.-Z. Glycine modulates synaptic NR2A- and NR2B-containing NMDA receptor-mediated responses in the rat visual cortex. *Brain Res*. 1190: 49-55, 2008.
19. Li, Y.H., Han, T.Z. and Meng, K. Tonic facilitation of glutamate release by glycine binding sites on presynaptic NR2B-containing NMDA autoreceptors in the rat visual cortex. *Neurosci. Lett.* 432: 212-216, 2008.
20. Li, Y.H., Wang, J. and Zhang, G. Presynaptic NR2B-containing NMDA autoreceptors mediate gluta-matergic synaptic transmission in the rat visual cortex. *Curr. Neurovasc. Res*. 6: 104-109, 2009.
21. Liu, L., Wong, T.P., Pozza, M.F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y.P. and Wang, Y.T. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304: 1021-1024, 2004.
22. Malenka, R.C. and Bear, M.F. LTP and LTD: An Embarrassment of Riches. *Neuron* 44: 5-21, 2004.
23. Mason, A. and Larkman, A. Correlations between morphology and electrophysiology of pyramidal neurons in slices of rat visual cortex. II. Electrophysiology. *J. Neurosci*. 10: 1415-1428, 1990.
24. Massey, P.V., Johnson, B.E., Moult, P.R., Auberson, Y.P., Brown, M.W., Molnar, E., Collingridge, G.L. and Bashir, Z.I. Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J. Neurosci*. 24: 7821-7828, 2004.
25. McBain, C.J. and Mayer, M.L. N-methyl-D-aspartic acid receptor structure and function. *Physiol. Rev*. 74: 723-760, 1994.
26. Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B. and Seeburg, P.H. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 256: 1217-1221, 1992.
27. Morishita, W., Lu, W., Smith, G.B., Nicoll, R.A., Bear, M.F. and Malenka, R.C. Activation of NR2B-containing NMDA receptors is not required for NMDA receptor-dependent long-term depression. *Neuropharmacology* 52: 71-76, 2007.
28. Neyton, J. and Paoletti, P. Relating NMDA receptor function to receptor subunit composition: limitations of the pharmacological approach. *J. Neurosci*. 26: 1331-1333, 2006.
29. Paoletti, P., Ascher, P. and Neyton, J. High-affinity zinc inhibition of NMDA NR1-NR2A receptors. *J. Neurosci*. 17: 5711-5725, 1997.
30. Perrett, S.P., Dudek, S.M., Eagleman, D., Montague, P.R. and Friedlander, M.J. LTD induction in adult visual cortex: Role of stimulus timing and inhibition. *J. Neurosci*. 21: 2308-2319, 2001.
31. Quinlan, E.M., Olstein, D.H. and Bear, M.F. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc. Natl. Acad. Sci. USA* 96: 12876-12880, 1999.
32. Rao, Y. and Daw, N.W. Layer variations of long-term depression in rat visual cortex. *J. Neurophysiol*. 92: 2652-2658, 2004.
33. Rittenhouse, C.D., Shouval, H.Z., Paradiso, M.A. and Bear, M.F. Monocular deprivation induces homosynaptic long-term depression in visual cortex. *Nature* 397: 347-350, 1999.
34. Sawtell, N.B., Huber, K.M., Roder, J.C. and Bear, M.F. Induction of NMDA receptor-dependent long-term depression in visual cortex does not require metabotropic glutamate receptors. *J. Neurophysiol*. 82: 3594-3597, 1999.
35. Schotanus, S.M. and Chergui, K. Long-term potentiation in the nucleus accumbens requires both NR2A- and NR2B-containing N-methyl-D-aspartate receptors. *Eur. J. Neurosci*. 27: 1957-1964, 2008.
36. Sheng, M., Cummings, J., Roldan, L.A., Jan, Y.N. and Jan, L.Y. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature* 368: 144-147, 1994.
37. Toyoda, H., Zhao, M.G. and Zhuo, M. Roles of NMDA receptor NR2A and NR2B subtypes for long-term depression in the anterior cingulate cortex. *Eur. J. Neurosci*. 22: 485-494, 2005.
38. Zhang, L., Meng, K., Li, Y.H. and Han, T.Z. NR2A-containing NMDA receptors are required for L-LTP induction and depotentiation in CA1 region of hippocampal slices. *Eur. J. Neurosci*. 29: 2137-2144, 2009.