

# NMDAR LTP and LTD induction: 2B or Not 2B... is that the question?

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**Abstract** NMDA receptor (NMDAR) dependent forms of synaptic plasticity are thought to play critical roles in many aspects of CNS function and dysfunction, from learning and memory to addiction. NMDARs are heteromeric tetramers principally comprised of two NR1 subunits and two of four varieties of NR2 subunits (NR2A–2D). Recently, it has been proposed that specific NR2 subtypes subserve distinct roles in NMDAR-dependent long-term potentiation (LTP) and long-term depression (LTD). Here, we will review this literature, and describe an existing countervailing hypothesis, the charge-transfer hypothesis, which postulates that the total charge transfer through NMDARs, rather than specific subunits, dictates the polarity of synaptic plasticity. We will propose that a modification of the charge-transfer hypothesis, to include the possible involvement of protein-protein interactions imparted by distinct NR2 subunits, best fits the existing data.

**Keywords** NMDA · synaptic plasticity · NR2A · NR2B · long-term potentiation · long-term depression · learning · memory

What are the molecular substrates for learning and memory? Intense research on this fundamental question has yielded two candidate mechanisms: a long lasting

enhancement of synaptic efficacy termed long-term potentiation (LTP) and a long-lasting reduction in synaptic efficacy termed long-term depression (LTD). It has been long surmised that these processes underlie memory formation, although direct evidence has been notably absent. Recent reports suggest that LTP and LTD can occur *in vivo* after a variety of behavioral conditioning paradigms [24, 31, 32, 38, 41, 44], reinforcing the need for an in depth mechanistic understanding of these processes.

The NMDA receptor (NMDAR) is a molecule that has figured prominently in both learning and synaptic plasticity. Specifically, the NMDAR controls the induction of certain forms of both LTP and LTD and particular forms of learning. It is crucial to note that while multiple NMDAR-dependent forms of exist, this article focuses solely on NMDAR-dependent forms of plasticity. NMDARs are members of the glutamate receptor family of ligand-gated ion channels, which include AMPA and kainate receptors [6]. NMDARs have two key properties that underlie their unique role in plasticity. The first is that they are permeable to calcium, a vital signaling molecule. The second is that at normal resting membrane potential they do not permeate ions because of a magnesium block of the pore. This allows these receptors to act as coincidence detectors requiring both postsynaptic membrane depolarization and presynaptic release of glutamate to be active.

Structurally, NMDARs are tetrameric assemblies composed of two NR1 subunits and two NR2 subunits [11], with the NR2A and NR2B subtypes being the most highly expressed and well-studied. Whereas NR1 splice variants [7] and NR3 subunits exist [35] and can have dramatic effects on NMDAR function, the majority of plasticity research has focused on the NR2 subunit and the ability of different NR2 subtypes to confer unique biophysical, pharmacological, signaling, and localization properties to

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the receptor [6]. These differences have been proposed to permit the NMDAR to induce two opposing forms of plasticity, LTP and LTD, in an NR2 subunit-specific fashion. We will focus first on critical differences between NR2A and NR2B subunits, then the proposed specific roles that these subunits play in the induction of LTP and LTD. We will then present an alternative explanation that is consistent with the majority of the results obtained to date, which is that there is no required subunit specificity for current flow through specific NMDARs for the induction of LTP and LTD. Rather, induction of plasticity requires a critical level of charge transfer through NMDARs and key protein–protein interactions.

### NR2A, NR2B: Key differences

One of the most apparent and widely appreciated differences between NR2A and NR2B containing NMDARs are their current-passing kinetic profiles. Diheteromeric NR2A containing NMDARs (two NR2A subunits and two NR1 subunits) have much more rapid decay rates compared to diheteromeric NR2B containing NMDARs [42]. Triheteromeric NMDARs (NR2A, NR2B, and two NR1 subunits) are thought to have a kinetic profile that is intermediate to diheteromeric populations [39], although a direct evaluation of microscopic kinetics of currents isolated from these receptors has not been performed. A recent study investigating the single-channel activities of NR2A and NR2B receptors proposed a kinetic model in which these receptors would promote differential temporal summation, which may have relevance to induction of plasticity [8].

Another major difference between NR2A and NR2B subunits is the protein–protein interactions in which they participate. For example, NR2B interacts with autophosphorylated CaMKII $\alpha$  with higher affinity than NR2A [34]. Furthermore, differences may exist in the relative localization of these subunits on the neuronal surface membrane, with the NR2B subunit exhibiting a greater enrichment at extrasynaptic sites when compared to the NR2A subunit [39]. However, these arguments are made at least in part based on experiments using suboptimal pharmacological tools, as will be discussed below. Furthermore, it is also clear that NR2A-containing receptors exist extrasynaptically [37], and NR2B-containing receptors exist synaptically [25], thus, in the best of cases this is not a binary distribution.

### NR2A, NR2B: Tools of the trade

Whereas genetic models and molecular tools can provide great insight into the involvement of NR2A and NR2B subunits in plasticity (and will be discussed below), over the

last few years pharmacological tools have been the most widely used tools for probing subunit selective function. There are several highly NR2B-selective allosteric antagonists available, notably ifenprodil and its higher affinity analogs, Ro 25-6981 and CPP101,606. The selectivity of these compounds, particularly Ro 25-6981 and CPP101,606, is excellent for NR2B-containing receptors [9, 26]; however, it has been noted that high concentrations of ifenprodil [46] can antagonize non-NR2B-containing receptors and other targets [16]. Further complicating the actions of these compounds is their ability to actually enhance NMDAR function at low levels of glutamate [9, 45].

NR2A selective antagonists have been more difficult to develop. Recently, a new compound, NVP-AAM077, was reported to have nearly 600-fold selectivity for NR2A vs NR2B when coapplied with the agonist [20]. However, a number of later studies have shown that the selectivity after preapplication of NVP-AAM077 is too low to allow for meaningful discrimination of NR2A vs NR2B contributions to synaptically elicited NMDAR currents [10, 28, 43]. Notably, Frizelle et al. [10] modeled the effects of NVP-AAM077 on both single and tetanically evoked NMDA excitatory postsynaptic currents composed of NR2A and NR2B diheteromers and found the selectivity too narrow under both circumstances.

It has further been shown that zinc can selectively inhibit NR2A-containing receptors at low concentrations (in nanomolar range) via an allosteric interaction with the N-terminal domain [29]. The concentration of zinc in the extracellular space is similar in range to the  $K_i$ , thus, it has been proposed that zinc exerts a tonic modulation on NR2A receptors. However, several factors act as confounders for using zinc as a subunit-selective compound, namely, the presence of a low-affinity binding site on the NR2B subunit [30], the pH dependence of the interaction [51], and the ability of zinc to act on signaling pathways independent of the NMDAR [33].

Another problem with the use of pharmacological tools in delineating the contributions of specific NR subunits is that the bulk of the studies examining the actions of these selective antagonists have been performed in expression systems focusing on diheteromeric receptors. However, strong evidence suggests the presence of endogenous triheteromeric receptors [21, 22, 39], yet little is known about their pharmacology. Recently, Hatton and Paoletti [12] used an elegant mutation strategy to isolate triheteromeric responses and examine their pharmacology in an expression system. These results suggested that N-terminal domain allosteric antagonists, ifenprodil and zinc, retain high affinity for triheteromeric receptors but with substantially reduced efficacy. Interestingly, when applied together these compounds can have a supraadditive effect. Because of this lowered efficacy, it is thus debatable whether existing pharmacological

tools are useful in assessing the role of current passing through triheteromeric receptors in neuronal function.

In addition to these pharmacological tools, a number of genetic models are available for the study of NR2A and NR2B. Whereas traditional NR2B knockouts produce early lethality [18], NR2A knockouts are viable and fertile [15]. Knockin mice generated with NR2 subunits lacking C-terminal tails have also proven useful [17], as have transgenic mice overexpressing NR2B [36]. Finally, a variety of viral-mediated transfer studies have been employed, as will be described below.

### LTP: Subunit selectivity or lack thereof?

#### Genetic and molecular studies

Several different genetic techniques have been employed to determine the contribution of individual NMDAR subunits to LTP. A global NR2A knockout generated by Mishina et al. [15] had both reductions in NMDAR currents and LTP in the hippocampus, although LTP was not ablated. A similar result was obtained with mice in which only the C-terminal domain of the NR2A subunit was deleted [17]. However, analysis of the NMDAR currents in these animals showed no difference in magnitude when compared to wild type, suggesting that the loss of an interacting protein may have caused the deficit of LTP. Further characterization of these animals showed that the deficit in LTP could be overcome with a stronger induction protocol, raising the idea that there is a threshold of activation or charge transfer necessary for induction of LTP.

Whereas no experiments have been undertaken investigating LTP in NR2B knockout animals, the effect of transgenic overexpression of NR2B has been shown to enhance LTP in the hippocampus [36]. Concomitant with this enhancement of LTP was an increase in the decay time of NMDAR-mediated currents and an increase in charge transfer. In keeping with this finding, antisense knockdown of NR2B subunit expression in the hippocampus impaired both LTP and spatial learning [5]. Utilizing a similar approach, Zhuo et al. [50] has shown that in the anterior cingulate, knockdown of NR2B receptors using siRNA can both reduce NMDAR-mediated currents and induction of LTP. Furthermore, in an elegant series of experiments, Barria and Malinow [1] used molecular tools to identify a critical role of the interaction between NR2B and CaMKII in plasticity. They showed that by transfecting hippocampal slice cultures with either a NR2A construct or a NR2B construct with diminished CaMKII binding they could dramatically reduce LTP, whereas transfection with a chimeric NR2A/B subunit containing only the NR2B CaMKII-binding domain rescues this loss of LTP. This

result, along with the studies examining the C-terminal knockouts of the NR2A subunit, highlights the importance of receptor subunits beyond current flow, as the intracellular bound protein proved to be the critical variable. Furthermore, these studies do not directly implicate one subunit vs another, but rather suggest that both NR2A and NR2B can play roles in induction of LTP.

#### Pharmacological studies

An early report using antagonists with selectivity for NR2A/B- vs NR2C/D-containing receptors suggested a more prominent role for NR2A/B receptors in the induction of LTP in the hippocampus [14]. Working in the cortex, Yoshimura et al. [49] noted the involvement of NR2B in pairing-induced LTP. Moreover, the authors found a developmental decline in NR2B expression that correlated with reduction of pairing-induced LTP, supporting the possibility that NR2B was critical for this form of plasticity.

Striking results were reported in 2004 when two groups reported that the purportedly NR2A-selective antagonist NVP-AAM077 could selectively block LTP in hippocampus [20] and visual cortex [23], whereas the NR2B-selective compound Ro 25-6981 had no effect. Unfortunately, as discussed above, problems of the properties of both of these ligands preclude interpretation of these results. First, the actions of the compound NVP-AAM077 are difficult to interpret because of the compound's lack of selectivity when applied before agonist, as in the case of an LTP experiment [43]. Furthermore, while we do not call in to question the selectivity of Ro 25-6981, the lack of effect of Ro 25-6981 only suggests that LTP is not impacted by the relatively selective inhibition of synaptic diheteromeric NR2B-containing receptors, as this compound has a reduced efficacy at triheteromeric receptors [12]. Finally, antagonism at NR2B-containing receptors would not be predicted to disrupt key NR2B-specific protein–protein interactions, such as with CaMKII.

Subsequent results obtained with these and other NMDAR antagonists reinforce the idea that the utility of these compounds is limited for interpretation of roles of NR2 subunits in neuronal function. For example, Berberich et al. [4] reported that LTP was induced in the hippocampus by high-frequency stimulation in the presence of lower concentrations of NVP-AAM077 that nonetheless still maximally inhibit diheteromeric NR2A-containing receptors. Furthermore, using an alternative LTP induction protocol, low-frequency stimulation (LFS) paired with postsynaptic depolarization, neither a “selective” concentration of NVP-AAM077 nor CP-101,606 impaired LTP. It should be noted that one critical difference is that Berberich et al. used mice whereas the original reports with NVP-AAM077 were in rats. Simultaneously, Weitlauf et al. [43]

showed that LTP persists in the bed nucleus of the stria terminalis of the NR2A knockout animal and that this LTP was still blocked by NVP-AAM077 in the NR2A knockout. Subsequent experiments have shown that the synaptically evoked NMDA current in the NR2A knockout is nearly completely blocked by Ro 25-6981 (T. Kash, unpublished results), suggesting NR2B-containing receptors can induce LTP in this region. Following up on their earlier work in the anterior cingulate cortex, Zhuo et al. [50] demonstrated that NR2B antagonists could partially block LTP produced by several different induction protocols. The authors also suggested, through the use of NVP-AAM077, that NR2A was involved in induction of LTP; unfortunately, the concentrations used in this study are nonselective. However, given the additive nature of the antagonists, the results are in line with a simple charge transfer requirement for induction of LTP.

Two recent reports have further expanded our understanding of this field. Collingridge et al. [2] showed that in the hippocampus of 2-week-old female rats Ro 25-6981 could reduce LTP. Given the concern that had arisen regarding the selectivity of NVP-AAM077, the authors performed a series of experiments that ultimately resulted in what appeared to be a concentration of NVP-AAM077 that was selective for NR2A. However, it remains a question as to how effective a concentration of drug found to be selective in a slow solution exchange recombinant system will be in determining subunit selectivity in a system such as brain slice with significant diffusion barriers. Using this concentration, they demonstrated that this concentration of NVP-AAM077 could also impair induction of LTP. Taken together, these data suggest that at this particular developmental stage both NR2A and NR2B are involved in induction of plasticity. Following up on an earlier paper demonstrating a lack of subunit selectivity for LTP in the mouse hippocampus, Berberich et al. [3] recently published a paper in which they examined the effect of a range of concentrations of different antagonists on a LFS LTP induction protocol. The results demonstrated that total charge transfer during induction was the critical variable for induction of LTP, rather than the specific subunit that was antagonized.

### **LTP conclusions: NMDAR–LTP can be elicited either through NR2A- or NR2B-containing NMDARs**

This brief review of studies investigating the subunit selectivity of LTP indicates that there is no clear evidence for a role for NR2A as a specific “LTP” NMDAR subunit across species, brain regions, or developmental stage. Rather than subunit selectivity, much of the results suggest a “charge transfer hypothesis,” where a minimal amount of calcium must flux into the postsynaptic cell to induce plasticity. The

idea that there is a requirement of charge transfer required for plasticity is in keeping with the model proposed by Lisman [19] in which postsynaptic calcium can trigger both LTP and LTD depending on the nature of the calcium signal. This is supported by several studies that demonstrated the ability of directly elevating postsynaptic calcium using a photolytic uncaging technique to induce a form of plasticity similar to LTP [27, 48]. However, several studies discussed above have demonstrated that binding of intracellular proteins also has a critical role in induction of LTP. One simple mechanistic explanation that integrates these findings is that LTP induction requires both of these events; a minimal level of calcium flux, which then activates CaMKII and other calcium-dependent signaling pathways. The ability in some systems to observe a subunit-selective induction in certain situations but not others is not entirely surprising given the dynamic regulation of synaptic NR2A and NR2B levels across brain regions and developmental stages. It has been suggested via modeling studies that NR2A and NR2B dimeromers have divergent charge transfer responses to similar tetanus protocols [8]. Thus, the selectivity that is seen with some induction protocols may reflect a more important role for charge transfer mediated by one receptor subtype based on that particular stimulation protocol.

### **LTD: Subunit or subcellular dependence?**

#### Genetic and molecular studies

Unfortunately, the application of genetic and molecular studies aimed at determining subtype selectivity of LTD induction has been relatively sparse when compared to LTP. In the NR2B knockout mouse, there was a near total lack of NMDAR-mediated synaptic currents and no ability to induce LTD [18]. Unfortunately, the authors did not examine the ability to induce LTP in these animals; however, given the lack of NMDAR currents it is reasonable to believe that this would be defective as well.

#### Pharmacological studies

The initial antagonist studies described above from the Sacktor lab, suggesting that NR2A/B are linked to LTP, also suggested that NR2C/D were linked to LTD [14]. However, the lack of selective antagonists has hampered this line of investigation, and the focus here will be centered on the role of the NR2B subunit. Morrisett et al. [13] reported that ifenprodil enhanced induction of LTD in the hippocampus of 2- to 3-week-old rats. The study by Liu et al. [20] in hippocampus stands in direct contrast to this result, showing that antagonism of NR2B abolished induction of LTD in the hippocampus of 3- to 4-week-old



rats. Similar to this finding, Toyoda et al. [40] demonstrated an NR2B-ligand-sensitive form of LTD. Most recently, three different groups published parallel studies with NR2B-selective ligands on hippocampal LTD, and were unable to replicate NR2B-ligand sensitivity of LTD [25]. Thus, these data suggest that likely subtle differences in the preparations between labs may influence the sensitivity of LTD to these drugs, reinforcing the idea that there is no absolute requirement for the recruitment of NR2B-containing receptors, and further consistent with the idea that the majority of these types of experiments are also consistent with the “charge transfer” hypothesis.

In all of the previously discussed LTD studies a stimulation protocol was used that was able to consistently produce NMDAR-dependent LTD. In several other studies a LFS protocol was only able to induce LTD in the presence of altered glutamate transport, induced either pharmacologically or via behavioral manipulations [23, 47]. In these studies, the authors concluded that extrasynaptic NMDARs were coupled to LTD induction. Furthermore, they demonstrated that both the LTD and the extrasynaptic NMDAR response was mediated via NR2B-containing receptors.

### LTD: Conclusions

Based on the available literature it is difficult to draw conclusions regarding the general role of NR2A and NR2B subunits in LTD. In the hippocampus, it could be suggested that there are distinct forms of NMDAR-dependent LTD, a form that can be induced without alterations in glutamate transport, and one that requires alterations in transport. Any differential pharmacology of these different forms could result from the different concentrations of glutamate at extrasynaptic sites. This is particularly interesting, given the ability of certain NR2B antagonists to actually enhance NR2B-mediated transmission when low concentrations of glutamate are present. One might predict that the inability of NR2B antagonists to inhibit LTD and in some cases enhance LTD is because of this pharmacological property, and that mechanistically this may be because of enhancing the actions of glutamate at extrasynaptic NMDARs. Finally, while several of these reports suggest that extrasynaptic NR2B-containing receptors are critical for induction of LTD, one cannot exclude the possibility that a similar charge transfer phenomena exists for solely extrasynaptic receptors.

### The future of plasticity research

Throughout this article, the focus has been primarily on the role of receptor subtypes in plasticity as revealed with antagonists, both selective and nonselective. The pharma-

cological approach is extremely valuable, but it has limits, in such that it focuses on merely one aspect of the NMDAR complex, charge transfer. Whereas we do favor the idea of the charge transfer hypothesis in which subtype selectivity observed may be determined by the innate receptor kinetics, there is clearly another story to be told, that of protein interactions. To make progress in understanding of these complex interactions, we believe that future studies should embrace innovative genetic and molecular techniques such as floxed NR2 subunits and receptor chimeras.

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### References

- Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* 48:289–301
- Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D (2006) Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. *Neuropharmacology* 52:60–70
- Berberich S, Jensen V, Hvalby O, Seeburg PH, Kohr G (2006) The role of NMDAR subtypes and charge transfer during hippocampal LTP induction. *Neuropharmacology* 52:77–86
- Berberich S, Punnakkal P, Jensen V, Pawlak V, Seeburg PH, Hvalby O, Kohr G (2005) Lack of NMDA receptor subtype selectivity for hippocampal long-term potentiation. *J Neurosci* 25:6907–6910
- Clayton DA, Mesches MH, Alvarez E, Bickford PC, Browning MD (2002) A hippocampal NR2B deficit can mimic age-related changes in long-term potentiation and spatial learning in the Fischer 344 rat. *J Neurosci* 22:3628–3637
- Cull-Candy SG, Leszkiewicz DN (2004) Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* 2004:re16
- Durand GM, Bennett MV, Zukin RS (1993) Splice variants of the N-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proc Natl Acad Sci U S A* 90:6731–6735
- Erreger K, Dravid SM, Banke TG, Wyllie DJ, Traynelis SF (2005) Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *J Physiol* 563:345–358
- Fischer G, Mutel V, Trube G, Malherbe P, Kew JN, Mohacsi E, Heitz MP, Kemp JA (1997) Ro 25-6981, a highly potent and selective blocker of N-methyl-D-aspartate receptors containing the NR2B subunit. Characterization in vitro. *J Pharmacol Exp Ther* 283:1285–1292
- Frizelle PA, Chen PE, Wyllie DJ (2006) Equilibrium constants for (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid (NVP-AAM077) acting at recombinant NR1/NR2A and NR1/NR2B N-methyl-D-aspartate receptors: Implications for studies of synaptic transmission. *Mol Pharmacol* 70:1022–1032
- Furukawa H, Singh SK, Mancusso R, Gouaux E (2005) Subunit arrangement and function in NMDA receptors. *Nature* 438:185–192
- Hatton CJ, Paoletti P (2005) Modulation of triheteromeric NMDA receptors by N-terminal domain ligands. *Neuron* 46:261–274

13. Hendricson AW, Miao CL, Lippmann MJ, Morrisett RA (2002) Ifenprodil and ethanol enhance NMDA receptor-dependent long-term depression. *J Pharmacol Exp Ther* 301:938–944
14. Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, Monaghan DT, Sacktor TC (2000) Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. *J Neurosci* 20:RC81
15. Kiyama Y, Manabe T, Sakimura K, Kawakami F, Mori H, Mishina M (1998) Increased thresholds for long-term potentiation and contextual learning in mice lacking the NMDA-type glutamate receptor epsilon1 subunit. *J Neurosci* 18:6704–6712
16. Kobayashi T, Washiyama K, Ikeda K (2006) Inhibition of G protein-activated inwardly rectifying K<sup>+</sup> channels by ifenprodil. *Neuropsychopharmacology* 31:516–524
17. Kohr G, Jensen V, Koester HJ, Mihaljevic AL, Utvik JK, Kvello A, Ottersen OP, Seeburg PH, Sprengel R, Hvalby O (2003) Intracellular domains of NMDA receptor subtypes are determinants for long-term potentiation induction. *J Neurosci* 23:10791–10799
18. Kutsuwada T, Sakimura K, Manabe T, Takayama C, Katakura N, Kushiya E, Natsume R, Watanabe M, Inoue Y, Yagi T, Aizawa S, Arakawa M, Takahashi T, Nakamura Y, Mori H, Mishina M (1996) Impairment of suckling response, trigeminal neuronal pattern formation, and hippocampal LTD in NMDA receptor epsilon 2 subunit mutant mice. *Neuron* 16:333–344
19. Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc Natl Acad Sci U S A* 86:9574–9578
20. Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304:1021–1024
21. Lu C, Fu Z, Karavanov I, Yasuda RP, Wolfe BB, Buonanno A, Vicini S (2006) NMDA receptor subtypes at autaptic synapses of cerebellar granule neurons. *J Neurophysiol* 96:2282–2294
22. Luo J, Wang Y, Yasuda RP, Dunah AW, Wolfe BB (1997) The majority of N-methyl-D-aspartate receptor complexes in adult rat cerebral cortex contain at least three different subunits (NR1/NR2A/NR2B). *Mol Pharmacol* 51:79–86
23. Massey PV, Johnson BE, Moulton PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J Neurosci* 24:7821–7828
24. McKernan MG, Shinnick-Gallagher P (1997) Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature* 390:607–611
25. Morishita W, Lu W, Smith GB, Nicoll RA, Bear MF, Malenka RC (2006) Activation of NR2B-containing NMDA receptors is not required for NMDA receptor-dependent long-term depression. *Neuropharmacology*
26. Mott DD, Doherty JJ, Zhang S, Washburn MS, Fendley MJ, Lyuboslavsky P, Traynelis SF, Dingledine R (1998) Phenylethanolamines inhibit NMDA receptors by enhancing proton inhibition. *Nat Neurosci* 1:659–667
27. Neveu D, Zucker RS (1996) Postsynaptic levels of [Ca<sup>2+</sup>]<sub>i</sub> needed to trigger LTD and LTP. *Neuron* 16:619–629
28. Neyton J, Paoletti P (2006) Relating NMDA receptor function to receptor subunit composition: limitations of the pharmacological approach. *J Neurosci* 26:1331–1333
29. Paoletti P, Ascher P, Neyton J (1997) High-affinity zinc inhibition of NMDA NR1-NR2A receptors. *J Neurosci* 17:5711–5725
30. Rachline J, Perin-Dureau F, Le Goff A, Neyton J, Paoletti P (2005) The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. *J Neurosci* 25:308–317
31. Rogan MT, Staubli UV, LeDoux JE (1997) Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390:604–607
32. Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. *Science* 308:83–88
33. Smart TG, Hosie AM, Miller PS (2004) Zn<sup>2+</sup> ions: modulators of excitatory and inhibitory synaptic activity. *Neuroscientist* 10:432–442
34. Strack S, Colbran RJ (1998) Autophosphorylation-dependent targeting of calcium/calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl-D-aspartate receptor. *J Biol Chem* 273:20689–20692
35. Sun L, Margolis FL, Shipley MT, Lidow MS (1998) Identification of a long variant of mRNA encoding the NR3 subunit of the NMDA receptor: its regional distribution and developmental expression in the rat brain. *FEBS Lett* 441:392–396
36. Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic enhancement of learning and memory in mice. *Nature* 401:63–69
37. Thomas CG, Miller AJ, Westbrook GL (2006) Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. *J Neurophysiol* 95:1727–1734
38. Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* 4:1217–1223
39. Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J Neurosci* 19:4180–4188
40. Toyoda H, Zhao MG, Zhuo M (2005) Roles of NMDA receptor NR2A and NR2B subtypes for long-term depression in the anterior cingulate cortex. *Eur J Neurosci* 22:485–494
41. Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 411:583–587
42. Vicini S, Wang JF, Li JH, Zhu WJ, Wang YH, Luo JH, Wolfe BB, Grayson DR (1998) Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. *J Neurophysiol* 79:555–566
43. Weitlauf C, Honse Y, Auberson YP, Mishina M, Lovinger DM, Winder DG (2005) Activation of NR2A-containing NMDA receptors is not obligatory for NMDA receptor-dependent long-term potentiation. *J Neurosci* 25:8386–8390
44. Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. *Science* 313:1093–1097
45. Whitemore ER, Ilyin VI, Konkoy CS, Woodward RM (1997) Subtype-selective antagonism of NMDA receptors by nylidrin. *Eur J Pharmacol* 337:197–208
46. Williams K (1993) Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol Pharmacol* 44:851–859
47. Yang CH, Huang CC, Hsu KS (2005) Behavioral stress enhances hippocampal CA1 long-term depression through the blockade of the glutamate uptake. *J Neurosci* 25:4288–4293
48. Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic [Ca<sup>2+</sup>]<sub>i</sub> elevation. *J Neurophysiol* 81:781–787
49. Yoshimura Y, Ohmura T, Komatsu Y (2003) Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex. *J Neurosci* 23:6557–6566
50. Zhao MG, Toyoda H, Lee YS, Wu LJ, Ko SW, Zhang XH, Jia Y, Shum F, Xu H, Li BM, Kaang BK, Zhuo M (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. *Neuron* 47:859–872
51. Zheng F, Erreger K, Low CM, Banke T, Lee CJ, Conn PJ, Traynelis SF (2001) Allosteric interaction between the amino terminal domain and the ligand binding domain of NR2A. *Nat Neurosci* 4:894–901