Neuron
Article

Rapid Bidirectional Switching of Synaptic NMDA Receptors

Camilla Bellone[1] and Roger A. Nicoll[1,*]
[1]Departments of Cellular and Molecular Pharmacology and Physiology, University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: nicoll@cmp.ucsf.edu
DOI 10.1016/j.neuron.2007.07.035

SUMMARY

Synaptic NMDA-type glutamate receptors (NMDARs) play important roles in synaptic plasticity, brain development, and pathology. In the last few years, the view of NMDARs as relatively fixed components of the postsynaptic density has changed. A number of studies have now shown that both the number of receptors and their subunit compositions can be altered. During development, the synaptic NMDARs subunit composition changes, switching from predominance of NR2B-containing to NR2A-containing receptors, but little is known about the mechanisms involved in this developmental process. Here, we report that, depending on the pattern of NMDAR activation, the subunit composition of synaptic NMDARs is under extremely rapid, bidirectional control at neonatal synapses. This switching, which is at least as rapid as that seen with AMPARs, will have immediate and dramatic consequences on the integrative capacity of the synapse.

INTRODUCTION

NMDARs are heteromeric cation channels composed of NR1 and NR2 subunits. These channels require the near simultaneous binding of glutamate and membrane depolarization to open, thus making this receptor the coincidence detector of pre- and postsynaptic activity underlying Hebbian synaptic plasticity. NMDAR channels display a high permeability to calcium, which, depending on the level of spine calcium, triggers either long-term potentiation (LTP) (high calcium) or long-term depression (LTD) (modest calcium) of AMPAR synaptic currents. The channel properties depend on the subunit composition of the receptor. All receptors require the presence of two NR1 subunits. The NR1 subunits in the hippocampus can assemble as heterodimers containing NR1/NR2A or NR1/NR2B or as heterotrimers containing NR1/NR2A/NR2B. NR2B receptors have a slower decay compared to NR2A receptors and are selectively blocked by the antagonist ifenprodil (Cull-Candy and Leszkiewicz, 2004). NR2B receptors are expressed early in development, while the expression of NR2A receptors increases during development (Monyer et al., 1994; Sans et al., 2000; Sheng et al., 1994). These developmental changes are accompanied by an acceleration of the decay of the NMDA EPSC and a decrease in ifenprodil sensitivity (Kirson and Yaari, 1996). In the neocortex, these changes occur during the critical period of visual development, and the switch is influenced by experience (Carmignoto and Vicini, 1992; Philpot et al., 2001). The discovery of subunit-specific antagonists for NMDARs, which are currently lacking for AMPARs, provides a powerful tool for studying possible subunit-specific trafficking of native synaptic receptors.

In synaptic plasticity, NMDAR activation initiates a cascade of events that results in the rapid recruitment of AMPA-type glutamate receptors (AMPARs) into the synapse. Such receptor dynamics is not limited to the brain, as the density of acetylcholine receptors at the neuromuscular junction is dependent on activity as well (Akaaboune et al., 1999). Thus, a general picture of excitatory synapses has emerged in which excitatory neurotransmitter receptors are highly dynamic. In striking contrast, NMDARs have been viewed as relatively fixed structural components of the postsynaptic density; NMDARs initiate plasticity and AMPARs express the plasticity. Yet, there is evidence in the literature suggesting that this scenario may be too simplistic. In cultured neurons, synaptic NMDARs can exchange with extrasynaptic receptors (Groc et al., 2006; Tovar and Westbrook, 2002). Moreover, changes in the number of receptors and subunits composition of NMDARs appear to be mediated by change in synthesis and degradation (Mu et al., 2003; Rao and Craig, 1997). In addition, it is well established that a switch in the subunit composition of synaptic NMDARs occurs during postnatal development and that the time course of this process is governed by experience (Barth and Malenka, 2001; Carmignoto and Vicini, 1992; Carroll and Zukin, 2002; Chavis and Westbrook, 2001; Hestrin, 1992; Philpot et al., 2001; van Zundert et al., 2004). However, little is known about the mechanisms involved in this developmental process. Here, we report that the subunit composition of synaptic NMDARs can quickly change in an activity-dependent manner at neonatal, but not mature, synapses. The change in subunit composition changes both the charge transfer and kinetics of synaptic NMDA EPSCs, which, in turn, would be expected to modify the activity requirements for evoking LTP and LTD.

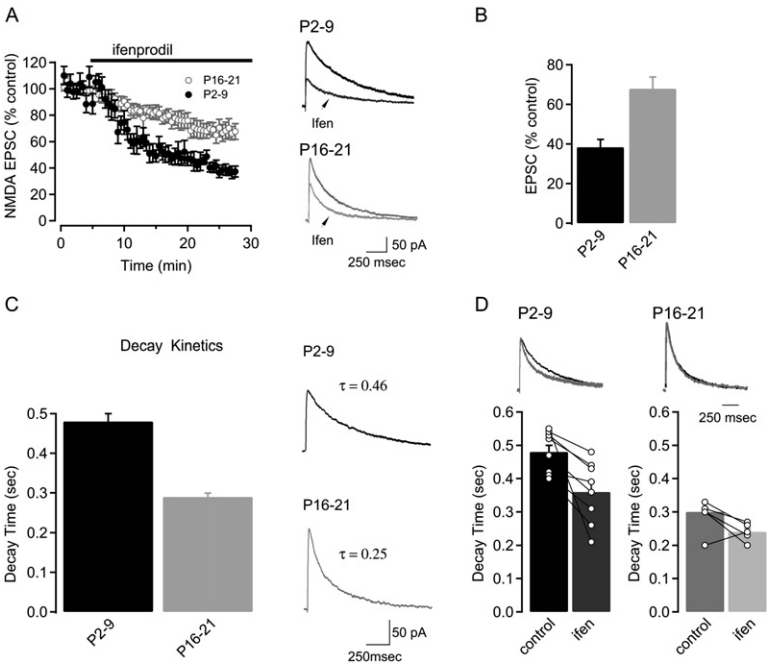

Figure 1. Developmental Change in the Relative Contribution of NR2A and NR2B Subunits to Synaptic NMDARs

(A) In the presence of NBQX (10 μM), ifenprodil (3 μM) depressed NMDA EPSCs recorded at +40 mV in young (P2-9; closed circles) and old (P16-21; open circles) rats. Representative sample traces of NMDA EPSCs from the two groups of rats before and 20 min after ifenprodil application are shown on the right.

(B) Bar graph representing average percentage of NMDA current remaining after ifenprodil application. (67.9% ± 6%, n = 6 in P16-21 group versus 38.3% ± 4%, n = 6 in P2-9 group; $p < 0.005$).

(C) Bar graphs showing the decay time constant of NMDA EPSC recorded in NBQX at +40 mV in young and old rats (0.48 ± 0.02 s, n = 11 versus 0.29 ± 0.01 s, n = 7, $p < 0.001$ Mann-Whitney test). Representative sample traces of NMDA EPSC for young and old animals.

(D) Ifenprodil treatment significantly decreased the time constant in young animals (0.48 ± 0.02 s versus 0.36 ± 0.03 s, n = 8, $p < 0.01$); in old animals, the decrease in decay time constant is less pronounced (0.29 ± 0.01 s versus 0.24 ± 0.01 s, n = 7, $p < 0.05$ Wilcoxon matched-pairs test). Sample scaled traces of NMDA EPSC from the two groups before and after ifenprodil are shown on the top.

Error bars = SEM.

RESULTS

In this study, we examined whether activity can influence the trafficking of NMDARs in hippocampal pyramidal cells. We compared the kinetics of NMDAR synaptic currents and their sensitivity to 3 μM ifenprodil in neonatal and mature hippocampal slices. At a concentration of 3 μM, ifenprodil provides a maximal and selective block of NR1/NR2B diheteromeric receptors in heterologous expression systems (Williams, 1993). As previously reported, the sensitivity of NMDA EPSCs to ifenprodil is substantially greater in neonatal slices compared to older slices (Kirson and Yaari, 1996) (Figures 1A and 1B). We next examined the effect of development on the kinetics of the NMDA EPSC. In parallel with the decreased sensitivity of the peak current to ifenprodil with age, the kinetics of the NMDA EPSCs becomes considerably faster (Figures 1C and 1D). The acceleration of the NMDA EPSC by ifenprodil in neonatal slices (Figure 1D) indicates that the decay time provides a simple assay for the subunit composition of the synaptic NMDARs.

Because the developmental switch of synaptic NMDAR subunit composition is influenced by experience, we wondered whether this switching process could be accelerated by activity in young animals. Indeed, in preliminary experiments in which we continuously monitored the NMDA EPSC at +40 mV over time in neonatal animals (2-9 days old), we noticed that the decay of the EPSC became faster (see Figure S1A in the Supplemental Data available with this article online). This acceleration did not occur if the recording pipette contained the calcium chelator BAPTA (Figure S1B) or if the EPSC was just sampled briefly at specific time points (Figure S1C). To study the effect of activity on the NMDA EPSC in a controlled manner, we stimulated two independent pathways and monitored the AMPA EPSC at −70 mV. We then induced LTP on one pathway, while using the other pathway as a control (Figure 2A). After establishing LTP of the AMPA EPSC, NBQX was applied to selectively block AMPARs, and the cell was depolarized to +40 mV to unblock the NMDARs. A comparison of the decay time between the control, naive pathway and the LTP pathway consistently revealed that the NMDA EPSC in the LTP pathway decayed more rapidly than the NMDA EPSC in the control pathway (0.33 ± 0.02 s in test pathway versus 0.45 ± 0.02 s in control pathway; n = 14, $p < 0.001$) (Figure 2B). In a separate set of experiments we found that the change in kinetics was still present 60 min after the induction of LTP (Figure 2B). We also demonstrated that the LTP of the AMPA EPSC was stable for at least an hour (Figure S1D). To determine whether this change in kinetics actually reflects a loss of NR2B-containing receptors, we then applied ifenprodil (Figure 2C). The NMDA EPSC in the LTP pathway was found to be much less sensitive to the antagonist. In addition, the effect of ifenprodil on the decay was diminished (Figure 2D), confirming the loss of NR2B-containing

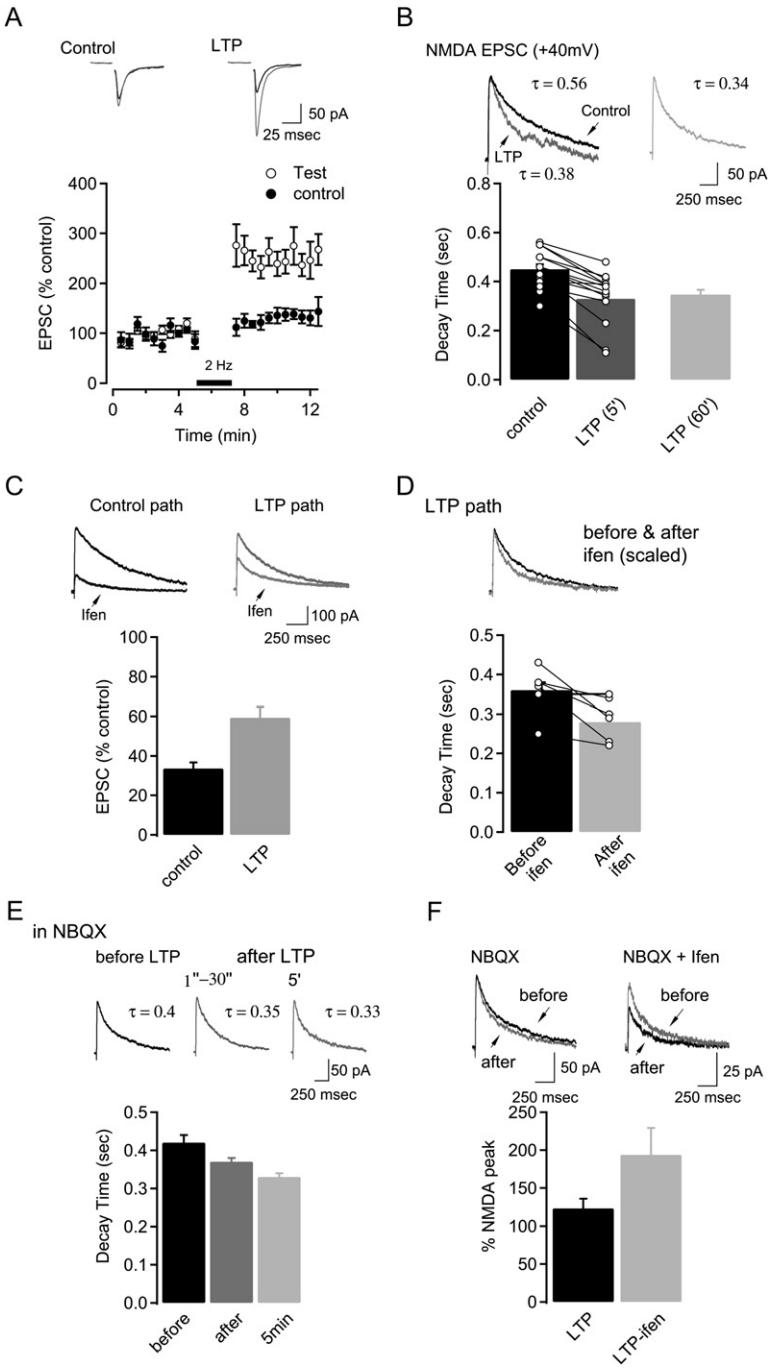

Figure 2. Long-Term Potentiation Inducing Stimuli Rapidly Switch the Subunit Composition of Synaptic NMDARs in Young Animals

(A) Summary of experiments (n = 14) showing LTP of AMPA EPSC, measured at −70 mV, induced by pairing the cells at 0 mV at a frequency of 2 Hz for 90 s. Sample traces of averaged AMPA EPSCs before and after LTP protocol for the control (unpaired) pathway (Control) and for the paired pathway (LTP) are shown on the top.

(B) Following the induction of LTP, NBQX was added and the cells were depolarized to +40 mV. In every experiment, the decay time of NMDA EPSCs in the LTP pathway is faster than that in the control pathway (0.45 ± 0.02 s versus 0.33 ± 0.02 s, n = 14, $p < 0.001$ Wilcoxon matched-pairs signed-ranks test). The decay time remains faster 60 min after LTP induction (0.35 ± 0.02 s, n = 5, $p < 0.05$ compared to control but not significantly different from LTP at 5 min). Scaled sample traces of NMDA EPSC measured at +40 mV in the two pathways are shown on the top; on the right, sample traces from representative experiment 60 min after LTP induction.

(C) Ifenprodil has less effect on the NMDA EPSC in the LTP pathway compared to the control pathway (59.39% ± 5.46.4% versus 33.67% ± 3.2%, n = 136, 811, $p < 0.05$ Mann-Whitney test) (in paired experiment 51.6% ± 6.95.8% versus 34.5% ± 4.53.74%, n = 7, $p < 0.05$). Representative sample traces from a two pathways experiment showing control and LTP pathway before and after ifenprodil are shown on the top.

(D) Ifenprodil still accelerates the decay of the NMDA EPSC on the LTP pathway, but to a smaller extent (0.36 ± 0.02 s versus 0.28 ± 0.02 s, n = 6, $p < 0.05$). Representative scaled averaged traces before (black) and after (gray) ifenprodil are shown on the top.

(E) In the same cell in the presence of NBQX (10 μM), we recorded traces before, 1–30 s after, and 5 min after the LTP protocol. A clear acceleration of the decay of the NMDA EPSC occurred immediately after the pairing protocol and is fully established by 5 min (0.42 ± 0.02 s before versus 0.37 ± 0.01 s, $p < 0.05$ and 0.33 ± 0.01 s, $p < 0.001$ after LTP induction; n = 23, 11, 12, Mann-Whitney test). Representative sample traces of NMDA EPSC at +40 mV before and either 1–30 s or 5 min after the LTP protocol are shown on the top.

(F) Bar graph representing the change in peak amplitude of NMDA EPSC at +40 mV in presence of NBQX. After LTP, the mean peak amplitude of NMDA EPSC does not change significantly compared to control condition (123.12% ± 12.2%, n = 11, $p > 0.05$). However, in the presence of ifenprodil, the peak amplitude is increased (193.9% ± 35.5%, n = 8; significantly different from 123.3% ± 12.8% n = 12 $p < 0.05$, Mann-Whitney test). Error bars = SEM.

receptors after LTP. LTP of the AMPA EPSC requires a rise in postsynaptic calcium. We therefore examined the effect of loading the postsynaptic cell with the calcium chelator BAPTA. In this condition, no change in the decay of the NMDA EPSC was seen after pairing (0.44 ± 0.02 s versus 0.40 ± 0.04 s; n = 6, data not shown).

The experiments thus far have relied on a cross-pathway comparison to determine the effects of LTP on NR2 subunit composition. We next carried out the entire experiment in the presence of NBQX so that we could compare the effect of LTP on the same pathway. In addition, this experiment allowed us to examine the time course of the

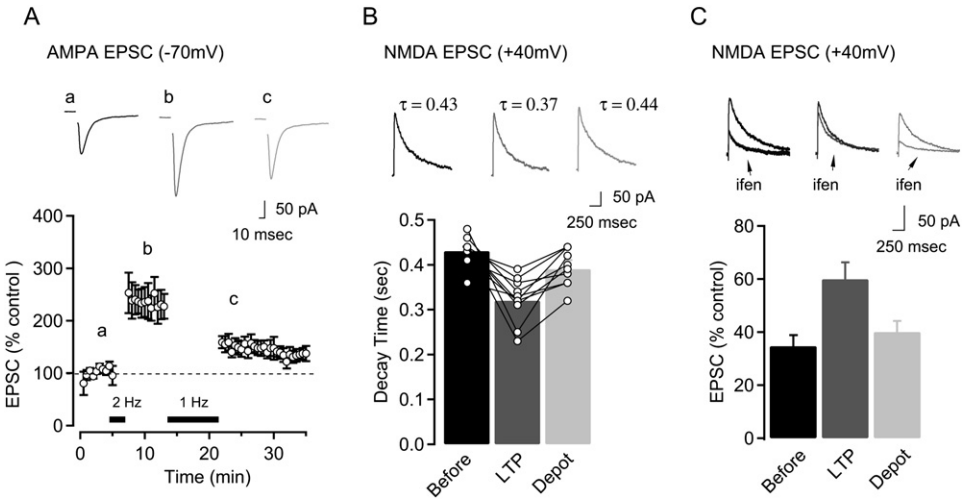

Figure 3. Depotentiation Protocol Reverses Synaptic NMDAR Subunit Composition

(A) Summary of experiments (n = 7) showing depotentiation of AMPA EPSCs (low-frequency stimulation [LFS] at 1 Hz for 7 min) 5 min after LTP induction. Representative sample traces of a single experiment at the time points indicated are shown on the top.

(B) In the same cell in the presence of NBQX, the fast decay time kinetics following LTP induction (0.32 ± 0.02 s versus 0.43 ± 0.01 s, n = 10, $p < 0.02$) are rapidly reversed after LFS and are not significantly different from baseline condition (0.39 ± 0.02 s versus 0.32 ± 0.02 s, n = 7, $p < 0.02$). Representative sample traces from a single experiment are shown on the top.

(C) Bar graph showing percentage of NMDA currents remaining in the presence of ifenprodil in control, LTP, and depotentiation conditions. After LFS, the average percentage of NMDA current remaining after ifenprodil application is less then after LTP (40% ± 4.2% versus 59.9% ± 6.4%, n = 5, 11, $p < 0.05$) but not significantly different from control inhibition. Representative sample traces from experiments for the different condition before and after ienprodil are shown on the top.

Error bars = SEM.

change in kinetics after LTP induction. A clear acceleration of the decay of the NMDA EPSC was evident immediately after the pairing protocol within the first 30 s and was fully established by 5 min (0.42 ± 0.02 s before versus 0.37 ± 0.01 s [1–30 s], $p < 0.05$ and 0.33 ± 0.01 s [5 min], $p < 0.001$ after LTP induction; n = 23, 11, 12) (Figure 2E).

Does LTP cause a selective loss of NR2B-containing receptors, or is there actually a replacement of the lost NR2B-containing receptors with NR2A-containing receptors? If there were simply a loss of NR2B-containing receptors, one would expect the size of the NMDA EPSC to decrease following LTP. However, on average, there was no change in the size of the NMDA EPSC (Figure 2F). This finding, then, favors a swapping of NR2B receptors for NR2A receptors. To test this idea directly, we repeated the experiments in the presence of ifenprodil and monitored only NR2A receptors. In this case, the size of the NMDA EPSC should increase after LTP, if LTP is associated with an insertion of NR2A receptors. Indeed, in the presence of ifenprodil the size of the NMDA EPSC increases following LTP (Figure 2F) (193.9 ± 35.5 pA, n = 8; significantly different from 123.3 ± 12.8 pA, n = 12; $p < 0.05$, Mann-Whitney test). Importantly, in the presence of ifenprodil LTP was not associated with a change in decay time (0.31 ± 0.02 versus 0.27 ± 0.03).

It is well established that, following the induction of LTP, synapses can be depotentiated with low-frequency stimulation (Fujii et al., 1991; O'Dell and Kandel, 1994). Can depotentiating stimuli reverse the subunit switching observed with LTP? We first did a series of experiments monitoring the AMPA EPSC to determine whether neonatal synapses are capable of being depotentiated. LTP was first induced by pairing at 0 mV. Following LTP, the synapses were stimulated at 1 Hz while holding the cell at −40 mV. A clear depotentiation was recorded (Figure 3A). It should be noted that in a separate set of experiments we show that the LTP in our conditions remains stable for at least an hour (Figure S1D). Having established that AMPA EPSCs can be depotentiated in the neonate, we repeated the entire experiment in the presence of NBQX. As shown in Figure 3B, LTP accelerated the EPSC decay in all cells, and this acceleration was reversed by depotentiation. These clear changes in kinetics were associated with a corresponding change in the sensitivity of the EPSC to ifenprodil (Figure 3C). We also considered the possibility that LTD in neonatal slices (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997) might, on its own, slow the NMDA EPSC. Despite generating robust LTD of the AMPA EPSC, we were unable to detect any change in the kinetics of the NMDA EPSC or a change in ifenprodil sensitivity after the induction of LTD (Figure S2).

A final question is whether the dramatic bidirectional plasticity we observe in young animals can also be seen in older animals. We recorded responses from two largely independent pathways and induced LTP in one of these pathways (Figure 4A). NBQX was then applied, and the membrane potential shifted to +40 mV. We observed no difference in the kinetics (Figure 4B) or the ifenprodil

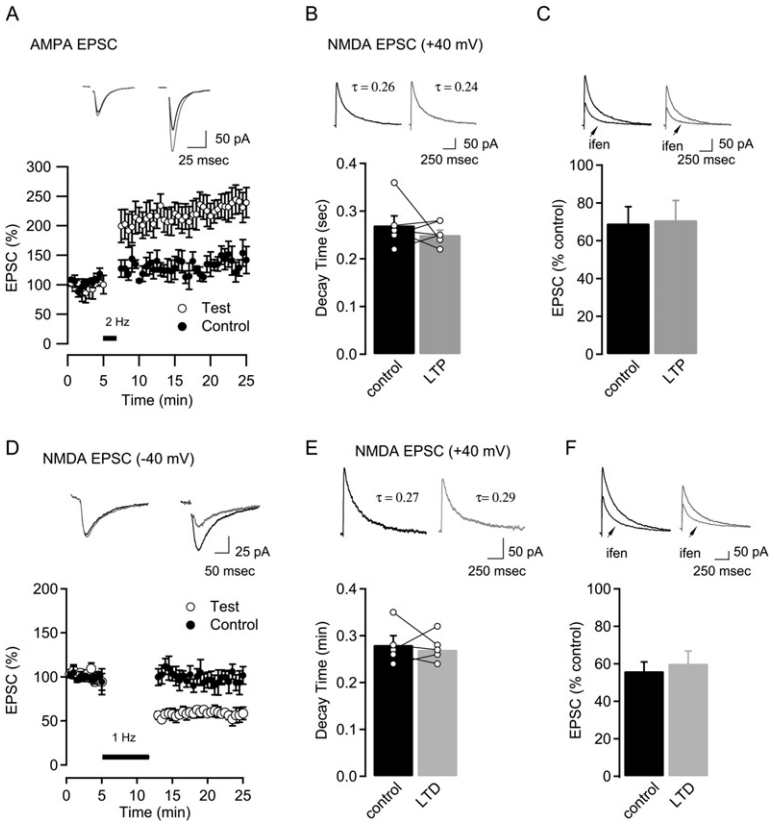

Figure 4. In Older Animals, Neither LTP nor LTD Can Modify Synaptic NMDAR Subunit Composition

(A) Summary of two pathway experiments (n = 7) showing LTP of the AMPA EPSC. Sample traces from a representative experiment in control and LTP pathways, taken prior to LTP induction and 5 min after induction, are shown on the top.

(B) There is no difference in the decay time between the control and LTP pathways (0.27 ± 0.02 s versus 0.25 ± 0.01 s, n = 6). Sample traces from a representative experiment.

(C) The effect of ifenprodil is the same on the two paths (69% ± 8.9% versus 70.8% ± 10.5%, n = 5).

(D) Summary of LTD experiments (n = 5) of the NMDA EPSC measured at −40 mV in NBQX. Representative sample traces of NMDA EPSCs for control and LTD pathway are shown on the top.

(E) Decay kinetics of NMDAR EPSC measured at +40 mV does not change after LFS protocol (0.28 ± 0.02 s versus 0.27 ± 0.02 s, n = 5).

(F) The effect of ifenprodil is the same on the two paths (56% ± 5% in control versus 60% ± 6.8% in test pathways n = 5, 8).

Error bars = SEM.

sensitivity (Figure 4C) following LTP. Perhaps this lack of effect is due to synapse maturation occluding the effects of LTP, i.e., all the NR2B subunits that can be removed had already been removed by maturation. In this case, one might expect that with LTD NR2B receptors could be recruited back to the synapse. To test this, we stimulated two independent pathways in the presence of NBQX and then induced LTD on one pathway by pairing 1 Hz stimulation while holding the cell at −40 mV (Selig et al., 1995). After establishing the presence of LTD on the NMDA EPSC, we depolarized the cell to +40 mV and compared the kinetics and ifenprodil sensitivity of the two pathways. We could detect no change in either the kinetics (Figure 4E) or the ifenprodil sensitivity (Figure 4F). Thus, the remarkable receptor switching seen in young animals is completely lost during development. These findings contrast with a study in which surface receptors were measured with biotinylation, and LTP-inducing stimuli were found to cause a change in subunit composition (Grosshans et al., 2002). Presumably, the effects observed in this study reflected shifts in extrasynaptic pools of receptors.

DISCUSSION

The present study establishes that at early ages synaptic NMDARs are at least as dynamic as AMPARs. LTP-inducing stimuli caused a switch in subunit composition within seconds and the change lasted for at least an hour. However, depotentiating stimuli rapidly reversed the change. Our data indicate that the activity-dependent loss of NR2B receptors is coincident with the simultaneous recruitment of NR2A receptors. How this swapping of receptors is coordinated is unclear, but is likely to be dependent on the differences in the C terminus of the two receptor subunits and the partners with which they interact (Barria and Malinow, 2005; Lavezzari et al., 2004; Lin et al., 2006; Steigerwald et al., 2000) as well as to differences in phosphorylation (Carroll and Zukin, 2002; Chen et al., 2006; Townsend et al., 2004).

NMDARs can rapidly internalize, especially during early synapse development (Washbourne et al., 2004), and removal of NMDARs during synaptic depression has been reported to be either dynamin dependent (Montgomery et al., 2005) or dynamin independent (Morishita et al., 2005; Sobczyk and Svoboda, 2007). PDZ protein interactions are thought to be critical for maintaining NMDARs at synapses, while interaction with AP-2 is an important determinant of the number of synaptic NR2B-containing NMDARs (Prybylowski et al., 2005). Lastly, lateral diffusion may also play an important role in controlling the number of synaptic NMDARs by allowing receptors to move between synaptic to extrasynaptic sites (Choquet and Triller, 2003; Tovar and Westbrook, 2002).

Ifenprodil is the first and best-characterized NMDAR antagonist shown to discriminate between NR2A- and

NR2B-containing receptors, being more than 100-fold more selective for NR2B- over NR2A-containing receptors (Williams, 1993). However, the effect of ifenprodil on triheteromeric receptors is not entirely clear. Hatton and Paoletti (Hatton and Paoletti, 2005) suggest that triheteromeric receptors have high affinity for ifenprodil but are inhibited to only a small extent. The use of 3 μM ifenprodil in our experiments, which has no effect on NR2A-containing receptors, ensures that any observed sensitivity arises from the presence of an NMDAR that contains an NR2B subunit. However, given the uncertainty of ifenprodil's effect on triheteromeric receptors, we cannot exclude the presence of these receptors in our experiments. The change in decay time caused by LTP can be fully accounted for by changes in NR2B content of synaptic receptors, because in the presence of ifenprodil no change in kinetics occurs during LTP.

The precise roles that NMDAR subunits play in synaptic plasticity are hotly debated (Bartlett et al., 2007; Liu et al., 2004; but see Berberich et al., 2005; Morishita et al., 2007). One possible role for subunit switching at neonatal synapses is the rapid acquisition of timing precision and refinement of coincidence detection. LTP at neonatal thalamocortical synapses has been shown to produce an improvement in the timing of neuronal output to a give synaptic input (Daw et al., 2006). Despite this controversy, it is clear that the immediate replacement of NR2B receptors with NR2A receptors will transform the synapse from one that has a broad window for spike-timing plasticity to one in which the window is greatly reduced.

EXPERIMENTAL PROCEDURES

Experiments were carried out according to the guidelines of UCSF Committee of Animal Research. Standard techniques were used to prepare transverse hippocampal slices (300 μm) from 2- to 21-day-old Sprague-Dawley rats, which were anesthetized with halothane. Slices were maintained at room temperature and allowed to recover for 1 hr before being transferred to the recording chamber, where they were submerged in a continuously superfusing solution at room temperature and equilibrated with 95% O_2 and 5% CO_2. The external solutions contained (in mM) 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM glucose, and 0.1 mM picrotoxin (pH 7.4). For the experiment of LTD in young animals, the concentration of $MgSO_4$ and $CaCl_2$ was 4 mM during the recording.

Evoked postsynaptic responses were induced in CA1 pyramidal cells by stimulating Schaffer collaterals in stratum radiatum (0.1 Hz) with monopolar glass pipettes. Studying possible activity-dependent changes in NMDA EPSCs is problematic because the very condition required to measure the NMDA EPSC also can be expected to initiate the process being investigated. We have designed two protocols to circumvent this problem. First, we designed a two-pathway experiment in which two stimulating electrodes were positioned on either side of the recording site in order to stimulate two nonoverlapping sets of synapses. After inducing LTP of the AMPA EPSC in one pathway, the cell was depolarized and the AMPA EPSC blocked. This allowed us to compare the NMDA EPSC of the LTP pathway to the NMDA EPSC of the naive control pathway. The second method was to briefly sample NMDA EPSCs in the presence of NBQX at different time points during the experiment, ensuring that the sampling was sufficiently brief as to not induce any changes (see Figure S1C). Somatic whole-cell voltage-clamp recordings were made from CA1 pyramidal cells using 2–6 ΩM electrodes. The internal solution contained (in mM) 115 $CsMeSO_4$, 20 $CsCl_2$, 10 HEPES, 2.5 $MgCl_2$, 4 NaATP, 0.4 NaGTP, 10 NaCreatine, and 0.6 EGTA (pH 7.2). Synaptic responses were collected with a Multiclamp 700B-amplifier (Axon Instruments, Foster City, CA), filtered at 2 kHz, digitized at 5 Hz, and analyzed online using Igor Pro software (Wavemetrics, Lake Oswego, OR). All data are expressed as mean ± SEM. Wilcoxon matched-pairs signed-ranks test was used for comparing paired observations; Mann-Whitney test was applied in the other case. Cells were held at −70 mV for AMPA EPSC recordings and at + 40 mV in presence of 10 μM NBQX for NMDA EPSC, except when otherwise indicated. To simplify the comparison of decay times across conditions, a single weighted decay measure (see Cathala et al., 2005) (referred in the text as decay time) was calculated from the area under the peak-normalized current for 1.3 s after the peak. To minimize possible voltage-clamp artifacts that can affect the kinetics of the NMDA EPSC, we limited the size of the EPSC to less than 200 pA. We also examined whether reducing the EPSC with APV altered the kinetics. APV (2.5 μM) reduced the EPSC to 64.6% ± 5.7% of control (n = 5), while the decay time did not change (before = 0.42 ± 0.02; after = 0.42 ± 0.02) (Figure S3). Presumably, the small size and limited dendritic branching of neonatal pyramidal cells minimizes space-clamp problems. Ifenprodil, NBQX, and AP-5 were obtained from Tocris Biosciences, UK. All other chemicals and drugs were obtained from Sigma.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/55/5/779/DC1/.

ACKNOWLEDGMENTS

We thank A. Tzingounis, A. Jackson, and members of the Nicoll lab for thoughtful discussion. R.A.N. is supported by grants from NIH.

Received: May 14, 2007
Revised: June 25, 2007
Accepted: July 27, 2007
Published: September 5, 2007

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