

Visual Experience and Deprivation Bidirectionally Modify the Composition and Function of NMDA Receptors in Visual Cortex

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Summary

The receptive fields of visual cortical neurons are bidirectionally modified by sensory deprivation and experience, but the synaptic basis for these changes is unknown. Here we demonstrate bidirectional, experience-dependent regulation of the composition and function of synaptic NMDA receptors (NMDARs) in visual cortex layer 2/3 pyramidal cells of young rats. Visual experience decreases the proportion of NR2B-only receptors, shortens the duration of NMDAR-mediated synaptic currents, and reduces summation of synaptic NMDAR currents during bursts of high-frequency stimulation. Visual deprivation exerts an opposite effect. Although the effects of experience and deprivation are reversible, the rates of synaptic modification vary. Experience can induce a detectable change in synaptic transmission within hours, while deprivation-induced changes take days. We suggest that experience-dependent changes in NMDAR composition and function regulate the development of receptive field organization in visual cortex.

Introduction

During a critical period of early postnatal life, neurons in the visual cortex gain and lose visual responsiveness as a function of sensory experience. For example, neurons in the visual cortex of animals reared in complete darkness respond sluggishly to light and lack the stimulus selectivity that is characteristic of normally reared animals (Blakemore and Van Sluyters, 1975; Fagiolini et al., 1994). However, this effect of dark-rearing can be rapidly reversed; within hours of light exposure (LE), many neurons acquire vigorous and selective responses to visual stimulation (Buisseret et al., 1978, 1982). Conversely, the robust visual responsiveness of cortical neurons in light-reared (LR) animals can be degraded by periods of visual deprivation. For example, binocular deprivation (BD) will, over the course of several days, cause a loss of visual responsiveness and a broadening of stimulus selectivity (Freeman et al., 1981). The fact that cortical physiology can be altered so dramatically

by manipulations of sensory experience, and the fact that these alterations have clear behavioral consequences (i.e., blindness and sight), has made the visual cortex a favorite model system for studying how experience modifies the brain. However, despite a wealth of descriptive data and many appealing theories on the consequences of various types of deprivation and experience, very little is known about the underlying synaptic modifications and their molecular basis.

Excitatory synaptic transmission in visual cortex is mediated by glutamate receptors, of which N-methyl-D-aspartate receptors (NMDARs) are one subtype. Over the course of early postnatal development, NMDAR-mediated excitatory postsynaptic currents (NMDAR-EPSCs) gradually shorten in duration (Carmignoto and Vicini, 1992; Hestrin, 1992; Flint et al., 1997; Livingston and Mooney, 1997; Ramoa and Prusky, 1997; White et al., 1999) and, in rat visual cortex, this change is prevented by rearing animals in the dark (Carmignoto and Vicini, 1992). Although NMDAR kinetics are regulated by many factors (Vicini et al., 1998; Shi et al., 2000), a putative molecular basis for the developmental shortening of the EPSC is a change in the subunit composition of the receptor (Monyer et al., 1994, 1992). NMDARs are heteromeric ion channels consisting of NR1 and NR2 subunits (McBain and Mayer, 1994). In the forebrain, NR2B is highly expressed at the time of birth and, over the course of the first several postnatal weeks, NR2A levels gradually rise. A functional consequence of altering the NR2A/B ratio is a change in the kinetics of EPSCs mediated by NMDARs. NR2A-containing NMDARs have shorter current durations than receptors comprised of the NR2B subunit (Monyer et al., 1994; Flint et al., 1997). Therefore, an appealing hypothesis is that an activity-dependent switch in the subunit composition of NMDARs accounts for the shortening of NMDAR currents in visual cortex during postnatal development.

Several recent findings in visual cortex suggest that NMDAR subunit composition is indeed regulated by activity. First, biochemical experiments have shown, at the level of both mRNA and protein, that the NR2A/B ratio increases in visual cortex over the first several postnatal weeks and that this change is delayed by dark-rearing (Nase et al., 1999; Quinlan et al., 1999a). Second, NMDAR-mediated field potentials (FPs) show heightened sensitivity to an NR2B-selective antagonist in visual cortex of dark-reared (DR) animals as compared with LR animals (Quinlan et al., 1999b), suggesting that the NR2A/B ratio in the population of synaptic NMDARs is lower than normal in the visual cortex of DR animals. Interestingly, the NR2A/B ratio is rapidly upregulated when DR animals are exposed to light. However, the functional consequences of these changes are unknown. In particular, it remains to be determined if the kinetic and dynamic properties of NMDAR-EPSCs are altered as a consequence of visual experience.

The work on the effects of dark-rearing and LE reviewed above suggests the exciting possibility that regulation of NMDAR composition and function could be a basis for experience-dependent visual cortical plastic-

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ity. However, dark-rearing is an unusual form of sensory deprivation because many aspects of cortical development are postponed until the animal experiences light (Cynader and Mitchel, 1980; Mower, 1991; Fagiolini et al., 1994). Therefore, it has not yet been established whether the changes observed after DR and LE are a response to sensory deprivation and subsequent experience or if they are responses to arrested cortical development.

In this paper, we describe experiments designed to test the hypothesis that changes in NMDAR subunit composition and function contribute to bidirectional, experience-dependent synaptic plasticity in visual cortex. We report that only 2 hr of visual experience in DR animals is sufficient to significantly alter the kinetics of NMDAR-EPSCs and to change the subunit composition of the complement of synaptic NMDARs. We also show that NMDAR subunit composition and function are bidirectionally modifiable and are altered by brief periods of visual deprivation in LR animals. Finally, we present evidence that the changes in NMDAR-EPSC kinetics have a profound functional impact on the dynamic properties of synaptic transmission. This is a novel demonstration of a molecular mechanism for bidirectional, experience-dependent synaptic changes in the mammalian brain.

Results

Three complementary methods were used to study the properties of isolated NMDAR-mediated synaptic responses in layers 2/3 of rat visual cortex. To assess the relative contribution of NR2B-containing receptors to synaptic transmission, we investigated the sensitivity of NMDAR-mediated field potentials (NMDAR-FPs) to NR2B-selective antagonists. To assess the potential functional impact of altered synaptic NR2B expression, we investigated the effects of experience and deprivation on the kinetics of NMDAR-EPSCs. Finally, we compared the short-term plasticity and temporal summation of responses mediated by NMDARs with those mediated by AMPA receptors (AMPA) in DR and LR animals.

Visual Experience and Deprivation Bidirectionally Modify the Subunit Composition of Synaptic NMDARs

We previously reported that NMDAR-FPs, evoked in layers 2/3 by layer 4 stimulation, show heightened sensitivity to ifenprodil in the visual cortex of DR animals. Here we have extended this analysis to the effects of brief BD in LR animals. We begin with a brief description of the approach (more detail is provided in the Appendix [<http://www.neuron.org/cgi/content/full/29/1/157/CD1>]), summarize and extend our previous findings, and compare these with the effects of BD (produced by placing LR animals in a darkroom).

Layer 2/3 field potentials typically have short response latencies, and the response is mediated by both the AMPA- and NMDA-type glutamate receptors (Figure 1A). We pharmacologically isolated the NMDAR-mediated component by bathing slices in a modified artificial cerebrospinal fluid (ACSF) solution (see Experimental Procedures). NMDAR-mediated potentials were small in size (~35% of the original field potential) and had a

longer response latency. The NMDAR-mediated field potentials were attenuated by application of NR2B-selective antagonists (3 μ M ifenprodil or 5 μ M CP101,606), and the remaining synaptic (calcium-dependent) response was entirely abolished by bath application of the NMDAR antagonist AP5 (100 μ M).

NMDAR-mediated FPs were monitored using probing stimulation consisting of 4 pulses separated by 30 s, given every 10 min (see Appendix [<http://www.neuron.org/cgi/content/full/29/1/157/CD1>] for rationale). When the response was stable for 30 min, the NR2B-selective antagonist was washed on for 90 min. The percent reduction of the response by the antagonist was determined by comparing the 30 min average immediately before adding the drug with the average of four consecutive sweeps collected 90 min after the drug application, when the field potential had completely equilibrated.

Figure 1B summarizes the effect of NR2B-selective antagonists on NMDAR-mediated FPs measured in slices from DR and LR animals (postnatal day [P] 21–28). Note that CP101,606 (5 μ M, 90 min) produces more inhibition of the FP in DR animals (47% \pm 3% reduction, $n = 12$ slices from 8 rats) compared to LR animals (35% \pm 2% reduction, $n = 15$ slices from 10 rats; t test, $p < 0.004$), confirming our previous findings using 3 μ M ifenprodil (Figure 1B, inset, replotted from Quinlan et al. [1999b]; also see Appendix [<http://www.neuron.org/cgi/content/full/29/1/157/CD1>]). To determine whether synaptic NMDAR subunit composition is bidirectionally modifiable, LR rats were given varying periods of BD, and ifenprodil sensitivity was tested at P26–P31. While 1 day of BD did not alter the ifenprodil sensitivity of NMDAR-FPs (Figure 1C; LR: 51% \pm 4% reduction, $n = 11$ slices from 8 rats; LR plus 1 day dark: 48% \pm 4% reduction, $n = 11$ slices from 6 rats; t test, $p = 0.58$), 5 days of BD significantly increased ifenprodil sensitivity (Figure 1D; LR: 45% \pm 3% reduction, $n = 16$ slices from 9 rats; LR plus 5 days dark: 55% \pm 4% reduction, $n = 13$ slices from 8 rats; $p < 0.05$). The slow effect of deprivation contrasts with the finding that just 2 hr of LE is sufficient to decrease ifenprodil sensitivity in DR rats (Figure 1D, inset; Quinlan et al., 1999b).

These data indicate that subunit composition can be bidirectionally modified by experience, although the time course for synaptic modifications differs. The fact that 5 days of BD is sufficient to lower the NR2A/B ratio in LR rats to a level similar to that in animals reared in complete darkness from birth suggests that the effect of DR on the NR2A/B ratio is not merely a reflection of arrested development. Rather, the synaptic NR2A/B ratio in visual cortex, as reflected in the sensitivity of NMDAR-FPs to ifenprodil, appears to accurately reflect the recent history of visual experience.

Visual Experience and Deprivation Bidirectionally Modify the Kinetics of NMDAR-EPSCs

To test whether differences in subunit composition are translated into functional differences in NMDARs, we examined the kinetics of NMDAR-mediated currents from layer 2/3 pyramidal cells, identified by their pyramidal shape, size, and prominent apical dendrite extending toward the pial surface. In all instances when Lucifer yellow was included in the internal recording solution,

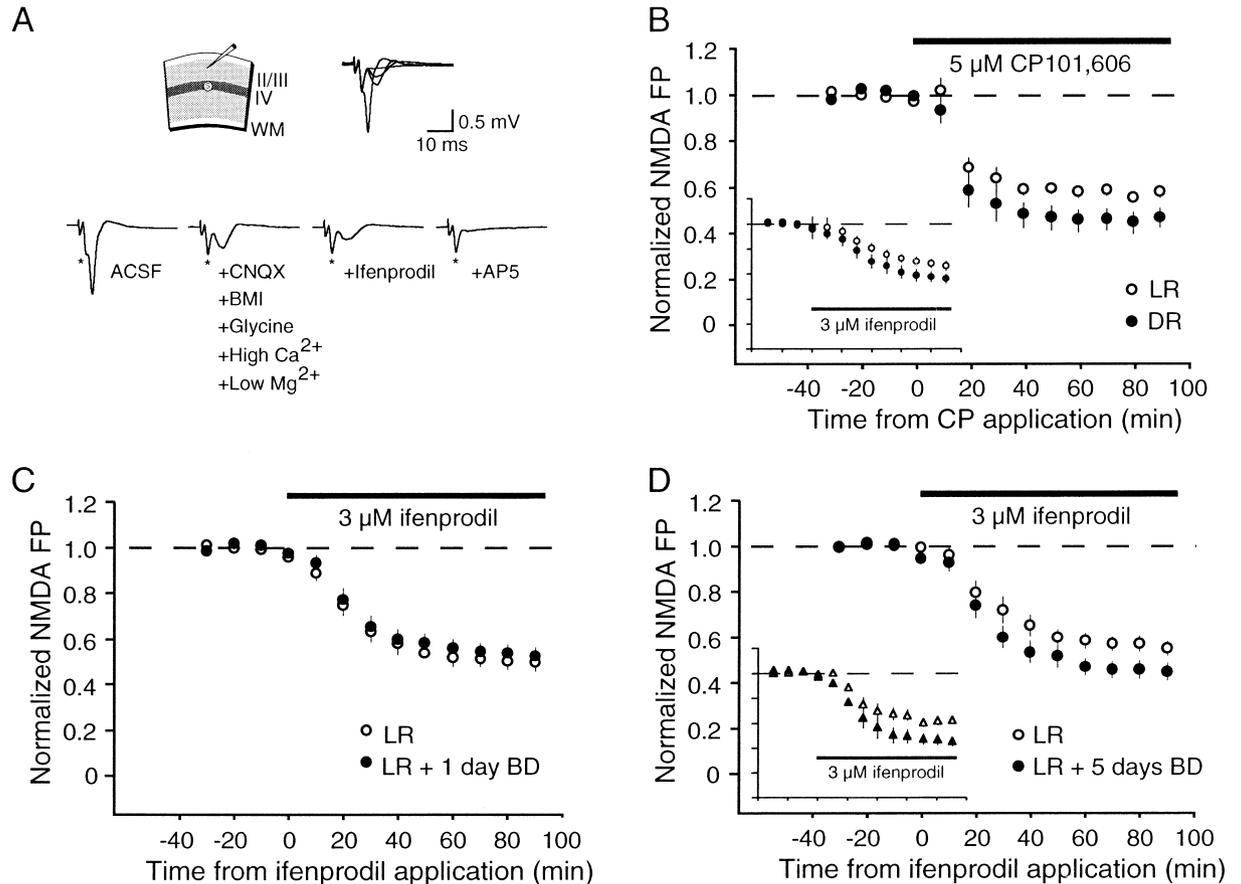


Figure 1. Visual Experience Bidirectionally Regulates the Sensitivity of Visual Cortex NMDAR-Mediated Potentials to NR2B-Selective Antagonists

Data are mean values averaged from the 4 pulses of probing stimulation (see Experimental Procedures).

(A) An example of the pharmacological isolation of NMDA-mediated potentials and the sensitivity to the NR2B-selective antagonist ifenprodil. AMPA and NMDA receptor-mediated field potentials (FPs) can be recorded from layers 2/3 and evoked by layer 4 stimulation, and NMDA-mediated potentials can be pharmacologically isolated (see Experimental Procedures). The proportion of NR2B-containing NMDARs can be assessed by ifenprodil sensitivity. AP5 application demonstrates isolation of NMDA-only mediated potentials. Note that the nonsynaptic component, indicated by asterisk, was unaffected.

(B) The NR2B antagonist CP101,606 more greatly attenuates NMDA potentials in DR visual cortex as compared to LR controls ($p < 0.004$). Similar results were observed using the NR2B antagonist ifenprodil (inset; Quinlan et al., 1999b).

(C and D) One day (C) of binocular deprivation (BD) does not alter ifenprodil sensitivity in LR rats, but (D) 5 days of BD significantly increases ifenprodil sensitivity ($p < 0.05$). In contrast to this slow modification, only 2 hr of light exposure (LE) is needed to reduce ifenprodil sensitivity in dark-reared rats (inset; DR = filled triangles, LE = open triangles; Quinlan et al., 1999b)

cells were confirmed to be layer 2/3 pyramidal cells using fluorescent microscopy. However, we can not exclude the possibility that some non-pyramidal cells were included in the study because histology was not performed on all cells.

In order to isolate NMDAR-mediated EPSCs, slices were bathed in an ACSF medium containing: CNQX to block non-NMDARs, picrotoxin to block GABA_A receptors, and saturating concentrations of the NMDAR coagonist glycine. In addition, ACSF containing high divalent cation concentrations was used in order to minimize polysynaptic activity. Cells were voltage-clamped at +40 mV to remove Mg²⁺ block. Stimulation was adjusted so that evoked currents were near 100 pA. Under these conditions, isolated NMDA-mediated currents demonstrated a typical “J” shaped I-V relation, had a reversal potential near 0 mV, and were blocked by the application

of the NMDAR antagonist AP5. Because current decays became correlated to series resistance at high series resistance, only cells with series resistance $< 30 \text{ M}\Omega$ were included in this study (average $R_s = 16.4 \pm 0.8 \text{ M}\Omega$).

To describe the deactivation kinetics of NMDAR-mediated EPSCs, 30–60 evoked EPSCs were averaged, and the current decays were described using the following formula:

$$I(t) = I_f \exp(-t/\tau_f) + I_s \exp(-t/\tau_s),$$

where I is the current amplitude, t is time, I_f and I_s are the peak amplitudes of the fast and slow components, respectively, and τ_f and τ_s are their respective time constants. A nonlinear regression in GraphPad Prism software (San Diego, CA) was used to fit decay curves. Small perturbations in the values of I_f , I_s , τ_f , and τ_s did not appreciably decrease the quality of the fit; therefore, the

Table 1. Effects of Visual Experience and Deprivation on the Kinetics of NMDAR-Mediated EPSCs

Experiment	Condition	n (cells)	I _f (%)	τ _f (ms)	τ _s (ms)	τ _w (ms)
DR versus LE	DR	11	58.0 ± 7.4	50.4 ± 6.7	210.9 ± 20.6	112.3 ± 6.9
	LE	9	59.4 ± 4.8	43.0 ± 4.9	166.0 ± 16.2	89.7 ± 6.5 ^a
LR versus BD	LR	25	63.4 ± 3.5	46.5 ± 2.8	215.1 ± 19.9	96.7 ± 5.4
	BD	18	56.7 ± 6.5	50.0 ± 5.5	237.2 ± 28.9	115.9 ± 7.5 ^a
LR versus DR	LR	16	60.9 ± 4.4	37.6 ± 3.3	170.1 ± 20.0	90.0 ± 8.7
	DR	26	43.8 ± 6.3	36.2 ± 5.5	194.5 ± 15.0	118.8 ± 6.6 ^b

^aSignificantly different, *p* < 0.04; *t* test.

^bSignificantly different, *p* < 0.02; *t* test.

exact values of each of these parameters should not be considered as particularly meaningful. A more robust measurement, introduced by Rumbaugh and Vicini (1999), is the weighted time constant (τ_w), calculated as:

$$\tau_w = \tau_f (I_f / (I_f + I_s)) + \tau_s (I_s / (I_f + I_s))$$

Therefore, we calculated τ_w for each neuron recorded in the present study and used these values for all statistical comparisons between groups (see Table 1).

Figure 2A presents the composite, normalized NMDAR-mediated EPSCs recorded in visual cortex of P21–P28 DR rats (*n* = 11 cells from 4 rats), and DR rats exposed to light for 2 hr (*n* = 9 cells from 4 rats). The value of τ_w in DR rats, 112.3 ± 6.9 ms, was significantly greater than that in LE rats, 89.7 ± 6.5 ms (*t* test, *p* < 0.04; Figure 2B). Thus, 2 hr of light exposure is sufficient to significantly shorten NMDAR current decay.

To test whether changes in NMDAR current duration could be bidirectionally modified, P26–P31 LR animals were either left in the normal light:dark cycle (*n* = 25 cells from 6 rats) or binocularly deprived of light (*n* = 18 cells from 4 rats). As compared to LR controls, 5 days of BD caused a significant increase in the duration of NMDAR-EPSCs (LR τ_w: 96.7 ± 5.4 ms; BD τ_w: 115.9 ± 7.5 ms; *t* test, *p* < 0.04; Figures 2C and 2D).

These experiments reveal that a synaptic consequence of visual experience is a shortening of NMDAR-EPSCs, which is reversed by sensory deprivation. Con-

sidered together with the field potential data, the results strongly suggest that a molecular mechanism for bidirectional experience-dependent synaptic plasticity in visual cortex is the regulation of the subunit composition and function of synaptic NMDARs.

Visual Experience Alters the Temporal Summation of NMDAR-EPSCs

Although the effects of sensory experience and deprivation on NMDAR current duration were reliable and consistent, the changes were relatively modest. However, in principle, even small changes could have a large cumulative impact on the responses to trains of stimuli. To examine this possibility, an additional series of experiments was performed in visual cortex of LR and DR animals. Stimulation was adjusted so that evoked NMDAR-EPSCs were near 100 pA. As suggested from the earlier experiments, currents were significantly faster in pyramidal cells from LR cortex as compared to cells from DR cortex (Table 1).

We used 10, 20, or 40 Hz trains of 11 stimulation pulses, given every 6 s for 3 min (for a total of 30 trains), to determine whether the experience-dependent differences in NMDAR subunit composition and current duration affected the summation of NMDA-mediated currents (Figure 3A). Current responses were averaged for all 30 trains, unless otherwise noted. Quantification was

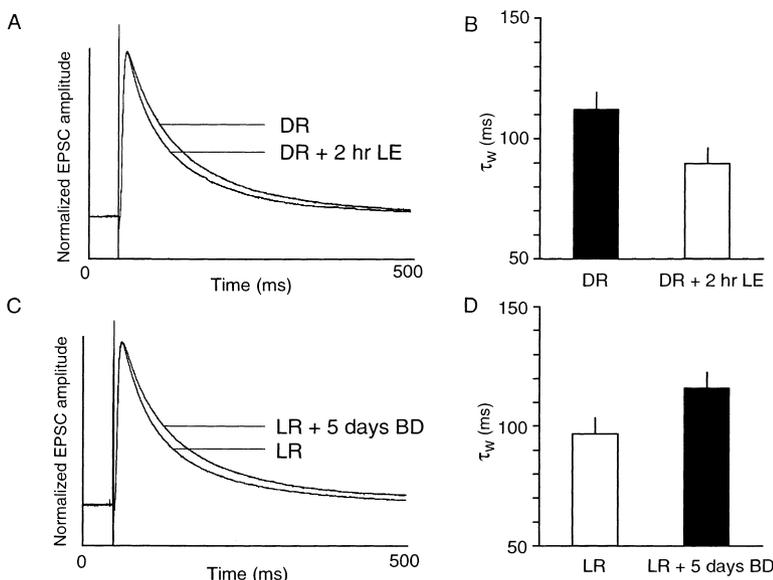


Figure 2. Visual Experience Bidirectionally Regulates NMDAR Current Durations

(A) Overlay of averaged and normalized NMDAR-EPSCs from layer 2/3 pyramidal cells in visual cortex of dark-reared (DR) rats and animals given just 2 hr of light exposure (LE). (B) The weighted time constant (τ_w) of NMDAR-EPSC decays recorded at +40 mV in cells from DR animals or animals given 2 hr LE. Two hours of LE is sufficient to shorten NMDAR currents (*p* < 0.04).

(C) Overlay of NMDAR-EPSCs in visual cortex of light-reared (LR) rats and LR rats given 5 days of binocular deprivation (BD).

(D) NMDAR current decays are significantly longer in pyramidal cells after 5 days of BD (*p* < 0.04).

performed by comparing the ratios of the average peak EPSC amplitude of each cell's response to the 11th pulse to the response to the 1st pulse; additional comparisons were made by normalizing the amplitude of the averaged first response to 1 and analyzing the area of the current response over time. At 10 Hz stimulation, the NMDA currents depressed slightly, and this depression did not differ between pyramidal cells recorded from LR ($n = 15$ cells from 5 rats; 11th pulse/1st pulse = 0.91 ± 0.06) or DR ($n = 24$ cells from 7 rats; 11th pulse/1st pulse = 0.91 ± 0.02) visual cortex (t test of 11th pulse/1st pulse, $p = 0.99$; t test of areas, $p = 0.22$). At 20 Hz stimulation, NMDAR-EPSCs facilitated, and, although nonsignificant, a difference began to emerge between LR ($n = 18$ cells from 6 rats; 11th pulse/1st pulse = 1.05 ± 7.6) and DR ($n = 25$ cells from 7 rats; 11th pulse/1st pulse = 1.10 ± 0.03) animals (t test of 11th pulse/1st pulse, $p = 0.58$; t test of areas, $p = 0.31$). However, 40 Hz stimulation revealed a dramatic effect of visual experience on NMDA-mediated currents; NMDAR-EPSCs exhibited much more summation in pyramidal cells from DR rats ($n = 26$ cells from 7 rats; 11th pulse/1st pulse = 1.56 ± 0.07) as compared to LR controls ($n = 16$ cells from 6 rats; 11th pulse/1st pulse = 1.29 ± 0.07 ; t test of 11th pulse/1st pulse, $p < 0.02$; t test of areas, $p < 0.02$).

In the course of these experiments, we noted that NMDAR-EPSCs exhibited rundown during the successive trains of 40 Hz stimulation. To determine whether differential rundown might contribute to the large differences in summation observed between LR and DR animals, the data were reanalyzed to examine current summation of the first 3 trains of 40 Hz stimulation, before substantial rundown appeared. Similar differences in current summation were observed whether the first 3 or all 30 trains were analyzed (see Figure 3A₃, inset), suggesting that rundown effects do not contribute to the significant differences in current summation observed between LR and DR animals.

Effect of Visual Experience and Deprivation on the Summation of AMPAR-Mediated EPSCs

Although these findings could be accounted for by the altered kinetics of unitary NMDAR-EPSCs, many factors contribute to the temporal summation of synaptic responses during repetitive stimulation. For example, the amount of synaptic facilitation or suppression during a train of stimuli varies systematically with the initial glutamate release probability (Debanne et al., 1996; Dobrunz and Stevens, 1997; Tsodyks and Markram, 1997), and this short-term plasticity has been shown to be experience dependent in some systems (Finnerty et al., 1999). It was therefore important to examine whether the effect of dark-rearing on the synaptic responses to repetitive stimulation was selective for NMDAR-mediated responses. To address this question, AMPAR-mediated currents were also examined in response to 10, 20, or 40 Hz stimulation as described above, except that CNQX and glycine were omitted from the bathing medium and AP5 (100 μ M) was included (Figure 3B). Because polysynaptic activity could be evoked under these conditions, stimulation intensities were reduced to evoke a monosynaptic EPSC of ~ 65 pA. Current trains invaded by strong polysynaptic activity were omitted

from analyses. Between 15–30 trains were averaged for each cell.

AMPA currents strongly depressed in response to 10 Hz stimulation, and no differences in current responses were observed in pyramidal cells from LR and DR visual cortex (LR, $n = 9$ cells from 3 rats, 11th pulse/1st pulse = 0.58 ± 0.05 ; DR, $n = 8$ cells from 3 rats, 11th pulse/1st pulse = 0.57 ± 0.06 ; t test of 11th pulse/1st pulse, $p = 0.95$). At 20 Hz stimulation, AMPAR-mediated currents exhibited an initial facilitation followed by strong synaptic depression, and no differences were observed in LR and DR animals (LR: $n = 14$ cells from 4 rats, 11th pulse/1st pulse = 0.66 ± 0.06 ; DR: $n = 14$ cells from 4 rats, 11th pulse/1st pulse = 0.70 ± 0.11 ; t test of 11th pulse/1st pulse, $p = 0.73$). While 40 Hz stimulation revealed a large difference in NMDA-mediated current summation between LR and DR pyramidal cells, AMPA currents similarly depressed in pyramidal cells from LR and DR visual cortex animals (LR: $n = 16$ cells from 4 rats, 11th pulse/1st pulse = 0.50 ± 0.04 ; DR: $n = 15$ cells from 4 rats, 11th pulse/1st pulse = 0.68 ± 0.11 ; t test of 11th pulse/1st pulse, $p = 0.11$). These findings suggest that the striking facilitation of NMDAR-EPSCs observed in DR animals is likely due to a postsynaptic modification of the NMDAR properties rather than a change in glutamate release probability.

Effect of Visual Experience and Deprivation on the Short-Term Plasticity of NMDAR-Mediated EPSCs

To further assess the possible contribution of presynaptic mechanisms to the experience-dependent modifications of NMDAR current summation, we analyzed the short-term plasticity of NMDAR-EPSCs by measuring the net amplitude of currents at 10, 20, and 40 Hz stimulation. Single exponential curves were fit by single-phase exponential decay, using GraphPad Prism software, to the 11 normalized net EPSC amplitudes described by the following formula:

$$I_{\text{net}}(t) = K \exp(-t/\tau_d) + PL$$

I_{net} is the net current amplitude, τ_d is the time constant of the synaptic depression, and PL is the plateau potential, or steady state, and $K + PL = 1$. At 40 Hz stimulation, a frequency where we observed dramatic experience-dependent consequences on temporal summation, short-term depression of NMDAR EPSCs did not differ significantly in cells from LR and DR rats (LR: $\tau_d = 52.0 \pm 13.0$ ms; DR: $\tau_d = 50.0 \pm 5.4$ ms; t test, $p = 0.87$; Figure 4A). Similarly, the rate of current depression did not differ between neurons from LR and DR rats at 10 and 20 Hz stimulation (LR @ 10 Hz: $\tau_d = 99.0 \pm 17.0$ ms; DR @ 10 Hz: $\tau_d = 88.9 \pm 7.6$ ms; t test, $p = 0.56$; LR @ 20 Hz: $\tau_d = 60.5 \pm 3.3$ ms; DR @ 20 Hz: $\tau_d = 71.6 \pm 7.5$ ms; t test, $p = 0.25$; Figure 6B). Finally, there were no significant differences in the steady-state current (plateau potential) between groups (average normalized steady-state amplitudes \pm SEM; LR @ 10 Hz = 0.50 ± 0.03 ; LR @ 20 Hz = 0.39 ± 0.02 ; LR @ 40 Hz = 0.24 ± 0.02 ; DR @ 10 Hz = 0.54 ± 0.02 ; DR @ 20 Hz = 0.39 ± 0.01 ; DR @ 40 Hz = 0.25 ± 0.02 ; $p > 0.05$). These data suggest that presynaptic neurotransmitter release is similar in cells from LR and DR subjects.

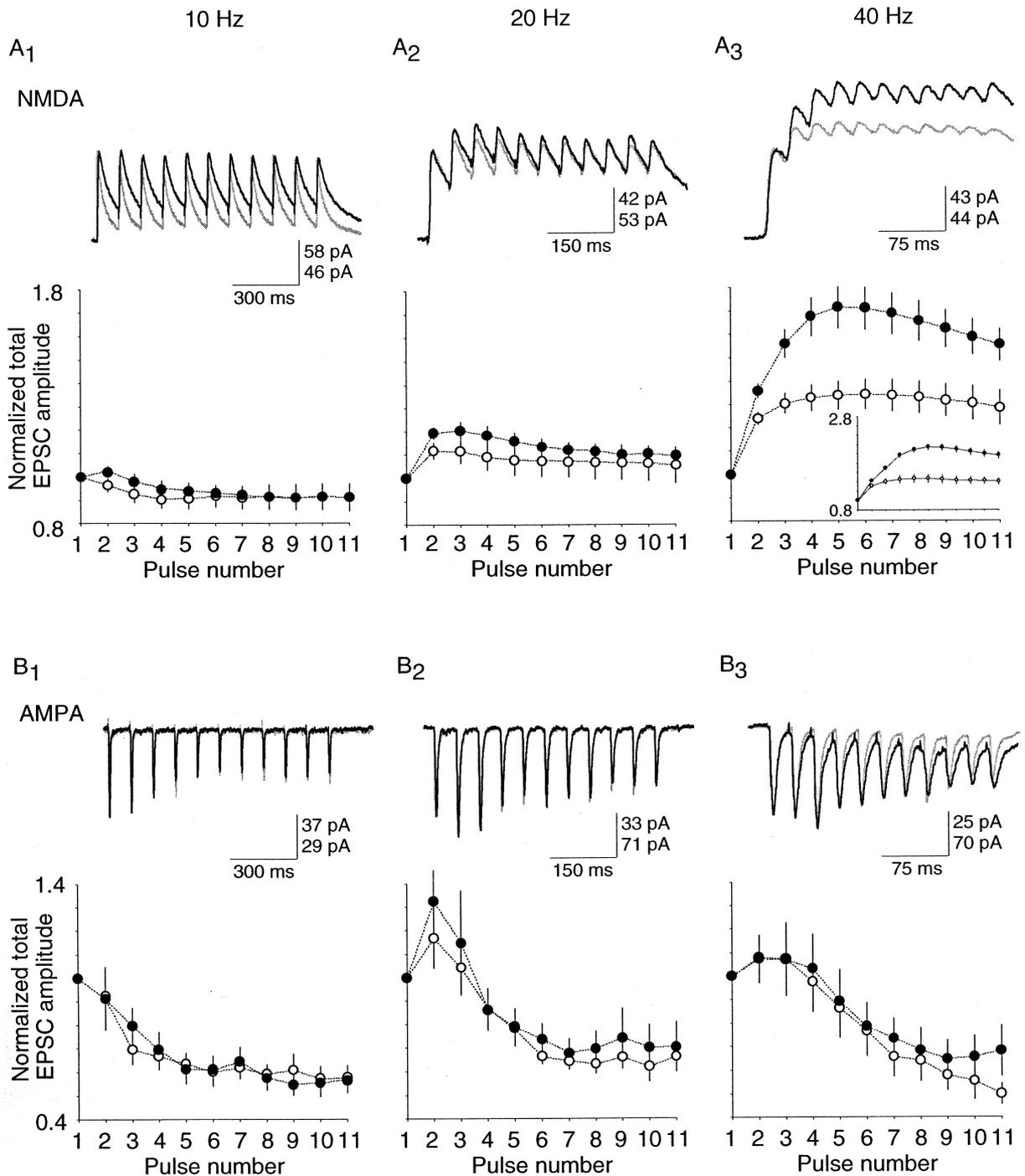


Figure 3. Visual Experience Alters the Temporal Summation of NMDAR-EPSCs but Does Not Affect the Temporal Dynamics of AMPAR-Mediated Currents

Example current traces recorded from layer 2/3 pyramidal cells from LR (light traces) and DR (dark traces) animals are shown above group averages from LR (open circles) and DR (filled circles) rats. Traces represent the average of 15–30 stimulation trains of 11 pulses at 10, 20, or 40 Hz. Group averages represent the averages of the peak current amplitude. All responses have been normalized to the peak amplitude of the 1st pulse.

(A) Pharmacologically isolated NMDAR-EPSCs, recorded at +40 mV, in response to 10 (A₁), 20 (A₂), or 40 (A₃) Hz stimulus trains. Note the dramatic increase in NMDAR-EPSC summation found in cells from DR rats as compared to LR controls after 40 Hz stimulation. The inset represents averages taken from only the first 3 stimulation trains, demonstrating that the effect was not due to differential rundown after 30 stimulus trains.

(B) AMPAR-mediated currents, recorded at –70 mV, in response to 10 (B₁), 20 (B₂), or 40 (B₃) Hz stimulus trains. Note that the responses of AMPAR-mediated currents do not differ among rearing conditions.

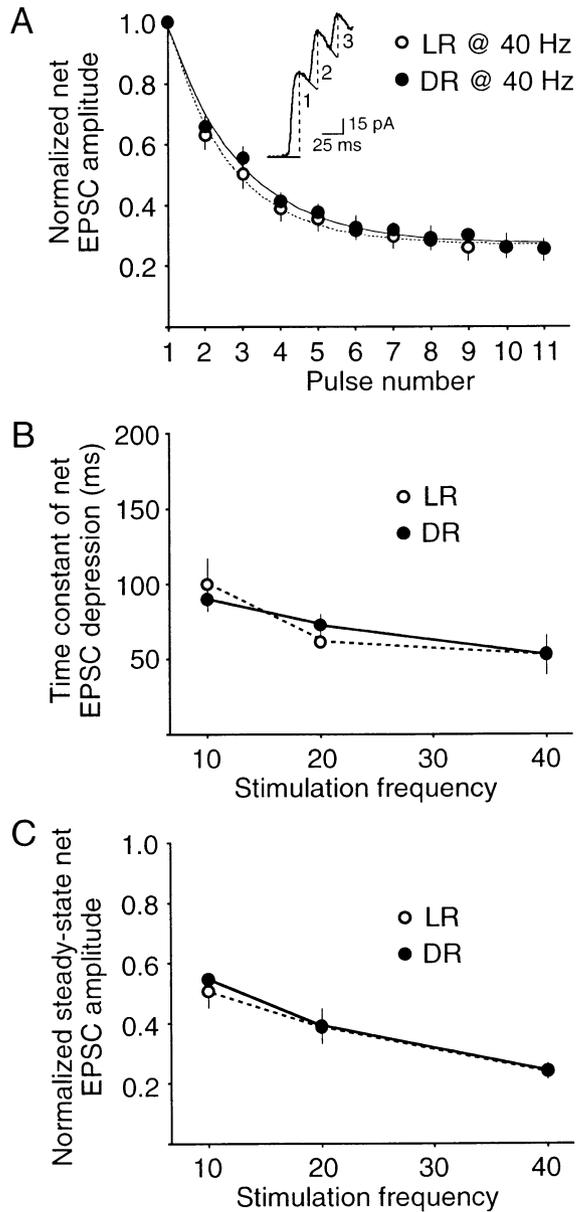


Figure 4. Visual Experience Does Not Alter Synaptic Depression of NMDAR-EPSCs

(A) Normalized NMDAR EPSC amplitudes and their single exponential curve fits in light-reared (LR) and dark-reared (DR) cortex in response to 40 Hz stimulation. The net amplitude was measured from the linear extrapolation from the tail of the preceding EPSC to the peak amplitude of the response (dashed lines in example). (B) Time constant of the synaptic depression for LR and DR animals plotted against frequency. (C) Normalized steady-state amplitude plotted against stimulation frequency for LR and DR animals.

These findings support the idea that the modest difference in NMDAR current decays is sufficient to account for the observed experience-dependent changes in NMDAR temporal dynamics. Consistent with this hypothesis, there is a significant positive correlation between the duration of NMDAR-EPSC decay and the current response during the stimulation train (described by

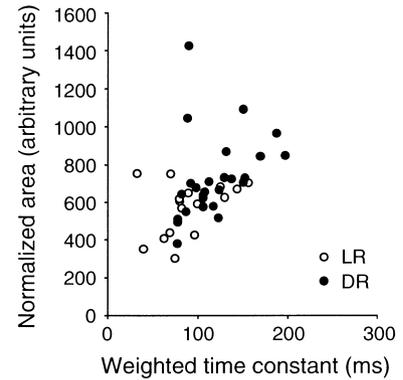


Figure 5. The Current Duration of Individual NMDAR EPSCs Correlates with the Charge Transfer during High-Frequency (40 Hz) Stimulation

NMDAR current duration, expressed as the weighted time constant, is positively correlated to the normalized charge transfer (area); the longer the current decay, the greater the charge transfer ($r = 0.43$, $p < 0.005$). Note the greater charge transfer in cells from dark-reared (DR) versus light-reared (LR) animals.

normalizing the amplitude of the averaged first pulse to 1 and measuring the area of the current response over time; Figure 5; $n = 42$ cells from 13 rats, $r = 0.43$, $p < 0.005$). Thus, these data suggest that the duration of NMDAR current responses in pyramidal cells is a strong determinant of the summation of currents to high-frequency stimulation.

A Simple Linear Model that Incorporates NMDAR Current Decay and Synaptic Depression Can Account for the Observed Differences in Temporal Summation

We next asked whether temporal summation of NMDAR currents could be predicted simply on the basis of the current decay and synaptic depression. We created a model based on simple linear addition of NMDAR-mediated EPSCs. Each EPSC has the form $I(t) = A(t)(I_f \exp(-t/\tau_f) + I_s \exp(-t/\tau_s))$, where $A(t)$ is the total amplitude of the pulse at time t , τ_f and τ_s are the respective time constants for the fast and slow components, I_f and I_s are the respective magnitudes of the fast and slow components of the current, and we assumed that $I_f + I_s = 1$. The amplitude, $A(t)$, changes with each pulse due to fast synaptic depression and can be defined by the expression $A(t) = K(f) \cdot \exp(t/\tau_d(f)) + PL(f)$, where $K(f)$, the time constant $\tau_d(f)$, and plateau value $PL(f)$ are all functions of the stimulation frequency. We assumed that $K(f) + PL(f) = 1$. As described above, for each cell we measured five independent parameters: I_f , τ_f , τ_s , K , and τ_d . Given these five parameters, we simulated a train of 11 NMDAR-mediated EPSCs for each cell and compared it to the experimentally recorded train. The ratios of the 11th pulse to the 1st pulse were compared in the simulated and experimental EPSC trains.

The model accurately described the experimentally observed temporal summation given by the 11th pulse (Figure 6; $n = 115$, $r = 0.46$, $p < 0.0000002$). The strong correlation between experimental and modeled data suggests that synaptic depression and NMDAR current

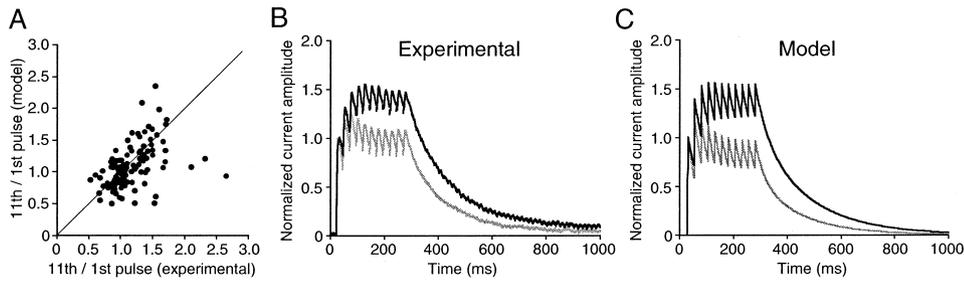


Figure 6. The Experimentally Observed Temporal Summation of NMDAR Currents Can Be Approximated using a Simple Linear Model Determined by the Current Decay and Synaptic Depression

(A) The experimental ratio of the 11th pulse to the first pulse plotted against that predicted by the model for each cell, using the empirically determined values for I_f , τ_f , τ_s , K , and τ_d . Data shown are for all cells from light- and dark-reared animals at 10, 20, and 40 Hz stimulation. A line with unitary slope is drawn as a reference.

(B) Experimental data demonstrating NMDAR current summation at 40 Hz in cells from dark- and light-reared animals (black and gray traces, respectively). Note that these cells differ primarily in NMDAR current duration rather than synaptic depression (light-reared cell: percent amplitude described by $I_f = 0.70$, $\tau_f = 32$, $\tau_s = 160$, $PL = 0.3$, $\tau_d = 32$; dark-reared cell: percent amplitude described by $I_f = 0.56$, $\tau_f = 44$, $\tau_s = 186$, $PL = 0.3$, $\tau_d = 40$; see Results for explanation of variables).

(C) The model for the two cells shown in (B) faithfully reproduces the observed temporal summation.

decay are strong determinants of temporal summation. The model also supports the conclusions that (1) a postsynaptic change in NMDAR current decay, in the absence of presynaptic changes in neurotransmitter release, is sufficient to account for the observed differences in temporal summation, and (2) NMDAR current duration more profoundly affects the magnitude of temporal summation at higher frequencies of stimulation as compared to lower frequencies.

Discussion

The major finding of this study is that the history of sensory experience is recorded by visual cortical synapses as a change in the subunit composition and functional properties of NMDARs. Visual experience is associated with decreased expression of NR2B-only receptors in the population of synaptic NMDARs and a shortening of NMDAR-EPSC duration. Visual deprivation exerts an opposite effect: increased contribution of NR2B-containing receptors and longer duration currents. Although the effects of experience and deprivation are reversible, the rates of synaptic modification are different. Experience can induce a detectable change in synaptic transmission within hours, while deprivation-induced changes take several days. We also find that modest differences in EPSC kinetics can have striking functional consequences. Because the short-term plasticity of synaptic transmission in layer 2/3 is not altered by visual deprivation, longer duration EPSCs show more summation with repetitive stimulation. Consequently, NMDAR-mediated synaptic transmission is greatly facilitated at high-stimulation frequencies in visual cortex of visually deprived animals.

While we have clearly demonstrated the bidirectional regulation of NMDAR subunit composition and function during the critical period of rat visual cortex development, it remains to be seen if experience-dependent changes in NMDAR composition occur in adult visual cortex. In any case, it is likely that the changes we have documented have a profound influence on visual cortical

development. Although their precise role continues to be debated (Hensch et al., 1998; Rittenhouse et al., 1999), it is well established that NMDAR-mediated responses make an essential contribution to the experience-dependent development of visual cortical receptive fields.

Alterations in the Sensitivity of NMDAR-FPs to NR2B-Selective Antagonists

Our data demonstrate that ifenprodil sensitivity of NMDAR-FPs is enhanced in layers 2/3 of visual cortex of DR and BD rats compared to LR and light-exposed DR animals. Several lines of evidence suggest that this difference is due specifically to changes in the proportion of NR2B-containing receptors. First, the effects of dark-rearing and visual experience were only observed using a range of ifenprodil concentrations at which the drug was selective for NR2B-containing receptors. Second, another more potent NR2B antagonist, CP101,606, yielded identical results. Third, AP5, an antagonist that does not discriminate between NR2 subunits, had comparable effects in visual cortex of DR and LR animals. Thus, the altered ifenprodil and CP101,606 sensitivity is not merely a consequence of a change in the total number of functional NMDARs, although we cannot exclude the possibility that this also occurs.

Native NMDARs may exist as NR1/NR2A and NR1/NR2B diheteromers or as NR1/NR2A/NR2B triheteromeric receptors (Sheng et al., 1994; Luo et al., 1997; Kew et al., 1998). Ifenprodil and CP101,606 selectively antagonize the NR2B-containing diheteromeric receptor but do not discriminate between the NR1/NR2A diheteromers and the NR1/NR2A/NR2B triheteromers (Brimecombe et al., 1997; Tovar and Westbrook, 1999). Thus, the simplest interpretation of our findings is that the population of visual cortical NR1/NR2B diheteromeric receptors, as a percentage of the total number of functional synaptic NMDARs, is increased by visual deprivation and reduced by visual experience.

Our findings are consistent with recent quantitative biochemical measurements of NR2A and NR2B protein expression in synaptoneurosomes prepared from visual

cortex (Quinlan et al., 1999a, 1999b). Those studies showed that visual experience increases the ratio of NR2A/B protein while visual deprivation reduces it. Moreover, the time course of the biochemical changes agrees perfectly with the changes in ifenprodil sensitivity. The close correlation between protein measurements and ifenprodil sensitivity suggests that the measured changes in protein are functionally significant and, indeed, causally related to the modifications in synaptic transmission reported here. In this context it is noteworthy that the biochemical changes were restricted to visual cortex, suggesting that the functional changes we have reported here are a specific consequence of visual experience and deprivation and not a global response to the stress of dark-rearing or sudden LE. Moreover, the fact that BD produces effects (both biochemical and functional) as pronounced as dark-rearing strongly supports the idea that the changes in NMDARs in visual cortex are specifically related to changes in the visual environment.

Notably, the most reliable effect of experience and deprivation on NMDAR subunit protein expression was a change in NR2A expression; NR2B levels were unchanged in DR animals as compared with LR controls. Experience-dependent delivery of NR2A-containing receptors to the synaptic membrane would be expected to reduce ifenprodil sensitivity of NMDAR-mediated responses by diluting the contribution of NR1/NR2B diheteromeric receptors to the total synaptic current. However, the fact that NR2A and NR2B proteins do not show reciprocal changes suggests that the total number of NMDARs might also be modified bidirectionally by experience and deprivation. Alternatively, NR2B protein might be removed from the synaptic membrane as NR2A-containing receptors are inserted, without being removed from the synaptoneurosomal fraction (Figure 7A). The relatively low resolution of our technique offers limited insight into this question. We observed no differences between DR and LR visual cortex with respect to the stimulation intensity required to evoke 80% of the maximal NMDAR-FP, the amplitude of the FP at this intensity, or the ratio of responses mediated by AMPARs versus NMDARs. However, a more refined analysis, using pairs of synaptically coupled neurons, will be required to fully address the possibility of experience-dependent change in the absolute contributions of NMDARs to synaptic transmission.

Bidirectional Modification of the Kinetics of NMDAR-Mediated Synaptic Responses

Previous studies have shown that the duration of NMDAR-EPSCs in the superior colliculus (Hestrin, 1992), the lateral geniculate nucleus (Ramoa and Prusky, 1997), and layer 4 of visual (Carmignoto and Vicini, 1992; Roberts and Ramoa, 1999) and somatosensory cortex (Crair and Malenka, 1995; Flint et al., 1997) progressively shorten over the course of postnatal development, coincident with changes in NR2A expression (Monyer et al., 1994; Sheng et al., 1994; Flint et al., 1997; Nase et al., 1999; Quinlan et al., 1999a; Roberts and Ramoa, 1999). The developmental change in NMDAR-EPSC kinetics in visual cortical layer 4 is reduced by dark-rearing (Carmignoto and Vicini, 1992). Our experiments confirm that

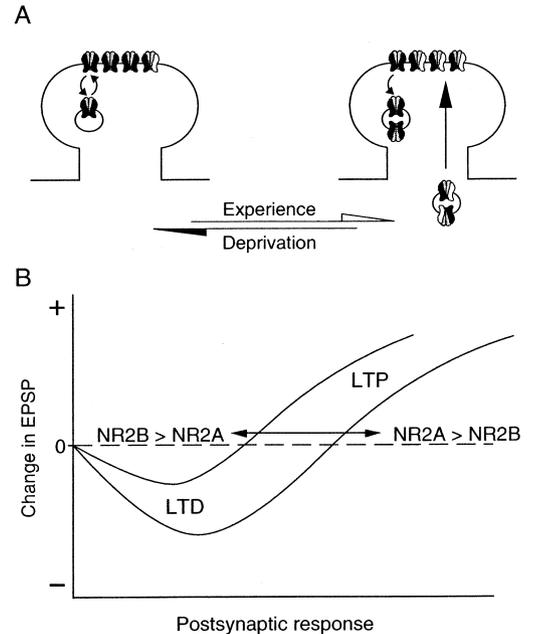


Figure 7. Molecular Basis for a Sliding Synaptic Modification Threshold in Visual Cortex

(A) Model to account for the observed bidirectional changes in ifenprodil sensitivity and NMDAR subunit expression. In the absence of visual experience, high constitutive expression of NR2B (black subunits) and reduced expression of NR2A (white subunits) leads to an increase in NR1/NR2B diheteromeric receptors at the synapse (and increased ifenprodil sensitivity). Visual experience triggers increased NR2A expression and the rapid delivery of NR1/NR2A/NR2B triheteromeric receptors to the synapse, compensated by a net loss of surface NR1/NR2B diheteromers (and reduced ifenprodil sensitivity).

(B) Model relating NMDAR subunit regulation to the properties of synaptic modification. The y axis represents the lasting change in synaptic strength following conditioning stimulation at different levels of integrated postsynaptic response (x axis). The curves are schematized from the data of Kirkwood et al. (1996). An increase in the NR2A/B ratio, as seen with light exposure after a period of dark-rearing, is proposed to be responsible for sliding the LTD-LTP crossover point (θ_m) to the right, thus decreasing the likelihood that synaptic strengthening will occur. Conversely, a fall in the NR2A/B ratio, as seen with binocular deprivation, slides θ_m to the left, favoring LTP over LTD. According to this model, the properties of synaptic modification depend upon the history of cortical activity, as originally proposed in the BCM theory, as a consequence of activity-dependent expression of NR2A-containing NMDARs at cortical synapses.

a similar difference between LR and DR animals can be observed in the neurons of visual cortical layers 2/3 and show that the effects of dark-rearing are not simply due to arrested cortical development in DR animals. Remarkably, only 2 hr of visual experience in DR animals is sufficient to significantly alter the kinetics of NMDAR-EPSCs. Moreover, BD of LR animals causes a prolongation of the EPSC that is comparable to that observed in animals reared from birth in complete darkness. Thus, the kinetics of the NMDAR-EPSCs in visual cortex are an accurate reflection of the recent history of visual experience.

The rate of current decay is determined by a variety of factors, such as agonist binding affinity and the rate of and recovery from desensitization (Lester et al., 1990;

Lester and Jahr, 1992). Notably, NR2B-containing receptors have a stronger binding affinity for glutamate than NR2A-containing receptors, and NR2B-containing receptors are also slower to desensitize and slower to recover from desensitization (Laurie and Seeburg, 1994; Priestley et al., 1995; Vicini et al., 1998). These studies support the idea that differences in EPSC decay are likely attributable to the subunit-specific properties of glutamate affinity and desensitization (Lester and Jahr, 1992; Laurie and Seeburg, 1994; Priestley et al., 1995; Vicini et al., 1998). We cannot rule out the possibility of additional experience-dependent modifications of NMDAR function (e.g., by phosphorylation/dephosphorylation; Shi et al., 2000); indeed, in the course of this study we discovered that isolated NMDAR-mediated responses are highly susceptible to rapid, activity-dependent modifications (see Appendix [<http://www.neuron.org/cgi/content/full/29/1/157/CD1>]). However, the close parallel between bidirectional changes in current duration, ifenprodil sensitivity, and NR2A/B protein expression strongly supports the conclusion that experience-dependent alterations of NMDAR subunit composition are a molecular basis for the observed changes in NMDAR-EPSC kinetics.

Changes in the Temporal Summation of NMDAR-Mediated Synaptic Responses

Our data demonstrate that dark-rearing enhances the temporal summation of NMDAR-mediated synaptic transmission in layer 2/3 pyramidal cells. When the complement of postsynaptic receptors remains constant, temporal summation appears to rely on the presynaptic probability of release and the properties of the readily releasable pool of neurotransmitters (Dobrunz and Stevens, 1997; Tsodyks and Markram, 1997). However, several lines of evidence suggest that the observed changes in NMDAR current summation in the present study are a postsynaptic consequence of the modest increase in NMDAR current duration. First, it is unlikely that presynaptic mechanisms can account for enhanced temporal summation of NMDAR-EPSCs, because no differences in the temporal dynamics of AMPAR-mediated responses were observed between visual cortex pyramidal cells from LR and DR animals. Second, short-term synaptic depression of NMDAR-EPSCs does not differ in cortex of LR and DR animals. Third, the duration of NMDAR currents is a strong predictor of the degree of facilitation to 40 Hz stimulation. Fourth, a simple linear model demonstrates that postsynaptic changes in NMDAR current duration are sufficient to account for the observed differences in temporal summation. Finally, other possibilities appear less likely. For example, subunit differences in calcium-dependent inactivation (Legendre et al., 1993; Krupp et al., 1996) or receptor phosphorylation (Shi et al., 2000) are unlikely to account for the observed differences in NMDAR function, as these effects were observed despite the presence of a strong calcium chelator, BAPTA, in the recording pipette. Moreover, it is unlikely that experience-dependent changes in magnesium or glycine sensitivity contribute to the findings; as all NMDA currents were recorded at +40 mV to remove magnesium block, there was no experience-dependent change in the current-voltage relationship,

and recordings were made in saturating levels of glycine. Thus, our data strongly suggest that a modest experience-dependent change in NMDAR composition and, thus, current duration, translates into a dramatic difference in short-term current summation at high frequencies of stimulation.

A previous study has demonstrated that partial whisker trimming can alter the short-term plasticity of synaptic AMPAR-mediated responses between layer 4 and 2/3 synapses in rat somatosensory cortex (Finnerty et al., 1999). These authors concluded that AMPAR-mediated EPSPs exhibited greater short-term synaptic depression at 5–10 Hz in “spared” cortical pathways as compared to “deprived” pathways, although no differences were observed at higher frequencies. In contrast, we failed to observe experience-induced shifts in AMPA-mediated currents in visual cortex. This difference might be attributed to the fact that the Finnerty et al. study had the advantage of using within animal controls to aid in the detection of relatively modest differences in short-term plasticity. Alternatively, there are likely differences in the types of experience-dependent plasticity that are induced in somatosensory and visual cortices, or there might be differences in competitive (partial whisker trimming) versus noncompetitive (dark-rearing) processes. In any case, because experience does not modify synaptic depression from layer 4 inputs to layer 2/3 pyramidal cells, longer NMDA-mediated currents in deprived animals lead to enhanced temporal summation.

Functional Significance

Experience and deprivation bidirectionally modify the visual responses of cortical neurons. Such modifications have a synaptic basis, but little is known of the underlying molecular mechanisms. We have been able to show that experience and deprivation bidirectionally alter the properties of visual cortical synaptic transmission, in part, by regulating the subunit composition of postsynaptic NMDARs. Little is known about how synaptic activity triggers a change in the synaptic expression of NMDARs *in vivo*, although the process is sensitive to blockers of NMDARs and protein synthesis (Quinlan et al., 1999b). Questions at this level of analysis are better addressed *in vitro*, where similar activity-dependent changes have been described (Gottmann et al., 1997; Tovar and Westbrook, 1999). Our results provide a necessary foundation for this reductionist approach, since we can now conclude that activity-dependent, bidirectional alterations in NMDARs are a form of naturally occurring synaptic plasticity in the brain.

There is evidence that experience-dependent modifications of NMDAR-mediated synaptic transmission can impact the visual responses of cortical neurons. Visual responses in DR animals show a heightened sensitivity to NMDAR blockade as compared with LR controls (Fox et al., 1991, 1992). The facilitated NMDAR currents during high-frequency stimulation could explain the greater contribution of NMDARs to visual responses in DR animals. Furthermore, reduced coincidence detection by the long-duration NMDAR-EPSCs might also contribute to the reduced stimulus selectivity of cortical neurons in DR animals.

It is important to point out that the changes in NMDAR properties observed here may also have a large impact on experience-dependent regulation of AMPAR-mediated responses. In many regions of the brain, including the neocortex, AMPAR-mediated transmission is potentiated as a consequence of a rise in intracellular calcium during strong NMDAR activation, a phenomenon called long-term potentiation (LTP; e.g., Kirkwood and Bear, 1994a; Isaac et al., 1997). Conversely, modest increases in calcium due to weak NMDAR activation can trigger a long-term depression (LTD) of synaptic transmission (e.g., Kirkwood and Bear, 1994b; Feldman et al., 1998). Our data suggest that alterations in the subunit structure of the NMDAR can substantially modify the calcium current that occurs in response to repetitive stimulation. The long-duration currents in visual cortex of DR animals predicts enhanced LTP and diminished LTD over a range of stimulation frequencies, and this is exactly what has been observed experimentally (Kirkwood et al., 1996). Moreover, the properties of LTP and LTD in visual cortex vary bidirectionally with brief LE and BD, in good agreement with the changes we observe in NMDARs (M. Rioult and M. F. B., unpublished data). We hasten to add, however, that the induction of plasticity can be modified by many means in addition to altered NMDAR kinetics, including changes in NMDAR-associated proteins (e.g., Husi et al., 2000; Sans et al., 2000) and inhibitory circuitry (e.g., Huang et al., 1999).

The idea that the properties of synaptic plasticity depend on the history of cortical activity was proposed initially by Bienenstock et al. (1982) in what is called the BCM theory. According to BCM, active synapses undergo LTD when the postsynaptic response is greater than zero (defined as the level of spontaneous activity) but less than a critical value, termed the modification threshold (θ_m); responses greater than θ_m lead to LTP of the active synapses. Another key assumption of the BCM theory was that the value of θ_m is not fixed but rather adjusts depending on the level of cortical activity. Elucidation of the voltage-dependent properties of NMDARs, and their special role in hippocampal LTP, led to the proposals that θ_m is related to the critical level of NMDAR activation that triggers LTP and that the value of θ_m adjusts by altering the properties of NMDARs (Bear et al., 1987). Our data are entirely consistent with this idea. We propose that one mechanism for the sliding synaptic modification threshold of the BCM theory is the experience-dependent, bidirectional regulation of NMDAR subunit composition (Figure 7B).

Experimental Procedures

Subjects

Male and female P21–P31 Long-Evans rats (Charles River) were used. All subjects were fed ad libitum. Control LR rats were raised on a 12:12 light:dark cycle, while DR rats were raised in complete darkness. BD or LE was performed by placing subjects in complete darkness or on the normal light:dark cycle, respectively, and was begun ~5 hours into the light:dark cycle.

Slice Preparation

Subjects were anesthetized with methoxyflurane vapor either in the dark (DR and BD groups) or the light (LR and LE groups). Rats were decapitated following the disappearance of corneal reflexes in compliance with the U.S. Department of Health and Human Ser-

vices and Brown University guidelines. Coronal slices were prepared (350 μm for patch-clamp recording and 400 μm for field potential recordings) from occipital neocortex as previously described (Kirkwood et al., 1993). We focused our study on layers 2/3, because these layers exhibit dramatic experience-dependent plasticity, field potentials are easily recorded from these layers, and layer 2/3 pyramidal cells are easily identified for whole-cell recordings using IR-DIC optics.

Extracellular Recordings

Field potential recordings were performed in a submersion recording chamber as previously described (Quinlan et al., 1999b). The amplitude of the field potential was used to monitor the magnitude of responses. Stable baseline responses were elicited two per min at 80% of maximal response. NMDAR-mediated responses were pharmacologically isolated in ACSF containing: 3 mM CaCl_2 , 0.1 mM MgCl_2 , 20 μM CNQX (Tocris), 1 μM glycine, and 0.5 μM bicuculline methiodide (see Quinlan et al., 1999b for details).

Probing stimulation, consisting of 4 pulses delivered at 30 s intervals, was given every 10 min to assess NMDAR-mediated field potentials (see Appendix [<http://www.neuron.org/cgi/content/full/29/1/157/CD1>] for rationale). Three micromolar ifenprodil (RBI) or 5 μM CP101,606 (a generous gift of Pfizer, Groton, CT) was used to block NR2B-containing receptors. These concentrations are in a range that produces nearly maximal inhibition of NR2B-containing NMDARs, has little effect on NR2A-containing NMDARs, and does not have nonspecific effects (Williams, 1993; Church et al., 1994; Chenard et al., 1995; Boeckman and Aizenman, 1996). As noted previously (Ramoia and Prusky, 1997), we found that wash-out of ifenprodil is slow and incomplete. Therefore, to calculate the percent inhibition of the NMDAR-mediated field potential by ifenprodil or CP101,606, we compared the 30 min average immediately before adding the drug with the average of 4 consecutive sweeps collected 90 min after the drug application.

The blockade of NMDAR field responses varied slightly in different batches of ifenprodil and in different groups of animals reared under the same conditions. Therefore, each experimental comparison was performed using interleaved control animals, from the same litter or an age-matched control, and the same batch of ifenprodil. It is not valid to make comparisons of ifenprodil sensitivity across groups from different experiments.

Voltage-Clamp Recordings

Slices were allowed to recover for 45 min at 35°C in a submersion chamber, containing oxygenated ACSF with 4 mM MgCl_2 and 4 mM CaCl_2 , and then moved to room temperature until use. For recording, slices were placed in a submersion chamber, maintained at 30°C, and perfused at 2 ml per min with oxygenated ACSF containing 4 mM MgCl_2 , 4 mM CaCl_2 , 1 μM glycine, 50 μM picrotoxin (Fluka), and 20 μM CNQX. Cells were identified using an Axioskop microscope (Zeiss) combined with infrared differential interference contrast optics (IR-DIC). Patch pipettes were pulled from thick-walled borosilicate glass. Open tip resistance were 4–8 M Ω when pipettes were filled with the internal solution containing 102 mM cesium gluconate, 5 mM TEA-chloride, 3.7 mM NaCl, 20 mM HEPES, 0.3 mM sodium guanosine triphosphate, 0.3 mM magnesium adenosine triphosphate, 0.2 mM EGTA, 10 mM BAPTA, and 5 mM QX-314 chloride (Alomone Labs, Jerusalem, Israel), with pH adjusted to 7.2 and osmolarity adjusted to ~300 mmol/kg with sucrose or ddH₂O. To stain cells, Lucifer yellow (2 mg/ml) was sometimes diluted in the internal recording solution. Voltage-clamp recordings were performed in the whole-cell configuration using a patch-clamp amplifier (Axoclamp 1D, Axon Instruments), and data were acquired and analyzed using a system from DataWave Technologies (Boulder, CO). Pipette seal resistances were typically >1 G Ω , and pipette capacitive transients were minimized prior to breakthrough. Series resistance was measured manually at the start of the experiment and was monitored throughout the experiment by giving a test pulse and measuring the amplitude of the capacitive current filtered at 30 kHz. Only cells with series resistance <30 M Ω were included in this study. No series resistance compensation was applied. Input resistance was monitored throughout the experiment by measuring the amplitude of the steady-state current, filtered at 2 kHz, evoked from a test pulse.

Input resistances did not differ between experimental groups and averaged $127 \pm 7 \text{ M}\Omega$. EPSCs were evoked from a stimulating electrode (concentric bipolar stimulating; $200 \mu\text{M}$ tip separation) placed in layer 4, and stimulation was given for $200 \mu\text{s}$ every 6 s. To examine functional changes in the summation of NMDA- and AMPA-mediated currents, 11 pulses of 10, 20, or 40 Hz trains of stimulation were given every 6 s for 3 min.

Drugs

Unless otherwise noted, drugs were purchased from Sigma.

Data Analysis

Statistics were done using t tests or ANOVA, with significance set at $p < 0.05$. Data are reported as means \pm SEM.

Acknowledgments

Special thanks to M. Barbarosie, K. Huber, and N. Sawtell for critical readings of the manuscript, to W. Wallace for performing the Lucifer yellow histology, and to E. Sklar and S. Meagher for assistance. H. Markram provided helpful advice. This work was supported in part by grants from the NIH, NSF, and HFSP.

Received May 30, 2000; revised December 5, 2000.

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