Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex *in vivo*

Elizabeth M. Quinlan[1], Benjamin D. Philpot[1], Richard L. Huganir[2] and Mark F. Bear[1]

[1] *Howard Hughes Medical Institute, Department of Neuroscience, Brown University, Providence, Rhode Island 02912, USA*

[2] *Howard Hughes Medical Institute, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA*

The first two authors contributed equally to this paper.

Correspondence should be addressed to M.F.B. (mark_bear@brown.edu)

Sensory experience is crucial in the refinement of synaptic connections in the brain during development. It has been suggested that some forms of experience-dependent synaptic plasticity *in vivo* are associated with changes in the complement of postsynaptic glutamate receptors, although direct evidence has been lacking. Here we show that visual experience triggers the rapid synaptic insertion of new NMDA receptors in visual cortex. The new receptors have a higher proportion of NR2A subunits and, as a consequence, different functional properties. This effect of experience requires NMDA receptor activation and protein synthesis. Thus, rapid regulation of postsynaptic glutamate receptors is one mechanism for developmental plasticity in the brain. Changes in NMDA receptor expression provide a mechanism by which brief sensory experience can regulate the properties of NMDA receptor-dependent plasticity in visual cortex.

The neuronal response to glutamate released at excitatory synapses depends on the complement of glutamate receptors in the postsynaptic membrane. Changes in receptor number, type and molecular composition can substantially alter the properties of synaptic transmission. During postnatal development, excitatory synaptic transmission is readily modified by sensory experience. Thus, it has been suggested that some forms of experience-dependent synaptic plasticity are associated with rapid changes in the complement of postsynaptic glutamate receptors. Most attention has focused on changes in the synaptic expression of AMPA receptors. However, NMDA receptors (NMDARs) are critical in triggering experience-dependent synaptic modifications, and changes in synaptic expression of these receptors could have a large effect on the properties of synaptic plasticity during development. In the present study, we addressed the possibility that experience rapidly regulates the synaptic expression of NMDARs in visual cortex *in vivo*.

NMDARs *in vivo* are heteromeric ion channels composed of NR1 and NR2 subunits. The subtype (A–D) of the NR2 subunit confers distinct functional properties to the receptor[1–7]. Receptors containing NR2B predominate in the neonatal forebrain, and over the course of development, these are replaced or supplemented with NR2A-containing receptors[2,8]. This subunit switch alters the channel properties such that the synaptic NMDAR-mediated currents shorten in duration[2,9]. The developmental shortening of NMDAR currents in visual cortical neurons is postponed when animals are deprived of vision[10], suggesting that the NR2A/B subunit composition of synaptic NMDARs differs between dark-reared animals and light-reared controls. We tested this hypothesis and then examined the effects of brief light exposure in dark-reared animals. Our results show that new NMDARs with a higher NR2A/B ratio are inserted into synaptic membrane within one hour of the onset of visual experience.

Results

To examine NMDAR subunit composition, we used quantitative immunoblotting for NR2A, NR2B and NR1 in synaptoneurosomes prepared from the visual cortices of postnatal day (P) 21–23 light-reared and dark-reared Long-Evans rats. Synaptoneurosomes are a biochemical fraction that is enriched for synaptic proteins[11]. This biochemical analysis showed that synaptoneurosomal NR2A protein levels were significantly higher in light-reared visual cortex than in dark-reared cortex (mean optical density (OD) ± s.e.m., light reared, 1077 ± 224; dark reared, 743 ± 152, paired *t*-test, $p < 0.005$, $n = 6$). In contrast, levels of NR2B and NR1 proteins were not affected by dark rearing (NR2B light reared, 1907 ± 175; dark reared, 1777 ± 133, paired *t*-test, $p > 0.05$, $n = 6$; NR1 light reared, 1656 ± 202; dark reared, 1656 ± 222, paired *t*-test, $p > 0.1$, $n = 8$). Thus, as predicted, a lower NR2A/B ratio (Fig. 1) is correlated with slower NMDAR current kinetics[10] in dark-reared visual cortex. The effect of dark rearing in visual cortex seems to be a specific consequence of sensory deprivation, as there were no detectable differences in NMDAR protein levels in the hippocampi of dark-reared or light-reared animals (NR2A light reared, 1009 ± 184 ; dark reared, 1067 ± 161, paired *t*-test, $p > 0.1$, $n = 6$; NR2B light reared, 873 ± 225; dark reared, 838 ± 221, paired *t*-test, $p > 0.1$, $n = 6$). To examine the effects of visual experience on synaptoneurosomal NMDARs, we exposed dark-reared animals to a normal 12:12 light:dark cycle for 24 or 48 hours. These experiments revealed that the reduction in synaptoneurosomal NR2A content in dark-reared animals could be completely reversed within one day of

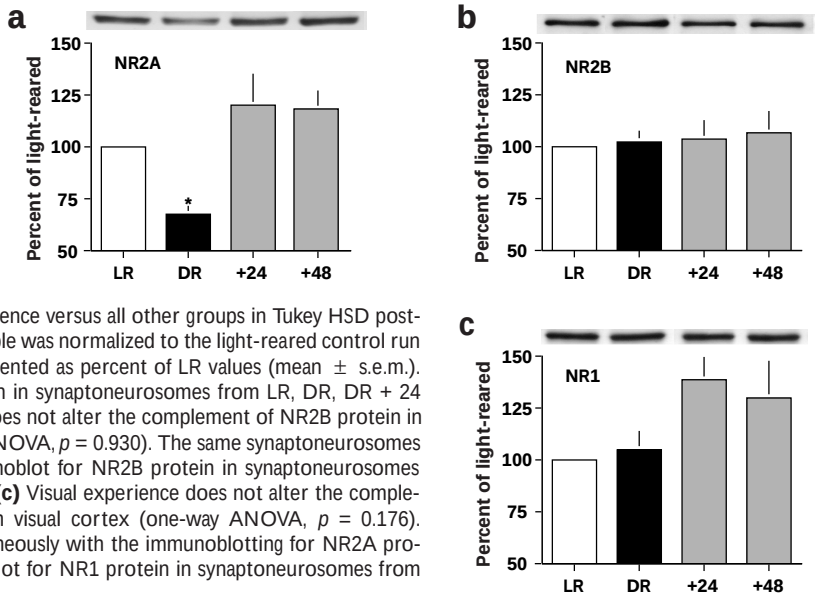

Fig. 1. Visual experience regulates the composition of NMDARs in synaptoneurosomes from visual cortex. Quantitative immunoblotting for NMDAR subunit proteins of synaptoneurosomes prepared from visual cortices of P21–23 rats raised in a normal (12:12) light:dark cycle (LR), in complete darkness (DR) or in darkness from birth followed by exposure to the normal light cycle for 24 (+24) or 48 (+48) h. **(a)** NR2A protein is reduced in visual cortex synaptoneurosomes from dark-reared animals, and this reduction is reversed by subsequent light exposure (one-way ANOVA. $p = 0.004$, *significant difference versus all other groups in Tukey HSD post-hoc comparison, $p < 0.05$). The O.D. from each sample was normalized to the light-reared control run on the same gel, and the summarized data are presented as percent of LR values (mean ± s.e.m.). Inset, representative immunoblot for NR2A protein in synaptoneurosomes from LR, DR, DR + 24 and DR + 48 visual cortex. **(b)** Visual experience does not alter the complement of NR2B protein in synaptoneurosomes from visual cortex (one-way ANOVA, $p = 0.930$). The same synaptoneurosomes were probed as in **(a)**. Inset, representative immunoblot for NR2B protein in synaptoneurosomes from LR, DR, DR + 24 and DR + 48 visual cortex. **(c)** Visual experience does not alter the complement of NR1 protein in synaptoneurosomes from visual cortex (one-way ANOVA, $p = 0.176$). Immunoblotting for NR1 protein was done simultaneously with the immunoblotting for NR2A protein depicted in **(a)**. Inset, representative immunoblot for NR1 protein in synaptoneurosomes from LR, DR, DR + 24 and DR + 48 visual cortex.

light exposure (Fig. 1). Again, there were no significant effects of visual experience on the levels of NR2B or NR1.

These biochemical data show that the ratio of NR2A/B protein at the synapse is affected by light deprivation and subsequent visual experience. However, it was critical to determine whether these differences in protein reflect differences in the composition of functional NMDARs at visual cortical synapses. To address this question, we examined the ifenprodil sensitivity of synaptically evoked, NMDAR-mediated field potentials in slices of visual cortex from P21–28 rats. Ifenprodil selectively blocks NR2B-containing NMDARs[12], so enhanced ifenprodil sensitivity indicates a lower NR2A/B ratio in the synaptic NMDARs. The NMDAR component of layer 2/3 field potentials was pharmacologically isolated in artificial cerebrospinal fluid (ACSF) containing reduced Mg^{2+} and blockers of AMPA and $GABA_A$ receptors (Fig. 2). Once a stable baseline was obtained, we applied 3 μM ifenprodil, a concentration that selectively blocks NR2B-containing NMDARs[12–14]. The percent inhibition by ifenprodil was calculated by comparing the baseline data with those collected 90 minutes after the onset of drug application. In our initial experiments, slices from dark-reared and light-reared animals were studied with the experimenter 'blind' to the rearing condi-

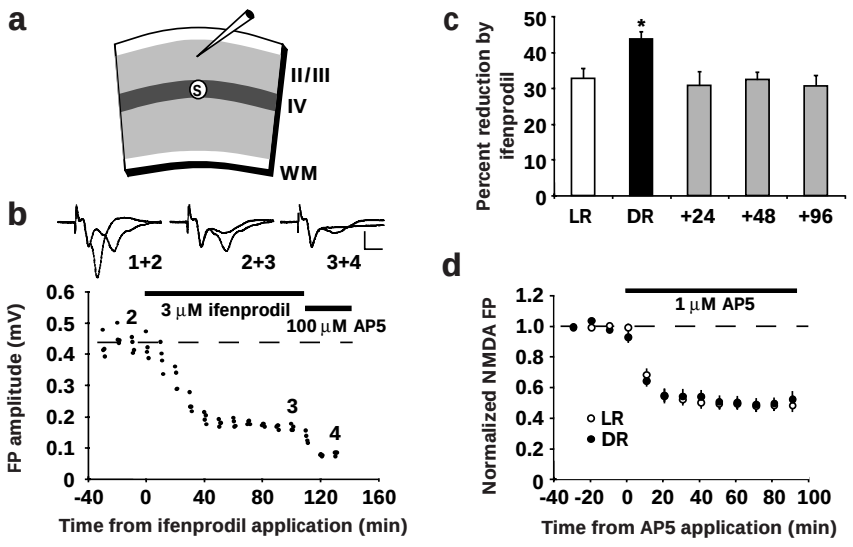

Fig. 2. The ifenprodil sensitivity of NMDAR-mediated field potentials in visual cortex is experience dependent. **(a)** The preparation. Slices of visual cortex were prepared from P21–28 rats, and synaptic field potentials were recorded in layers 2/3 in response to layer-4 stimulation. **(b)** Experimental design. NMDAR-mediated synaptic field potentials were isolated pharmacologically. To determine the contribution of NR2B-containing NMDARs to this response, we applied 3 μM ifenprodil. The remaining synaptic field potential was completely blocked by 100 μM AP5 (DL-2-amino-5-phosphonovaleric acid), confirming that the response reflects NMDAR-mediated currents. (The residual AP5-insensitive negativity is non-synaptic and is not affected by the rearing history of the animal.) This representative example was recorded from a slice taken from a P26 dark-reared rat. The field potentials shown are averages of four consecutive responses taken at the indicated times. (Trace 1 is the AMPAR-dominated field potential recorded before isolation of the NMDAR component.) Throughout the experiment, there was no change in the non-synaptic component of the evoked response. Scale bar, 0.2 mV and 5 ms. Dashed line, 30-minute average of the NMDAR-mediated field potential amplitude before ifenprodil application. **(c)** NMDAR-mediated responses in dark-reared rats are more sensitive to ifenprodil than light-reared controls, and the effect of dark rearing is reversed by 24, 48 or 96 h in a normal light cycle. Data are mean (± s.e.m.) reduction after 90-minute ifenprodil application relative to the 30-minute baseline average. *Significant difference versus all other groups in Tukey HSD post-hoc comparison, $p < 0.05$. **(d)** Partial blockade of NMDARs with AP5, which does not distinguish between NMDAR subtypes, reveals no difference between dark-reared and light-reared groups.

tions. This experiment revealed a significant increase in the ifenprodil sensitivity of NMDAR-mediated responses in dark-reared visual cortex (n = 12 slices from 7 rats) as compared to light-reared controls (n = 15 slices from 7 rats; t-test, $p < 0.05$). To confirm the selectivity of this effect, we repeated the experiment using a subsaturating concentration of AP5 (1 μM) that produces a partial block of NMDAR responses, but does not distinguish among the NMDAR subunits. Unlike ifenprodil, the AP5-sensitivity of NMDAR responses was comparable in the dark-reared (n = 7 slices from 4 rats) and light-reared (n = 8 slices from 4 rats) groups (t-test, p = 0.49). There were also no significant differences in the absolute magnitude of the AMPA-receptor-dominated or NMDAR-mediated field potentials between light-reared and dark-reared groups, or in the stimulation intensity required to elicit 80% of the maximal response.

To test whether visual experience could reverse the effects of dark rearing, we examined ifenprodil sensitivity in a second series of slices taken from animals that were light reared (n = 27 slices from 13 rats), dark reared (n = 26 slices from 13 rats, including those used for the 'blind' portion of the study) or dark reared and then exposed to 24 (n = 14 slices from 8 rats), 48 (n = 15 slices from 7 rats) or 96 hours (n = 10 slices from 5 rats) of the normal light cycle (Fig. 2). Exposing dark-reared rats to a regular light cycle was sufficient to restore the light-reared phenotype (one-way ANOVA, $F_{4,91} = 4.85$, $p = 0.001$). The data demonstrate that 24 or more hours of visual experience is sufficient to abolish the effect of dark rearing.

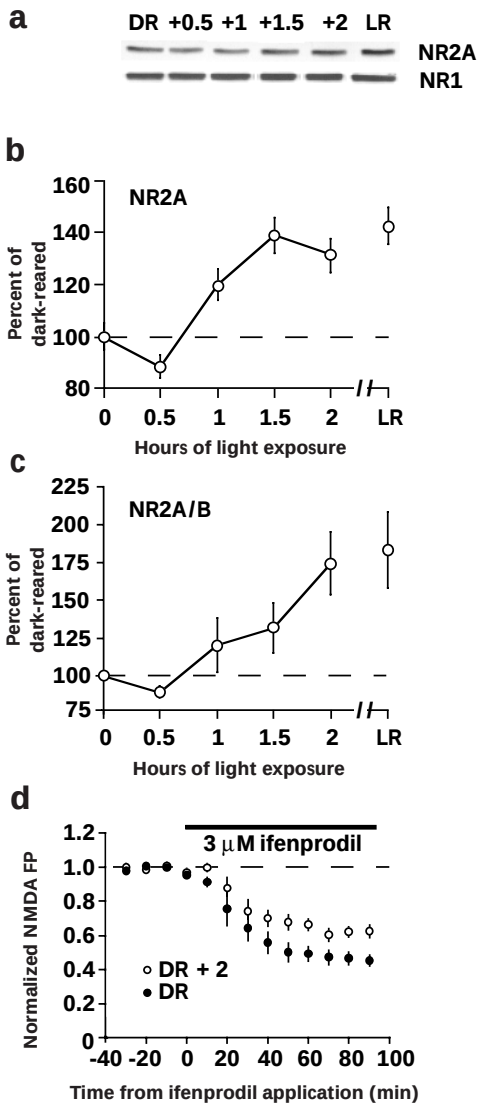

Fig. 3. Brief light exposure induces a rapid change in synaptic NMDAR composition and function in visual cortex. **(a)** Representative immunoblots for NR2A and NR1 proteins in synaptoneurosomes prepared from visual cortices of rats raised in complete darkness (DR), DR plus 0.5, 1, 1.5 or 2 h of light, or in a normal light cycle (LR). **(b)** Brief light exposure increased NR2A protein from synaptoneurosomes of visual cortex (one-way ANOVA, $p < 0.05$). Each sample is normalized to the dark-reared control run on the same gel (dashed line), and the summarized data are presented as percent of DR values (mean ± s.e.m.). **(c)** Brief light exposure increases NR2A/B from synaptoneurosomes of visual cortex (one-way ANOVA, $p < 0.05$). For each sample, the signal for NR2A/NR2B is calculated before normalizing to the dark-reared control run on the same gel (dashed line). Summarized data are presented as percent of DR values (mean ± s.e.m.). **(d)** Brief light exposure decreases the sensitivity of NMDAR potentials to ifenprodil. Normalized NMDAR-mediated field potentials in dark-reared rats (filled circles) or dark-reared rats exposed to light for two hours (DR + 2, open circles). Data are mean value averaged for the four pulses of probing stimulation. After two hours of light, NMDAR-mediated field potentials in visual cortex slices are less sensitive to ifenprodil than slices from dark-reared rats (t-test at 90 min after ifenprodil, $p < 0.01$). Error bars indicate s.e.m. Dashed line, normalized 30-minute baseline.

Both the biochemical and the electrophysiological results show that the synaptic NMDARs have a lower NR2A/B ratio in dark-reared visual cortex than in light-reared cortex. These findings were not entirely unexpected, as they are consistent with previous reports on the effect of light deprivation on the development of NMDAR response kinetics and ifenprodil sensitivity in the visual pathway[10,15]. However, it was a surprise that NMDARs in dark-reared cortex completely recovered the light-reared phenotype with one day of light exposure. Thus, our next step was to explore the minimum visual experience required to change NMDAR composition. Dark-reared animals were exposed to 0.5, 1, 1.5 or 2 hours of a normal lighted environment, and changes in NR2A, NR2B and NR1 protein were measured in visual cortical synaptoneurosomes. These experiments showed that light exposure for as little as one hour induced a significant increase in NR2A protein (Fig. 3a and b). Again, the levels of NR2B and NR1 were not significantly changed by light exposure. Thus, within two hours of light exposure, the NR2A/B ratio reached the light-reared value (Fig. 3c).

To address the question of whether the rapid change in synaptoneurosomal protein reflects an alteration in the composition of functional synaptic NMDARs, we examined the effects of brief light exposure on the ifenprodil sensitivity of NMDAR-mediated responses (Fig. 3d). Two hours of visual experience (n = 10 slices from 6 rats) was sufficient to produce a significant reduction in ifenprodil sensitivity compared to dark-reared controls (n = 6 slices from 5 rats; t-test, $p < 0.05$), consistent with a change in the NR2A/B ratio. Taken together, the data show that two hours of visual experience is sufficient to significantly alter the molecular composition (and therefore function) of synaptic NMDARs in visual cortex. To our knowledge, this is the first demonstration that sensory experience (versus deprivation) can induce a change in the complement of postsynaptic glutamate receptors *in vivo*.

Experience-dependent modifications of synaptic responses in visual cortex have been shown to require NMDAR activation[16,17]. We therefore were interested to know whether the experience-induced regulation of NMDAR composition was itself dependent on NMDAR activation in the visual pathway. To address this question, we injected dark-reared animals in the dark with the competitive NMDAR antagonist CPP (3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid, 10–15 mg per kg, i.p.) 30 minutes before exposing them to light for two hours. Subse-

quent electrophysiological analysis of the ifenprodil sensitivity of NMDAR-mediated responses in visual cortex revealed no significant differences between the CPP-injected, dark-reared group (n = 10 slices from 6 rats) and the CPP-injected group receiving light exposure (dark-reared +2; n = 13 slices from 8 rats; t-test, p = 0.47). Thus, CPP treatment completely prevented the experience-dependent modification of synaptic NMDARs (Fig. 4a). Immunoblot analysis confirmed that the light-induced increase in NR2A protein was also completely blocked in the visual cortex of CPP-injected animals (Fig. 4b; raw mean O.D. ± s.e.m., dark reared, 1010 ± 116; dark reared + 2 h light, 936 ± 120; one-tailed t-test, p = 0.34). Thus, NMDAR activity is necessary for the experience-dependent insertion of NR2A-containing synaptic NMDARs.

In a final series of experiments, we examined whether the experience-dependent increase in synaptic NR2A requires protein synthesis. Animals were injected in the dark with the mRNA translation inhibitor cycloheximide (1 mg per kg, i.p.) 30 minutes before receiving one hour of light exposure. This treatment completely blocked the experience-induced increase in NR2A protein (Fig. 4c; raw mean OD ± s.e.m., dark reared, 946 ± 46; dark reared + 1 h light, 941 ± 62; n = 4, one-tailed t-test, p = 0.50).

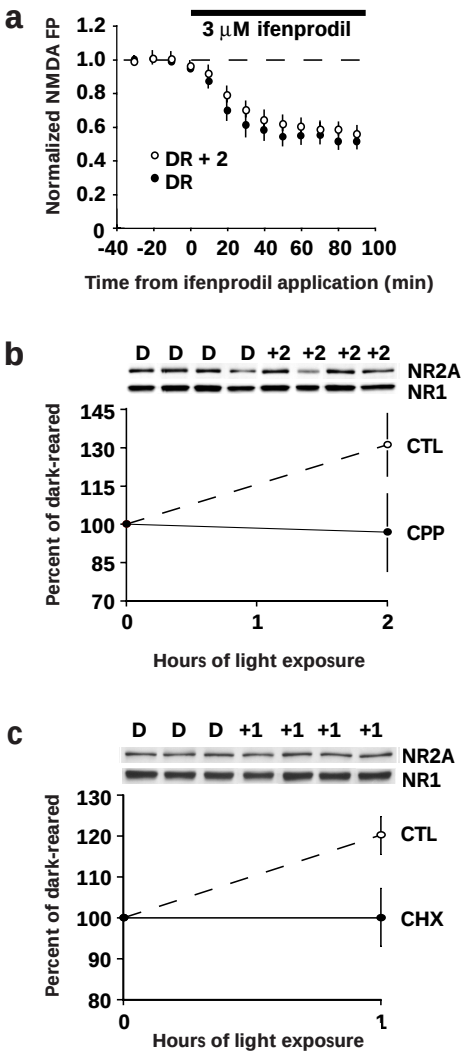

Fig. 4. Treatment of animals with CPP, a competitive antagonist of NMDARs, or cycloheximide, an inhibitor of mRNA translation, blocks the experience-induced increase in NR2A/B. **(a)** Effect of ifenprodil on the magnitude of normalized NMDAR-mediated field potentials in visual cortex slices taken from rats injected in the dark with CPP. Mean value averaged for the four pulses of probing stimulation in the DR (filled circles) and DR + 2 (open circles) groups. **(b)** Immunoblots of NR2A and NR1 proteins of synaptoneurosomes prepared from visual cortices of dark-reared rats treated with CPP (D) and from visual cortex of animals exposed to light for 2 h (+2) starting 30 min after CPP treatment. Summary data show that light exposure did not change the level of NR2A subunit following CPP treatment (solid line). For comparison, NR2A data from visual cortex in DR and DR + 2 without CPP (dashed line) are shown. Each sample was normalized to the mean dark-reared value, and the summarized data are presented as percent of DR values (mean ± s.e.m.). **(c)** Immunoblots of NR2A and NR1 proteins of synaptoneurosomes prepared from visual cortices of dark-reared rats treated with cycloheximide (D) and from visual cortex of animals exposed to light for 1 h (+1) starting 30 min after cycloheximide (CHX) treatment. Light exposure produced no change in the level of NR2A subunit following CHX treatment (solid line). For comparison, NR2A data from visual cortex in DR and DR + 1 without CHX (dashed line) are shown.

Discussion

Studies *in vitro* have suggested that some developmental changes in glutamate receptors are activity dependent. For example, growing neuronal cultures under conditions of heightened[18] or reduced[19] activity can cause a change in the surface expression of synaptic AMPA receptors. Likewise, NMDAR subunit expression[14,20] and clustering at postsynaptic sites[21] *in vitro* are regulated by presynaptic activity and postsynaptic NMDAR activation. However, these changes invariably have required days for their expression, too slow to account for rapid, activity-dependent synaptic modifications[22]. Thus, the finding that only one to two hours of visual experience can alter the complement of postsynaptic glutamate receptors in visual cortex *in vivo* is of considerable interest. Such a change is fast enough to contribute to the rapid synaptic modifications that have been reported in the visual cortex of dark-reared animals exposed to light[23–25].

A model consistent with the results of our study is that sensory experience regulates, via NMDAR activation, the synthesis and postsynaptic surface expression of NR2A-containing NMDARs in the visual cortex *in vivo*. This proposed mechanism must be viewed as tentative because our manipulations of NMDARs and protein synthesis were systemic, and might have exerted their effects at sites other than the visual cortex. However, the idea that NMDAR subunit expression can be controlled by NMDAR activity receives strong support from studies using cultured cerebellar slices. In this preparation, postsynaptic NMDAR activation (for several days and coupled with neuregulin) drives expression of the NR2C subunit, which, in turn, regulates the phenotype of NMDARs in granule cells[20] . There is also a precedent for experience-dependent regulation of mRNA translation in the visual cortex. Specifically, brief visual experience stimulates the rapid polyadenylation and translation of α-CaMKII mRNA[26]. The localization of NR2A and NR2B mRNA to neurites of cultured neurons[27] raises the intriguing possibility that experience-dependent regulation of NR2A synthesis could occur in dendrites[28] in response to synaptic activation.

What are the functional consequences of changing the subunit composition of NMDARs in visual cortex? In visual cortex, as elsewhere, the amount of calcium passing through activated NMDARs can determine whether a synapse undergoes long-term potentiation (LTP) or long-term depression (LTD)[29]. The experience-dependent increase in the NR2A/B ratio and the concomitant shortening of synaptic NMDAR currents are likely to have a significant impact on the properties of synaptic plasticity. Shortening NMDAR currents would be expected to alter the LTD-LTP 'modification threshold' (θ_m), making LTD more likely and LTP less likely in response to a given amount of synaptic activation[30]. Indeed, studies of LTD and LTP in visual cortex reveal precisely this change in dark-reared animals exposed to

light[31]. Theoretical investigations suggest that these activity-dependent adjustments of θ_m are crucially involved in cortical development by maintaining the network of modifiable synapses within a useful dynamic range[32]. We suggest that one molecular mechanism for such experience-dependent modifications of synaptic plasticity in visual cortex is the regulation of NMDAR subunit composition.

METHODS

Immunoblot analysis. Male and female P21–23 Long-Evans rats (Charles River) were anesthetized with methoxyflurane vapor either in the dark (dark-reared) or the light (light-reared and light-exposure groups) and were decapitated following disappearance of corneal reflexes in compliance with the U.S. Department of Health and Human Services and Brown University guidelines. Light exposure was begun 5–7 h into the light cycle. Synaptoneurosomes were prepared using a procedure adapted from ref. 11. The primary visual cortex was rapidly dissected in ice-cold dissection buffer (212.7 mM sucrose, 2.6 mM KCl, 1.23 mM NaH_2PO4, 26 mM $NaHCO_3$, 10 mM dextrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.02 mM CNQX and 0.1 mM AP5, saturated with 95% O_2 and 5% CO_2) and immediately homogenized in ice-cold homogenization buffer (10 mM HEPES, 1 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 0.1 mM PMSF, 10 mg per liter leupeptin, 50 mg per liter soybean trypsin inhibitor and 100 nM microcystin). Tissue was homogenized in a glass–glass tissue homogenizer (Kontes, Vineland, New Jersey), and the homogenate was passed sequentially through two 100-μm-pore nylon mesh filters, followed by a 5-μm-pore filter, and centrifuged at 1000 × g for 10 min. The resulting pellets were resuspended in boiling 1% SDS and stored at –80°C. Equal amounts of synaptoneurosome protein, determined using the BCA assay (Pierce, Rockford, Illinois), were resolved on 7.5% polyacrylamide gels, transferred to nitrocellulose and probed with either anti-NR2A or anti-NR2B polyclonal antibodies (1:1000; ref. 6) or anti-NR1 monoclonal antibody (1:1000, clone 54.1, Pharmingen, San Diego, California), followed by the appropriate secondary antibody coupled to horseradish peroxidase (1:3500, Sigma, St. Louis, Missouri) in Tris-buffered saline, pH 7.3, containing 1% bovine serum albumin and 0.1% Triton X-100 (Sigma). Visualization of immunoreactive bands was produced by enhanced chemiluminescence (Amersham ECL) captured on autoradiography film (Amersham Hyper ECL). Digital images produced by densitometric scans of autoradiographs on a ScanJet IIcx (Hewlett Packard) with DeskScan II software (Hewlett Packard) were quantified using NIH Image 1.60 software. The intensity of each band was determined relative to a baseline immediately above and below the band within the same lane, and normalized to light-reared or dark-reared controls run on the same gel.

Electrophysiology. Slice electrophysiology experiments were done as described[33]. Briefly, rats were deeply anesthetized with inhalation anesthetic methoxyflurane and decapitated. The brain was removed, dissected and sliced in dissection buffer as described above, with the exceptions that the buffer contained 3 mM $MgCl_2$, 1 mM $CaCl_2$ and 5–10 mM kynurenic acid instead of CNQX and AP5. Slices were allowed to recover for 1–2 h at room temperature in artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 5 mM KCl, 1.25 mM Na_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 10 mM dextrose, saturated in 95% O_2, 5% CO_2. For recording, slices were placed in a submersion recording chamber, maintained at 30°C and perfused with ACSF at a rate of 2 ml per min. Extracellular electrodes (filled with ACSF; 1.0 MΩ) were used to monitor field potentials evoked with a stimulating electrode (concentric bipolar tungsten). The magnitude of responses was monitored by the amplitude of the field potential. Stable baseline responses were elicited two per min at 80% of maximal response. NMDAR-mediated responses were pharmacologically isolated in artificial cerebrospinal fluid (ACSF) containing 3 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.1% DMSO, 20 μM CNQX, 1 μM glycine and 0.5 μM bicuculline methiodide. Probing stimulation consisting of 4 pulses delivered at 30-second intervals was given every 10 min to assess NMDAR-mediated field potentials. Because of studies demonstrating inhibition of NR2A-containing NMDARs by zinc[34], we did pilot studies to demonstrate that there was no tonic inhibition of NMDARs by zinc in our slice preparation (data not shown). NMDAR blockade was achieved by the bath application of 100 μM DL-2-amino-5-phosphonovaleric acid (AP5; Sigma). Ifenprodil (3 μM; RBI) was used to block NR2B-containing receptors. This concentration of ifenprodil is in a range that produces nearly maximal inhibition of NR2B-containing NMDARs, has little effect on NR2A-containing NMDARs and does not affect voltage-dependent calcium channels[12–14]. Dose–response curves done for this study also determined that this concentration is specific to antagonism of the NMDAR (data not shown). As noted previously[15], 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, we compared the 30-minute average immediately before adding the drug with the average of 4 consecutive sweeps collected 90 minutes after drug application, when the ifenprodil effect had completely equilibrated. A similar difference between light-reared and dark-reared cortex was found using another NR2B-selective antagonist, CP-101,606-27 (5 μM, Pfizer; data not shown).

Drug injections. To block NMDA receptors or protein synthesis, we injected animals in the dark (i.p.) with either CPP or CHX. CPP-injected animals were awake and alert. However, a fraction of the animals displayed slightly reduced locomotor activity. CHX-injected animals were awake and alert, and displayed no overt illness or distress 1.5 h after injection. However, we did note in pilot studies that animals were visibly sluggish at 2.5 h after the injection. Therefore, we restricted analysis to the earlier time point. Synaptic transmission and neuronal excitability remain normal for many hours following the blockade of protein synthesis with CHX[35].

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