The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex

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Bidirectional synaptic plasticity during development ensures that appropriate synapses in the brain are strengthened and maintained while inappropriate connections are weakened and eliminated. This plasticity is well illustrated in mouse visual cortex, where monocular deprivation during early postnatal development leads to a rapid depression of inputs from the deprived eye and a delayed strengthening of inputs from the non-deprived eye. The mechanisms that control these bidirectional synaptic modifications remain controversial. Here we demonstrate, both in vitro and in vivo, that genetic deletion or reduction of the NR2A NMDA receptor subunit impairs activity-dependent weakening of synapses and enhances the strengthening of synapses. Although brief monocular deprivation in juvenile WT mice normally causes a profound depression of the deprived-eye response without a change in the non-deprived eye response, NR2A-knockout mice fail to exhibit deprivation-induced depression and instead exhibit precocious potentiation of the non-deprived eye inputs. These data support the hypothesis that a reduction in the NR2A/B ratio during monocular deprivation is permissive for the compensatory potentiation of non-deprived inputs.

The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex

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Bidirectional synaptic plasticity has been studied in slice recordings of visual cortex in the form of long-term potentiation (LTP) and long-term depression (LTD), whereby synapses strengthen and weaken in response to stimulation (6). These activity-dependent modifications can be modeled by a learning rule whereby high levels of post-synaptic activation (evoked electrically by high-frequency stimulation) induce LTP and smaller levels of post-synaptic activation (evoked electrically by lower-frequency stimulation) induce LTD (7). The crossover point from synaptic weakening to strengthening is called the modification threshold ($\theta_m$). An important feature of this model is that the value of $\theta_m$ is not fixed; rather, its value can “slide” as a function of the history of post-synaptic activation. According to the BCM theory, closing the dominant contralateral eye first leads to depression of the deprived synapses, followed by a leftward shift in $\theta_m$ caused by the reduction in average cortical activity. This shift in $\theta_m$ is permissive for the subsequent increase in the responses to the non-deprived, ipsilateral eye (5).

A wealth of data now indicate that deprivation and experience during early postnatal development can indeed modify the plasticity threshold. For example, a period of complete darkness lowers the plasticity threshold such that LTP is enhanced and LTD is attenuated across a range of stimulation frequencies (8–10). These observations demonstrate that the susceptibility of synapses to plastic changes in visual cortex modifies in relation to their history of experience-driven activity.

Data suggest that the shift in the $\theta_m$ is caused by a change in NMDA receptor (NMDAR) function (9), and regulation of the molecular composition of the NMDA receptor provides a powerful means to achieve this change. The NMDA receptor is a heteromer that contains the obligatory NR1 subunit and a mixture of NR2A-D subunits that alter receptor properties (11, 12). At birth, most cortical NMDARs contain the NR2B subunit (11). NR2A subunit levels gradually increase with development and reach a maximal expression between the peak and end of juvenile plasticity (13, 14). This switch from predominantly NR2B to NR2A subtypes is experience-dependent and reflects the recent history of visual experience (15–17). During MD, after the initial depression of deprived-eye responses, there is a transient reduction in the NR2A/B ratio that slightly precedes open-eye response potentiation (18). Because lowering the NR2A/B ratio reduces the threshold for inducing LTP in mouse visual cortex (10), it has been proposed that activity-dependent regulation of NR2A and/or NR2B receptor expression is the molecular basis for the “sliding” $\theta_m$.

In the current study we examined the connection between NMDAR subunit composition and the qualities of bidirectional synaptic plasticity in the visual cortex of NR2A KO, heterozygote (Het), and WT mice. We confirm in layer IV that reducing NR2A expression shifts to lower frequencies both the LTP threshold and the optimal stimulation for LTD. In response to MD, visually evoked potentials (VEPs) evoked in vivo through the deprived eye fail to depress normally in NR2A mutants. Instead, an ocular dominance shift occurs by precocious potentiation of responses through the non-deprived eye. These data support the hypothesis that experience-dependent modifications...
in the NR2A/B ratio at synapses provides a powerful in vivo mechanism for regulating subsequent induction of plasticity.

Results

Effect of NR2A Gene Dosage on the Synaptic \( \theta_m \) in Layer IV of Mouse Visual Cortex. The goal of this study was to determine how decreasing the NR2A/B ratio alters the LTD/LTP \( \theta_m \) in vitro and compare this with changes in the properties of naturally occurring plasticity in the visual cortex in vivo as a consequence of MD. We examined this question using mice with targeted disruption of one or both alleles of the NR2A gene (19). Because NR2A mutant mice do not display compensatory alterations in NR1 or NR2B subunit expression in visual cortex at the ages of interest, reducing NR2A expression effectively changes the NR2A/B ratio (10).

The bidirectional changes in visual responsiveness that occur after MD were established using VEPs recorded in layer IV of visual cortex (5). Current source density (CSD) analysis in vivo has confirmed that changes in the amplitude of layer IV VEPs reflect changes in synaptic current sinks in this layer (20, 21). However, previous studies of LTD and LTD in NR2A mutant mice were performed in layer III (10), and it is now understood that there are significant laminar differences in the mechanisms of visual cortical plasticity in mice (21, 22). Therefore, our study began with an analysis of the effect of NR2A gene dosage on synaptic plasticity in layer IV of slices of mouse visual cortex. A CSD analysis confirmed that the negative extracellular field potential (FP) evoked in layer IV of mouse visual cortex by white matter (WM) stimulation in vitro reflects a current sink qualitatively similar to the VEP in vivo [supporting information (SI) Fig. S1]. Therefore, we attempted to replicate, in layer IV FPs, the effects previously described in layer III of reducing NR2A on the LTD/LTP threshold (10).

To test whether the plasticity threshold was altered by reducing NR2A gene dosage, we examined the consequences of a stimulation protocol (1 Hz for 15 min) in KO and Het mutants that typically result in LTD in normally reared WT mice (aged between postnatal days 21 and 28). Following collection of a baseline, 1 Hz stimulation produced reliable depression in WT mouse (Fig. 1A; 83.03% ± 2.56% of baseline, \( n = 8 \) slices from 7 mice). However, as previously shown in layer III of the KO mouse, we discovered that 1 Hz stimulation causes LTP of layer IV FP amplitudes in mice lacking NR2A (Fig. 1A; 111.39% ± 2.33% of baseline, \( n = 9 \) slices from 7 mice). Moreover, in the NR2A Het mice, 1 Hz stimulation resulted in a modest depression of synapses (Fig. 1A; 93.77% ± 5.33% of baseline, \( n = 9 \) slices from 7 mice) that was intermediate between the WT (\( P = 0.034 \)) and NR2A KO (\( P = 0.001 \)) values. Importantly, basal synaptic transmission was comparable between genotypes (Fig. 1A), and there was no correlation between baseline FP amplitude and the percent change in synaptic transmission following 1 Hz stimulation (Fig. 1A). There was also no effect of genotype on AMPA receptor-mediated miniature excitatory post-synaptic currents (mEPSCs) in layer IV neurons (NR2A KO, 18.58 pA ± 1.76, \( n = 10 \) neurons from 3 mice; WT, 18.90 pA ± 1.30, \( n = 13 \) neurons from 3 mice). Together, these results support previous conclusions that the LTD/LTP threshold is proportional to the level of NR2A expression in mouse visual cortex (10).

To confirm that the plasticity observed in the NR2A mutants was still NMDAR-dependent, we repeated the experiment in the presence of the competitive NMDAR antagonist D-2-amino-5-phosphonopentoic acid (APV; 50 \( \mu M \)). In addition to the expected effect of APV on LTD in WT mice (\( n = 5 \) slices from 3 mice) (22), we found that blocking NMDARs prevented both the residual LTD in the Het mice (\( n = 5 \) slices from 3 mice) and the LTP induced by 1 Hz stimulation in the KO animals (\( n = 5 \) slices from 3 mice; Fig. 1D).

Finally, to confirm that reducing NR2A caused a change in the induction requirements for LTD/P rather than a dose-dependent loss of LTD, we repeated the experiment using 0.5 Hz stimulation—a frequency that was shown previously to be optimal for LTD induction in visually deprived animals (9). We found that 900 pulses at 0.5 Hz elicited reliable and statistically significant depression in mice of all genotypes, with the greatest effect in the NR2A KO mice (Fig. 1; NR2A KO, 70.56% ± 6.41% of baseline, \( n = 10 \) slices from 5 mice; Het, 79.05% ± 6.86%, \( n = 6 \) slices from 3 mice; WT, 84.43% ± 5.64%, \( n = 6 \) slices from 3 mice). Taken together, these data lead us to conclude that lowering the NR2A/B ratio shifts the stimulation-response curve to the left, and the degree of this shift is proportional to the amount of NR2A present in visual cortex.

Effect of NR2A Gene Dosage on the Ocular Dominance Shift Following MD in Layer IV of Mouse Visual Cortex. We next examined the impact of altered NR2A and synaptic plasticity on ocular dominance plasticity in layer IV of mouse visual cortex. Electrodos were chronically implanted in layer IV of the binocular zone in primary visual cortex. Baseline VEPs were measured at postnatal days 27 to 29, and the eyelid of the eye contralateral to the experimental hemisphere was sutured closed. After 3 days of MD, the sutured eye was opened, the animal was allowed to recover from anesthesia, and VEPs were again recorded. We assessed ocular dominance plasticity by determining the ratio of contralateral to ipsilateral eye responses (C/I ratio), which is normally approximately 2:1 at baseline and decreases after MD to approximately 1:1. Our results show that NR2A KO and Het mice, as well as their WT litter-mates, exhibit a normal shift in the C/I ratio (Fig. 24; day 0, 1.67 ± 0.21; day 3, 0.89 ± 0.13 in KO, \( n = 8 \), \( P = 0.01 \); day 0, 1.75 ± 0.09; day 3, 1.05 ± 0.14 in Het, \( n = 9 \), \( P < 0.01 \); day 0, 1.95 ± 0.22; day 3, 0.90 ± 0.13 in WT, \( n = 10 \), \( P < 0.001 \)), similar to what has been reported previously (23). The degree of the shift is indistinguishable among the 3 genotypes (Kruskall-Wallis test, \( n = 27 \); \( P = 0.81 \)). However, upon closer examination of the deprived and non-deprived eye responses, we discovered profound differences in

![Fig. 1. Loss of NR2A lowers the LTP threshold and the optimal LTD stimulation frequency. (A) Averaged data (±SEM) demonstrate that 1 Hz stimulation (900 pulses) induces LTD in WT and Het mice and LTP in NR2A KO mice. (A) Baseline waveforms averaged across all individual experiments summarized in (A), (WT = 8; Het = 9; KO = 7). Note that averaged waveforms are of comparable size and shape in all 3 genotypes. (A) Scatter plot of individual baseline FP amplitudes of each genotype and percent change of synaptic transmission following 1 Hz stimulation. Note that the effect of 1 Hz stimulation is not correlated with initial response amplitude. (B) Averaged data demonstrating that bath-applied APV prevents the effects of 1 Hz stimulation in all genotypes. (C) Averaged data demonstrate that stimulation at 0.5 Hz (900 pulses) yields LTD in all genotypes, with maximal effect in NR2A KO mice.](pnas.0808104106.F1)
the qualities of the ocular dominance shift between the genotypes (Fig. 2B). As previously reported (5), we found that deprived-eye responses in WT mice were significantly depressed (Fig. 2B; day 0, 211.2 ± 17.1 μV; day 3, 127.6 ± 30.5 μV, n = 10, P = 0.002), and non-deprived eye responses remained at baseline levels (day 0, 114.5 ± 13.2 μV; day 3, 151.9 ± 26.4 μV, n = 10, P = 0.07). In stark contrast to WT mice, the deprived-eye responses in NR2A KO mice were unchanged (Fig. 2B; day 0, 185.2 ± 19.7 μV; day 3, 202.6 ± 21.4 μV, n = 8, P = 0.55), whereas the non-deprived eye responses dramatically potentiated (day 0, 118.5 ± 11.6 μV; day 3, 247.9 ± 34.3 μV, n = 11, P = 0.005). Results in the Het mice were intermediate: there was still a significant depression of the deprived eye (Fig. 2B; day 0, 221.3 ± 14.7 μV; day 3, 180.0 ± 26.5 μV, n = 9, P = 0.04) and a slight but statistically significant potentiation of the non-deprived eye responses (day 0, 129.1 ± 10.4 μV; day 3, 175.2 ± 14.9 μV, n = 9, P = 0.03).

These findings are consistent with the idea that reducing the NR2A/B ratio promotes the depression-induced adjustment of the BCM $\theta_m$ and thereby enhances open-eye response potentiation and reduces deprived-eye response depression in vivo, similar to what we observed in the slice experiments. However, an alternative explanation is that the shift occurs normally, but

is superimposed on an exaggerated global upward scaling of responses caused by visual deprivation. To investigate the possibility of enhanced synaptic scaling in response to deprivation, we recorded VEPs before and after 3 days of binocular lid suture in NR2A KO and WT litter-mates. An increased homeostatic scaling response should lead to substantially increased visual responses after binocular deprivation (BD).

Our results show that the C/I ratios of both NR2A KO and WT mice do not change following this visual manipulation (Fig. 3A; day 0, 2.16 ± 0.19; day 3, 1.92 ± 0.20 in KO, n = 7; day 0, 2.34 ± 0.15; day 3, 2.57 ± 0.43 in WT, n = 7; P = 0.31). More importantly, BD did not affect the VEP amplitudes of contralaterally projecting eyes (Fig. 3B; day 0, 228.4 ± 16.2 μV; day 3, 232.0 ± 35.5 μV in KO, n = 7; day 0, 236.5 ± 35.7 μV; day 3, 220.8 ± 35.1 μV in WT, n = 7; P = 0.72) nor the VEP amplitudes of ipsilaterally projecting eyes (Fig. 3B; day 0, 111.9 ± 11.5 μV; day 3, 121.6 ± 12.0 μV in KO, n = 7; day 0, 99.2 ± 12.2 μV; day 3, 87.0 ± 6.8 μV in WT, n = 7; P = 0.26). These data indicate that reduction of NR2A does not promote synaptic scaling in response to 3 days of visual deprivation.

Open-Eye Potentiation in WT Mice Requires NMDAR Activation. In WT mice, MD for >5 days causes potentiation of visual responses that we hypothesize is enabled by a deprivation-induced decrease in the NR2A/B ratio (18). This hypothesis rests on the assumption that response potentiation is an NMDAR-dependent form of Hebbian synaptic plasticity (24, 25). The alternative hypothesis is that responses increase by global upward scaling (26), a process that has been shown to be independent of NMDAR activation (27). To distinguish among these hypotheses, we designed experiments in which NMDARs were blocked pharmacologically during the time span when response potentiation occurs (Fig. 4A).

Following 3 days of MD, which allowed for deprived-eye depression, either (R, S)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP; 10 mg/kg) or saline solution were injected i.p. every 6 h over the course of 4 additional days of MD. The saline solution controls showed a normal response to 7 days of MD. First, deprived-eye responses were significantly depressed.
that the NR2A/B ratio specifies the value of the synaptic responses instead. Our results are consistent with the hypothesis normally have no effect cause precocious potentiation of re-

patterns of synaptic activity through the open eye that normally cause depression of

cortex. Reduced NR2A expression shifts the LTD/LTP thresh-
dependent bidirectional synaptic plasticity in layer IV of visual
subunit can dramatically alter the qualities of NMDAR-

Our data show that even a graded reduction of the NR2A
blockade of response potentiation with an NMDAR antagonist

NR2A/B ratio is significantly reduced after 5 days of MD (18).
NR2A and NR2B expression also occur during the course of
partially experience-dependent, as even brief episodes of visual

time of adolescence. This developmental profile is at least
how the subunit composition of NMDARs varies during the
developmental loss of NMDAR-dependent LTD observed in many
regions of the brain. However, the validity of these findings is
now being questioned because these studies were conducted
using non-specific concentrations of NR2A-selective antagonists
(34). Moreover, recent data contradict the initial findings that
NR2A and NR2B play distinct roles in regulating the polarity of
synchronic plasticity (35–39). Finally, accumulating evidence (10,
33, 36, 40), including findings from the present study, demon-
strate that LTP can be induced in NR2A KO mice, suggesting
that a synaptic requirement of NR2A for LTP is overly simplistic.
The current findings best fit the theory that NMDAR subunit
composition regulates a sliding threshold for bidirectional syn-
aptic plasticity (7, 31). As previously demonstrated in layer III
(10), we find in layer IV that reducing NR2A expression shifts the
optimal LTD stimulation frequency leftward and enables LTP at low stimulation frequencies. It has been suggested
previously that the decrease in NR2A/B protein that normally occurs between 3 and 5 days of MD enables the potentiation of the non-deprived eye by shifting the $\theta_m$ to the left (18). Our

The current findings are consistent with this theory. However, rather than setting the threshold per se, reducing NR2A appears to remove a constraint on how fast it can adjust, so that 3 days of contralateral-eye MD is sufficient to cause potentiation of the ipsilateral eye responses. Additional mechanisms for adjusting the threshold independently of NR2A could include regulation of NR2B (18) and/or the total number of NMDARs at the synapse (10), among other possibilities (41, 42).

The current data are relevant to the recent debate over whether the compensatory potentiation of the non-deprived eye after MD reflects a process analogous to input-specific LTP enabled by metaplastic adjustment of the $\theta_m$ (5, 20), or a cell-wide process of homeostatic synaptic scaling (26, 43). Scaling is a phenomenon that does not require NMDAR activation (27), so the OD plasticity phenotype in the NR2A mutant mice is unlikely to result from altered scaling. Moreover, consistent with findings in adult mice (20, 44), we find that the response potentiation caused by 7 days of MD in juvenile mice requires NMDAR activation. Therefore, the current findings implicate metaplasticity rather than scaling as the mechanism for deprivation-induced response potentiation, at least in layer IV.

In conclusion, our data support the hypothesis that the experience-dependent regulation of the NR2A/B ratio is critical for adjusting the threshold for synaptic modifications, both in vitro and in vivo. These data suggest that lowering the NR2A/B ratio might provide a permissive milieu for strengthening weak
cortical inputs. An exciting possibility is that manipulation of this ratio, either experimentally or pharmacologically, could be exploited therapeutically to promote synaptic rewiring after brain injury or disease.

Materials and Methods

Subjects. Mice deficient in NR2A were supplied by S. Nakanishi (Kyoto, Japan). The mice were developed by replacing the region spanning the M2 transmembrane segment of NR2A subunits with the neomycin resistance gene as previously described (19). A pathogen-free line was re-derived on a C57BL/6 background by Charles River Laboratories. WT (+/+), heterozygotes (+/-), and NR2A-KO (-/-) mice were used between postnatal days 21 and 28 for in vitro experiments and between postnatal days 24 and 36 for in vivo experiments. Subjects were fed ad libitum and reared in normal lighting conditions (12 h/12 h light/dark cycle). There was no significant difference in AMPA receptor-mediated responses across genotypes, as evidenced by the facts that (i) the baseline VEP amplitudes were not different (Figs. 2B and 3B), (ii) the baseline FPs evoked in layer IV by WM stimulation were not different (Fig. 1A), and (iii) the stimulation intensities required to evoke a half-maximal FPs was not different. Whole-cell recordings of AMPA/NMDAR ratios in layer IV cells revealed no difference between KO and WT, suggesting a normal level of NMDAR expression at these ages (data not shown). As described previously, changes in synaptic strength were measured by comparing the synaptic current amplitudes elicited by stimuli of similar size, in the presence and absence of AMPA receptor block (20).

Cortical Slice Preparation. Following an overdose of barbiturates (+/−), mice were decapitated upon disappearance of corneal reflexes in compliance with the US Department of Health and Human Services. The brain was rapidly removed and immersed in ice-cold dissection buffer (composition in mM, NaCl, 87; KCl, 2.5; NaH2PO4, 1.25; NaHCO3, 25; sucrose, 75; dextrose, 10; ascorbic acid, 1.3; MgCl2, 7; and CaCl2, 0.5) bubbled with 95% O2 and 5% CO2. The visual cortex was rapidly removed and 350-μm coronal slices were cut using a vibrating microtome (model VT1000S, Leica). Slices recovered for 15 min in a submersion chamber at 32 °C filled with warmed artificial cerebral spinal fluid (ACSF: 124 mM NaCl, 5 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 1 mM MgCl2, 2 mM CaCl2, and 10 mM dextrose, saturated with 95% O2 and 5% CO2) and then cooled gradually to room temperature until use.

Extracellular Electrophysiology. Slices were transferred to an interface recording chamber maintained at 30 °C and perfused with ACSF at a rate of 2.5 mL/min. A stimulation electrode (concentric bipolar tungsten) was positioned in layer 6/VWM, and a glass recording electrode (−1 MΩ) filled with ACSF was positioned in layer IV. The magnitude of responses evoked by a 200-μs pulse was monitored by the amplitude of the FP. Stimulation intensity was adjusted to elicit half the maximal response, and stable baseline responses were elicited every 30 s. The resulting signals were filtered between 0.1 Hz and 3 kHz, digitized at 10 kHz on an IBM-compatible computer using pCLAMP 9.2 software (Molecular Devices). After achieving a stable baseline (<5% drift) for 15 min, slices were stimulated with 900 pulses at 1 Hz or with 900 pulses at 0.5 Hz. FP amplitudes were recorded every 30 s for 45 min following the cessation of the stimulation protocol. The concentration used for bath application of D-APV was 50 μM. Control and experimental subjects were run in an interleaved fashion. Objective criteria (baseline drifts ≤5% and proper waveform alignment) were applied as inclusion criteria for further analysis. The data were normalized, averaged, and reported as mean ± SEM. Changes in synaptic strength were measured by comparing the average response amplitude 35 to 45 min after conditioning stimulation to the preconditioning baseline response.

CSD Analysis. CSD analysis was performed to determine the spatio-temporal pattern of current sinks and sources evoked in layer IV by bipolar stimulation at the layer VI/VWM boundary of primary visual cortex. The glass recording electrode (~1 MΩ) filled with ACSF was tracked down through the layers in 100-μm steps. At each recording depth, ten 200-μsec pulses were delivered by bipolar stimulation (Isolated Pulse Stimulator model 2100; A-M Systems) and the responses were averaged. At the completion of the recording session, the recording electrode was lifted along the z-plane and its tip immersed in Fluoro Willie polystyrene microspheres and returned to its recording site to verify layer IV localization. The section was then mounted on gelatin-coated slides and fluorescently stained for Nissl substance (Neurotrace; Molecular Probes).

From the FPs collected, the corresponding one-dimensional (i.e., depth) CSD profile was constructed according to the method described by Mitzdorf (45) by using a spatial differentiation grid of 200 μm. A full account of the theoretical basis of CSD analysis has previously been presented (45, 46).

mEPSC Recordings. Slices were maintained in ACSF containing (in mM) 142 NaCl, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 1 MgCl2, 2 CaCl2, and 20 K-glucose, saturated with 95% O2 and 5% CO2 (315 mOsm, pH 7.25). Recording electrodes were filled with internal containing (in mM) 20 KCl, 100 K gluconate, 10 MgCl2, 5 dithiothreitol, 20 spermidine, 200 K-ASCA, and 10 EGTA, and pH, adjusted to 7.25 and osmolarity adjusted to 300 mOsm. AMPA receptor-mediated mEPSCs were recorded in the presence of blockers for voltage-gated sodium channels (tetrodotoxin; 200 nM), GABA receptors (bicuculline; 50 μM), and NMDARs (D,L-AP5; 100 μM). The internal recording solution contained 1 μM MK801 and mEPSCs were recorded at negative holding potentials (~80 mV). Events were first identified using an automatic template detection program (pCLAMP; Molecular Devices) and then manually verified so that only events with a monotonic rise time and exponential decay were included in the analysis. More than 100 events were analyzed for each data point for each cell.

In Vivo Electrophysiology. VEP recordings were conducted in awake mice as described previously (5). Mice were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine i.p. and placed dorsal side up on a warm stage. After a 10-min stabilization period, mice were placed in a stereotaxic head frame and anesthesia was maintained by inhalation of isoflurane (2.5% isoflurane) for 15 min followed by 1 μL of 1% lidocaine; 1 μL lidocaine was delivered into the eye. Mice were then returned to the stereotaxic head frame for recording. VEPs were evoked by either horizontal or vertical stimuli and recorded at 0.05 cycles/degree. VEPs were evoked by either horizontal or vertical stimuli. As described previously, stimuli of orthogonal orientations were presented before and after MD to avoid the phenomenon of stimulus-selective response potentiation (5, 48).

Visual stimuli consisted of full-field sine-wave gratings of 0% and 100% contrast, square reversing at 1 Hz, and presented at 0.05 cycles/degree. VEPs were evoked by either horizontal or vertical stimuli. As described previously, stimuli of orthogonal orientations were presented before and after MD to avoid the phenomenon of stimulus-selective response potentiation (5, 48).

Visual stimuli were presented to both eyes randomly. A total of 100 stimuli were used for each condition. VEP amplitude was quantified by measuring trough-to-peak response amplitude, as described previously (20).

Statistics. Global ANOVAs with a repeated measures factor were run with post-hoc analyses (Fisher protected least significant difference) to test for statistical significance among multiple groups. Data are expressed as mean ± SEM, and significance was placed at P < 0.05.

Drugs. Unless otherwise noted, drugs were purchased from Sigma.

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