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Instructive Effect of Visual Experience in Mouse Visual Cortex

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Summary

We describe a form of experience-dependent response enhancement in the visual cortex of awake mice. Repeated presentations of grating stimuli of a single orientation result in a persistent enhancement of responses evoked by the test stimulus. Response potentiation is specific to the orientation of the test stimulus, develops gradually over the course of several training sessions, and occurs in both juvenile and adult mice. The stimulus-selective response potentiation (SRP) can mask deprivation-induced response depression in adult mice. SRP requires NMDA receptor activation and is prevented by viral delivery of a peptide that interferes with AMPA receptor trafficking. SRP may reveal the mechanisms involved in certain forms of perceptual learning.

Introduction

Under appropriate behavioral conditions, repeated exposure to specific sensory stimuli can persistently modify the brain to improve perception of these stimuli. Understanding this type of perceptual learning is important because it can reveal mechanisms of implicit memory formation, and it might be exploited to promote rehabilitation after brain damage (Karni and Bertini, 1997). Evidence has accumulated over the past 15 years that the neural substrate for perceptual learning can include plasticity of responses in primary sensory cortex. For example, Karni and Sagi (1991) originally reported human perceptual learning with characteristics strongly suggesting localization to primary visual cortex, and this conclusion is now supported by functional imaging of human V1 (Furmanski et al., 2004). Although many properties of this type of experience-dependent plasticity in primary visual cortex (e.g., the requirements for attention and a consolidation period) have been deduced by elegant experiments in primates and humans, the fundamental neural mechanisms remain largely unknown (Ghose, 2004; Gilbert et al., 2001).

Here we describe a robust phenomenon in the primary visual cortex of the mouse—called stimulus-selective response potentiation (SRP)—that should be useful for the mechanistic dissection of cortical plasticity resulting from repeated exposure to sensory stimuli. The discovery of SRP was a fortuitous outcome of an effort to establish a chronic recording method to study ocular dominance plasticity in adult mice (Sawtell et al., 2003). Our approach was to record in awake, head-restrained mice the cortical response evoked by a high-contrast horizontal grating, before and after manipulations of visual experience such as monocular lid closure. Our progress was slowed by what appeared to be instability in the baseline recording. Typically, the response to the grating progressively grew over successive recording sessions, often taking over a week to stabilize. Only after completion of our first study (Sawtell et al., 2003) did we realize that this change was not caused by "drift" of the recording electrode, but rather by the selective potentiation of responses to the experienced stimuli. (Our subsequent studies of ocular dominance plasticity have avoided this complication by taking only a single baseline measurement and by using orthogonal gratings for testing the consequences of monocular deprivation [Frenkel and Bear, 2004].)

In this paper we describe the properties of SRP in mouse visual cortex and how SRP alters a fundamental conclusion of our previous study of adult ocular dominance plasticity (Sawtell et al., 2003). We also show that SRP is prevented by pharmacological blockade of NMDA receptors and by viral delivery into the visual cortex of a peptide that interferes with AMPA receptor trafficking, suggesting a possible mechanism. SRP is a striking example of how experience can modify primary sensory cortex and provides a simple paradigm for further investigation of the cortical mechanisms that may underlie perceptual learning and implicit memory.

Results

Properties of a Stimulus-Specific Response Potentiation in Mouse Visual Cortex

We used chronic visually evoked potential (VEP) recordings to monitor changes in the strength of cortical responses over the course of repeated visual testing (Porciatti et al., 1999a; Sawtell et al., 2003). We chose a recording depth of 450 μm for our chronic recording experiments because VEPs recorded at that depth have the maximum negativity and shortest latency, corresponding to a current sink layer IV (Sawtell et al., 2003).

Unless otherwise specified, electrodes were implanted in primary visual cortex at P24–P25, the age used routinely in this lab for studies of ocular dominance plasticity (Figure 1A). After habituation to the restraint, VEPs were recorded at P28 in fully awake, head-restrained mice in response to square-reversing sinusoidal gratings of 0.05 cycles/deg (Figure 1B). We first

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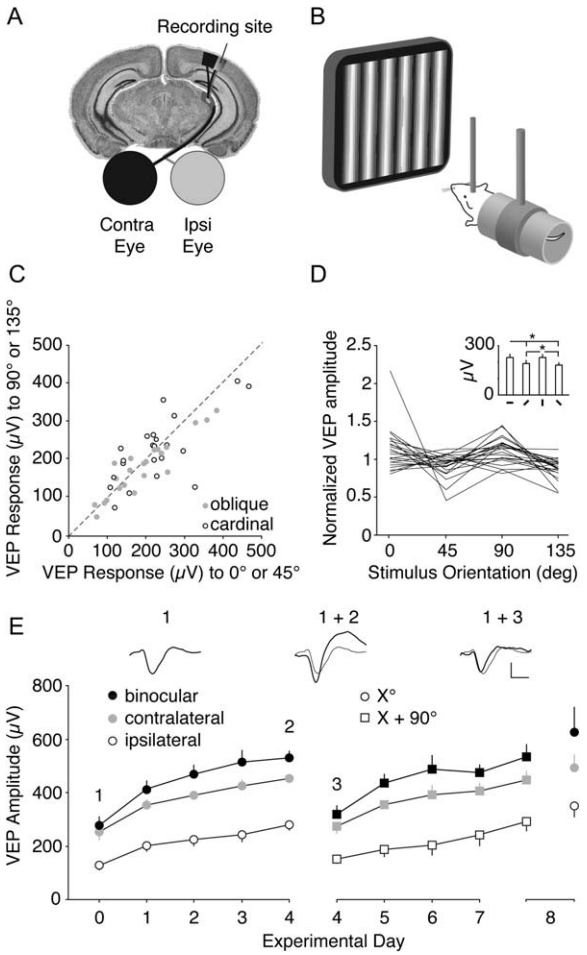

Figure 1. Stimulus-Selective Response Potentiation

(A) VEPs were recorded from the binocular region of primary visual cortex (gradient gray) at a depth that yields the maximum negative-going potential.

(B) Schematic of the recording setup. The mouse was placed in the restraint apparatus 20 cm away from the midline of the monitor. Recordings were made in nonanesthetized head-restrained animals.

(C and D) In naive mice, orthogonal orientations evoke similar responses. In (C), responses to orthogonal orientations are plotted on separate axes. Gray circles represent responses to oblique stimuli (45° and 135°) and open circles represent responses to cardinal stimuli (0° and 90°). The dashed line is drawn at 45 degrees. In (D), responses to four tested orientations are plotted as lines for individual mice. Amplitudes of individual animals are normalized to the average of responses to four orientations of that animal. Inset shows the VEP average of all mice for tested orientations.

(E) Daily exposure to a grating stimulus of a single orientation selectively potentiates the amplitude of VEPs to that orientation. Representative traces of binocular VEPs are shown for days indicated by numerals. SRP occurs regardless of the grating orientation used (X = 0° [n = 5], X = 90° [n = 1], X = 45° [n = 2]). Scale bar, 100 μV, 50 ms.

performed experiments to assess the orientation tuning of VEPs elicited by binocular stimulation in animals naive to the stimuli. Mouse visual cortex lacks orientation columns, and accordingly, we consistently found that gratings of orthogonal orientations elicit very similar VEPs. Responses to vertical (0°) and horizontal (90°) orientations were identical, as were the responses to 45° and 135° orientations (Figure 1C). As reported in other species (Coppola et al., 1998; Wang et al., 2003), we also found that VEPs to the cardinal orientations (0° and 90°) were significantly larger than those to the oblique orientations (Figure 1D) (n = 22; VEP amplitude for 0° is 227 ± 20 μV, for 90° is 228 ± 19 μV, for 45° is 191 ± 19 μV, and for 135° is 181 ± 16 μV; repeated measure ANOVA $F_{(3,21)} = 8.47$; $p < 0.0001$; Fischer post hoc test: $p > 0.05$ for comparisons between orthogonal orientations and $p < 0.01$ for other comparisons).

During our initial characterization of SRP, we used sinusoidal gratings of 0.05 cycles/deg and 100% contrast, phase-reversing at 1 Hz. In each session, responses to 400 contrast reversals (200 complete cycles) were averaged, and the time elapsed between sessions was 24 hr. We found that repeated presentations of gratings of a single orientation (termed X° because the absolute value was varied randomly from animal to animal) resulted in a potentiation of the evoked responses to that stimulus, which became evident during the subsequent testing sessions (Figure 1E, circles) (n = 8; repeated measure ANOVA $F_{(4,7)} = 27.6$; $p < 0.0001$). SRP appeared to reach saturation after three to four sessions. The usual measure of VEP amplitude is the difference between the negative and positive peaks (Porciatti et al., 1999b; Sawtell et al., 2003), but separate analysis of each VEP component revealed comparable and statistically significant potentiation ($p < 0.0001$). This response enhancement was not due to drift in the preparation, as subsequent testing with stimuli of an orthogonal orientation (X + 90°) elicited a response comparable in amplitude to the initial VEP (orientation X°).

Following induction of SRP to gratings of orientation X°, daily presentations of orientation X° were discontinued, and for the next 5 days SRP was induced with gratings at X + 90° (Figure 1E, square symbols). Subsequent testing with stimuli of the original orientation (X°) revealed that the response to this orientation was still potentiated (Figure 1E, circle on day 8). Therefore, the modifications that underlie SRP to gratings of one orientation are persistent and unaffected by subsequent induction of SRP to an orthogonal orientation. The magnitude of SRP to the new orientation was as robust as it was to the first (two-way repeated measure ANOVA $F_{(1,14)} = 0.05$; $p > 0.05$ for comparison between X° and X + 90° over time; $F_{(1,4)} = 1.7$; $p > 0.05$ for the interaction effects of orientations and days), despite the fact that the age of the animals at this point was past some estimates of the critical period in mice (P33) (Gordon and Stryker, 1996). Indeed, as will be shown below, significant SRP is observed in mice $\geq$P60.

The experiments illustrated in Figure 1 show that SRP can be induced serially to two different orientations without interference. We next asked if SRP could be induced to different orientations simultaneously. Mice were exposed daily to gratings of four different orientations, and we observed that the responses to each of these stimuli increased significantly (Figure 2A). We also exposed animals daily to a checkerboard stimulus and found a variable increase in the response to this stimulus, but no change in the response to oriented gratings (Figure 2B).

We next performed a series of parametric studies to better understand the characteristics of SRP. The progression of SRP was similar whether using 100, 200, or 400 stimuli per session. The progression of SRP also

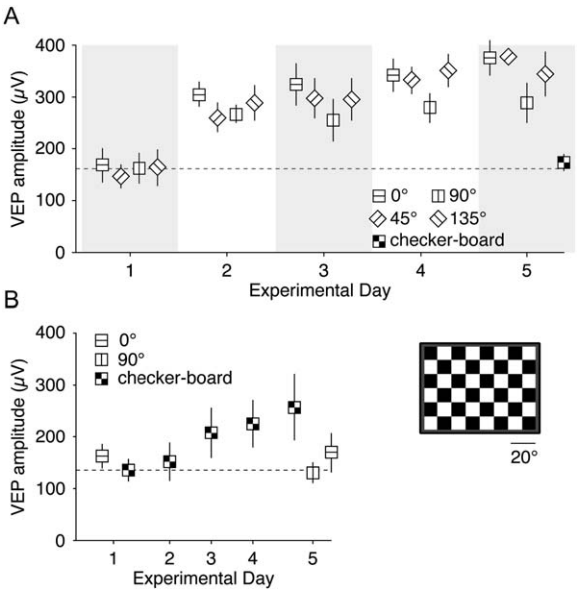

Figure 2. SRP to Different Stimuli Can Occur Simultaneously and Does Not Transfer to Novel Stimuli

(A) VEP amplitude increases over time in response to binocular presentation of four orientations during the same recording session (n = 6). The increase in VEP amplitude does not transfer when a novel checkerboard pattern is presented on day 5. The dashed line indicates the average VEP response to all four orientations on day 1.
(B) Repeated binocular presentation of a checkerboard pattern (200 stimuli per session, 0.05 cycles/deg, reversing at 1 Hz) produces a variable increase in VEPs (n = 5) that does not transfer to gratings of vertical or horizontal orientations tested on day 5. The dashed line indicates VEP amplitude to checkerboard stimulation on day 1.

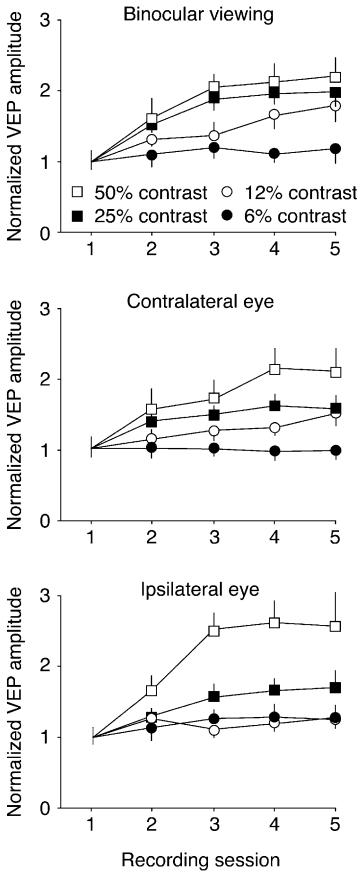

Figure 3. Cooperativity

Daily recording sessions were performed for 5 days with 200 stimuli per viewing condition per session. Mice were exposed to stimuli of various contrasts ranging from 6% (n = 6) to 50% (n = 5) with 12% (n = 6) and 25% (n = 6) as intermediate values. VEP amplitude is normalized to the first recording session.

appeared to be largely independent of both inter-session intervals ranging from 12–96 hr and the temporal frequency of grating patterns reversing from 0.1–1 Hz (data not shown). Interestingly, SRP did depend on stimulus contrast. Low-contrast (6% or 12%) grating stimuli failed to elicit SRP (Figure 3; n = 6 for each group; $F_{(1,10)} = 2.59$; $p > 0.05$), suggesting SRP induction requires a threshold response magnitude that must be exceeded during stimulus exposure. Further increases in stimulus contrast led to an increase in the magnitude of SRP (n = 6 for 25% group and n = 5 for 50% group; $F_{(1, 9)} = 26.7$; $p < 0.0001$).

Experiments with 400 stimuli per session were analyzed to see if SRP could be detected within a session (Figure 4A). Although significant SRP was observed between sessions, no response enhancement was observed during the course of stimulation. This finding suggests that stimulation sets in motion a process that takes time to be expressed as SRP.

We also performed experiments to examine whether inter-ocular transfer of SRP occurs. On the first day of recording, ipsilateral eye VEPs were collected in response to stimuli of orientation X°, whereas contralateral eye VEPs were elicited by stimuli of orientation X + 90° (Figure 4B, day 0). On the subsequent days, SRP was induced by stimulating ipsilateral eye with gratings of X° (open circles in Figure 4B). On the last day of recording, contralateral VEPs were collected in response to X° gratings (filled circle in Figure 4B). SRP induced by the monocular exposure to X° gratings through the ipsilateral eye failed to transfer to contralateral eye. This finding is remarkable in light of the fact that mouse visual cortex has few, if any, neurons that respond exclusively to the ipsilateral eye—virtually every neuron has some response to the contralateral eye (Drager, 1975; Gordon and Stryker, 1996). The eye- and orientation-selectivity of the effect strongly suggest a change in the strength of geniculo-cortical transmission as the basis for SRP.

Single-Unit Correlates of SRP

The VEP data are most simply explained by a modification of synaptic transmission between thalamus and cortex. We were next interested to know how the output of cortical neurons might be influenced by this change. By performing single-unit recording, we could address several different questions. Does a greater proportion of neurons respond to the experienced stimulus after SRP? Does the average response to the experienced stimulus increase after SRP? Is the orientation tuning of neurons selective to the experienced stimulus influenced by SRP?

We acutely recorded single-unit activity from ten naive mice and 14 mice in which SRP was induced by 5 days of daily exposure to vertically oriented gratings (90°).

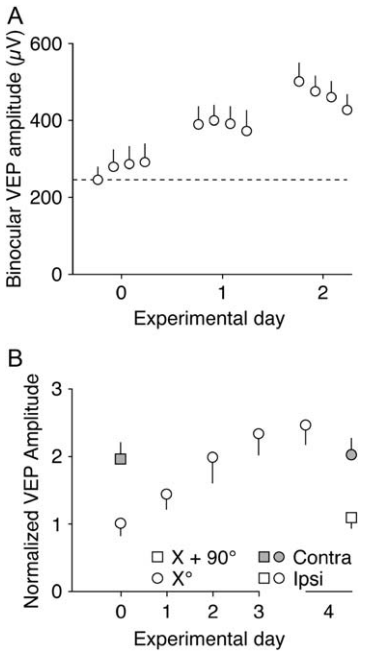

Figure 4. Hysteresis and Input-Specificity

(A) SRP does not occur within a single recording session, but emerges between sessions. Animals were presented with 400 stimuli during each recording session. Each symbol represents the average of 100 VEPs. There is no significant intra-session change in VEP amplitude but a significant inter-session change.

(B) SRP is specific to the eye of pre-exposure. Monocular presentations of stimuli of orientation X° to the ipsilateral eye (open circles) do not lead to SRP in the contralateral eye (filled circle). Filled symbols represent contralateral VEPs, open symbols represent ipsilateral VEPs. Squares indicate VEPs in response to X + 90°, circles indicate VEPs in response to 0°. VEP amplitudes are normalized to the ipsilateral response on day 1.

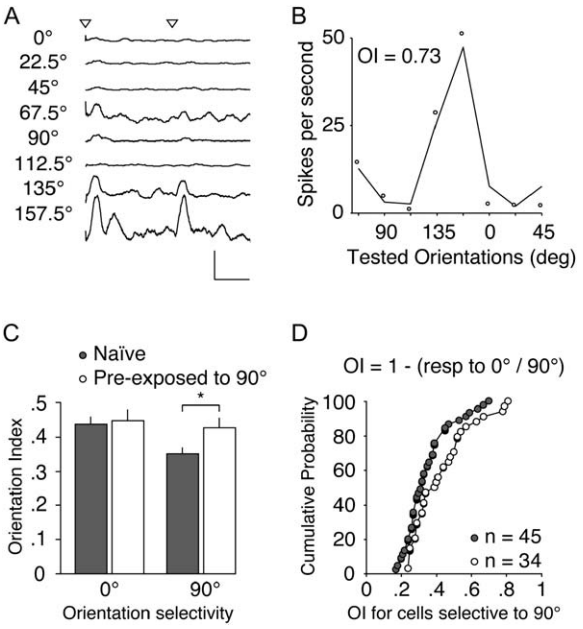

Figure 5. Single-Unit Recordings

(A) Peristimulus time histogram of a recorded unit in response to eight stimuli. Firing rate is plotted as a function of time. Time of stimulus reversal is indicated by arrows. Scale bar, 50 Hz, 200 ms.

(B) Orientation tuning profile for unit shown in (A). Open circles correspond to the actual firing rate. Black line is a fitted curve (see Experimental Procedures).

(C and D) Changes in orientation index following SRP. (C) Comparison of orientation indices for cells selective to 0° and 90° from naive mice and mice pre-exposed to 90°. (D) After SRP to 90° stimuli, the orientation index distribution is shifted to the right in cells selective for 90°, indicating a greater response to the experienced orientation relative to the orthogonal.

Extracellular recordings of single units were conducted in fully awake, head-restrained mice using custom made eight-electrode microwire bundles that were advanced in ≥100 μm steps to sample from neurons at different cortical depths. In a subset of mice, chronic VEP electrodes were implanted in the hemisphere opposite the one in which single-unit recordings were to be done to monitor the progression of SRP. For each encountered unit, spikes were recorded in response to random presentation of full-field gratings of eight different orientations (0°, 22.5°, 45° …157.5°). Spontaneous activity was assessed by interleaved presentations of a uniform full-field gray screen, as well as a black screen. An example of unit responses is shown in Figures 5A and 5B.

Each recorded unit (n = 416 for naive group and 369 for pre-exposed group) was classified as orientation selective, panorientational, or nonresponsive (see Experimental Procedures). No significant differences in the proportions of these cell types were observed in mice having undergone SRP versus naive mice; nor was there a significant difference in the fraction of orientation-selective units favoring 90° (Table 1). There was also no difference in the mean peak firing rate of neurons selective to 90°. However, there was a statistically significant difference in the orientation index (OI), a measure of relative response to preferred and orthogonal orientations (Figures 5C and 5D). After SRP, the OI of neurons selective to 90° shifted to values closer to 1, indicating a greater response to the experienced orientation relative to the orthogonal (0°).

Molecular Requirements for SRP

Other forms of response enhancement, including long-term potentiation (LTP) induced in the rodent visual cortex by thalamic stimulation (Heynen and Bear, 2001) and increases in open-eye VEP amplitude following monocular deprivation (MD) (Sawtell et al., 2003), require

Table 1. Unit Properties after SRP, Expressed as Mean Values Per Animal ± SEM

Parameter	Naive (10 mice, 416 units)	Pre-exposed to 90° (14 mice, 369 units)
Orientation-selective units (%)	40 ± 5.4	47 ± 3.4
Panorientational units (%)	24 ± 2.5	20.4 ± 3.1
Nonresponsive units (%)	36 ± 5.3	32.5 ± 4.1
Units responsive to 90° (%)	33.3 ± 3	28.6 ± 4
Units selective to 90° (%)	9.27 ± 2.1	8.16 ± 1.8
Mean firing rate to 90° of units selective to 90° (spikes/s)	24.4 ± 2.5	18.1 ± 2.0
Mean firing to 0° of units selective to 90° (spikes/s)	17.1 ± 2.0	11.7 ± 1.6
Orientation index of units selective to 90°	0.35 ± 0.02	0.43 ± 0.03*

* $p < 0.05$.

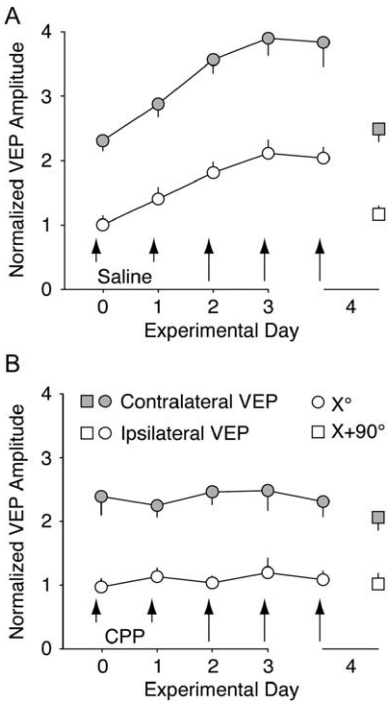

Figure 6. NMDA Receptor Dependence

(A and B) Pharmacological blockade of SRP in juvenile mice. Mice were injected (indicated by arrows) with either saline (n = 9) or the competitive NMDA receptor antagonist CPP (n = 11) 2.5 hr prior to each recording session for 5 days. In saline-injected mice (A) SRP is readily expressed, whereas SRP is blocked in CPP treated animals (B). Data are normalized to the ipsilateral VEP amplitude recorded on day 1.

activation of NMDA receptors. Similarly, we found that systemic administration of the NMDA receptor antagonist CPP completely abolished SRP in juvenile mice, whereas it remained present in saline-treated controls (Figure 6; CPP group, n = 11; ANOVA, $F_{(5,10)} = 1.09$, $p > 0.05$ for contra; $F_{(5,10)} = 0.6$, $p > 0.05$ for ipsi; Saline group, n = 9; $F_{(5,8)} = 17.9$, $p < 0.0001$ for contra; $F_{(5,8)} = 29.4$, $p < 0.0001$ for ipsi). These results were further corroborated by a retrospective analysis of chronic VEP recordings conducted in mice with a postadolescent, cortex-specific deletion of the obligatory NR1 subunit of the NMDA receptor. NR1 deletion in layers 2–4 prevents deprivation-induced potentiation of open-eye VEPs in these mice (Sawtell et al., 2003). SRP is also blocked in these mice (unpublished data), suggesting that cortical NMDA receptors are necessary for SRP induction.

One well-studied mechanism for increasing excitatory synaptic strength is an increase in the number of glutamate receptors on the postsynaptic membrane (see Luscher et al., 2000 and Malinow et al., 2000 for reviews). Induction of LTP increases the number of postsynaptic AMPARs, and there is evidence that this process is mediated by intracellular interactions with the carboxy-tail of the GluR1 subunit (Hayashi et al., 2000). We hypothesized that this mechanism is also responsible for SRP expression. Viral transfection of the C-terminal domain of GluR1 (GluR1-CT) has been shown to block the new insertion of AMPA receptors upon induction of LTP in cultured hippocampal slices (Shi et al., 2001) and following sensory experience in somatosensory cortex in vivo (Takahashi et al., 2003). Therefore, we tested our hypothesis by virally expressing GluR1-CT in visual cortex in order to prevent activity-dependent delivery of AMPARs following visual stimulation.

Initial experiments were conducted to determine if HSV-mediated infection with GluR1-CT over 3 days results in a change in VEP amplitude. The visual cortex was injected with either a GluR1-CT vector or a GFP-only vector, and chronic electrodes were implanted at P26. Electrode placement within the virally infected area was verified histologically in every infected animal (Figures 7A1–7A3). GFP expression was apparent 24 hr postinfusion and reached its maximum expression 36–70 hr postinfusion. Hence, VEPs were recorded at 12 hr postinjection to collect responses at the beginning of viral gene expression and again at 96 hr postinfection (Figure 7B1). Effects of repeated testing were avoided by using orthogonal gratings on the second recording session (Frenkel and Bear, 2004). No differences in VEP amplitudes were observed across recording sessions as a result of viral infection (Figures 7B2–7B4; HSV-GFP group [n = 5] VEP amplitudes: contra on 1st recording 140 ± 30 μV, on 2nd recording 125 ± 25 μV, $p > 0.05$ paired t test; ipsi on 1st recording 67 ± 12 μV, on 2nd recording 59 ± 12 μV, $p > 0.05$; HSV-GluR1-CT group [n = 6] VEP amplitudes: contra on 1st recording 138 ± 15 μV, on 2nd recording 120 ± 19 μV, $p > 0.05$; ipsi on 1st recording 74 ± 8 μV, on 2nd recording 66 ± 11 μV, $p > 0.05$). VEP amplitudes in infected animals were not significantly different from those recorded in uninfected control animals (n = 6) (Figures 7B2–7B4, contra VEP on 1st recording 175 ± 11 μV, on 2nd recording 188 ± 22 μV; ipsi VEP on 1st recording 85 ± 7 μV, on 2nd recording 87 ± 10 μV; ANOVA $F_{(2,28)} = 2.0$, $p > 0.05$ for main effect between three animal groups).

To test whether SRP expression requires the activity-dependent delivery of AMPARs, mice were chronically implanted on P26 after the visual cortex was injected with either an HSV-GFP-GluR1-CT vector or a GFP-only vector. Additional control animals were chronically implanted but did not receive intracortical infusions of virus. Testing with gratings of a single orientation was begun 48 hr postinfection and continued for 3 consecutive days (Figure 7C1). SRP was observed in noninjected controls and in mice infected with the GFP-only vector (Figures 5C2 and C4, n = 6 for both groups; $F_{(1,10)} = 33.59$, $p < 0.0001$ for noninfected group; $F_{(1,10)} = 15.1$, $p < 0.0001$ for GFP-infected mice), but was completely blocked in mice receiving the GluR1-CT vector (Figure 5C3, n = 7, $F_{(1,12)} = 0.77$, $p > 0.05$). The fact that SRP was prevented by local cortical expression of the GluR1-CT peptide confirms that SRP originates in visual cortex and strongly suggests that its expression requires the delivery of AMPA receptors to cortical synapses.

Interaction of SRP and Adult Ocular Dominance Plasticity

In a previous study we demonstrated that 5 days of MD in adult mice results in a potentiation of synaptic responses evoked by stimulation of the nondeprived eye (Sawtell et al., 2003). In those experiments we recorded baseline VEPs until VEP amplitude was stable for at least four sessions. A retrospective analysis of all pre-MD data revealed that, in many mice, VEP amplitudes

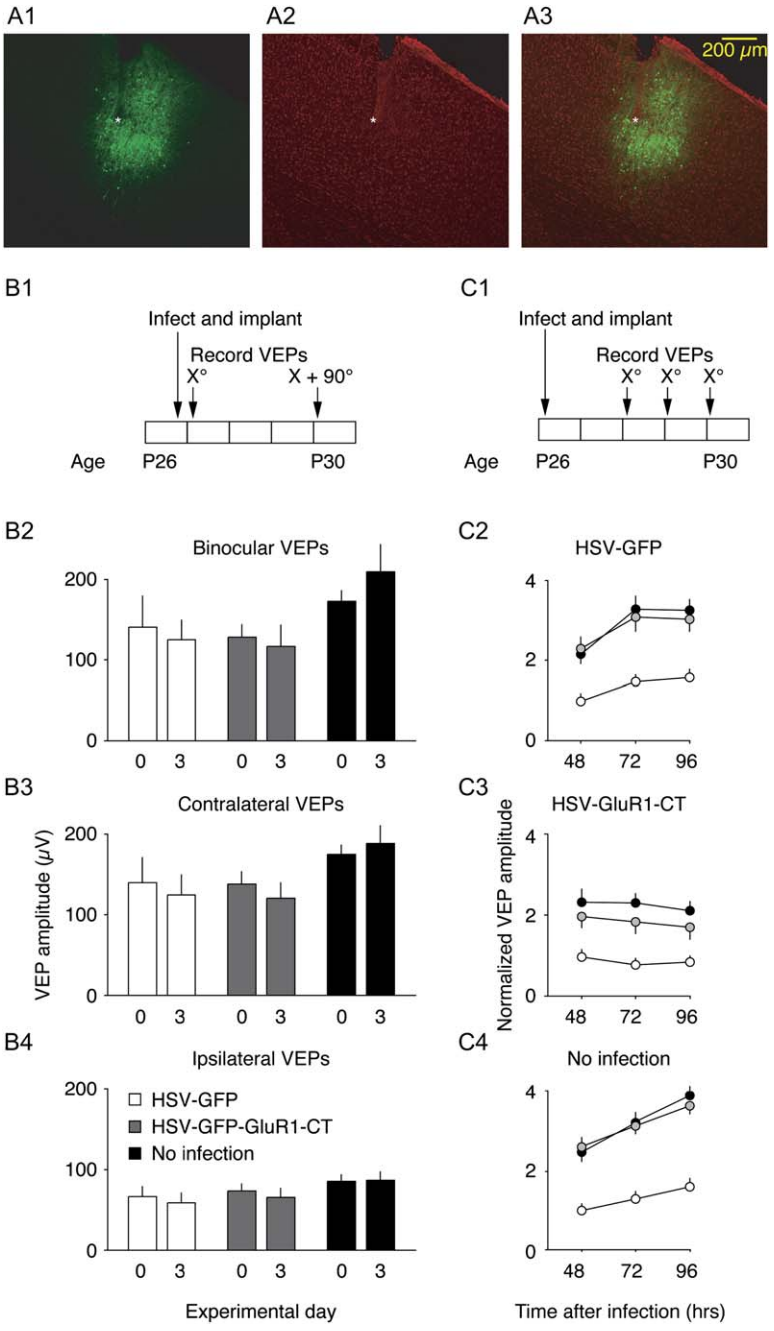

Figure 7. Block by Viral Expression of the GluR1 C-Tail

(A1–A3) Histological verification of electrode placement and viral infection. (A1) Image of GFP staining. (A2) Image of Nissl staining. (A3) Overlay of GFP- and Nissl-stained images. White star indicates electrode tip.

(B) Viral infection with GluR1-CT does not result in a rundown of VEP amplitude over 4 days. (B1) Experimental design to test for VEP rundown. The first recording was made 12–16 hr after initial infection (grating stimuli of X° orientation were used); the last recording session was performed 4 days postinfection (grating stimuli of X + 90° orientation were used). Animals virally infected with GFP (open bars, n = 5) and GFP+GluR1 (gray bars, n = 6) did not differ from noninfected control (black bars, n = 6).

(C) SRP is blocked in animals receiving cortical viral infection of GluR1-CT for 3 days. (C1) Experimental design. Daily recordings began 2 days postinfection. Grating stimuli of X° orientation were used for 3 days. Only the viral construct containing GluR1-CT blocked SRP ([C3], n = 7), whereas SRP occurred in animals infected with GFP only ([C2], n = 6) and in noninfected control animals ([C4], n = 6).

increased over the course of the first several testing sessions before eventually saturating (data not shown). In the present study, we performed additional experiments to confirm that SRP continues to be expressed in adult mice (>P60) and to investigate the relationships between SRP and the changes induced by MD. VEPs were recorded in two groups of adult mice tested daily for 6 days with pattern-reversing grating stimuli of a single orientation. In both groups, VEPs evoked by both ipsilateral- and contralateral-eye stimulation increased significantly over the course of the first three to four testing sessions (Figure 8; day 1 VEP amplitudes: contra 173 ± 14 μV, ipsi 78 ± 9 μV [Figure 8A, n = 11]; contra 211 ± 25 μV, ipsi 82 ± 11 μV [Figure 8B, n = 6]; day 6 VEP amplitude: contra 245 ± 27 μV, ipsi 149 ± 22 μV [Figure 8A]; contra 293 ± 23 μV, ipsi 158 ± 17 μV [Figure 8B]; ANOVA $F_{(3,30)} = 38.8$; $p < 0.0001$). A subset of mice was tested with gratings of an orthogonal orientation on day 6 (data not shown). As in juvenile mice, response potentiation was specific to the orientation of the test stimulus. After the sixth session, the mice in the first group were returned to their home cages, while mice in the second group were deprived of vision through the contralateral eye by monocular lid suture. A final testing session was conducted 7 days later. VEP amplitudes remained elevated and comparable to the posttraining values in nondeprived mice (Figure 8A; contra VEP amplitude 233 ± 27 μV, ipsi VEP amplitude

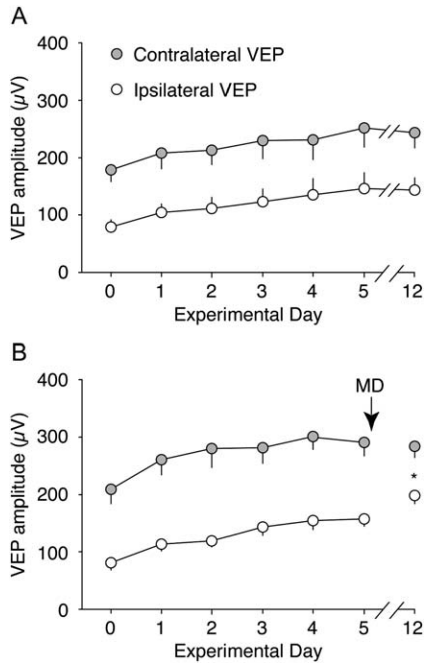

Figure 8. Interaction of SRP and Ocular Dominance Plasticity in Adult Mice

(A and B) SRP is expressed in adult mice and does not prevent potentiation of open-eye responses following MD. Two groups of mice were pre-exposed to stimuli of the same orientation for 6 consecutive days. After VEP amplitude stabilized, one group ([A], n = 11) was placed in their home cages for 1 week; a second group ([B], n = 6) underwent MD for 7 days. VEP amplitude was still elevated in mice with normal visual experience ([A], day 12). However, open-eye VEPs increased after 7 days of MD ([B], open circle on day 12); no depression of the deprived-eye response was detected ([B], gray circle on day 12). *p < 0.05.

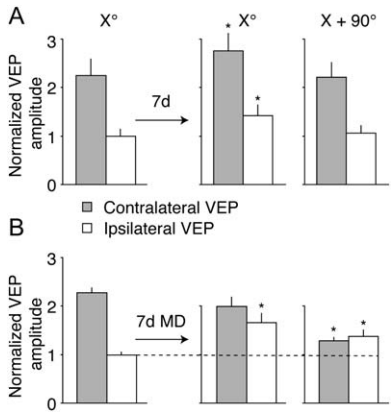

Figure 9. SRP Masks Deprivation-Induced Depression of Visual Responses in Adult Mice

(A) VEPs remain stable over 7 days of normal visual experience (n = 7) when orthogonal stimuli are used to compare responses on day 0 and day 7. When tested with previously exposed stimuli, VEPs increase significantly.

(B) The effects of 7 days of MD are shown (n = 7). Using orthogonal stimuli on day 0 and day 7 reveals a significant decrease in deprived-eye VEP amplitude. This decrease is masked when visual testing is performed using stimuli of a previously exposed orientation (X°). VEP amplitude was normalized to the ipsilateral VEP on day 1 of recording. Asterisks indicate significant difference (p < 0.05) compared to pre-MD baseline.

134 ± 22 μV), suggesting that, as in juvenile mice, synaptic changes underlying SRP in adult mice are persistent. Seven days of MD resulted in a significant further potentiation of VEPs evoked by stimulation of the nondeprived eye (Figure 8B, for ipsi VEPs ANOVA $F_{(5,6)} = 28.4$; $p < 0.0001$; Fischer post hoc test $p < 0.05$ when comparing post-MD data with pre-MD data). These data are consistent with our previous findings in adult mice that MD enables open-eye potentiation.

A recent report by Tagawa et al. (2005) suggests that monocular deprivation in adult mice also results in the weakening of the deprived eye input, an effect that we had not observed in our previous study (see also Hofer et al., 2006). We considered the possibility that prior induction of SRP may prevent or mask deprivation-induced depression of deprived-eye responses. To examine this question, experiments were performed in which VEPs were recorded twice in each mouse, 7 days apart. A grating of a single orientation (X°) was used to evoke VEPs on day 0, and gratings of both X° and X + 90° were used on day 7 to assess the magnitude of SRP induced during the first session. In one group of mice (n = 7), the contralateral eyelid was sutured closed under anesthesia after the initial recording session and opened immediately before the second session. A second, control group (n = 7) received the same regimen of anesthesia, but were not monocularly deprived. Recordings in the controls revealed that the VEP amplitude recorded on day 0 to orientation X° was identical to the VEP amplitude recorded on day 7 to orientation X + 90° (Figure 9A), as expected from previous experiments in young mice (cf. Figure 1). In comparison, day 7 recordings of VEP amplitude to orientation X° showed clear and significant evidence of SRP. In the monocularly deprived mice (Figure 9B), the VEP amplitudes on day 7 to orientation X + 90° were substantially different from those recorded on day 0 to orientation X°: the non-deprived-eye response was significantly potentiatied (VEP amplitude pre-MD: 96 ± 6 μV, post-MD: 133 ± 13 μV; $p < 0.05$), and the deprived-eye response was significantly depressed (VEP amplitude pre-MD: 219 ± 10 μV, post-MD: 124 ± 7 μV; $p < 0.001$). The deprived-eye response depression was masked by SRP, however, when the VEPs were recorded at the original orientation (X°).

These experiments in adult mice, taken together, suggest that MD does indeed induce deprived-eye response depression (Figure 9), but that this effect of MD can be prevented by prior saturation of SRP (Figure 8).

Discussion

The visual cortex has long been used as a model to study the effects of the sensory environment on the structure and function of the brain. To date, most progress has been made understanding the effects of visual *deprivation* (see, e.g., Heynen et al., 2003; Maffei et al., 2004). Much less is known about how visual *experience* modifies the cortex, particularly at a mechanistic level. Indeed, the basic question of whether selective visual experience actually augments responses in primary visual cortex to the experienced stimuli during development aroused considerable controversy in the past. One view, inspired by the observation of orientation

selectivity in newborn macaques (Wiesel and Hubel, 1974) and visually inexperienced kittens (Sherk and Stryker, 1976), is that orientation preferences are genetically determined and are therefore likely to be nonmodifiable. An opposing view is that preferences result from experience with oriented visual stimuli and can change when, for example, an animal is exposed to a visual environment in which lines of a narrow range of orientations predominate (Blakemore and Cooper, 1970; Hirsch and Spinelli, 1970). The current consensus is that visual experience is not needed for initial development of orientation preference in kitten visual cortex, but is crucial for its maintenance (Chapman et al., 1999), and that selective experience can be "instructive," biasing the cortex to have greater and more frequent responses to the orientations experienced more often (Sengpiel et al., 1999).

Our findings strongly support the notion of an instructive effect of visual experience in mouse visual cortex. Particularly remarkable is how few stimuli are required to induce SRP—as little as 10 min per day of selective visual experience across 3–4 days was sufficient to reach a saturated level of potentiation in the awake mouse. Although the absolute change in VEP amplitude appeared to decline by P60 (cf. Figures 1 and 8), SRP certainly is not confined to an early postnatal critical period.

We discovered SRP by recording VEPs, believed to reflect averaged synaptic currents (Logothetis, 2003; Mitzdorf, 1985), in cortical layer 4, which receives monosynaptic input from the lateral geniculate nucleus. The observation that SRP induced through the ipsilateral eye does not transfer to the contralateral eye strongly suggests a modification of synaptic transmission early in the visual pathway, before inputs from the two eyes are mixed. However, the fact that SRP is orientation selective suggests the locus of change is cortical, since orientation selectivity first emerges in primary visual cortex. A cortical locus for SRP is confirmed by experiments in which it was prevented by local manipulations of NMDA and AMPA receptors. Together, the data strongly suggest that SRP of VEPs reflects a modification of excitatory geniculo-cortical synaptic transmission in layer 4.

To assess the impact of SRP on cortical output, unit recording experiments were conducted acutely in two separate groups of mice, one naive and the other preexposed for several days to a grating of orientation 90° to induce SRP. A limitation of these experiments is that, unlike the VEP recording method, comparisons had to be made across rather than within animals using population statistics. In addition, the use of microwire bundles, while greatly increasing the rate of sampling over the limited duration of a recording session, biased our sample toward the deep layers of cortex (Shuler and Bear, 2006). In the population of neurons selective to 90°, comparison of the average spiking with either the 90° or the orthogonal 0° grating revealed no statistically significant differences between the two groups, likely due to the high degree of variability in spiking rates across cells and animals. However, the OI calculation reduces variability by normalizing the 90° response to the 0° response for *each neuron* (conceptually similar to calculating ocular dominance). Comparison of OI values across the groups did reveal a statistically significant effect, meaning that there is a greater difference between the responses to 90° and 0° in the animals with SRP than in controls.

Interesting properties of SRP include the requirement for a threshold level of stimulus contrast, the apparent absence of compensatory or competitive changes in responses to orthogonal stimuli, the stability and persistence of the change, and the fact that SRP did not appear within a session, but was clearly present when tested as few as 12 hr later. These properties are strikingly similar to those described for some forms of human perceptual learning (Karni and Bertini, 1997). For example, visual skill learning can be highly specific for a single parameter, strongly modulated by attention and behavioral arousal, very stable over time, and slowly evolving, taking hours between sessions to be expressed. Of course, we have not determined if our mice have "learned" anything; this determination obviously would require behavioral analysis that is beyond the scope of the current study (and not easily accomplished in mice). However, it is well established that in alert animals, including humans, repeated exposure to visual stimuli can induce perceptual learning (a lowered threshold to detect these stimuli), and that this correlates with increased activation of primary visual cortex by these stimuli (Furmanski et al., 2004). Therefore, we consider it likely that SRP reveals a neural basis for perceptual learning.

Previous attempts to understand the neural correlates of perceptual learning in monkey primary visual cortex used an orientation discrimination task (Ghose et al., 2002; Schoups et al., 2001). With practice, monkeys (like humans) show a substantial improvement over the course of several months with properties suggesting a modification of V1. Consistent with our findings, Schoups et al. found a subtle change in the tuning of V1 neurons selective to the trained orientation (but see Ghose et al., 2002). However, no change was observed if the monkeys were passively stimulated in an unattended region of the visual field, and no change was detected in layer 4. In considering these apparent differences with our findings, it is important to note we did not show the grating stimuli at the same time the mice were asked to focus attention elsewhere. Our observations of the mice during the recording session suggest they were aroused and attentive to the visual stimulation. Moreover, the laminar organization of monkey visual cortex, particularly layer 4, is radically different from mouse. Unlike monkeys, mice have orientation-selective neurons in thalamorecipient layer 4, lack ocular dominance columns, and do not have segregated magnocellular and parvocellular input streams.

The advantages of the mouse for mechanistic studies of visual cortical plasticity are so obvious that they hardly need to be mentioned. Although there is much more to learn, we have already been able to substantially advance the understanding of the molecular basis for experience-dependent plasticity underlying SRP. Based on properties observed in humans, Karni and Sagi (1991) suggested a reductionist model for perceptual learning, involving Hebbian increases in synaptic strength in primary visual cortex that require a consolidation period to become manifest. The leading experimental paradigm for Hebbian modifications is LTP, and the key properties of LTP nicely match those of SRP, including input

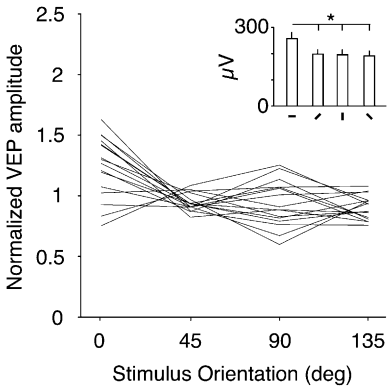

Figure 10. Orientation Preference in Adult Mice as Assessed by VEP Recordings Is Biased toward Horizontal Stimuli

Normalized VEP amplitude to four tested orientations is plotted for each individual mouse. Amplitudes for each animal were normalized to the average response to the four orientations presented to that animal. Inset is the mean (± SEM) VEP amplitude for all mice across all four orientations. *p < 0.05. These findings may be compared to those obtained in juvenile mice (Figure 1).

specificity, cooperativity, and persistence. Moreover, at many cortical synapses, induction of LTP requires strong activation of NMDA receptors, and expression of LTP requires the delivery of AMPA receptors containing the GluR1 subunit (Malinow et al., 2000). Our experiments reveal that SRP shares identical molecular requirements.

SRP appears to be a naturally occurring form of LTP, but one could still question the broader significance of a phenomenon that requires the "forced" viewing of visual stimuli never before experienced by a mouse. We were therefore interested to find that unlike the equivalent VEPs evoked in young mice by horizontal and vertical gratings, in adults, the VEPs were significantly larger to horizontal gratings than to all other orientations (Figure 10). It is tempting to speculate that the emergence of a preferential response to horizontal stimuli with age reflects ongoing synaptic plasticity in a visual environment statistically dominated by horizontally oriented contours (Ruderman, 1994).

Experimental Procedures

Electrode Implantation

Mice were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine i.p., and a local anesthetic of 1% lidocaine hydrochloride was injected over the scalp. For purposes of head fixation, a post was fixed to the skull just anterior to bregma using cyanoacrylate and a further application of dental cement. For VEP experiments, small (<0.5 mm) burr holes were made in the skull overlying the binocular visual cortex (3 mm lateral of lambda), and tungsten microelectrodes (FHC, Bowdoinham, ME) were inserted 450 μm below the cortical surface. For single-unit experiments, a plastic well of 1 to ~2 mm in diameter (0.5 mm thick) was attached to the intact skull overlaying the binocular visual cortex of one hemisphere, and a tungsten electrode was implanted in the other hemisphere. Reference electrodes were placed bilaterally over prefrontal cortex. Electrodes were secured in place using cyanoacrylate, and the entire exposure was covered with dental cement. Animals were monitored postoperatively for signs of infection or discomfort and were allowed at least 24 hr recovery before habituation to the restraint apparatus.

VEP Recording Procedure

VEP recordings were conducted in awake mice. Mice were habituated to the restraint apparatus prior to the first recording session. The animals were alert and still during recording. Visual stimuli were presented to left and right eyes randomly. A total of 100 to 400 stimuli were presented per each condition. VEP amplitude was quantified by measuring trough to peak response amplitude, as described previously (Frenkel and Bear, 2004; Huang et al., 1999; Sawtell et al., 2003). Responses to stimuli of 0% contrast were also collected to measure activity not evoked by patterned visual stimuli.

Visual Stimuli

Visual stimuli consisted of full-field sine wave gratings (0.05 cycles/deg) of varying contrast (0%–100%) generated by a VSG2/2 card (Cambridge Research System, Cheshire, UK) and presented on a computer monitor suitably linearized by γ correction. VEPs were elicited by either horizontal, vertical, or oblique (45° or 135°) bars. For single-unit experiments, full-field gratings of eight different orientations (0°, 22.5°, 45°…157.5°), a gray screen of equal luminance to grating stimuli and a black screen were randomly presented. A total of 32 stimuli from each condition were presented during single-unit experiments. The display was positioned 20 cm in front of the mouse and centered on the midline, thereby occupying 92° × 66° of the visual field. Mean luminance, determined by a photodiode placed in front of the computer screen, was 27 cd/m^2.

Single-Unit Recording

On the day of recording, mice previously implanted with a well (see above) were anesthetized by inhalation of 2%–3% isoflurane (IsoFlo 2%–3%) and placed under a surgical microscope. A small (~0.5 mm) burr hole was made in the skull area enclosed in the well. Mice were then placed in the recording apparatus. After fully recovering from anesthesia (5–10 min), a custom-made, eight-electrode bundle (Tungsten H-formvar wire 20 μm outer diameter, California Fine Wire Company, CA) was lowered through the neocortex using a stereotaxic arm. Electrodes were lowered until neuronal discharges were observed. A recording system (Plexon, Inc., Dallas, TX) was used to collect single neuron spike activity in response to visual stimulation. The output from each electrode was split and connected to preamplifiers, band-pass filtered and sent to an oscilloscope, an audio monitor, and a host PC that ran the data acquisition software. Single units were discriminated offline using Offline Sorter software (Plexon, Inc.).

Statistical Analysis of Single-Unit Data

Neuronal responses to grating stimuli were computed as the mean firing rate observed during two 100 ms windows corresponding to the stimulus-induced activity (20 to 120 ms from the onset of each phase of the stimulus). Spontaneous neuronal activity was computed as the mean firing rate over the same windows in responses to the black screen and equiluminant screen. The black screen was better for spontaneous activity measurements; therefore, responses to equiluminant stimuli were ignored for statistical analyses. ANOVA was performed across nine conditions (responses to eight oriented stimuli and the black screen) followed by Tukey-Kramer post hoc test. A cell was classified as nonresponsive if none of the responses to oriented stimuli differed from the responses to the black screen. If any of the responses to oriented stimuli differed from responses to the black screen, further ANOVA was performed across eight conditions (responses to orientated stimuli only). If no significant main effect was detected, a cell was classified as panorientational. Cells that had a significant main effect were classified as orientation selective and further analyzed for the orientation selectivity.

Quantitative Analyses of Orientation Tuning

Orientation selectivity was computed by fitting parametric curves to neuronal responses as a function of the stimulus orientation (adapted from Albright, 1984). These fits were made using a Gaussian function of the following type:

$$r_i = a + b * e^{-0.5 * ((x_i - x_o)/s)^2}$$

where a represents the minimum firing rate, b represents the difference between the maximum and minimum firing rate, x_o represents the preferred orientation, s represents the standard deviation of the fitted Gaussian, and r_i represents the firing rate for a stimulus moving in a given direction x_i. The Gaussian function that achieved the best fit

to the neuronal responses in the eight tested orientations was determined for each tuning curve using an iterative least-squared-residuals algorithm. Parameters of the fitted Gaussian were used to compute measures that characterize orientation tuning: differential response, bandwidth, and orientation index. Differential response was the difference between the fitted maximum and minimum responses (parameter *b* of the fitted Gaussian). Bandwidth was the full width of the tuning curve at one-half of the distance between the maximum and minimum responses (i.e., 2.355 *s*, where *s* is a parameter of the fitted Gaussian). The OI reflects the ratio of response strength in the preferred orientation relative to that in the orthogonal orientation (90° from preferred). This index was calculated by the following equation: OI = 1 − ([response to the orthogonal orientation]/[response to the preferred orientation]).

Viral Construct/Delivery

For the GluR1 C-tail construct, a PCR product that encodes the entire intracellular domain of GluR1 (aa 809–889) was cloned into a pEGFP-C1 (primers: 5′cctcgagccgagttctgctacaaatcc-3′, 5′-accggttt acaatcctgtggctccc-3′). The NheI- BamHI fragment which encodes EGFP-GluR1 C-tail fusion protein was then cloned under the HSV-1 IE 4/5 promoter of the pHSVPrPUC amplicon vector. These constructs were then transfected into packaging cell line 2-2 cells and processed for viral packaging (Carlezon and Neve, 2003). For intracortical viral injections, animals were prepared for electrode implantation as described earlier. After a small hole was made in the skull overlaying the binocular area in V1, a glass micropipette attached to a syringe pump (WPI, Inc) was inserted to a depth of 450 μm. The dural surface was then covered by a thin layer of NeuroSeal (NeuroNexus Technologies, MI). A small volume of HSV-GFP or HSV-GluR1-CT (typical titer of 5×10^7 IU/ml) was injected at a rate of 0.1 μl/min. A total volume of 1–1.5 μl was injected. After the injection, the position of the micropipette was held for 5 min before being withdrawn. Animals were subsequently implanted with a recording electrode in the site of injection as previously described. The experimenter was blind to the contents of the pipette.

CPP Injections

Mice were injected i.p. with either 10 mg/kg CPP (Sigma) or saline 2.5 hr before each VEP recording. The intraperitoneal injections of 10 mg/kg of CPP were previously shown to block the induction of LTP in vivo (Davis et al., 1997). No obvious change in the behavior was observed following CPP injections. Saline and CPP injections were performed blind.

Histology

Histology was performed on all brains injected with either HSV-GFP or HSV-GluR1-CT. Electrode position in mice with chronic implants was routinely determined by making an electrolytic lesion and staining with Cresyl Violet.

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