Abstract

Orientation selectivity of primary visual cortical neurons is an important requisite for shape perception. Although numerous studies have been previously devoted to a question of how orientation selectivity is established and elaborated in early life, how the susceptibility of orientation plasticity to visual experience changes in time remains unclear. In the present study, we showed a postnatal sensitive period profile for the modifiability of orientation selectivity in the visual cortex of kittens reared with head-mounted goggles. When goggle rearing (GR) started at P16-P30, 2 weeks of GR induced a marked over-representation of the exposed orientation, and 2 more weeks of GR consolidated the altered orientation maps. GR that started later than P50, in turn, induced the under-representation of the exposed orientation. Orientation plasticity in the most sensitive period was markedly suppressed by cortical infusion of NMDA receptor antagonist. The present study reveals that the plasticity and consolidation of orientation selectivity during the critical period are significantly modulated by visual experience in early life.

Introduction

Neuronal circuits in the mammalian visual cortex maintain certain dynamic mechanisms of structural and functional modification in the early postnatal period as well as in adulthood. Typically, monocular deprivation in early postnatal days leads to a shift in the dominant responses of primary visual cortical neurons to the non-deprived, experienced eye [1-4]. A deficit of vision caused by retinal damage is compensated for by rewiring of cortical circuits even in adult animals [5]. A different type of plasticity has been found with regard to the orientation selectivity of visual cortical neurons: it is currently thought that the orientation selectivity is innately generated [6], and then maintained [7] and elaborated [8] by visual experience in early postnatal days. Experimentally, this orientation plasticity is induced by exposure of young animals to retinal images restricted to a single orientation [9-16]. However, results to date are variable, reflecting the various methods used for inducing and detecting the orientation plasticity. This variability has made it difficult to delineate the postnatal critical period during which orientation plasticity manifests.

Recent technological progress in optical imaging of intrinsic signals [17-21] and data processing methods for noise reduction [22,23] now provide an efficient method, complementary to conventional unit recording, for revealing overall features of the cortical distribution of orientation selectivity. In addition, head-mounted cylindrical-lens-fitted goggles [24] provide an efficient tool for restricting visual experience to a single orientation. We previously reported that attaching goggles to a kitten continuously without interruption by dark-rearing episodes was particularly effective in causing modification of orientation selectivity [15].

In this study, we used these technical improvements to determine the critical period for orientation plasticity in cat primary visual cortex. It has been shown that infusion of an NMDA receptor antagonist to the visual cortex suppresses the expression of orientation selectivity [25,26]. It is further expected that NMDA-receptor-mediated long-term potentiation (LTP) at synapses to visual cortical neurons optimally activated by the exposed orientation is involved in the over-representation of that orientation. We confirmed that cortical infusion of an NMDA receptor antagonist nearly abolished the modifiability of orientation selectivity during the critical period. We also addressed an important question of how the orientation map modified by single-orientation exposure is consolidated afterwards.

Results

Normal rearing

Figure 1 shows three orientation polar maps (Figs. 1a, c and e) and corresponding orientation histograms (Figs. 1b, d and f), obtained from normally reared kittens of different ages. At P29, the relative size of responsive domains is largest for horizontal orientation (0° or equivalently 180°) and smallest for vertical orientation (90°) (Fig. 1b). The sample-averaged orientation histogram across 10 normally reared kittens in the age range of P26-32 exhibited a bias toward horizontal orientation (Fig. S1a...
The horizontal bias diminished at P51 (Fig. 1d), and shifted to a weak vertical bias at P94 (Fig. 1f). The sample-averaged orientation histogram across 15 normally reared kittens of P33–84 showed a weak bias toward vertical orientation (Fig. S1b online). A one-way repeated measures ANOVA on the relative sizes of cortical domains indicates non-uniform orientation distribution (p = 0.0001). The following pairs showed significant differences: 90° and 30°, 90° and 150°, and 90° and 0° (p = 0.0011, p<0.0001 and p = 0.014, respectively; post hoc analysis by Tukey’s HSD test), but the other pairs did not show significant differences.

Figure 1g shows the relative domain size for 90° (red open circles) and for 0° (blue open squares) repeatedly measured in 23 normally reared kittens, as a function of the postnatal day of imaging. The red and blue curves were obtained by a smoothing procedure to show slight but significant age-dependent changes of orientation selectivity in normal kittens; the horizontal bias was prominent before P35, but thereafter it was taken over by the vertical bias.

**Goggle rearing**

Here, we first describe the results of two weeks of goggle rearing (GR). Measurements in goggle-reared kittens were completed within 5 hrs after the removal of the goggles. Immediately after the 2-week GR started at P16–P17, the over-representation of horizontal orientation was exclusive (Figs. 2g and h), whereas that of vertical orientation was somewhat moderate (Figs. 2a and b). For GR that started at P27–P29, the over-representation of vertical orientation also became nearly exclusive (Figs. 2c and d). The representation of horizontal orientation was still predominant, although that of unexposed orientations appeared (Figs. 2i and j). The imbalance between vertical and horizontal orientations at P16–P17 may reflect, at least partly, the horizontal bias detected in normal kittens (Figs. 1a and b). When 2-week GR was started at P49–54, the induced over-representation of the exposed orientation, either vertical or horizontal, diminished drastically (Figs. 2e, f, k and l). A close examination of Figs. 2e and f revealed that the effect of vertical GR was reversed to a slight under-representation of the vertical orientation.

Figure 3 shows which changes in the orientation representation during 1-w GR also sufficiently modify orientation selectivity if adequately timed. In Fig. 3a, single-orientation maps of a kitten at P28 after normal rearing show a patchy organization for stimulus orientations (0° and 90°). After 1-w vertical GR from P31 to P38 in the same kitten, patchy organization became more prominent for the exposed orientation (90°), and the domain size increased particularly around the posterior part of the visual cortex. In contrast, patchy organization for the unexposed orientation (0°) almost disappeared. To quantify these changes, profiles of response strengths were measured along the line (7.2 mm long) drawn anteroposteriorly in the single-orientation maps (0°; Fig. 3b; 90°; Fig. 3c) and compared between the data from before and after GR. Figure 3d depicts the net decrease or increase of response strengths caused by GR. Our results show that vertical GR decreases response strengths at 0° by 1.5×10^{-5}±1.2×10^{-5} (s.e.) per pixel on average (Figs. 3b and d), whereas those at 90° increase by 1.1×10^{-7}±1.5×10^{-7} (s.e.) (Figs. 3c and d). This implies that, in local response strengths, the suppression occurring for the unexposed orientation is comparable with or even larger than the enhancement occurring for the exposed orientation. Such suppression and enhancement (Fig. 3d) together account for the marked over-representation of the exposed orientation, observed in the orientation polar maps (Figs. 2a, c, g and i).

---

**Figure 1.** Orientation selectivity in normally reared kittens. Data are shown for postnatal days indicated. (a), (c) and (e): orientation polar maps. Color and brightness indicate preferred orientation and orientation selectivity. White curves delineate functionally defined area 17. Scale bars, 2 mm. Color code is placed below e. (b), (d) and (f): orientation histograms. Height of bins indicates the relative size of cortical domains preferentially responding to stimulus orientations. Horizontal lines indicate the relative size of iso-orientation domains for a uniform orientation representation. The same bin is doubly depicted at 0° and 180° for symmetric representation. Cortical coordinate placed on c: A, anterior; P, posterior; R, right; and L, left. (g): the relative size of cortical domains preferentially responding to vertical orientation (red dots) and horizontal orientation (blue dots) plotted against the postnatal day of optical imaging repeated in 23 normally reared kittens. Two curves were obtained using Stineman’s smoothing procedure. doi:10.1371/journal.pone.0005380.g001

online). A one-way repeated-measures ANOVA on the relative sizes of cortical domains for 6 stimulus orientations supports this observation (p = 0.0001). The relative size of domains representing 0° was significantly larger than that of the other orientations, except for 150° (p<0.0001 for 60° and 90°; p = 0.0002 for 120°; p = 0.0005 for 30°; ANOVA, post-hoc analysis by Tukey’s HSD test). This indicates that orientation representation is biased toward the horizontal orientation for very young normal kittens. This is analogous to the innate bias toward the contralateral eye, as has been known in ocular dominance [27].
Critical period of orientation selectivity

Figure 4 illustrates the time profile of the sensitivity for the modification of orientation selectivity as revealed by 2-week GR in 18 kittens. Here, we quantified the sensitivity as the relative size of the cortical domains for the exposed orientation, which is normalized by the representation bias in normal kittens (Fig. 1g) at respective days of optical imaging (see legend of Fig. 4). The sharp enhancement obtained for vertical- and horizontal-orientation exposures are generally consistent with each other, except for a discrepancy at 2–3 postnatal weeks. Taken together with the horizontal bias observed in normal kittens as mentioned above, this discrepancy in the sensitivity profiles may suggest that the innate bias toward horizontal orientation is inherent to neuronal mechanisms that determine orientation selectivity. The critical period can be defined as the postnatal period during which 2-week GR applied (started and/or ended) effectively causes the modification of orientation selectivity. Thus defined, the critical period starts 2 weeks after birth and lasts for 6 weeks.

Figure 4 also shows that the critical period is followed by a late phase of under-representation of the exposed orientation for GR starting between P55 and P151. We also observed the case of an adult cat reared with vertical goggles from P396 for one month; this cat showed a small relative size of cortical domains representing the exposed orientation (0.11) for 90°. Therefore, continuous single-orientation exposure after the critical period, even in adulthood, leads to the under-representation of the exposed orientation.

NMDA receptor antagonist blocks modifiability of orientation selectivity

To test whether NMDA receptors contribute to the critical period of orientation selectivity, we infused the left visual cortex with an NMDA receptor antagonist, D(-)-2-Amino-5-phosphopentanoic acid (D-AP5), by an osmotic minipump system [28]. In three kittens, we carried out 1-week concurrent GR and minipump infusion.

In the kitten starting GR and D-AP5-infusion at P24, the darkness in the orientation polar map in the D-AP5-infused hemisphere (Fig. 5a lower) indicates an overall reduction of orientation selectivity. This contrasts with the almost full representation of the exposed vertical orientation in the non-infused (control) right hemisphere (Fig. 5a upper). The response-strength maps in Figs. 5b, f and j show that, although visual responses completely disappeared within the diameter of 1–2 mm from the site of D-AP5-infusion (red dots), low levels of visual responses were preserved outside of this unresponsive region. In orientation histograms, the D-AP5-infused hemisphere showed a weak over-representation of vertical orientation (Fig. 5c), as contrasted to the virtually full representation in the control hemisphere (Fig. 5d). Similar results were obtained in the kitten with GR and D-AP5 infusion starting at P30 (Figs. 5e–h). In the other kitten started at P37 at the late critical period (Fig. 4), D-AP5 was virtually ineffective (Figs. 5i–l).

Figure 6 illustrates the sensitivity profile of orientation selectivity for 1-week GR. The critical period defined for 1-week GR (grey dots) rapidly ended before P40, earlier than the end of the critical period defined for 2-week GR (Fig. 4). The ineffectiveness of D-AP5 infusion at P57 on the induction of over-representation of the exposed orientation seems consistent with the end of the critical period for 1-week GR. The sensitivity was drastically suppressed in the D-AP5-infused hemispheres of kittens goggle-reared from P24 and P30 (blue dots), whereas the D-AP5-untreated hemispheres still exhibited large sensitivity (yellow dots) comparable to that for goggle-reared kittens without D-AP5 infusion (grey dots).
In Fig. 7, we illustrate how the once-induced over-representation of the exposed orientation changes afterwards. On the left half of the diagram, the normalized relative sizes of cortical domains representing exposed vertical (circles) or horizontal (squares) orientations are plotted at the end of 2-week GR (solid symbols) and also at the end of the succeeding normal rearing (hollow symbols), respectively. Plotted points for identical kittens are linked by the lines. In 4 kittens for which 2-week GR started at P29, 32, 37 and 39, 3-day normal vision immediately before the second optical imaging eliminated the over-representation; the normalized relative domain sizes returned to 0 (level of normal kittens). However, in 4 kittens in which 2-week GR started relatively earlier at P16, P21 and P25, recovery during the succeeding 3-days of normal vision was partial.

On the right half of the diagram, triangles plot for 4–6 week GR. In 4 kittens with long-term vertical GR started at P21–P25 (triangles), the over-representation of the exposed orientation was retained at moderate levels between 0.39 and 0.64 in the first optical imaging. As tested in two of these kittens (P24–P73; P25–P74), the over-representation was preserved even after 3 weeks of normal rearing. A similar tendency was observed in another kitten in which horizontal GR started at P23 and switched to normal rearing at P51. To summarize, recovery of GR-induced changes are time-dependent in threefold: 1) when 2-week GR covers the relatively late phase of the critical period (at P29–39), normal rearing quickly eliminates the over-representation; 2) when 2-week GR covers the relatively early phase of the critical period (earlier than P29), the diminution of the over-representation is moderate; and 3) GR for 4 weeks or more outlasting the critical period acts to...
Discussion

In this study, we applied a combination of head-fixed cylindrical-lens goggles and optical imaging of intrinsic signals to investigate postnatal orientation plasticity in kitten primary visual cortex. By measuring the over-representation of exposed orientation using optical imaging at the end of 1- or 2-week GR, we revealed a brief period exhibiting a sharp increase in the sensitivity of orientation modifiability between postnatal 3–5 weeks (Fig. 4 and Fig. 6). This increase was nearly abolished after 1-week infusion of D-AP5 (Fig. 6). The presently delineated critical period for orientation plasticity overlaps the most sensitive period for ocular dominance plasticity (postnatal 4–5 weeks) [29]. It may be worth mentioning that the method presently applied to kittens would have broad application to various animal species. We recently reported an over-representation of the exposed orientation in goggle-reared young rats [30], in which individual neurons showing orientation selectivity are distributed in the visual cortex without grouping in the form of orientation maps.

NMDA-dependent orientation plasticity

Neuronal mechanisms underlying the increased responses to the exposed orientation have yet to be investigated, but based upon the wealth of knowledge about cortical synaptic plasticity so far accumulated, the following conjecture may be made. In cortical synapses, activity-dependent LTP could be induced at synapses with postsynaptic NMDA receptors by the coincidence of pre- and postnatal activity. This would provide a mechanism for the increased responses to the exposed orientation, and would also allow for the preservation of the moderate over-representation of the exposed orientation.

Figure 5. Effect of NMDA receptor antagonist on orientation plasticity. (a), (e) and (i): orientation polar maps constructed immediately after concurrent GR and D-AP5 infusion for 1 week in three kittens for different onset ages of GR. (b), (f) and (j): response-strength maps constructed by summing all single-orientation maps in the respective animals. Brightness indicates response strength. White dots in a, e and i, and red dots in b, f, and j indicate locations of infusion centers of D-AP5 in the left-hemisphere or saline in the right hemisphere. (c), (g) and (k), and (d), (h) and (l) show orientation histograms for the D-AP5-treated visual cortex and for the control visual cortex, respectively. Other conventions are as in Fig. 1.

doi:10.1371/journal.pone.0005380.g005

Figure 6. Effects of D-AP5 infusion on the sensitivity profile. Sensitivity profiles are shown for control and D-AP5-treated hemispheres, separately. For reference, we plot the sensitivity profile for 1-week GR without D-AP5 infusion obtained from 6 kittens. Symbols are defined in the figure.

doi:10.1371/journal.pone.0005380.g006
NMDA-independent orientation plasticity

The decrease of responses to unexposed orientations does not involve NMDA receptor function and possibly is mediated by a process such as NMDA-independent LTD. One possible mechanism of the reduction of responses to unexposed orientations may be activity-dependent LTD induced by the activation of cannabinoid receptors located at presynaptic sites [36,37], but the elucidation of the mechanism requires further investigation.

Modifiability of orientation selectivity after the critical period

The presently revealed brief critical period for orientation modifiability (Fig. 4) contrasts with the long tail phase of ocular dominance plasticity [29]. This discrepancy suggests different neural mechanisms of ocular dominance and orientation plasticity. In this regard, it may be worth noting that the over-representation of the exposed orientation shifts over to under-representation after the critical period for orientation plasticity (Fig. 4). There may be opposing forces that expand and contract the area of cortical domains representing the exposed orientation in early life. If it is the case, the end of the critical period may be determined by the age at which the contraction dominates the expansion, resulting in the rapid decrease of the sensitivity profile for orientation plasticity different from the long tail of the sensitivity profile for ocular dominance plasticity.

In accordance with the above-mentioned under-representation of the exposed orientation, decreased neuronal responses to the exposed orientation have been shown in unit recording experiments. An early experiment on adult cats performed by Creutzfeldt and Heggelund revealed a decrease in the number of neurons optimally responding to vertical orientation exposed in a striped environment for 1 hr, twice a day during 2 weeks [38]. As speculated by these authors, adaptation-dependent response modification may be a mechanism of orientation plasticity after the end of the critical period. A recent experiment has shown a similar form of orientation-dependent adaptation in the orientation tuning curves of neurons, which rapidly changed in adult cats when one orientation was presented more frequently than the other orientations [39]. However, the time scale of such adaptation is distinct in the order of that of the under-representation observed in the present study. A question remains on whether common mechanisms of adaptive response reduction work in wide range of time scales.

On the other hand, it has been reported that intracortical microstimulation induced the expansion of an iso-orientation domain around the stimulation site in adult cats [40]. In addition, the pairing of single-orientation visual stimulation and the injection of depolarizing current to visual cortical neurons shifted preferred orientations towards the exposed orientation in adult cats as well as in kittens [41]. These findings seem to be contradictory to the under-representation of the exposed orientation observed in the present study. Considering that our goggle-reared cats after the end of the critical period simply experienced a striped environment for 1 hr, twice a day during 2 weeks [38].

Persistence of modified orientation selectivity

Goggle rearing for 1 or 2 weeks modifies orientation preferences to nearly match the exposed single orientation, but such changes quickly diminish upon returning kittens to normal rearing (Fig. 7). Extension of GR for 4 weeks or longer is required for obtaining a persistent over-representation of exposed orientation, which was preserved at least 4 weeks after the kittens were returned to normal vision (Fig. 7).

Very recently, Ohzawa et al. (SFN Abstract 2007-A-109015) performed single-unit recordings in cats that were reared with our
goggles for vertical orientation exposure. The persistent, modest over-representation of the exposed orientation observed after long-term GR is supported by recordings using the subspace reverse-correlation-mapping technique [42]. They have shown that a substantially increased number of neurons in cat areas 17 and 18 responded optimally to the exposed orientation after long-term (>43 days) GR started at P21–28. This effect was robust, irrespective of the length of normal rearing intervals (0–50 days) before single-unit recording.

Comparisons with previous studies

There have been two hypotheses about the effect of visual experience on orientation plasticity. One is the selection hypothesis and the other the instruction hypothesis. In the former hypothesis, after single-orientation exposure, neurons innately selective for unexposed orientations just decrease their responses to the unexposed orientations without changes of preferred orientations. In the latter hypothesis, neurons selective for unexposed orientations change their preferred orientations towards the exposed orientation. Blakemore and Cooper [9] supported the instruction hypothesis, because they found preferred orientations of recorded units were strongly biased toward the exposed orientation in single-unit recording in cats that had experienced striped environment. Later, Stryker et al. [11] found in cats that had experienced parallel lines with opaque goggles [10] that single-orientation exposure changed a large portion of units nonselective or unresponsive, although responsive units preferring for the exposed orientation relatively increased. Particularly, they observed an orderly arrangement of selective units according to preferred orientation along the electrode tracks, as found in normal cats, but clustering of nonselective or unresponsive units frequently appeared.

In our optical imaging on kittens exposed to a single orientation, stimulus-related intrinsic signals in response to unexposed orientations were reduced in cortical domains originally selective for the unexposed orientations (Fig. 3b), and a proportion of pixels without orientation selectivity tended to increase [15], consistently with single-unit recording by Stryker et al. [11]. However, stimulus-related intrinsic signals in response to the exposed orientation tended to increase in these domains (Figs. 3c and d), resulting in the changes of orientation preference. As seen in Fig. 5C of Stryker et al. [11], it seems that nonselective/unresponsive units were rather distributed randomly, even if they might be weakly clustered along the track. In addition, this figure showed a wide range of a constant preferred orientation around the exposed horizontal orientation from 3–6 mm in the track distance. Therefore, this figure does not definitely support that neurons innately selective for unexposed orientations decrease selectivity or responsiveness without changes of their preferred orientations.

In the single-unit recordings by Ohzawa et al. (SFN Abstract 2007-A-109015) on cats reared with our goggles for vertical orientation exposure, when they penetrated electrodes into the medial bank, they also recorded responsive units in these cats fewer than in normal cats, as in Stryker et al. [11]. Among the responsive units, most units were selective for the exposed orientation, but intriguingly the number of units selective for the orthogonal horizontal orientation was larger than that of units selective for oblique orientations (Fig. S3 online). The presence of the orthogonally oriented units may not be accounted for by the selection hypothesis alone, because horizontally oriented units should be most unlikely detected if the lack of experience decreases selectivity or responsiveness. On the contrary, in the instruction hypothesis, preferred orientations closer to the exposed orientation may be shifted more effectively towards the exposed orientation, and the orthogonally oriented units can possibly remain with their preferred orientations unchanged due to the largest separation in the preferred orientation from the exposed orientation, as we have previously postulated a possible mechanism for orientation modification (Fig. 6 in [15]).

Differences of experienced patterns during single-orientation exposure may be worth noting. Stryker et al. [11] exposed kittens to stationary lines through their goggles. Carlson et al. [16] also presented stationary stripe patterns with various spatial frequencies to monocularly deprived infant monkeys. To examine the effect of exposure to stationary oriented stimuli, we have tried to rear 4 kittens chronically with spherical-lens-fitted goggles for exposure to a stationary stripe with a spatial frequency of about 0.5 and 0.15 c. p. d [24]. Although the exposed orientation was over-represented at the first optical imaging experiments after 2- or 3-week GR in 3 kittens, the under-representation of the exposed orientation occurred in the other kitten, in which the orthogonal orientation was over-represented (Fig. S4 online). Even in the kittens showing the over-representation of the exposed orientation, the layouts of orientation preferences were labile during prolonged GR. In 3 of the 4 kittens, the over-representation disappeared or changed to the under-representation after long-term GR. Such labile alteration of orientation maps is characteristic of exposure to a stationary oriented pattern. This is contrasted with the finding that orientation maps altered by exposure to a dynamic single orientation through cylindrical-lens-fitted goggles are consolidated preserving the modest over-representation of the exposed orientation (Fig. 7). It should be noted that the instability in orientation map alteration for stationary stripe pattern exposure was not due to the repeated optical imaging, because orientation maps altered by rearing with cylindrical-lens-fitted goggles changed gradually in each optical imaging, and were finally stabilized at the moderate over-representation of the exposed orientation. The fact that Carlson et al. [16] recorded units selective for the orthogonal orientation to the exposed orientation in the open eye may be such labile modification of orientation selectivity induced by the stationary stripe pattern exposure. The disappearance of the orientation selectivity modification for prolonged exposure to stationary oriented stimuli is suggested to have a weak impact on the structural modification of orientation maps. This may be consistent with the fact that Stryker et al. [11] observed gradual and progressive changes of the preferred orientation of selective units along the electrode track in kittens exposed to stationary lines, which are similar to those observed in normal cats.

Conclusion

Orientation plasticity appears to have two phases: First, the visual cortical circuit is rendered highly modifiable in orientation selectivity during a brief postnatal critical period for 6 weeks; and second, modified orientation selectivity consolidates during continuous long-term single-orientation exposure. The combined application of GR and optical imaging to not only cats but also rodents would be instrumental to further analyses of molecular and cellular mechanisms underlying this unique system phenomenon, orientation plasticity.

Materials and Methods

Preparation of animals

We used 58 kittens (normal: 23; gonad-reared: 35) obtained from the institutional colony. The procedures of surgery and optical imaging were approved by the Institutional Animal Research Committee at RIKEN (No. H13-B040), and performed in accordance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Science” of the Japanese
Physiological Society. We performed chronic optical imaging repeatedly twice or three times for 12 among 35 goggle-reared kittens to see the recovery or consolidation of altered orientation maps, and for 15 among 23 normally reared kittens to see the age-dependent changes of orientation representation bias. We made efforts to minimize the animals’ suffering and to reduce the number of animals examined as much as possible. We refrained from performing optical imaging experiments within 7 days after surgery, and from repeating optical imaging on identical animals at an interval shorter than 7 days. After experiments, we sacrificed animals injecting the overdose of pentobarbital (50–100 mg/kg, i.v.).

Goggles for single-orientation exposure

We used goggles fitted with planoconvex acrylic cylindrical lenses (lens thickness, 10.0 mm; lens aperture diameter, 15.0 mm; lens power, +67 D), through which the animals were able to see elongated images of their environments [24]. We used two types of goggles: v- and h-goggles, which elongated visual images vertically and horizontally, respectively.

Surgical procedure

Surgery was conducted according the procedure described in our previous papers [14,15]. Initial anesthesia was induced using ketamine hydrochloride (5.0 mg/kg, i.m.) following sedation with medetomidine hydrochloride (0.1 mg/kg, i.m.). The animals were fixed on a stereotaxic apparatus and were artificially ventilated with a 60:40% mixture of N₂O and O₂ containing 0.5–1.0% isoflurane. Heart rate, end-tidal CO₂ concentration, and rectal temperature were continuously monitored during surgery. A metal head holder for fixing the goggles and a metal chamber for optical imaging were cemented on the animal’s skull using dental resin, and from repeating optical imaging on identical animals at an interval shorter than 7 days. After experiments, we sacrificed animals injecting the overdose of pentobarbital (50–100 mg/kg, i.v.).

Analysis of optical imaging data

The analysis methods that we used were described in a previous paper [15]. It is noteworthy to explain the methods in some detail here to show that observed map changes are attributable to biological changes rather than artificial changes originating from our analysis methods. One trial of optical imaging was composed of six frames (duration of each frame, 1 s). To extract stimulus-related intrinsic signals, we subtracted signals recorded in the first frame (without stimulus presentation) from those signals recorded in succeeding frames with stimulus presentations. Then, we averaged the subtracted signals over the 4th to 6th frames for each trial. Next, we applied the generalized indicator function method to these averaged signals [22], which efficiently excluded noisy signals originating from volume and oxygenation changes in thick blood vessels and spatially slowly varying fluctuations of signals inherent in the recorded intrinsic signals. It should be noted that the image data processing based on the generalized indicator function method underestimates the effects of over-representation of exposed orientation induced by single-orientation exposure, because the data processing method eliminates spatially slowly varying point/space components of intrinsic signal [21], which may partially contain responses to the exposed orientation. Having excluded the spatially slow noise components, we summed the stimulus-related signals over all trials for each stimulus orientation and applied Gaussian low-pass filtering with a 150-μm standard deviation to eliminate high-frequency noise. In this way, we constructed a single-orientation map for each stimulus orientation. We also defined an integrated response-strength map by summing single-orientation maps over all stimulus orientations.

To determine the preferred orientation at each pixel inside the recorded area, we used the vector sum method [19], which is based on the Fourier analysis in the circular symmetric orientation dimension. Thus, at each pixel, we obtained the preferred orientation and the modulation amplitude in the second harmonic component, which is regarded as orientation selectivity. The orientation polar map was constructed with the preferred orientation and orientation selectivity as color and brightness, respectively.

For further analysis, we discarded pixels eliciting response strengths lower than a half of the response strength averaged over all pixels inside the recorded area. According to this criterion, the domains containing the remaining pixels nearly lined up with functionally defined area 17, which was exclusively activated by
stimuli of a 0.5-c.p.d. spatial frequency. To construct an orientation histogram, we counted the number of pixels involved in each orientation, 30˚ width, and normalized them by the total number of pixels involved in all orientations.

Supporting Information

Figure S1 Sample-averaged orientation histograms of normal kittens. (a, b): Orientation histograms obtained by averaging 10 normal kittens (P26–32), and 15 normal kittens (P45–84), respectively. *: p<0.05; **: p<0.005; ***: p<0.001 (post hoc analysis by Tukey’s HSD test). In this analysis, we used data obtained from repeated optical imaging on 9 animals of the age of P26–32 and 6 animals of the age of P33–84 as different samples. Inmate orientation bias changes in the early stage of postnatal development. To investigate GR-induced alteration of orientation representation, it is important to examine such innate changes in normally reared kittens. Preferred orientation is significantly biased toward horizontal orientation at P26–32 (a). However, this bias tends to change toward weak but significant vertical-orientation bias (b).

Found at: doi:10.1371/journal.pone.0005380.s001 (3.83 MB TIF)

Figure S2 Effects of D-AP5 infusion in single-orientation maps. Single-orientation maps of a kitten, in which 1-week GR and concurrent D-AP5 infusion were started at P24 and P30, respectively. Corresponding cortical domains in the right control hemisphere are delineated by blue lines and those in the left D-AP5-treated hemisphere are delineated by red lines. The observation that response strength to the unexposed orientation decreases during GR (Fig. 3) raises the question of whether NMDA receptor activation is involved in this response reduction. To answer this question, we used single-orientation maps of the kitten in which 1-week GR concurrent with D-AP5 infusion into the left hemisphere started at P 24. Responses to any orientations were weak, as seen inside of the regions delineated by red lines.

Note, however, that these responses in the D-AP5-infused left hemisphere are of about the same intensity as the responses to unexposed orientations 0˚, 30˚ and 150˚ in the right hemisphere (enclosed by blue lines). These findings indicate that the blockade of NMDA receptors does not affect the decline of response strengths to unexposed orientations. Hence, it is unlikely that an NMDA receptor is involved in the reduction of responses to unexposed orientations.

Found at: doi:10.1371/journal.pone.0005380.s002 (4.48 MB TIF)

Figure S3 Orientation histogram obtained from single-unit recordings along the tracks when electrodes were penetrated into the medial bank of 3 cats exposed to vertical orientation. By courtesy of Dr. Ohzawa.

Found at: doi:10.1371/journal.pone.0005380.s003 (2.06 MB TIF)

Figure S4 Time course of the normalized relative area of the exposed orientation in kittens exposed to a stationary stripe pattern. Blue: horizontally oriented stripe of 0.15 cpd; Red: vertically oriented stripe of 0.15 cpd; Sky blue: horizontally oriented stripe of 0.5 cpd; Pink: vertically oriented stripe of 0.5 cpd. The origin in the day of GR indicates the onset day when GR was started.

Found at: doi:10.1371/journal.pone.0005380.s004 (5.47 MB TIF)

Acknowledgments

We thank Y. Akimoto and K. Ozawa for their assistance in the experiments and maintenance of all the kittens examined. We also thank M. Ito for his critical reading and many helpful comments on the manuscript.

Author Contributions

Conceived and designed the experiments: ST. Performed the experiments: ST TT KO KI. Analyzed the data: ST TT JR. Wrote the paper: ST.

References

1. Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philos Trans R Soc Lond B Biol Sci 274: 377–409.
2. Kasamatsu T, Pettigrew JD (1976) Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens. Science 194: 206–209.
3. Isa NP, Trachtenberg JT, Chapman B, Zuo KR, Snyder MP (1999) The critical period for ocular dominance plasticity in the Ferret’s visual cortex. J Neurosci 9: 6965–6978.
4. Gordon JA, Snyder MP (1996) Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J Neurosci 16: 3274–3286.
5. Gilbert CD, Wiesel TN (1992) Receptive field dynamics in adult primary visual cortex. Nature 356: 150–152.
6. Hubel DH, Wiesel TN (1965) Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J Neurophysiol 28: 994–1002.
7. Crair MC, Gillespie DC, Stryker MP (1998) The role of visual experience in the development of columns in cat visual cortex. Science 279: 566–571.
8. Blakemore C, Van Shyters RC (1975) Inmate and environmental factors in the development of the kitten’s visual cortex. J Physiol 249: 663–716.
9. Blakemore C, Cooper GF (1970) Development of the brain depends on visual environment. Nature 228: 477–478.
10. Hirsch HV, Spinelli DN (1970) Visual experience modifies distribution of horizontally and vertically oriented receptive fields in cats. Science 168: 869–871.
11. Stryker MP, Sherk H, Leventhal AG, Hirsch HV (1978) Physiological consequences for the cat’s visual cortex of effectively restricting early visual experience with oriented contours. J Neurophysiol 1: 896–909.
12. Rauschecker JP, Singer W (1983) The effects of early visual experience on the cat’s visual cortex and their possible explanation by Hebb synapses. J Physiol 310: 215–239.
13. Sengpiel F, Stavinski P, Bonhoeffer T (1999) Influence of experience on orientation maps in cat visual cortex. Nature Neurosci 2: 727–732.
14. Tanaka S, Ribo T, Miyashita M (2004) Roles of visual experience and intrinsic mechanism in the activity-dependent self-organization of orientation maps: Theory and experiment. Neural Networks 17: 1363–1373.
15. Tanaka S, Ribo T, Imamura K, Tani T (2006) Orientation-restricted continuous visual exposure induces marked reorganization of orientation maps in early life. NeuroImage 30: 462–477.
16. Carlson M, Hubel DH, Wiesel TN (1986) Effects of monocular exposure oriented lines on monkey striate cortex. Dev Brain Res 25: 71–81.
17. Grünvald A, Lüke E, Frostig RD, Gilbert CD, Wiesel TN (1986) Functional architecture of cortex revealed by optical imaging of intrinsic signals. Nature 342: 361–364.
18. Ratliff EH, Grünvald A (1991) A tandem-lens epifluorescence microscope: hundred-fold brightness advantage for wide-field imaging. J Neurosci Methods 36: 127–137.
19. Bonhoeffer T, Grünvald A (1991) Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns. Nature 353: 429–431.
20. Bonhoeffer T, Kim D-S, Malinek D, Shoham D, Grünvald A (1995) Optical imaging of the layout of functional domains in area 17 and across the area 17/18 border in cat visual cortex. Eur J Neurosci 7: 1973–1988.
21. Gilbert CD, Das A, Iro M, Kapadia W, Westheimer G (1996) Spatial integration and cortical dynamics. Proc Natl Acad Sci USA 93: 615–622.
22. Yokoo T, Knight BW, Sirioch L (2001) An optimization approach to signal extraction from noisy multivariate data. NeuroImage 14: 1309–1326.
23. Ribo T, Tanaka S, Tanaka H, Aijima A (2006) Online analysis method for intrinsic signal optical imaging. J Neurosci Methods 153: 8–20.
24. Tanaka S, Tani T, Ribo T, Yamazaki T (2007) Chronically mountable goggles for persistent exposure to single orientation. J Neurosci Methods 160: 206–214.
25. Kleinschmidt A, Bear MF, Singer W (1987) Blockade of “NMDA” receptors disrupts experience-dependent plasticity of kitten striate cortex. Science 238: 355–358.
26. Bear MF, Kleinschmidt A, Gu Q, Singer W (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J Neurosci 10: 909–925.
27. Wiesel TN, Hubel DH (1963) Single-cell responses in striate cortex of kittens deprived of vision in one eye. J Neurophysiol 26: 1003–1017.
20. Kasamatsu T, Schmidt EK (1997) Continuous and direct infusion of drug solutions in the brain of awake animals: implementation, strengths and pitfalls. Brain Res Protocols 1: 57–69.

21. Olson CR, Freeman RD (1980) Profile of the sensitive period for monocular deprivation in kittens. Exp Brain Res 39: 17–21.

22. O’Hashi K, Miyashita M, Tanaka S (2007) Experience-dependent orientation plasticity in the visual cortex of rats exposed to a single orientation. Neurosci Res 58: 86–90.

23. Rauschecker JP (1991) Mechanisms of visual plasticity: Hebb synapses, NMDA receptors, and beyond. Physiol Rev 71: 387–415.

24. Buller AL, Monaghan DT (1997) Pharmacological heterogeneity of NMDA receptors: characterization of NR1a/NR2D heteromers expressed in Xenopus oocytes. Eur J Pharmacol 320: 87–94.

25. Fagiolini M, Katagiri H, Miyamoto H, Mori H, Grant SGN, et al. (2003) Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. Proc Nat Acad Sci USA 100: 2854–2859.

26. Tran DH, Gong R, Tang S-J (2007) Differential roles of NR2A and NR2B subtypes in NMDA receptor-dependent protein synthesis in dendrites. Neuropharmacol 53: 252–256.

27. Mower GD, Chen L (2003) Laminar distribution of NMDA receptor subunit (NR1, NR2A, NR2B) expression during the critical period in cat visual cortex. Brain Res Mol Brain Res 119: 19–27.

28. Huang Y, Yasuda H, Sazuki A, Tsuji T (2008) Roles of endocannabinoids in heterosynaptic long-term depression of excitatory synaptic transmission in visual cortex of young mice. J Neurosci 28: 7074–7083.

29. Liu C-H, Heynen AJ, Hussain Shaler MG, Bear MF (2008) Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58: 340–345.

30. Creutzfeldt OD, Heggelund P (1973) Neural plasticity in visual cortex of adult cats after exposure to visual patterns. Science 180: 1025–1027.

31. Dragoi V, Sharma J, Sur M (2000) Adaptation-induced plasticity of orientation tuning in adult visual cortex. Neuron 28: 287–298.

32. Godde B, Leonihardt R, Gords SM, Dine H (2002) Plasticity of orientation preference maps in the visual cortex of adult cats. Proc Nat Acad Sci USA 99: 6352–6357.

33. Fregnac Y, Shulz D, Thorpe S, Bienenstock E (1988) A cellular analogue of visual cortical plasticity. Nature 335: 367–370.

34. Ringach DL, Sapiro G, Shapley R (1997) A subspace reverse-correlation technique for the study of visual neurons. Vision Res 37: 2455–2464.

35. Tsumoda K, Yamane Y, Nishizaki M, Tanifuji M (2001) Complex objects are represented in macaque inferotemporal cortex by the combination of feature columns. Nature Neurosci 4: 832–838.

36. Movshon JA, Thompson ID, Tolhurst DJ (1978) Spatial and temporal contrast sensitivity of neurons in areas 17 and 18 of the cat’s visual cortex. J Physiol 218: 101–110.

37. Oliki K, Matuda Y, Ajima A, Kim D-S, Tanaka S (2000) Arrangement of orientation pinwheel centers around area 17/18 transition zone in cat visual cortex. Cereb Cortex 10: 593–601.

38. Imamura K, Kasamatsu T, Shirotawa T, Ohashi T (1999) Restoration of ocular dominance plasticity mediated by adenosine A1 receptor blockade in adult visual cortex. Proc R Soc Lond B Biol Sci 266: 1507–1516.