Cell-specific restoration of stimulus preference after monocular deprivation in the visual cortex

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Monocular deprivation evokes a prominent shift of neuronal responses in the visual cortex toward the open eye, accompanied by functional and structural synaptic rearrangements. This shift is reversible, but it is unknown whether the recovery happens at the level of individual neurons or whether it reflects a population effect. We used ratiometric Ca²⁺ imaging to follow the activity of the same excitatory layer 2/3 neurons in the mouse visual cortex over months during repeated episodes of ocular dominance (OD) plasticity. We observed robust shifts toward the open eye in most neurons. Nevertheless, these cells faithfully returned to their pre-deprivation OD during binocular recovery. Moreover, the initial network correlation structure was largely recovered, suggesting that functional connectivity may be regained despite prominent experience-dependent plasticity.

How do mature cortical circuits achieve a stable representation of sensory input while maintaining their capacity to adapt to changes in an animal’s sensory environment? In the binocular visual cortex, afferent inputs from the two eyes converge onto single neurons. Monocular deprivation (MD) shifts ocular dominance (OD) toward the nondeprived eye. This OD shift can be induced in juvenile carnivores (1, 2) and primates (3), as well as in rodents (4–10). In mice, OD shifts also occur in adults and are reversible (7, 11–13), providing the opportunity for longer-lasting experiments and the study of converging phases of experience-dependent plasticity at the single-cell level. Due to technical limitations, however, longitudinal assessments of OD plasticity have so far been based on either short-term single-cell recordings (14), recordings from different animals (15, 16), or repeated population recordings lacking single-cell resolution (7, 11–13, 17). It is therefore not yet clear how individual neurons shift their functional properties in response to prolonged perturbations of sensory input, and whether their initial stimulus selectivity is lost or maintained after recovery. In this study, we measured the changes in the response properties of single neurons during MD, recovery from MD, and repeated MD.

We performed ratiometric Ca²⁺ imaging (fig. S1), using the genetically encoded Ca²⁺ indicator GCaMP6s (18), which we coexpressed with the bright structural marker mRuby2 (19) under control of the CamKIIα promoter, using adeno-associated virus. This allowed us to follow visually evoked activity of the same excitatory (fig. S2) layer 2/3 neurons in the binocular cortex of adult mice over months during multiple episodes of OD plasticity (Fig. 1A).

A substantial fraction (64%) of excitatory layer 2/3 neurons responded to eye-specific drifting grat ing stimuli of varying orientation with significant changes in their somatic Ca²⁺ concentration (Figs. 1, B and C, and 2C). We closed the lid of the eye contralateral to the recorded hemisphere for 5 to 8 days under conditions that facilitate adult OD plasticity (see supplementary methods). The majority (62%) of all continuously responsive cells showed significant OD changes (Fig. 2, B and C), and most cells shifted toward the nondeprived eye. Contrary to what classical theories would predict, ~10 to 20% of the cells responded more strongly to deprived-eye stimulation after MD (Fig. 2B, fig. S3, and supplementary text).

The overall number of visually responsive cells decreased significantly after MD (Fig. 2C). As in previous reports using a similar deprivation paradigm in adult mice (8, 9), we observed strong deprived-eye depression and more moderate open-eye strengthening (Fig. 2D). Unlike OD plasticity in juvenile mice (10), a major driving force was deprived-eye depression of initially contratermally dominated neurons lacking prominent competing open-eye input (Fig. 2D). Whereas the sum of the eye-specific changes in response strength remained largely constant in originally binocular neurons (Fig. 2D), cells with a strong contralateral bias were prone to be silenced during MD, explaining the decrease in responsive neurons.

OD plasticity is based on substantial modifications to visual cortex circuits, including changes in excitatory and inhibitory synapse number and strength (4, 5, 13, 20–22). Deprived-eye depression and open-eye strengthening leave deprived-eye inputs at a competitive disadvantage during binocular recovery. Both factors may render it unlikely that eye-specific responses recover precisely to their original values in single cells. Instead, recovery may be mediated by compensatory changes at the network level, irrespective of the initial selectivity of individual neurons. Alternatively, some “memory trace” of the initial eye-specific input strength may guide individual neurons to return to their original response properties after MD.

We allowed 8 to 10 days of recovery with normal binocular vision and assessed the eye-specific responsiveness of significantly plastic cells (MD shift magnitude >1σ of baseline OD fluctuations). The population of plastic neurons recovered fully (Fig. 2E). Compensatory changes of neurons other than the plastic cells are therefore unnecessary to explain recovery from MD. To assess the degree to which individual neurons regain their initial OD after MD, we compared the OD after recovery with the pre-MD OD of plastic neurons. Single-cell OD just before MD and after recovery were significantly correlated [Pearson’s correlation coefficient (r) = 0.51], although individual neurons showed deviations from the unity line of perfect recovery (Fig. 2F and fig. S4A).

To gauge the magnitude of MD-induced changes and the degree of single-cell recovery, we quantified the variability of eye-specific response properties under baseline conditions (Fig. 2, G and H, and fig. S5). Population OD was stable over ~8 days of baseline recording (three imaging sessions) and showed a characteristic contralateral bias (fig. S5B). To assess the stability of single-cell tuning, we measured the functional properties of the same repeatedly imaged neurons over different time intervals (4 days and 12 to 14 days). If neurons in the binocular visual cortex would continuously and randomly change their response properties in a process akin to a random walk, one would expect drift, in which net changes in response properties accumulate over time. However, we found no systematic difference between the changes in OD, orientation preference, and orientation selectivity for the short and long measuring interval (Fig. 2H and fig. S5, C to H), indicating the presence of mechanisms ensuring the constancy of neural responses over time.

Still, baseline variability was considerably higher than expected from measurement uncertainty alone (Fig. 2H, within-session correlation). Thus, similar to but less pronounced than in the somatosensory cortex (23), neurons in the visual cortex show response variation even during normal sensory experience. The difference may be related to the different rates of synaptic turnover in these sensory areas (4, 24). For the same cells, baseline variability was higher in awake mice than under anesthesia (fig. S7).

The single-cell OD index (ODI) deviated prominently from baseline after MD (Fig. 2H and fig. S4). The baseline-recovery correlation of individual plastic neurons, however, was not different from the 4-day within-baseline correlation (Fig. 2H), and the distribution of pairwise ODI differences was equally indiscernible from the baseline (fig. S4). Orientation tuning was largely unaffected by MD (fig. S6).

Having shown that the OD of individual cells is restored to its original value within the bounds set by baseline variability, we next asked whether the underlying eye-specific inputs also regain their original strengths. On the population level, ipsi- and contralateral response magnitudes were largely reestablished after recovery (Fig. 3A). To quantify the degree to which the combined eye-specific changes during recovery mirror the changes
during MD on the single-cell level, we expressed
the change in ipsi- and contralateral single-cell
responsiveness during MD and recovery as vec-
tors, and used the angular difference between
these shift trajectories as a metric for the sim-
ilarity between initial shift and subsequent re-
covery (Fig. 3B). Recovery of OD in individual
dominant cell respond after the second
MD periods, a contralaterally (cell 1) and an ipsilaterally (cell 2) dominated cell
exhibit repeated deprived-eye depression or open-eye strengthening, respect-
ively, and show response recovery during binocular vision in between (scale
bars ΔR/R0 = 200%; 10 s; for further examples, see figs. S5A, S6B, and S10C).
(C) Sorted structural (left) and functional (right) cell maps of individual
neurons imaged over 10 sessions (26 continuously responsive cells of a single
animal showing a significant OD shift during the first MD). OD is depicted as the
pixel-wise peak fluorescence ratio change in response to ipsi- and contralateral
eye preferred grating direction \(\text{ODI} = \frac{(\Delta R/R_{\text{contra}} - \Delta R/R_{\text{ipsi}})(\Delta R/R_{\text{contra}} + \Delta R/R_{\text{ipsi}})}{2} \). Red hues indicate ipsilateral dominance (ODI < 0), and blue hues
indicate contralateral dominance (ODI > 0). Pixel intensity is scaled by
response amplitude. Cells are sorted by cell identity (horizontally) and by ODI
of the last pre-MD session (vertically, descending ODI; red dots: example
neurons shown in Fig. 3, D and E).

We found a broad distribution of pairwise noise and signal correlations with low average corre-
lation values (figs. S9, A and D). To assess the stability of network activity patterns, we followed
the similarity of correlation structures throughout the plasticity episodes. We correlated the
matrices of pairwise correlation coefficients with each other (29) to obtain a similarity measure (Fig.
4A). MD induced a drop in both signal and noise correlation matrix similarity in comparison to the
last baseline session. After MD signal and noise correlation similarity returned to original values
(Fig. 4B). Thus, specific network activity patterns are altered by MD, but recovery from MD renders
them indistinguishable from those before MD. Therefore, the correlation structure of neuronal
activity patterns, and thus perhaps functional connectivity, seem to be remarkably resilient to
massive perturbations of sensory input.

One explanation for this remarkable stability in the mature visual cortex may be that a subset of
stable synaptic connections is protected from being overwritten by plasticity, thereby providing the
“tuning backbone” that on one hand counters the degradation or drift of eye-specific responses
during baseline and on the other hand guides pre-
cise recovery after major plasticity episodes (Fig.
2H). Indeed, recent data suggest that cells with correlated responses form functional subnetworks

Fig. 1. Ratiometric long-term single-cell imaging of repeated OD plastici-
Fig. 1. Ratiometric long-term single-cell imaging of repeated OD plasticity. (A) Timeline of the experiment. We performed chronic ratiometric Ca\(^{2+}\)
imaging (mRuby2-P2A-GCaMP6s) of the somata of excitatory layer 2/3 neu-
rons in a small volume of the binocular visual cortex in adult mice [rapid
sequential acquisition of a 185 × 185 × 100 µm volume; four slices at an
image plane depth increment \(\Delta z_{\text{slice}}\) of 25 µm, frame rate 7.5 Hz, scale bar
40 µm]. The same image locations and cells were revisited for up to
2 months during multiple episodes of OD plasticity (contralateral eye MD (contra
MD), binocular recovery, and repeated contra MD). (B) Ca\(^{2+}\) signals (flu-
orescence ratio changes, \(\Delta R/R_0\)) in two example neurons in response to ipsi-
and contralateral eye drifting grating stimulation (12 directions, four repetitions)
over selected sessions covering all OD plasticity episodes. After each of two MD
periods, a contralaterally (cell 1) and an ipsilaterally (cell 2) dominated cell

Both MD and recovery lead to prominent struc-
tural and functional synaptic rearrangements
(4, 5, 13, 20–22), which are indicative of major
network rewiring. This raises the question of to
what extent only single-cell responses but also connectivity among cells are recovered after
such phases of dramatic functional plasticity. We
analyzed the structure of pairwise correlations in
trial-to-trial fluctuations (i.e., noise correlations)
and mean stimulus responses (i.e., signal correla-
tions) over time (Fig. 4). Pairwise noise correlations
in spike trains are often used as a proxy for func-
tional connectivity (25, 26). Pairwise signal corre-
lations, in turn, measure evoked response similarity
and are a more comprehensive measure of neu-
ronal feature selectivity than are unidimensional
tuning indices such as ODI (27). Pairs of neurons
showing high signal correlation have a high prob-
ability of being directly connected (28).

Both single-cell OD changes during the first
and second MD were, again, highly correlated
on the single-cell level (Fig. 3F) and could not
simply be explained by a population-wide effect
(Fig. 3F, shuffled data). Therefore, individual
eurons underwent repeated plastic changes, with
highly reproducible bidirectional modifications in
eye-specific inputs, over repeated periods of
experience-dependent plasticity (MD, recovery,
second MD).

It has been proposed that the synaptic con-
nections that have been established during an
early MD episode are reused during a second
MD (4, 11). To test whether the same neurons
undergo repeated plasticity and to assess whether
these cells repeat the previously observed eye-
specific changes, we performed a second MD 2
weeks after the last recovery session (Fig. 3, D
to F). Single-cell OD changes after the second
MD correlated significantly with the OD changes
induced by the first MD in the same neurons
(Fig. 3E). The relative contributions of ipsi- and
that are interconnected by exceptionally strong synapses (29). It is tempting to speculate that these connections remain stable even under conditions that are interconnected by exceptionally strong associations for reversible plastic modifications (4). Our data clearly show that experience-dependent plasticity of neurons in the visual cortex occurs on a cell-by-cell basis. Many of the experience-dependent changes are carried by a subpopulation of plastic neurons, which regain their original response properties after plasticity is reversed and follow the same eye-specific shift trajectories when challenged with a second deprivation episode. However, many neurons are resilient to plastic changes and some even change their response properties in the opposite direction of what classical theories would have predicted. The role of the counterintuitive shifters is less clear, but neurons resilient to plastic changes could be important for providing stability of the cortical network in the face of constantly occurring changes and adaptations. Neurons that change strongly but recover precisely, together with neurons not susceptible to plastic changes, may be the visual cortex’s solution to the opposing needs for plasticity and stability.

Fig. 2. Full recovery of eye-specific tuning after MD. (A) Example time courses of single-cell ODI over baseline, contralateral eye MD, and recovery (gray lines: continuously responsive individual cells; black line: mean ± SEM, n = 15 cells, 1 animal). (B) Single-cell ODI distribution during baseline (mean of three pre-MD sessions; ODI = 0.29 ± 0.47, SD) and after 5 to 8 days of contralateral eye MD (post-MD: ODI = 0.08 ± 0.67, SD, n = 456 cells, 10 animals, P < 10\(^{-12}\), Wilcoxon signed-rank test). The lines connect individual neurons; line color indicates shift significance expressed in units of standard deviations over baseline fluctuations. Colored histogram bins indicate class definitions for contralateral (blue), binocular (black), and ipsilateral (red) cells. (C) Left, fraction of neurons showing a significant MD-evoked ODI change (n = 456 cells). Right, fraction of responsive and unresponsive neurons before and after MD (P < 10\(^{-6}\), n = 1245 cells, χ\(^2\) test). (D) Difference in eye-specific responsiveness before and after MD (gray shading: ±SEM) as a function of pre-MD ODI (n = 593 cells continuously responsive during three baseline sessions, grouped into pre-MD ODI sextiles comprising similar numbers of cells [98 to 99 cells per class], paired t tests; OD group classes are indicated by the color bar on the x axis). (E) Population ODI recovery of plastic neurons (ODI change >1σ of baseline fluctuations; mean ± SEM; n = 133 cells from 8 animals continuously responsive during baseline, MD, and recovery; baseline versus MD P < 10\(^{-7}\), baseline versus recovery P > 0.09, Wilcoxon signed-rank test; gray lines: individual cells). (F) Single-cell ODI correlation of significantly plastic neurons before MD and after recovery (pre-MD versus recovery Pearson’s r = 0.51, P < 10\(^{-9}\), n = 133 cells, 8 animals). (G) Correlation of the same cells during baseline sessions spaced 4 days apart (r = 0.56, P < 10\(^{-12}\)). Histograms in (F) and (G) show the distributions of pairwise differences (not drawn to scale; not significantly different, P = 0.35, two-sample Kolmogorov-Smirnov test, n = 133 cells, 8 animals; fig. S4). (H) Left, baseline 4-day and 12- to 14-day eye-specific visual tuning correlations of all responsive neurons (n = 738 cells, 11 animals, 4-day versus 12- to 14-day P > 0.65, Fisher’s r-to-z transformation). To estimate the influence of trial-to-trial variability, we show the distribution of bootstrapped within-session correlations (black line). Right, correlation of the same plastic neurons during baseline (sessions 4 days apart), baseline and MD (fig. S4), and baseline and recovery (n = 133 cells, 4-day versus MD P < 0.006; 4-day versus recovery (Rec.) P > 0.59, Fisher’s r-to-z transformation). Correlations are presented with 95% confidence intervals (CIs). Here and in the following figures, *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 3. Precise reversion and repetition of eyespecific changes during recovery and repeated MD. (A) Population recovery of eyespecific response amplitudes of plastic neurons (mean ± SEM; n = 133 cells, 8 animals; contra: baseline versus MD P < 10−10, baseline versus recovery P > 0.3; ipsi: baseline versus MD P < 0.0002, baseline versus recovery P < 0.02; gray lines: individual cells; paired t-test). (B) Schematics illustrate the calculation of the difference between the directions of initial shift and recovery (upper panel) or between shifts induced by the first and second MD (lower panel; see supplementary methods). (C) Single-cell angular differences (θMD,recovery) between MD and recovery shift trajectories. The difference of 0° indicates perfectly sign-inverted shift in joint ipsi- and contralateral responsiveness after recovery (gray bars; angular differences, line: bootstrapped angular differences with shuffled cell IDs between MD and recovery sessions ± 95% CI; n = 828 cells). (D) Example time course of single-cell ODI over baseline, MD, recovery, and repeated contra MD [gray lines: individual, continuously responsive cells; black line: mean ± SEM, n = 36 cells, 1 animal; dark red and light red lines indicate example neurons shown in (E) and in Fig. 1C (red dots)]. (E) Correlation of single-cell ODI shift (post-MD − pre-MD ODI) between first and second MD (Pearson’s r = 0.42, P = 0.009, n = 38 cells, 2 animals; green symbols: cells significantly plastic during first MD). (F) Single-cell angular differences (θMD1,MD2) between MD1 and MD2 shift trajectories. A difference of 0° indicates identical shifts in joint ipsi- and contralateral responsiveness during first and second MD (line: bootstrapped angular differences with shuffled cell IDs between MD1 and MD2 sessions ± 95% CI; n = 165 cells). Significance in (C) and (F) is assessed by comparing matched cell IDs against the 95, 99, and 99.9% CIs of bootstrapped angular differences from scrambled cell IDs.

Fig. 4. Recovery of correlation structure. (A) Example pairwise signal (rsignal) and noise (rnoise) correlation structure of 15 consistently responsive neurons of a single animal imaged over baseline, MD, and recovery sessions. For visualization of correlation structure only, cells are clustered and sorted independently for rsignal and rnoise on the last pre-MD session (supplementary methods). The same sort matrix is used for all other time points. As a measure of correlation structure similarity, we used Pearson’s r of the correlation matrices with baseline session 3 (ρbaseline). (B) Average session-wise similarity of rsignal and rnoise structures with the last baseline session (mean ± SEM). Both signal and noise correlation structure similarity show a drop after MD (rsignal, P = 0.11, rnoise: P = 0.011, n = 9 animals, Wilcoxon signed-rank test), but after recovery both rsignal and rnoise correlation structures are not statistically different from baseline (rsignal: P = 0.11, rnoise: P = 0.38, n = 7 animals).
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June 9, 2016
Science 352 (6291), 1319-1322. [doi: 10.1126/science.aad3358]

Editor's Summary

Ocular dominance plasticity reconsidered
How neuronal circuits are established and reformed during development and learning is unclear. One idea is that cortical circuits have virtually unlimited plasticity and are rebuilt routinely from random components. An alternative view is that some of these connections are more or less preformed and rigid. Working in mice, Rose et al. looked at how visual cortical neurons change their response after monocular deprivation. After recovery, the response properties of the neurons returned to their pre-deprivation pattern. Thus, it is not the strong connectivity backbone but perhaps inhibitory and weaker connections that are changed temporarily during sensory deprivation, whereas a core circuitry returns to default mode even after several days of altered activity.

Science, this issue p. 1319