Synaptic Scaling and Homeostatic Plasticity in the Mouse Visual Cortex In Vivo

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SUMMARY

Homeostatic plasticity is important to maintain a set level of activity in neuronal circuits and has been most extensively studied in cell cultures following activity blockade. It is still unclear, however, whether activity changes associated with mechanisms of homeostatic plasticity occur in vivo, for example after changes in sensory input. Here, we show that activity levels in the visual cortex are significantly decreased after sensory deprivation by retinal lesions, followed by a gradual increase in activity levels in the 48 hr after deprivation. These activity changes are associated with synaptic scaling, manifested in vitro by an increase in mEPSC amplitude and in vivo by an increase in spine size. Together, these data show that homeostatic activity changes occur in vivo in parallel with synaptic scaling.

INTRODUCTION

Homeostatic plasticity is believed to be essential in maintaining a target firing rate in neurons, preventing too high or too low activity levels caused by synaptic strengthening or weakening due to long-term potentiation (LTP) and depression (LTD) or pathological conditions (Turrigiano et al., 1998; Burrone et al., 2002; Turrigiano and Nelson, 2004). In the most commonly studied form of homeostatic plasticity, a global reduction of neuronal activity leads to synaptic scaling, where increases in miniature excitatory postsynaptic current (mEPSC) amplitude are hypothesized to result from the insertion of a similar fraction of new 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid receptors (AMPA receptors) into all of a neuron’s synapses, thus preserving their relative weights (Turrigiano et al., 1998; Turrigiano and Nelson, 2004). In either cortical or hippocampal cultures, synaptic scaling accompanies the homeostatic restoration of activity levels to those observed before activity blockade (Turrigiano et al., 1998; Burrone et al., 2002). Synaptic scaling and other homeostatic mechanisms, such as changes in neuronal excitability and alterations in the excitation/inhibition balance, have been demonstrated ex vivo in acute slices after sensory deprivation (Desai et al., 2002; Goel et al., 2006; Goel and Lee, 2007; Maffei and Turrigiano, 2008; Gao et al., 2010; Lambo and Turrigiano, 2013), and evoked responses have been shown to increase in vivo during the critical period after long-term deprivation (Mrsic-Flogel et al., 2007; Kaneko et al., 2008). It remains unclear, however, whether or not cortical activity levels are restored in vivo by homeostatic mechanisms such as synaptic scaling.

Here we employ complete, bilateral retinal lesions to address this question in mouse visual cortex in vivo. We chose this deprivation paradigm because we expected it to cause a marked reduction in cortical activity by removing both visually evoked and spontaneous retinal activity from the sensory periphery (whereas other deprivation paradigms, such as dark rearing, only remove visually evoked activity). We demonstrate that after the complete removal of retinal input, activity in the visual cortex of awake mice is strongly decreased. After this initial activity drop, we find that cortical activity gradually increases over a period of 48 hr, coincident with synaptic scaling, observed as an increase in average mEPSC amplitude measured in slices prepared from animals that have previously undergone visual deprivation by retinal lesions. This synaptic scaling is paralleled by an increase in layer 5 pyramidal neuron spine size in vivo. Spine size has been shown to correlate with the number of AMPA receptors in the spine (Matsuzaki et al., 2001; Bélique et al., 2006). Together, these data suggest that synaptic homeostatic mechanisms are triggered by a decrease in activity levels in vivo and that these mechanisms are associated with a subsequent increase in cortical activity.

RESULTS

To investigate how neuronal activity adjusts after input removal in vivo, we measured activity levels using repeated two-photon imaging in the visual cortex of awake, head-fixed mice that were free to run on a spherical treadmill (Dombec et al., 2007; Keller et al., 2012). Mice expressed the genetically encoded calcium indicator GCaMP3 (Tian et al., 2009) and, in a second set of experiments, GCaMP5 (Akerboom et al., 2012). We repeatedly measured activity from the same layer 2/3 and layer 5 neurons 48 and 24 hr before retinal lesions and at time points 6, 18, 24 and 48 hr postlesion (Figures 1, S1A, and S1B available online;
Figure 1. Changes in Visual Cortical Activity Levels In Vivo

(A) Top: mean-normalized maximum projection (see Experimental Procedures and Figure S1I) of the same region of layer 5 cells (460 μm below the surface) for time points before (-48 hr, -24 hr) and after (+6 hr, +18 hr, +24 hr) retinal lesion. Bottom: individual ΔF/F traces of highlighted neurons in the top panels.

(B) Same as (A), for a region in layer 5 of a sham-lesioned control animal.

(legend continued on next page)
51 imaging regions, 2,249 cells). At 6 hr postlesion, overall cortical activity levels were significantly decreased relative to sham-lesioned controls (anesthesia and ocular atropine application, without laser ablation of the retina, Figure 1A). Activity levels then increased over the 24–48 hr after retinal lesions (Figures 1 and S1). This general trend of a lesion-induced activity drop followed by rapid recovery over 24–48 hr held true over a range of thresholds to determine activity levels (Figure S1C) and was also observed with different measures, including mean integrated fluorescence (a measure of average spiking activity over the entire imaging period, Figure 1C) and the fraction of cells active over the entire imaging period (Figure S1D, see Experimental Procedures). Note that activity levels at 6 hr after sham lesion in control animals are also reduced compared to prelesion levels. Plotting the difference in activity levels between control and lesion animals at each time point (Figure S1E) revealed that the recovery of activity levels in the lesioned animals occurred between time points 18 and 24 hr.

We found that despite the removal of all retinal input, activity levels in visual cortex were still relatively high immediately following lesions, consistent with previous work demonstrating that a substantial fraction of activity in the visual cortex is not related to visual input (Gallant et al., 1998; Vinje and Gallant, 2000; Fiser et al., 2004; Iturri et al., 2012; Keller et al., 2012). Specifically, neuronal activity in the visual cortex has been shown to be both modulated (Niell and Stryker, 2010; Anderson et al., 2011; Ayaz et al., 2013) and driven (Keller et al., 2012) by locomotion of the animal. To verify that the observed drop in average cortical activity was not simply the result of the decrease in overall motor activity after visual deprivation, we examined cortical activity levels during episodes of locomotion. Indeed, changes in activity levels followed the same overall trend (decreased activity at 6 hr, increasing at 24–48 hr) during locomotion (Figures S1F and S1G). Furthermore, we measured the fraction of time that the animals spent running before and after lesions. We found no difference between lesioned animals and sham-lesioned controls (Figure S1H), except at 6 hr post-lesion when lesioned animals were more active than sham-lesioned animals yet had lower cortical activity levels. These results show that the changes in cortical activity are caused by removal of retinal input rather than reduced locomotion.

Having observed a recovery in activity levels in visual cortex, we next investigated whether established mechanisms of homeostatic plasticity were underlying these changes. We thus performed whole-cell recordings from layer 5 pyramidal neurons in acute slices prepared from visual cortex of adult animals at 6, 18, 24, and 48 hr after complete retinal lesions. We measured amplitude and frequency of mEPSCs and found that mEPSC amplitude was not changed relative to sham-lesioned controls either 6 or 18 hr after a complete retinal lesion (Figure 2A). In line with previous results from cortical cultures (Turrigiano et al., 1998) and from layer 4 (Desai et al., 2002) and layer 2/3 cells (Desai et al., 2002; Goel et al., 2006; Maffei and Turrigiano, 2008, but see Lambo and Turrigiano, 2013) in visual cortex slices, there was a significant increase in the amplitude 24–48 hr (Figures 2A and 2B) after silencing both retinae, indicating an increase in the strength of excitatory synapses. We noted a transient decrease in mEPSC frequency 18 hr after input removal (Figure 2C), which is consistent with reduced cortical activity measured with GCaMP at 18 hr after lesioning (Figure 1). There was, however, no significant change before or after that time point (Figures 2C and 2D). The increase in mEPSC amplitude 24–48 hr after deprivation parallels the changes in activity observed in vivo, suggesting that synaptic scaling contributes to the changes in cortical activity.

We also assessed the effect of visual deprivation on two other proposed homeostatic mechanisms—changes in neuronal excitability and the level of inhibition—in layer 5 cells. To test for a potential increase in intrinsic excitability, we measured the voltage threshold for inducing an action potential (AP). There was no detectable change in excitability following lesions (voltage threshold for inducing an AP in lesioned mice relative to controls: 18 hr, 117% ± 11%, \( p < 0.3 \); 24 hr, 126% ± 10%, \( p < 0.09 \); 48 hr, 113% ± 15%, \( p < 0.3 \); mean ± SD, t test). To determine whether the overall level of inhibition was reduced—which could also lead to increased activity levels in excitatory cells—we investigated whether there was a change in miniature inhibitory postsynaptic currents (mIPSCs) onto layer 5 pyramidal cells (in the same recordings as in Figures 2A–2D). Neither mIPSC amplitude—a correlate of inhibitory synapse strength—nor mIPSC frequency—a measure for the number of inhibitory synapses—changed in the first 24 hr following retinal lesions (Figure 2E). However, as we have previously reported (Keck et al., 2011), mIPSC frequency in layer 5 pyramidal cells decreased at 48 hr (Figure 2E), consistent with a loss of inhibitory synapses (Keck et al., 2011), without a change in mIPSC amplitude. This result suggests that inhibition is reduced by either a loss of inhibitory synapses (as in Keck et al., 2011) or presynaptic plasticity of inhibitory synapses, e.g., an increase in release failures. Thus, neither changes in excitability nor altered levels of inhibition seem to contribute strongly to the observed homeostatic increase in activity during the first 24 hr after input removal.

Having found synaptic scaling of excitatory synapses in vitro, we next wanted to determine whether it also occurs in vivo. Previous work indicates that increases in spine volume measured in fixed tissue may reflect synaptic scaling (Wallace and Bear, 2004), and numerous studies have demonstrated a clear correlation of dendritic spine size with both synapse strength and the number of synaptic AMPA receptors (Matsuzaki et al., 2001; Noguchi et al., 2005, 2011; Asadian et al., 2007; Zito et al., 2009), which, by their insertion and removal, are thought to underlie synaptic scaling (Turrigiano et al., 1998). We therefore used spine size, measured in vivo, as a proxy for synapse strength. We used chronic two-photon imaging in adult mice expressing GFP under the thy-1 promoter.

(C) Mean integrated fluorescence at each time point, normalized to the mean integrated fluorescence during the first two time points before lesion (black) or sham lesion (red). Mean of all cells is shown (L2/3, L5, GCaMP3, GCaMP5). (For C, 6 hr \( p = 10^{-4} \), 18 hr \( p < 10^{-4} \), 24 hr \( p = 0.9 \), 48 hr \( p = 0.03 \), \( * p < 0.05 \), \( ** p < 0.01 \), \( \text{t test}; n = 12 \) animals, 51 regions; number of cells: 2,249; layer 5: \( n = 533 \) in lesion, \( n = 573 \) in control; layer 2/3: \( n = 606 \) in lesion, \( n = 538 \) in control.) Error bars, SEM. See also Figure S1.
M-line [Feng et al., 2000]) to image layer 5 pyramidal cells’ dendrites and spines located in the upper layers (1 and 2/3) of monocular visual cortex before and after complete bilateral retinal lesions (Figure 3A). While spine density did not change during the 48 hr following input removal (Figure 3B), we found a clear increase in spine size (approximated by integrated spine brightness, see Experimental Procedures), which became evident 24 hr after the retinal lesion (Figure 3C) and was maintained 48 hr postlesion, thus following the same time course as the changes in mEPSC amplitude and the increases in cortical activity in vivo. This result suggests that synapse size increases after a drop in cortical activity in vivo.

To further characterize the relationship between structural changes in vivo and functional changes in vitro, we determined whether the magnitude of changes in spine size was sufficient to explain the changes in mEPSC amplitudes. We first examined the distribution of the relative changes of spine size and mEPSC amplitude 24 hr following retinal lesions. Both distributions changed significantly after lesions (Figures 3D and 3E) and, importantly, the distributions of spine size and mEPSC amplitude changed in a similar way, such that while the distributions of spines and mEPSC amplitudes in lesioned mice were significantly different from the respective control distributions (lesion versus control distribution: spines, K-S test, p < 0.05, mEPSC amplitude, K-S test, p < 0.05), they were not significantly different from one another (lesion distributions: spines versus mEPSC amplitude, K-S test, p > 0.9).

Second, one of the basic premises of synaptic scaling is its multiplicative nature (Turrigiano et al., 1998; Turrigiano and Nelson, 2004). To determine whether the changes observed here are multiplicative, we multiplied the normalized control distribution of the mEPSC amplitudes by the necessary constant to scale the mean of the control distribution to match the lesion distribution mean (1.24). While the means of these two distributions would by definition be the same, the changes would only be multiplicative if the entire distribution was overlapping, indicating that all of the amplitudes had “scaled” by the same factor. This is exactly what we found, as the scaled control distribution was statistically indistinguishable from the lesion distribution (K-S test, p > 0.3; Figure 3F), indicating that mEPSC amplitudes scaled multiplicatively, as has been reported previously (Turrigiano et al., 1998).

In order to make the same measurements for the spine size changes, we first measured the control distribution of
spine sizes 24 hr after sham lesions, normalized to 48 hr before, i.e., spine size changes over time. This control distribution provides the baseline level of spine size fluctuations in vivo. To determine whether the lesion-induced changes in spine size beyond these baseline fluctuations were multiplicative, we multiplied the control distribution by the constant required to match the mean of the normalized lesion distribution (1.22). Like in the mEPSC measurements, the means of these two distributions would by definition be the same, but the control distribution would be multiplicatively scaled if it overlaps the
lesion distribution. Again, we found that the scaled control distribution and the lesion distribution were statistically indistinguishable (K-S test, p > 0.3, Figure 3G), indicating that the two distributions differ only by a multiplicative scaling factor. Thus, these results suggest that the relative changes in spine size following lesions scale multiplicatively, when compared to the relative changes in spine size over the same time course in control animals. Combined, these data show that structural and functional changes not only follow the same time course but that synaptic scaling also modifies their distributions in a similar way, further strengthening the view that the observed changes in structure reflect the measured functional changes in synaptic strength.

**DISCUSSION**

We have used a combination of two-photon imaging and electrophysiology to investigate homeostatic plasticity in the adult visual cortex in vivo. In behaving mice, we found that cortical activity levels were strongly decreased after complete retinal lesions and that they gradually recovered over 24–48 hr after the onset of deprivation. Over the same time course, we observed two homeostatic mechanisms—synaptic scaling, and, during the later phase, a reduction of inhibition. Synaptic scaling manifested itself as an increase in mEPSC amplitude, which we found to be paralleled in timing and magnitude by increases in spine size in vivo. These data provide additional support for the hypothesis that functional changes reflect structural changes and suggest that homeostatic mechanisms may be associated with the increase of cortical activity levels in vivo.

**Relationship between Structure and Function**

Increases in mEPSC amplitude are hypothesized to occur by the insertion of AMPARs into all of a neuron’s synapses (Turrigiano et al., 1998; Turrigiano and Nelson, 2004). In turn, the number of synaptic AMPARs is correlated with spine size (Matsuzaki et al., 2001; Béique et al., 2006; Zito et al., 2009). Therefore, the fact that the increase in spine size observed in our experiments occurs over the same time course as the increased mEPSC amplitude offers additional support for AMPAR insertion as the basis for synaptic scaling. On the other hand, a change in mEPSC frequency is often associated with a change in the number of excitatory synapses impinging onto a cell (Turrigiano et al., 1998; Turrigiano and Nelson, 2004). Thus, one might have expected to see a transient decrease in spine density to correlate with the drop in mEPSC frequency observed 18 hr after retinal lesions, which was not the case. One possible explanation for this discrepancy is that, while changes during synaptic scaling have been suggested to occur postsynaptically (Wierenga et al., 2005), recent work suggests that there may be a presynaptic component, particularly in mature neurons (Han and Stevens, 2009). As a result, a reduction in mEPSC frequency could be explained by a decrease in presynaptic release frequency, which would go undetected in our postsynaptic structural measurements.

We found temporally coordinated changes in spine size and mEPSC amplitude (Figure 3). However, spine size was determined in the distal apical dendrites, while the patch-clamp recordings, made at the soma, are likely to reflect more proximal inputs because of space-clamp limitations. We nonetheless believe that both measures capture the same underlying process, namely multiplicative synaptic scaling. Key to this concept is that synaptic scaling affects all of a neuron’s synapses, maintaining the relative synaptic weights of the inputs (Turrigiano et al., 1998; Desai et al., 2002; Goel et al., 2006). Accordingly, it should in theory not matter from which population of a neuron’s synapses the sample is drawn.

**Relationship between Homeostatic Mechanisms and Activity Changes In Vivo**

While homeostatic mechanisms—specifically synaptic scaling—have been shown to underlie homeostatic restoration of activity levels in cortical (Turrigiano et al., 1998) or hippocampal (Burrone et al., 2002) cultures, it had not been tested whether synaptic scaling or other homeostatic mechanisms are associated with changes in cellular activity levels in vivo. Here, in behaving mice expressing a genetically encoded calcium indicator, we show that activity levels in the visual cortex are decreased after retinal input removal. We believe that this decrease in activity measured at 6 and 18 hr postlesion is predominantly a result of the removal of all retinal activity (both visually evoked and spontaneous), possibly combined with presynaptic changes that we measured as a decrease in mEPSC frequency at 18 hr. Note, however, that we cannot rule out that cortical activity levels are influenced by rapid plasticity in these first 6–18 hr. We then showed that synaptic scaling occurs with the precise time course over which cortical activity levels increase, which we interpret as a homeostatic response to this activity loss. These data demonstrate in vivo that homeostatic mechanisms correlate with changes in cellular activity levels.

Consistent with previous studies showing that cells in visual cortex (Livingstone et al., 1996; Gallant et al., 1998; Vinje and Gallant, 2000; Fiser et al., 2004; Keller et al., 2012) or the LGN (Linden et al., 2009) show substantial activity unrelated to visual input, the removal of all visual input by complete lesions of both retinae decreased activity in visual cortex of behaving mice by only 50%–60%. This limited decrease in activity was, however, clearly sufficient to evoke homeostatic mechanisms after deprivation, showing that homeostatic changes can occur without completely silencing the cortex. As studies of homeostatic plasticity thus far have either completely removed activity—typically in culture by TTX application (Turrigiano et al., 1998; Burrone et al., 2002)—or did not measure activity levels after deprivation (Desai et al., 2002; Goel and Lee, 2007; Maffei and Turrigiano, 2008; Gao et al., 2010; Lambo and Turrigiano, 2013), these results provide important evidence that mechanisms of homeostasis also occur when activity levels are changing more moderately.

The homeostatic mechanisms demonstrated with our in vivo paradigm—synaptic scaling and, with a delay, reduced inhibition—probably play a role in the observed increases in cortical activity; however, they are unlikely to be solely responsible. Given that activity is decreased, but not eliminated, after complete visual deprivation and that inputs from other brain regions are probably still active, we expect that other mechanisms of synaptic modification—such as LTP and LTD—are still ongoing.
In line with this hypothesis, we have previously reported—using the same deprivation paradigm—that the turnover of spines, which is typically associated with synapse specific (or nonhomeostatic) plasticity (Trachtenberg et al., 2002; Zuo et al., 2005; Holtmaat et al., 2006), increases 72 hr after deprivation (Keck et al., 2008).

Taking these data together, we suggest the following scenario: immediately after a complete lesion of both retinae, cortical activity decreases to approximately half the original value. Synaptic scaling then manifests itself after 24 hr, at which time mEPSC amplitudes and spine sizes have increased, followed by a decrease in inhibition after 48 hr. The overall increase in synaptic strength, together with a reduction in inhibition, leads to a nearly complete restoration of cortical activity levels; however, feedforward inputs are not restored. Thus, after 72 hr, dendritic spine turnover increases (Keck et al., 2008), potentially reflecting the search for novel active inputs and further circuit rearrangement.

**EXPERIMENTAL PROCEDURES**

An extended description of the experimental procedures is included in the Supplemental Experimental Procedures. All experimental procedures carried out at the Max Planck Institute of Neurobiology were performed in accordance with the institutional guidelines of the Max Planck Society and the local government (Regierung von Oberbayern). All experimental procedures carried out at the Friedrich Miescher Institute in Basel were approved by the Veterinary Department of the Canton of Basel-Stadt, Switzerland.

**Retinal Lesions**

Complete retinal lesions were carried out as described previously (Keck et al., 2008).

**Electrophysiology**

At 6, 18, 24, or 48 hr after the retinal/sham lesion, coronal slices were prepared from C57BL/6J mice. Visualized whole-cell patch-clamp recordings of layer 5 pyramidal neurons were performed at room temperature (24°C). mEPSCs or mIPSCs were recorded in voltage clamp at ~70 mV (corrected for liquid junction potential). mEPSCs and mIPSC analysis was done with custom software, blind to the experimental condition. Events were detected based on amplitudes greater than 5 pA and 20%–80% rise times of less than 1 ms (Desai et al., 2002).

**Intrinsic and Two-Photon Structural Imaging**

Experiments were carried out as described previously (Keck et al., 2008). Cranial windows were implanted (Holtmaat et al., 2009) in adult mice expressing enhanced GFP (eGFP) under the thy-1 promoter (GFP-M line [Feng et al., 2000]). The visual cortex was localized using intrinsic signal imaging prior to retinal lesions. Apical dendrites in layer 1 and 2/3 (0–150 μm below the pial surface) of layer 5 cells in monocular visual cortex were imaged using a custom-built two-photon laser-scanning microscope. Chronic imaging was carried out under anesthesia 24 and 48 hr before and 6, 18, 24, and 48 hr after a retinal/sham lesion. Analysis was done blind to the experimental condition on raw, three-dimensional image stacks. Integrated spine brightness (reflecting the search for novel active inputs and further circuit rearrangement) was calculated as described previously (Keller et al., 2005), increases 72 hr after deprivation (Trachtenberg et al., 2002; Zuo et al., 2005; Holtmaat et al., 2006), increases 72 hr after deprivation (Keck et al., 2008).

**Awake Two-Photon Functional Imaging**

Mice were injected with AAV2/1-hsyn1-GCaMP3 or, in a separate set of mice, AAV2/1-ef1a-GCaMP5 between P39 and P65. At the time of virus injection, a cranial window was implanted (Holtmaat et al., 2009). Functional calcium imaging was performed with a custom-built two-photon microscope. Head-fixed animals were free to run on a spherical treadmill, as described previously (Keller et al., 2012). Experiments consisted of four alternating 5 min blocks in which the mouse either received visual feedback coupled to locomotion or the stimulation screens were off (i.e., darkness). Data were full-frame registered using a custom registration algorithm. Cells were selected based on mean and mean-normalized maximum projections of the data. Cellular activity was calculated using either integrated fluorescence or binary classification into active and nonactive cells (for details, see Supplemental Experimental Procedures). Our results were quantitatively consistent for a range of activity thresholds.

**Statistics**

As stated in the text, time-matched sham-lesioned controls were compared to lesioned animals using either a Kolmogorov-Smirnov (K-S) test for cumulative distributions, an ANOVA with Bonferroni post hoc test, a Student’s t test, or either a Mann-Whitney test or Wilcoxon rank-sum test for nonnormally distributed data.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.08.018.

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