Neuron, Volume 80

Supplemental Information

Synaptic Scaling and Homeostatic Plasticity

in the Mouse Visual Cortex In Vivo

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Figure S1, related to Figure 1: A, B) Mean integrated fluorescence at each time point, normalized to the mean during the first two time points before lesion for A) layer 2/3 (black) and layer 5 (green); B) GCaMP3 (black) or GCaMP5 (grey). Mean of all cells is shown. C) Mean integrated fluorescence at each time point for lesioned animals, normalized to the mean during the first two time points. Each line shows a different threshold value used for analysis. D) Fraction of active cells at each time point, normalized to the average number of cells active during the first two time points before lesion (black) or sham-lesion (red) (\(p<0.05\), \(p<0.01\)). E) Difference between lesion and control animals’ cortical activity levels measured by integrated brightness at each time point. Calculated by subtracting the black line (lesion) from the red line (control) in Figure 1C. F) Absolute activity before and after lesions (black) or sham-lesion (red) for running in the light (solid) or running in the dark (dashed). G) Mean integrated fluorescence at each time point, normalized to the mean during the first two time points before lesion (black) or sham-lesion (red), exclusively during locomotion. Mean of all cells is shown (L2/3, L5, GCaMP3, GCaMP5). \(6\) hours \(p=0.0001\), \(18\) hours \(p=0.0001\), \(24\) hours \(p=0.077\), \(48\) hours \(p=5\times10^{-7}\). \(\ast\) \(p<0.01\). t test. H) Running activity only transiently decreases at \(6\) h following retinal lesions. Fraction of time spent running, before and after lesions (black) or sham-lesions (red), \(n=12\) animals, \(p<0.05\). I) Mean (left) and mean-normalized maximum (right) projection of the same imaging site. Insets show a close up of the outlined region. Red dotted lines illustrate the selection of the regions of interest (ROI) used to calculate activity of individual cells. Typically these ROIs did not include the nucleus.
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

Adult mice (P100-120) were anaesthetized with ketamine (0.21 mg per gram of bodyweight) and xylazine (0.015 mg per gram of bodyweight), or, in a subset of animals, a mixture of medetomidine, midazolam and fentanyl. Both retinae were completely photo-coagulated by multiple confluent laser lesions (infrared laser, 400-1000mW, 200ms, 300 μm) through a laser-adapted operating microscope. Lesions were directly aimed to the optic disc and in multiple concentric circles to its surround in order to destroy all retinal ganglion cell fibers. Atropine (1 μl of 0.5% atropine diluted to 1:2000) was applied to the eye for 15 minutes before laser treatment to dilate the pupil and facilitate lesioning. Sham-lesioned mice received the same anaesthesia and atropine treatment, without the laser photo-coagulation.

Electrophysiology

At 6, 18, 24 or 48 hours after the retinal/sham lesion, C57BL/6J mice were deeply anaesthetized with ketamine and xylazine and transcardially perfused with 10 ml ice-cold artificial cerebral spine fluid (ACSF, see below), after which
the brain was rapidly removed and placed for 2 minutes in ice-cold ACSF saturated with 95% O₂/5% CO₂. Coronal slices (300μm thick) containing both hemispheres of the primary visual cortex were cut using a Vibratome 3000 (Leica). Slices were incubated in a submersion holding chamber for 30 minutes at 34°C and then at least 30 minutes at room temperature (24°C) before recording.

Visualized whole-cell patch clamp recordings of layer 5 pyramidal neurons were performed at room temperature (24°C) in a submersion recording chamber mounted on an upright microscope stage with infrared differential interference contrast optics. The cortical layer and cell type were confirmed visually before recording and, in a subset of experiments, post hoc by biocytin staining. Patch pipettes had a tip resistance of 3-5 MΩ and were filled with intracellular solution (see below). Miniature excitatory postsynaptic currents (mEPSCs) or miniature inhibitory postsynaptic currents (mIPSCs) were recorded in O₂/CO₂ saturated ACSF with 1μM Tetrodotoxin (TTX) and 250μM Trichlormethiazide (TCM). After obtaining whole cell access, neurons were voltage clamped at -70mV (corrected for liquid junction potential) using an Axopatch 200B amplifier (Molecular Devices). mEPSCs and mIPSCs were recorded and digitized using in-house software (LabVIEW). Neurons with changes in membrane potential or input resistance of more than 10% during the recording were excluded from analysis.

In a subset of experiments, 10 μM (3-(2-carboxypiperazine-4-yl))-propyl-1-phosphonic acid (CPP) to block N-methyl-d-aspartate (NMDA) receptors and 10 μM 2,3-dihydro-6-nitro-7-sulphamoyl-benzo(f) quinoxaline (NBQX) to block
(R,S)-3-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptors were added to the bath, eliminating all mEPSCs, without affecting the frequency or amplitude distributions of mIPSCs. In a separate subset of experiments, addition of the γ-aminobutyric acid (GABA_A) blocker bicuculline (10 μM) had no effect on the frequency or amplitude distribution of the mEPSCs, but eliminated all mIPSCs.

**Solutions**

Standard ACSF contained (in mM) 126NaCl, 25NaHCO_3, 25Glucose \times \text{H}_2\text{O}, 3.5KCl, 1NaH_2PO_4 \times \text{H}_2\text{O}, 0.5MgSO_4 \times 7\text{H}_2\text{O} and 1CaCl_2 \times 2\text{H}_2\text{O} (osmolarity approx. 325 milliosmol/kg). The internal solution contained (in mM) 120K-Gluconat, 10KCl, 20HEPES, 5NaCl and 12Mg^{2+}-ATP (pH 7.20, osmolarity 292 milliosmol/kg).

**Intrinsic and two-photon structural imaging**

Cranial windows were implanted (Holtmaat et al., 2009) in ketamine (0.14 mg per gram of bodyweight) and xylazine (0.01 mg per gram of bodyweight) anaesthetized C57BL/6J mice expressing enhanced green fluorescent protein (eGFP) under the thy-1 promoter in a subset of excitatory neurons (GFP-M line (Feng et al., 2000)). All imaging was carried out at least 30 days post surgery. The visual cortex was mapped using intrinsic signal imaging prior to retinal lesions as described previously (Keck et al., 2008), and all two-photon imaging was targeted to the monocular visual cortex using the surface blood vessels as landmarks.
Two-photon imaging was carried out as described previously (Keck et al., 2008). Briefly, the apical dendrites in layer 1 and 2/3 (0-150 μm below the pial surface) of layer 5 cells were imaged using a custom built two-photon laser scanning microscope. High-resolution image stacks (1024x1024 pixels, 91x91μm, 0.5 μm z-step) were obtained using Fluoview software (Olympus) and a Mai Tai Ti:sapphire laser (Spectra-Physics) tuned to 912 nm. Laser power in all experiments was less than 50mW at the brain surface. With Ketamine/Xylazine anesthesia (70% of surgical dosage), chronic imaging was carried out 24 and 48 hours before and 6, 18, 24 and 48 hours after a retinal lesion/sham lesion. Lower resolution images (512x512 pixels, 350 x 350 μm, 2.5 μm z-step) were taken to localize dendritic stretches (in order to locate the same regions) and cell bodies (to ensure the imaged cells were indeed layer 5 pyramidal cells).

**Awake two-photon functional imaging**

*Subjects and imaging.* Data were collected from 12 adult (P95-P166) C57BL/6J mice. Mice were injected with AAV2/1-hsyn1-GCaMP3, or in a separate set of mice AAV2/1-ef1α-GCaMP5 between P39 and P65. At the time of virus injection, 5 mm circular glass cover slips were implanted to sit flush with the skull, or in a subset of mice, to rest on the brain without additional pressure. Experiments were carried out 3 to 15 weeks post transfection. Due to the increased sensitivity of GCaMP5 (Akerboom et al., 2012) compared to GCaMP3 (Tian et al., 2009), we expect to detect more of the action potentials in experiments using GCaMP5, which could explain why we see differences in the levels of activity changes following sensory deprivation. Otherwise, the dynamic ranges for GCaMP3 and
GCaMP5 are similar, both can be excited to threshold by multiple spikes per second, and both dyes saturate at a few tens of spikes per second.

Functional calcium imaging was performed with a custom built two-photon microscope. Illumination source was a Spectra Physics MaiTai eHP Laser with a DeepSee prechirp unit (<70 fs pulse width, 80 MHz repetition rate) or a Coherent Chameleon Vision S. We used an excitation wavelength of 910 nm, and a 525/50 emission filter (BrightLine HC 525/50). The scanhead was based on an 8 kHz (in some experiments, 4 kHz) Cambridge Technology resonant scanner, which we used in bidirectional mode. This enabled frame rates of 40 Hz at 400 x 600 pixels. In later experiments, to increase yield, we in addition used a high power objective Z-piezo stage (P-726, Physik Instrumente) to move the objective down in steps of 20 µm between frames and return to the initial position after four frames. With this system we acquired data at 4 different depths, reducing the effective frame rate from 40 Hz to 10 Hz. In the best case, this setup allowed for imaging to a depth of up to 750 µm, which in turn permitted us to image both from layers 2/3 and 5 while keeping laser power at the brain surface always below 50 mW. We used a Nikon 16x 0.8 NA objective. Data were acquired with a 250 MHz digitizer (National Instruments, NI 5762) and pre-processed with a custom programmed FPGA (National Instruments, NI PXIe-7965R).

To ease head-fixation, animals were briefly (approx. 10 seconds) anaesthetized with isoflurane. Mice were then allowed to adjust to head-fixation and the spherical treadmill (see below), with visual feedback for 10 to 30 minutes. Experiments consisted of alternating 3 minute blocks in which either the mouse
received coupled visual feedback or the stimulation screens were turned off (i.e. darkness). Each condition was repeated twice. Four regions per animal were typically recorded, which on average took 90 minutes.

*Trackball and visual stimulation.* Head-fixed animals were free to run on a spherical treadmill based on the design of Dombeck et al. (Dombeck et al., 2007) (air supported polystyrene-foam ball). Details were described previously (Keller et al., 2012). Briefly, rotation of the ball around the vertical axis was restricted with a pin. Visual stimuli were presented on 2 screens arranged at an angle of 60 degrees relative to each other in front of the mouse that covered approximately 190 degrees in the horizontal axis and 55 degrees (to the front) to 65 degrees (to the sides) in the vertical axis of visual space. This arrangement of screens was intended to simulate visual flow similar to that experienced when running between two walls. Visual stimuli presented on the screen were full field vertical gratings. Motion of full field gratings was controlled by the mouse's movement on the ball (forward running induced movement of the gratings in the opposite direction). To ensure a baseline level of locomotion, animals were encouraged to run once per three minutes (in the first 30 seconds of the alternating light and dark periods) with three repetitions of either air puffs or manual stimulation.

**Data analysis**

*Electrophysiology.* mEPSC and mIPSC analysis was done with in-house software (Matlab). Analysis was done 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 (as in Desai et al., 2002).
**Structural Imaging analysis.** To calculate spine density, all spines that were clearly distinguishable in any imaging plane (including z) were included for a total number of 5698 spines from 24 cells in 10 animals over 6 time points. Analysis was done blind to the experimental condition on raw, 3-dimensional image stacks using ImageJ.

Integrated spine brightness as a measure for spine size was calculated as described previously (Hofer et al., 2009). Briefly, only spines that extended from the dendrite in the x-y plane were included. In the best focal section, the intensity of all pixels comprising the spine was added, and the background from an area nearby (devoid of any GFP-labeled structures) was subtracted. The spine intensity was then normalized by the intensity of the adjacent dendrite to account for differences in overall intensity between imaging sessions. To calculate relative changes in spine size at each time point (Figure 3C), the size was normalized to the initial spine size at 48 hours before lesion/sham-lesion. Only spines that were present at all time points were included in the analysis.

**Functional Imaging analysis.** Data were full frame registered using a custom written registration algorithm. Cells were selected based on mean and mean-normalized maximum projections of the data by hand (typically the nucleus was excluded from the selection). Mean-normalized maximum projections were calculated by normalizing the maximum projection, calculated on a running average of 20 frames, by the mean projection. Use of mean-normalized maximum projection ensured the inclusion of all active cells, even ones that were not
visible in the mean projection. This biased our cell selection towards active cells. Fluorescence traces were calculated as average fluorescence of pixels lying within the cell in each frame. To remove slow signal changes in raw fluorescence traces the 8-percentile value of the fluorescence distribution in a +/-15 s window was subtracted from the raw fluorescence signal. ∆F/F signals were calculated by dividing raw fluorescence signal by the median of each cell’s fluorescence distribution.

Cellular activity was calculated using either integrated fluorescence or binary classification into active and non-active cells as follows: To calculate integrated fluorescence, the ∆F/F signal was low pass filtered and all fluorescence above a threshold of 10% ∆F/F was integrated. The use of a threshold was necessary to exclude a potential influence of brain motion related fluorescence changes. For binary classification of cells as active or non-active, the ∆F/F signal was low pass filtered and thresholded at 100% ∆F/F. Cells were deemed active if they crossed threshold at least once. The percentage of cells active at a given time point as a fraction of all cells active at any time point was calculated per region. Our results were qualitatively consistent for a range of thresholds (see Figure S1C).

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 non-normally distributed data. Cumulative distribution function for mEPSCs was calculated.
such that binning started at the value of the ‘scaling factor’ (1.24 for mEPSCs) multiplied by the threshold for detection (5pA for mEPSCs).

References:


