

## SUPPORTING ONLINE MATERIAL

### METHODS

#### *Whisker deprivation.*

All whisker deprivation experiments were carried out with an equal number of deprived and control Wistar rats from the same litter. Subsequently control and deprived animals from the same litter were analysed as interleaved experiments. Deprived animals had their right-side whiskers from A-, B- and C-rows (and  $\alpha$ ,  $\beta$ ,  $\gamma$  straddler whiskers) trimmed daily to keep the whisker hairs shorter than 2 mm. In one series of experiments the D- and E-rows (and  $\gamma$ ,  $\delta$  straddler whiskers) were trimmed instead to generalize the conclusions to different whisker trimming patterns. In some litters the whiskers of the deprivation group of animals were initially plucked giving a four-day period before regrowth of whiskers (upon which they were then trimmed). Whisker deprivation began at P7. Experimental analysis of barrel cortex synaptic circuitry began after a minimum of 10 days of deprivation.

#### *In vivo voltage-sensitive dye imaging and whole-cell recording.*

Methods for *in vivo* whole-cell recordings and VSD imaging followed previous descriptions (S1, S2). Rats were anaesthetised with urethane (1-2 mg/g) and placed in a stereotaxic apparatus whilst maintaining body temperature at 37°C. A craniotomy was performed on the left hemisphere over primary somatosensory barrel cortex centered relative to bregma at posterior 2.5 mm and lateral 5.5 mm. Extreme care was taken at all times not to damage the cortex, especially during the removal of the dura. For experiments not involving imaging, small craniotomies of ~0.5 mm diameter were prepared and these did not require agarose stabilisation.

For voltage-sensitive dye imaging experiments the craniotomy was ~3 mm diameter and the exposed cortex was bathed for 2 hours in 0.1mg/ml RH1691 dissolved in Ringers solution containing (in mM): 135 NaCl, 5 KCl, 5 HEPES, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>. The cortex was subsequently washed for 15 minutes to remove unbound dye, covered with 1% agarose and covered with a glass cover slip shaped to extend little wider than the craniotomy allowing access for whole-cell recording electrodes from both left and right. Voltage-sensitive dye imaging was triggered at a fixed point of the electrocardiogram to allow subtraction of heart beat artifacts. Epifluorescent images

(excitation 630 nm, emission >665 nm) were collected every 2.4 ms with a Fuji Deltaron HR 1700 camera via a tandem lens setup. The spatiotemporal dynamics of the whisker-evoked responses could be analysed during an experiment using custom written routines in IgorPro and aligned with the blood vessel pattern, allowing targeted whole-cell recordings from specific regions of the barrel cortex. The VSD signal was obtained from an average of 20 stimulus repetitions with an interstimulus interval of one minute and between each stimulus a sweep was recorded without a stimulus.  $\Delta F/F$  was calculated for each image. Sensory responses were quantified in several ways. For quantification of VSD responses at 12ms poststimulus, three consecutive image frames were averaged to reduce noise. The response amplitude was quantified during the time interval 9.6ms to 14.4ms poststimulus relative to the prestimulus baseline which was quantified during the period -4.8ms to 0ms before stimulus onset. For quantification of VSD responses at 50ms poststimulus, five consecutive image frames were averaged. The response amplitude was quantified during the time interval 45.6ms to 57.6ms poststimulus relative to the prestimulus baseline which was quantified during the period 12ms to 0ms before stimulus onset. To calculate asymmetry of the sensory response in C2 and E2 barrel columns, the VSD signal amplitudes relative to the prestimulus values were calculated in 200  $\mu\text{m}$  x 200  $\mu\text{m}$  sized regions of interest centered on the respective barrel columns. One dimensional profiles of VSD signals were also calculated relative to the prestimulus values by quantifying the VSD amplitude along a line of width 400  $\mu\text{m}$  running parallel to the arcs of barrel cortex and through the center of the D2 barrel.

Whole-cell pipettes were advanced through the agarose and into the neocortex with a positive pressure 200 mm Hg until the electrode tip was around 50  $\mu\text{m}$  before the location of where the whole-cell recording would ideally be located. The positive pressure was subsequently reduced to 30 mm Hg and the pipette was advanced in steps of 2  $\mu\text{m}$  until the resistance of the pipette increased suddenly indicating contact with a cell. Suction was subsequently applied until a  $G\Omega$  seal was formed and then the whole-cell recording configuration was established by slowly ramping the pressure to increasingly negative values. Whole-cell pipettes had resistances of  $5M\Omega$  filled with a solution containing (in mM): 135 potassium gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, and 0.3  $\text{Na}_3\text{GTP}$  (adjusted to pH 7.2 with KOH). Biocytin (2mg/ml) was included in the intracellular solution to allow the

morphology of the neurons to be analysed. At the end of the experiment, during which a neuron had been filled with biocytin through the whole-cell recording, the rat was transcardially perfused with PBS followed by 4% paraformaldehyde. After overnight fixation, 100  $\mu\text{m}$  thick brain slices were cut in a plane tangential to the pia. The slices containing layer 4 were stained for cytochrome oxidase to visualise the barrels. Subsequently the biocytin filled neurons were visualised by diaminobenzidine reaction with avidin horseradish peroxidase conjugation. Slices were mounted using moviol and neuronal morphology reconstructed using NeuroLucida software.

*In vitro voltage-sensitive dye imaging and whole-cell recording.*

Paracoronaral brain slices (350  $\mu\text{m}$  thick) were prepared to visualise the A-E rows of the posterior medial barrel subfield (S3). In one set of experiments slices were cut tangentially to the pia (500  $\mu\text{m}$  thick) containing upper layer 4 to identify the barrels and the lower  $\sim$ 400  $\mu\text{m}$  of layer 2/3. All experiments were performed at 35°C with a bath solution containing (mM): 125 NaCl, 25 NaHCO<sub>3</sub>, 25 glucose, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 CaCl<sub>2</sub>, and 1 MgCl<sub>2</sub> bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. For voltage-sensitive dye imaging the slices were bathed in 0.1 mg/ml RH155 for 15 minutes and washed for a further 10 minutes before imaging. Electrical stimulation was delivered via a glass micropipette to the center of a D-row layer 4 barrel and the change in voltage-sensitive dye absorption at 750 nm was measured at 0.6ms time resolution with the Fuji Deltaron HR1700 attached to a Zeiss Axioskop using a 10x lens. Whole-cell pipettes had resistances of 5M $\Omega$  filled with a solution containing (in mM): 135 potassium gluconate, 4 KCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, and 0.3 Na<sub>3</sub>GTP (adjusted to pH 7.2 with KOH). Biocytin (2mg/ml) was routinely included in the intracellular solution to allow the morphology of the neurons to be analysed. Loose-patch stimulation of single presynaptic neurons was performed with 5M $\Omega$  pipettes filled with extracellular solution, with loose seals of  $\sim$ 50M $\Omega$  obtained by suction, and 5 ms current injection of 10-100 nA delivered with an Axoclamp 2B amplifier allowing observation of the evoked action potential.

## **SUPPLEMENTAL DATA**

One might predict that if we reversed the pattern of spared and trimmed whiskers we would also reverse the direction of the spread of the VSD signal so that cells in columns representing the spared whiskers would again preferentially signal to each other. To test this, we made experiments where half of the animals had the D- and E-rows trimmed from P7 and the other half served as controls without whisker trimming. As before the animals were trimmed daily until analysed at P > 18. The C2-whisker was stimulated, which is represented in the non-deprived cortex at the border to the deprived area of the cortex. The spread of the sensory-evoked VSD signal observed in B-columns vs D-columns was quantified at 50 ms following stimulation. In animals with all whiskers intact stimulation of the C2 whisker evoked sensory responses initially restricted to the C2-column that later spread into B2 and D2 columns with a B2/D2 ratio of  $0.76 \pm 0.04$  (n = 5). In the E- and D-row trimmed animals stimulation of the C2-whisker evoked larger responses in the B-row column with a B2/D2 ratio of  $1.15 \pm 0.06$  (n = 5). Thus in a second partial deprivation paradigm, whisker trimming again biased the expansion of sensory responses towards cortical columns representing spared whiskers.

## **REFERENCES**

- S1. C.C.H. Petersen, A. Grinvald, B. Sakmann, *J. Neurosci.* **23**, 1298 (2003)
- S2. M. Brecht, A. Roth, B. Sakmann, *J. Physiol.* **553**, 243 (2003)
- S3. G.T. Finnerty, L.S.E. Roberts, B.W. Connors, *Nature* **400**, 367 (1999)