Synaptic Changes in Layer 2/3 Underlying Map Plasticity of Developing Barrel Cortex

Carl C. H. Petersen,1,2* Michael Brecht,1 Thomas T. G. Hahn,1 Bert Sakmann1

The functional and anatomical rearrangements of cortical sensory maps accompanying changes in experience are not well understood. We examined in vivo and in vitro how the sensory map and underlying synaptic connectivity of the developing rat barrel cortex are altered when the sensory input to the cortex is partially deprived. In the nondeprived cortex, both the sensory responses and synaptic connectivity between columns were strengthened through an increase in the synaptic connection probability between L2/3 pyramids in adjacent columns. This was accompanied by a selective growth of L2/3 pyramid axonal arbors between spared columns. In contrast, deprived and nondeprived cortical columns became weakly connected in their L2/3 pyramid connections.

Activity-dependent cortical plasticity contributes to organizing the representation of sensory information in well-ordered maps. Experience-dependent plasticity has been examined extensively in the visual cortex (1–3). Such experiments and related ones in somatosensory cortex have indicated that electrical activity and competition between the different sensory inputs can contribute to specifying the map (4). However, the difficulty in defining the visual cortical map in vitro and lack of an anatomical reference point in vivo have so far limited the study of what changes occur at the level of synaptic connections between specific classes of neurons in different cortical layers (5, 6). The neocortical somatosensory map representing the whiskers of rats is exceptionally well delineated by the layer 4 barrel pattern, which is isomorphic to the layout of the whiskers on the rodent snout (7). Barrels can be visualized in acute, living brain slices, allowing the neocortical circuits of specific regions of the sensory map to be investigated in detail (8, 9).

Large-scale representational plasticity can be induced by simply trimming or plucking whiskers (8, 10–20). Receptive fields can expand when all or all but one of the whiskers are trimmed (10–14), but subtle differences in behavior can reverse the expansion into a contraction (14). In a “pairing” paradigm of two neighboring spared whiskers, sensory responses are enhanced between the spared whiskers (15). However, the detailed synaptic and anatomical changes underlying specifications of cortical maps are not clear. We combine in vivo analysis of response properties and sensory maps with in vitro analysis of synaptic connectivity to derive a mechanistic view of how synaptic changes lead to alterations in a sensory map. We used a partial deprivation protocol related to the pairing paradigm, in which we spared whiskers of rows D and E (thus, these could be considered as “paired” whisker rows) while trimming whiskers of rows A, B, and C (Fig. 1, A and B). Deprivation began at postnatal day 7 (P7) and whiskers were trimmed every day for at least 10 days. The neuronal networks activated by the spared D-row whiskers are particularly interesting to investigate because they are flanked by cortex deprived of sensory input on one side and nondeprived cortex receiving normal sensory input on the other.

First, large-scale changes in the representation of D-row whiskers in layer 2/3 were investigated in vivo by voltage-sensitive dye (VSD) imaging with RH1691 (Fig. 1, C and D). Fluorescence signals from this indicator under our experimental conditions correlate very closely with subthreshold postsynaptic potentials (PSPs) in layer 2/3 pyramidal neurons (21, 22). The D2 whisker was deflected for 2 ms, and the first sensory response in layer 2/3 was observed 12 ms later in the D2 column. This rapid initial response was not different between animals with intact whiskers and partially deprived animals, suggesting that the afferent signaling pathway from the whisker to the cortex is unaffected by the deprivation. The initial VSD signal was limited to the lateral extent of the D2-barrel column (Fig. 1, C to F). During the next tens of milliseconds, the response expands over a much larger area of cortex through local intracortical synaptic activity (21, 22), and it is this later part of the response that is substantially altered by sensory deprivation. We examined the asymmetrical spread of synaptic excitation across the columns representing the arcs of whiskers because the C columns are deprived but the E columns are not. In animals with intact whiskers, the signal spread from the D columns preferentially toward the C columns. For these control animals, the ratio of the E2 to C2 VSD response was 0.75 ± 0.22 (mean ± SEM, n = 7; measured at 50 ms after stimulus and quantified across a 200-μm area centered on the E2 or C2 column, respectively). This bias toward the C-row column was reversed in partially deprived animals such that excitation evoked by stimulation of the D2 whisker spread preferentially toward the E2 column with a ratio E2/C2 = 1.84 ± 0.28 (mean ± SEM, n = 9; significantly different from control animals, P < 0.05, Student’s t test).

Next, we investigated the cellular basis of such large-scale changes in the spatiotemporal dynamics of cortical sensory maps with in vivo whole-cell recordings from barrel-related L2/3 pyramidal neurons (21–23). In one set of in vivo experiments, whole-cell recordings were targeted stereotaxically toward the region of the barrel cortex representing the posterior B- and C-row whiskers. The average PSP amplitude evoked by stimulating D-row whiskers measured at 50 ms was reduced to about half in deprived animals [averaged across all stimulated whiskers (D1 to D4): 1.63 ± 0.24 mV (n = 17) in animals with intact whiskers versus 0.9 ± 0.24 mV (n = 16) in partially deprived animals, P < 0.05, Student’s t test; average for the best D-row whisker: 4.41 ± 0.62 mV (n = 17) in control animals versus 2.32 ± 0.55 mV (n = 16) in partially deprived animals, P < 0.05, Student’s t test].

In the second set of experiments, whole-cell recordings were targeted in vivo to the C2 and E2 columns based on the location of the D2 column determined functionally by VSD imaging. In each animal, whole-cell recordings of L2/3 pyramidal neurons were made from both the C2 and the E2 columns (Fig. 1, G to N), allowing comparison of the relative response amplitudes to D2-whisker stimulation in each animal. Care was taken to record from neurons located at equal distances from the center of the D2 column. In the C2 column of partially deprived animals, we again observed a reduction in amplitude of PSPs evoked by D2 whisker stimulation [9.2 ± 0.96 mV (n = 8) in control animals versus 3.7 ± 1.1 mV (n = 8) in partially deprived animals; P <
Furthermore, we found an increase in the amplitude of PSPs recorded in the E2 column of the spared cortical area of the partially deprived animals \([6.2 \pm 1.0 \text{ mV (n=8) in control animals versus } 9.4 \pm 2.0 \text{ mV (n=8) in partially deprived animals; } P < 0.05, \text{ Student's } t \text{ test}].\) The ratio of response amplitudes E2/C2 was 0.70 \(\pm 0.33 \) (n=8) for animals with intact whiskers but 2.54 \(\pm 0.86 \) (n=8) for animals with partial whisker trimming \((P < 0.05).\) The whole-cell data from individual L2/3 pyramids are thus consistent with the VSD imaging results from the population of L2/3 cells. Thus, the layer 2/3 pyramidal neuron sensory map is specified during development to preferentially distribute sensory information to the nearby cortical columns processing the most similar input, which in this experimental paradigm are the neighboring nondeprived cortical columns [see also (25) for alternative whisker-trimming paradigms].

Having defined the experience-dependent induction of asymmetry of the sensory response in vivo, we next investigated whether such a change could be demonstrated using in vitro slices of barrel cortex. First, we imaged the spread of excitation with VSD RH155 in thick tangential brain slices comprising \(\pm 100 \mu \text{m of upper layer 4 and } \pm 400 \mu \text{m of layer 2/3}\) (Fig. 2, A and C). Electrical stimulation of the D2 barrel evoked a VSD response restricted primarily to the stimulated column with a much more limited spread of excitation into adjacent columns than was observed in vivo. The spread of excitation into adjacent columns was quantified, and an asymmetry along the arc was observed. Responses in partially deprived animals (but not in animals with intact whiskers) preferentially spread into the spared E-row column \((\text{ratio of VSD signal E2/C2 was } 0.87 \pm 0.067 \text{ (n=19) in partially deprived animals but } 0.58 \pm 0.067 \text{ (n=8) in control animals.}\)
animals with intact whiskers and $1.54 \pm 0.23$ ($n = 21$) in animals with partial whisker-trimming; $P < 0.05$, Student’s $t$ test).

To investigate the laminar structure of the activity-dependent differences in local circuits between animals with intact and partially trimmed whiskers, we used coronal cortical slices in which barrels representing whisker rows A to E could be visualized (8). Stimulation of layer 4 in the D column with an extracellular electrode (26) evoked an early column-restricted response in both control (Fig. 2B) and partially deprived animals (Fig. 2D). The VSD image measured during the first 10 ms of the response did not differ between control and partially deprived cortices. Later, the VSD signal spread laterally within layer 2/3 (but not layer 4), and this late lateral spread in layer 2/3 differed between animals with intact and partially trimmed whiskers. In partially deprived animals, activity spread preferentially toward the spared E row ($E/C = 0.97 \pm 0.11$ ($n = 20$) for control animals; $E/C = 1.43 \pm 0.19$ ($n = 20$) for deprived animals; $P < 0.05$, Student’s $t$ test). Thus, the observed change in the sensory map is likely to occur primarily through synaptic alterations of the layer 2/3 neuronal network.

To examine changes at the level of individual synaptic connections, we made simultaneous triple recordings in L2/3 between pyramids located in C, D, and E columns (Fig. 3). Care was taken to locate the postsynaptic neurons at an equal distance from the center of the D column, where presynaptic neurons were stimulated. Action potentials were evoked in individual pyramidal neurons located in the D column in the loose-patch configuration (9) to test for synaptic connections onto the two target neurons recorded in the whole-cell configuration located in the C column (or the L2/3 C-D septum-related region) and E column (or L2/3 D-E septum-related region). A total of 1034 pairs of neurons were tested (532 pairs in 12 animals with intact whiskers, 502 pairs in 12 partially deprived animals). The mean unitary excitatory postsynaptic potential (EPSP) amplitude of excitatory connections originating from D-column pyramids was largest with C columns in animals with intact whiskers. In contrast, excitatory synaptic connectivity between D and E columns was stronger in animals with partially trimmed whiskers (27). The probability of finding neuron pairs connecting D to E columns was increased by 81% ($0.10 \pm 0.02$, $n = 12$ control animals; $0.18 \pm 0.06$, $n = 12$ partially deprived animals; $P < 0.05$, Student’s $t$ test) in the spared columns of partially deprived animals without a significant change in mean unitary EPSP amplitude. In contrast, the mean unitary EPSP amplitude decreased by 44% ($181 \pm 58 \mu V$, $n = 12$ control animals; $102 \pm 19 \mu V$, $n = 12$ partially deprived animals; $P < 0.05$, Student’s $t$ test) for connections between the D- and the deprived C-row column in partially deprived animals, without a significant change in the rate of finding connected pairs.

Finally, anatomical reorganization can accompany cortical plasticity (3, 12, 16, 17, 28). We therefore investigated possible changes in the development of axonal and dendritic arbors of pyramidal neurons induced by partial sensory deprivation.

We made three-dimensional computer-aided reconstructions of the dendritic and axonal arbors of L2/3 pyramidal neurons recorded in vivo that had their soma located in the D2 column (Fig. 4). The dendritic
arbor branches of D-row pyramids projecting into the spared E columns, leading to a prominent expansion of the axonal density profile into the E-row territory (white contour lines in Fig. 4). The axonal length in the C and E columns was quantified for each cell, and an asymmetry index was calculated ranging from -1 (only axon in C columns, none in E columns) to +1 (only axon in E columns, none in C columns). In control animals, this axonal asymmetry index was $-0.21 \pm 0.30 (n = 8)$, showing that in animals with all whiskers intact, the axons of D columns preferentially target the C columns. In partially deprived animals, the axonal asymmetry index favored the E column with a value of $0.24 \pm 0.12 (n = 8)$, which is significantly different from that of control animals ($P < 0.05$, Student’s t test).

30 APRIL 2004 VOL 304 SCIENCE www.sciencemag.org

For D-column pyramidal cells, the total axonal length in the E columns in partially deprived animals increased to 210% of the value in control animals. In contrast, the total axonal length in the C row was reduced by 23%. Independent of sensory experience, we observed a $\sim 5$-$\mu$m spacing between boutons (30), suggesting that the quantification of axonal length may provide a reasonable estimate of changes in anatomical synaptic connectivity. These changes in total axonal length were accompanied by differences in the number of axonal branch points. The spared E-row columns of partially deprived animals had 335% more branch points than E-row columns of control animals. In contrast, the deprived C row had 46% fewer branch points (31). The activity-dependent regulation of axonal growth during development thus appears to be mediated primarily by the number of branches, rather than the length of individual axonal segments.

These anatomical measurements thus suggest that layer 2/3 pyramidal neurons in the D columns of partially deprived animals establish more synapses with pyramidal neurons in the E columns. This anatomical change during development is very likely to contribute to the increased functional connectivity observed by our electrophysiological and VSD-imaging measurements. The effects of partial sensory deprivation examined here cover a critical period in the development of layer 2/3 barrel cortex, which coincides with intense axonal collateralization of the pyramids in L2/3. Indeed, the same deprivation protocol used in this study but applied to adult rats does not generate measurable changes in the sensory map with voltage-sensitive dye (32). The experience-dependent specification of a sensory map during early postnatal development described here also differs from another form of map plasticity, in which areas representing active inputs expand at the expense of those with inactive inputs. In our experimental paradigm, the deprived cortex may instead be recruited by the competing somatosensory signals carried by the paralemniscal/septal signaling pathway (19). The mechanisms that we describe here may also underlie the formation of axonal arbor patches in the cat primary visual cortex. Here, cortical regions receiving similar orientation-selective inputs are anatomically connected to each other (33–35).

In summary, we find that the experience-dependent specification of a sensory map during development is based on at least two cellular mechanisms. The decreased connectivity of L2/3 pyramidal neurons in cortical areas receiving uncorrelated input results mainly from a functional change in synapses characterized by decreased unitary EPSP amplitudes. In contrast, the increased connectivity between areas receiving correlated synaptic input is a consequence of a selective increase of axonal arborization of L2/3 pyramids.

References and Notes
24. The amplitude of the sensory responses in the second set of experiments with whole-cell recordings was larger than that in the first set of experiments because these neurons were located specifically in the C2 (or E2) columns, whereas in the first set of experiments the neurons were targeted through stereotaxic coordinates to a less-defined B/C-row region of barrel cortex.
25. Materials and methods are available as supporting material on Science Online.
27. In control animals, 38 connections were found from D- to C-row pyramidal neurons with a mean unitary EPSP amplitude of 155 $\mu$V. Originating from the same presynaptic D-row pyramidal neurons, 27 connections were found onto the E-row pyramidal neurons with a mean unitary EPSP amplitude of 121 $\mu$V. In deprived animals, 35 connections were found from D- to C-row pyramidal neurons with a mean unitary EPSP amplitude of 108 $\mu$V and 46 connections from D- and E-row pyramidal neurons with a mean unitary EPSP amplitude of 101 $\mu$V.
29. The asymmetry index was calculated as $[2 \times \frac{\text{length of axon in E row}}{\text{[length of axon in E row]}} - 1$. We furthermore also measured this asymmetry index in neurons recorded in vitro, finding consistent results with our in vivo measurements. In control animals in vitro, the asymmetry index was $0.24 \pm 0.18 (n = 8)$. Whereas in deprived animals in vitro, the asymmetry index was $0.21 \pm 0.08 (n = 8)$.
30. From eight in vivo control cells with soma in D row: E-row axon has 0.203 $\pm 0.008$ boutons/ $\mu$m and C-row axon has 0.205 $\pm 0.006$ boutons/ $\mu$m. From eight in vivo deprived cells with somata in D row: E-row axon has 0.222 $\pm 0.006$ boutons/ $\mu$m and C-row axon has 0.213 $\pm 0.008$ boutons/ $\mu$m. These measurements from in vitro filled cells are not significantly different ($P > 0.1$, Student’s t test). We also quantified our in vitro reconstructions. From eight in vitro control cells with soma in D row: E-row axon has 0.205 $\pm 0.009$ boutons/ $\mu$m and C-row axon has 0.198 $\pm 0.011$ boutons/ $\mu$m. From eight in vitro deprived cells with soma in D row: E-row axon has 0.219 $\pm 0.018$ boutons/ $\mu$m and C-row axon has 0.199 $\pm 0.013$ boutons/ $\mu$m. These measurements from in vitro filled cells are not significantly different ($P > 0.1$, Student’s t test).
31. Axonal branch points of D-row pyramidal neurons reconstructed following in vivo recordings were quantified within the C row and the E row. In control animals, D-row pyramids ($n = 8$) had 4.6 $\pm 1.5$ nodes in the C row and 2.3 $\pm 0.6$ nodes in the E row. In deprived animals, D-row pyramids ($n = 8$) had 2.5 $\pm 0.8$ nodes in the C row and 10 $\pm 2.4$ nodes in the E row.
36. We are grateful to D. Feldman, D. Simons, and R. Aronoff for helpful comments on an earlier version of this manuscript. We also thank D. Feldmeyer, F. Helmchen, M. Larkum, H. Spors, and J. Waters for useful discussions and technical advice. We are grateful to A. Grinvald for the gift of voltage-sensitive dye RH1691. Thanks are also due to M. Kaiser for staining of slices and S. Wiegert for constructing the morphology of the neurons.
Retraction

IN THE REPORT "SYNAPTIC CHANGES IN LAYER 2/3 UNDERLYING MAP PLASTICITY OF DEVELOPING BARREL CORTEX" (1), we concluded that functional and anatomical changes in layer 2/3 underlie different forms of cortical map plasticity. It was pointed out to us by a reader that the anatomical analysis contains errors. Although these errors did not affect the main conclusions, we reanalyzed the data set. Re-analysis confirmed that whisker stimulation evokes a cortical response, which spreads preferentially to neighboring, nondeprived cortical columns as originally reported. However, the reported difference between the axonal fields in control and deprived animals was not statistically significant. Further, the deprivation-induced decrease in unitary EPSP amplitude was also not statistically significant. Thus, major conclusions of the Report are no longer supported, and we retract the Report. We apologize for any confusion that we may have caused to the readers of Science.

CARL C. H. PETERSEN,1 MICHAEL BRECHT,2 THOMAS T. G. HAHN,3 BERT SAKMANN3
1The Laboratory of Sensory Processing, Brain and Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne CH-1015, Switzerland. 2Department of Neuroscience, Erasmus University, Dr. Molewaterplein 50, NL-3015 Rotterdam, Netherlands. 3Department of Cell Physiology, Max-Planck-Institute for Medical Research, Jahnstrasse 29, Heidelberg D-69120, Germany.

Reference

GALEX and UV Observations

IN HIS ARTICLE "ULTRAVIOLET ASTRONOMERS FACE LOSS OF VISION" (News Focus, 25 June, p. 1899), Govert Schilling makes the important point that we will soon lose our view of the ultraviolet (UV) sky unless we preserve or replace the few existing UV space missions. However, for the Galaxy Evolution Explorer (GALEX), the future now looks brighter than the fall 2005 end date stated in the article. GALEX received the top ranking in the April 2004 Senior Review of Astronomy and Physics Mission Operations and Data Analysis Programs (1). NASA accepted the recommendation for “completion of the prime mission in FY05 and FY06, with an extended mission covering... FY07-FY08” [(1), p. 4]. GALEX is healthy and carries no consumables, so we hope it will be capable of observing well beyond 2008.

Schilling’s article discusses the importance of UV observations. Emphasizing this, the NASA Review lists three areas in which GALEX surveys are particularly significant to the astrophysics community. The first is synergy with FUSE and HST, UV missions that can follow up on sources identified by GALEX. Second, the wide-area GALEX legacy database of the 135- to 280-nm UV sky promises to be “one of the most important data sets in astrophysics in this decade” [(1), p. 4]. Third, the GALEX Guest Investigator (GI) program will broaden GALEX scientific impact well beyond the primary science of star formation history in galaxies.

Finally, we wish to qualify Schilling’s caveat about the dearth of glorious UV images. The wealth of GALEX images are both beautiful and scientifically compelling (2). The GALEX images trace star formation in a profound variety of physical settings, as well as many otherwise invisible physical processes important in understanding galaxy formation and evolution in the local and early universe.

PETER G. FRIEDMAN AND D. CHRISTOPHER MARTIN
California Institute of Technology, MS 405-47, 1200 East California Boulevard, Pasadena, CA 91125, USA.

References
2. See samples at www.galex.caltech.edu/imagegallery.html.

Clinical Trials or Exploitation?

THE CHANGING LANDSCAPE OF RESEARCH and the market pressures are causing a shift of medical experiments by U.S. entities overseas, where bureaucracy is less rigorous, patients are more eager to enroll, and costs are significantly lower. Ethical concerns about international trials and the protection of subjects have been heightened (1, 2). Nonetheless, little has been done to prevent underprivileged communities from being left out of clinical and scientific benefits after having served as test subjects. This happens in 33% of the studies conducted overseas (3); after a successful trial, the sponsor does not market the product locally. In the United States, patients tested for a new product continue to receive it either through the market or by applying to special programs. Sponsors should be required to market the new drug in the country where the trial was carried out, and to do so considering local economy, health care coverage, and purchasing power. This calls for a more direct involvement of local institutions. This would allow such institutions to not only protect individual patient rights, but also gain expertise and become more competitive.

The GALEX observation of M31 is a mosaic of 10 GALEX images with FUV and NUV displayed in blue and red, respectively. The image shows blue regions of young, hot, high-mass stars tracing out the spiral arms where star formation is occurring and the central orange-white "bulge" of old, cooler stars formed long ago. The star-forming arms of M31 are unusual in being quite circular rather than the usual spiral shape. Many other regions of star formation can be seen far outside the main body of the galaxy. The image shows several smaller companion galaxies. These include M32, a dwarf elliptical galaxy directly below the M31 central bulge and just outside the spiral arms, and M110, which is above and to the right of the M31 center. M110 has an unusual FUV bright core in an otherwise "red" old star halo.
Synaptic Changes in Layer 2/3 Underlying Map Plasticity of Developing Barrel Cortex
Carl C. H. Petersen, Michael Brecht, Thomas T. G. Hahn and Bert Sakmann

Science 304 (5671), 739-742.
DOI: 10.1126/science.1096750