Circuit Analysis of Experience-Dependent Plasticity in the Developing Rat Barrel Cortex

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Summary

Sensory deprivation during a critical period reduces spine motility and disrupts receptive field structure of layer 2/3 neurons in rat barrel cortex. To determine the locus of plasticity, we used laser scanning photostimulation, allowing us to rapidly map intracortical synaptic connectivity in brain slices. Layer 2/3 neurons differed in their spatial distributions of presynaptic partners: neurons directly above barrels received, on average, significantly more layer 4 input than those above the septa separating barrels. Complementary connectivity was found in deprived cortex: neurons above septa were now strongly coupled to septal regions, while connectivity between barrel regions and layer 2/3 was reduced. These results reveal competitive interactions between barrel and septal circuits in the establishment of precise intracortical circuits.

Introduction

Whiskers map topographically onto the primary somatosensory cortex of rodents (Woolsey and Van der Loos, 1970), where neurons in each cortical column are driven best by the column’s principal whisker, and more weakly by surrounding whiskers (Armstrong-James and Fox, 1987; Simons, 1978). Neurons in the layer 4 barrels are the targets of thalamocortical axons from the ventral posterior medial nucleus (VPM). Between barrels are septa (Woolsey and Van der Loos, 1970). Septa are associated with a separate thalamocortical system involving the medial division of the posterior nucleus (POM) (Koralek et al., 1988; Chmielowska et al., 1989; Lu and Lin, 1992), which resembles the anterior pulvinar of other mammals (Cusick and Gould, 1990; Krubitzer and Kaas, 1992; Sherman and Guillery, 2000).

Barrels develop early, during postnatal days (P) 0–5 (O’Leary et al., 1994; Agmon et al., 1993; Jhaveri et al., 1991) and, once formed, persist despite drastic sensory manipulations (Rice and Van der Loos, 1977; Schlaggar et al., 1993). Barrels thus provide a stable cytoarchitectonic map against which experience-dependent changes in cortical circuits can be measured (Fox, 1992).

The majority of intracortical synaptic circuitry is generated after barrels have formed. Between P10 and P15, cortical synaptic density increases 5-fold (Micheva and Beaulieu, 1996), and sensory-evoked synaptic potentials increase in amplitude several-fold (Stern et al., 2001). This burst of synaptogenesis coincides with a period of rapid spine turnover and motility (Lendvai et al., 2000). At this time, rats also begin to use their whiskers actively (Welker, 1964), suggesting that the wiring of intracortical circuitry might be influenced by sensory experience. Consistent with this hypothesis, unilateral full-field sensory deprivation reduces spine motility (Lendvai et al., 2000) and disrupts receptive field structure (Stern et al., 2001) of layer 2/3 neurons. On the other hand, receptive fields of layer 4 neurons are stable. These in vivo observations suggest that synaptic circuits involving layer 2/3 neurons are shaped by sensory experience (Stern et al., 2001; Feldman, 2001). However, the circuitry plasticity underlying changes in receptive fields is not known and could be complex, since layer 2/3 neurons receive input from a number of sources, including layer 4 neurons in barrels and septa, layer 5 neurons, and other layer 2/3 neurons.

Here we used laser scanning photostimulation (LSPS) (Callaway and Katz, 1993; Katz and Dalva, 1994; Roerig and Chen, 2002; Schubert et al., 2001) to measure the distributions of presynaptic partners of layer 2/3 neurons. We find that these cells receive different types of inputs, depending on their locations with respect to barrels and septa. Experience-dependent differences in the spatial distributions of these inputs could explain receptive field plasticity at the level of cortical synaptic circuits.

Results

Mapping Synaptic Connectivity with Laser Scanning Photostimulation

For sensory deprivation, all the whiskers on one side of the snout were trimmed daily starting at P9 (Stern et al., 2001). Several days later (P14–P16) acute brain slices were cut, orthogonal to barrel rows (Figure 1A) (Finnerty et al., 1999). In these slices, barrels and septa can readily be visualized (Agmon and Connors, 1991) (Figure 1B), allowing us to target our recordings to layer 2/3 pyramidal neurons at specific horizontal locations with respect to barrels and the septa. We recorded from cells directly above barrels (Feldmeyer et al., 2002) or septa.

We mapped the locations of neurons presynaptic to layer 2/3 cells in acute brain slices using laser scanning photostimulation (LSPS) by glutamate uncaging (Figures 1C–1E). Connectivity was assayed by focally uncaging glutamate with a UV laser while whole-cell recording from a layer 2/3 neuron (Figure 1C). Uncaging can elicit three types of responses in the recorded (postsynaptic) neuron (Figure 1D). (i) When the laser spot overlaps with the membrane of the recorded cell, glutamate receptors are activated by uncaged glutamate, resulting in direct stimulation. (ii) When the laser induces spiking in a synaptically coupled presynaptic neuron, synaptic transmission occurs, resulting in synaptic stimulation. (iii) In other cases, action potentials can be elicited in neurons...
not monosynaptically connected to the recorded neuron. Importantly, under our conditions these neurons did not cause spiking in other neurons that may be connected to the recorded neuron, and therefore the recorded synaptic events reflect mono- and not disynaptic (or higher order) coupling of presynaptic neurons (see below). Thus, we can use LSPS to map the spatial distribution of synaptic input of a recorded neuron. Such “input maps” were constructed by uncaging in 256 spots at 50 μm spacing. To avoid local accumulation of glutamate and the resulting desensitization of receptors, the laser scan pattern was designed so that locations visited close in time were relatively well separated in space (Figure 1E).

Resolution of Mapping by Photostimulation
To interpret input maps, it is important to characterize the effective resolution of LSPS. The effective resolution for mapping synaptic responses is defined by the spatial distribution of uncaging sites that produce action potentials in a neuron. It therefore depends on both the structure and membrane characteristics of particular classes of neurons (Dantzker and Callaway, 2000; Schubert et al., 2001), together with the type and concentration of caged compound and the intensity and shape of the stimulating light beam. It is important to note that the synaptic mapping resolution is related to, but distinct from, the resolution for mapping direct responses on individual dendrites, which is approximately 10–20 μm (our unpublished data) (Dodt et al., 1999). To characterize the synaptic mapping resolution in our system, we used loose-seal recording to detect spikes, thus avoiding perturbations introduced by membrane rupture (Figure 2A). We mapped the distributions of locations at which glutamate uncaging could produce action po-

Figure 1. Laser Scanning Photostimulation by Glutamate Uncaging to Map Circuitry in Brain Slices

(A) Vibrissae were trimmed unilaterally (left) from P9 through P14–P16, when across-row slices were cut from the contralateral hemisphere (middle), yielding up to five adjacent barrels in individual slices (right).
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tentials (excitation profiles) for excitatory neurons in layers 2/3, 4, and 5 of control and deprived animals. All cells appeared under DIC optics to have pyramidal morphology except for layer 4 cells, most of which (19/20) had more stellate than pyramidal morphology (Lübke et al., 2000; Petersen and Sakmann, 2000).

For most cells, a single perisomatic location dominated the excitation profile, with contributions from two to four other nearby locations (Figure 2B). Average excitation profiles (n = 7–10 cells per group) portray the resolution of photostimulation (distribution of excitability) (Figures 2C–2J). The vast majority of active potentials appear close to the soma; 80% of the action potentials occurred within a mean equivalent radius of 100 μm, as calculated by averaging the equivalent radii of the 80% lines for each of the six cell groups (Figures 2C–2J). We did not find sites of excitability in layer 1 (Dantzker and Callaway, 2000; Schubert et al., 2001).

We also used another measure of the spatial distribution of excitability, averaging for each cell the mean distance to the soma of each action potential (Figure 2K). Calculated this way, the mapping resolution for layer 2/3 neurons was slightly better than for layer 4 and 5 neurons. The photoexcitability of control and deprived neurons was comparable (Figure 2L). In particular, there were no differences in layer 4 barrel and septal cells. Overall, deprived neurons had a slight tendency (not significant) to be less excitable than control neurons; because these small differences could not account for the changes observed in synaptic mapping experiments, they were not used to scale synaptic map data in this study.

The excitability profiles (Figure 2B) further indicated that spiking occurs only upon direct stimulation, and not through excitation of synaptically coupled neurons. Synaptically driven spiking, if common, would have been detected as isolated “hotspots” away from the cell, which was not found (Figure 2). Moreover, comparison of excitation maps obtained with synaptic transmission blocked (nominally 0 mM Ca²⁺ in the bath) or enabled (4 mM Ca²⁺) showed no changes in excitability (see uncaging, measured by loose-seal voltage recordings from control (C, E, G, and I) and deprived cells (D, F, H, and J) in layer 2/3 (C and D) (n = 7 cells each), layer 4 barrel cells (E and F) (n = 10 cells each), layer 4 septal cells (G and H) (n = 9 cells each), and layer 5 (I and J) (n = 8 cells each). In each average excitation profile, 50% of spikes occurred within the area bounded by the inner white contour line and 80% of spikes lay within the outer red line.

(A) Loose-seal recordings from a control layer 2/3 pyramidal cell, showing action potentials elicited by photostimulation. The trains with most spikes occurred when the UV beam was closest to the soma.

(B) For the same cell, a spatial profile of excitability (excitation profile) was made by color coding the number of action potentials elicited at each stimulus site (within a temporal window of 75 ms from the stimulus). The stimulation pattern consisted of 64 sites in an 8 × 8 array (50 μm spacing), and the area mapped is indicated by the inner white box in Figure 2B. The triangle marks the soma position.

(C–J) Average excitation profiles for different cell types in control and deprived animals. The soma was always in the center of the mapped area (triangles). Pixels represent the average number of action potentials evoked at that position by scanned glutamate uncaging, measured by loose-seal voltage recordings from control (C, E, G, and I) and deprived cells (D, F, H, and J) in layer 2/3 (C and D) (n = 7 cells each), layer 4 barrel cells (E and F) (n = 10 cells each), layer 4 septal cells (G and H) (n = 9 cells each), and layer 5 (I and J) (n = 8 cells each). In each average excitation profile, 50% of spikes occurred within the area bounded by the inner white contour line and 80% of spikes lay within the outer red line.

(K) Analysis of excitation profiles: resolution of photostimulation. For each cell, an estimator of the spatial distribution of excitability was calculated as:

\[ R = \frac{\sum r \times n}{C} \]

where for each site yielding excitation r is the distance to the soma and n is the number of spikes. The plot shows, as a function of cell type (L23, layer 2/3 pyramidal cells; L4*, layer 4 barrel cells; L4s, layer 4 septal pyramidal cells; L5, layer 5 pyramidal cells), average values of R for control (blue) and deprived (red) cells. Values of this estimator were on average somewhat smaller in layer 2/3, and generally less than 100 μm.

(L) Analysis of excitation profiles: excitability to photostimulation. For each cell, the total number of action potentials (APs) was counted. The plot shows, as a function of cell type, the average number of APs/cell for control (blue) and deprived (red) cells. Layer 4 barrel (L4*) and septal (L4s) cells did not differ significantly from each other or with deprivation.

Figure 2. Excitation Profiles and the Resolution of Photoactivation by Glutamate Uncaging

(A) Loose-seal recordings from a control layer 2/3 pyramidal cell, showing action potentials elicited by photostimulation. The trains with most spikes occurred when the UV beam was closest to the soma.

(B) For the same cell, a spatial profile of excitability (excitation profile) was made by color coding the number of action potentials elicited at each stimulus site (within a temporal window of 75 ms from the stimulus). The stimulation pattern consisted of 64 sites in an 8 × 8 array (50 μm spacing), and the area mapped is indicated by the inner white box in Figure 1B. The triangle marks the soma position.

(C–J) Average excitation profiles for different cell types in control and deprived animals. The soma was always in the center of the mapped area (triangles). Pixels represent the average number of action potentials evoked at that position by scanned glutamate uncaging, measured by loose-seal voltage recordings from control (C, E, G, and I) and deprived cells (D, F, H, and J) in layer 2/3 (C and D) (n = 7 cells each), layer 4 barrel cells (E and F) (n = 10 cells each), layer 4 septal cells (G and H) (n = 9 cells each), and layer 5 (I and J) (n = 8 cells each). In each average excitation profile, 50% of spikes occurred within the area bounded by the inner white contour line and 80% of spikes lay within the outer red line.

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(L) Analysis of excitation profiles: excitability to photostimulation. For each cell, the total number of action potentials (APs) was counted. The plot shows, as a function of cell type, the average number of APs/cell for control (blue) and deprived (red) cells. Layer 4 barrel (L4*) and septal (L4s) cells did not differ significantly from each other or with deprivation.
Experimental Procedures), ruling out the further possibility that some perisomatic sites represent synaptic driving. Based on the data of Figure 2 (and additional mapping over several-fold larger areas; our unpublished data), we estimate that disynaptic driving accounts for <1% of all synaptic responses (Dantzker and Callaway, 2000; Schubert et al., 2001). Therefore, input maps recorded under the conditions used here, with divalent cations present at increased concentrations in the bathing medium, report the spatial distribution of monosynaptic inputs to a recorded neuron with <100 μm resolution. Hence mapping by LSPS is well suited to analyze cortical circuitry and experience-dependent changes therein, with subcolumnar and sublaminar resolution.

Layer 2/3 Neurons above Barrels Are Strongly Coupled to Layer 4
Layer 4 of barrel cortex is anatomically and functionally segmented into barrels and septa (Woolsey and Van der Loos, 1970; Kim and Ebner, 1999; Brecht and Sakmann, 2002). Is this segmentation transferred onto layer 2/3? For the barrels, previous studies predict strong coupling between layer 4 barrels and layer 2/3 cells in the same column (Petersen and Sakmann, 2000, 2001; Feldmeyer et al., 2002; Laaris and Keller, 2002).

We recorded input maps for layer 2/3 cells centered above barrels (Figure 3). We find that most of these layer 2/3 cells do receive prominent input from the barrel directly below (e.g., Figures 3A and 3B). However, there was significant additional structure in maps of single neurons (Figures 3–5). Strongly connected sites often occurred as sharp peaks (e.g., Figure 3, white arrows) close to the recorded neuron or within the same column. Sparse and weaker input also arose at more distant sites. These input maps were highly consistent across repeated measurements as demonstrated by low standard deviations in most pixels (Figure 3C). The EPSCs evoked at some of the sites of strongest input appear to reflect the excitation of more than one neuron connected to the postsynaptic cell (Figure 3E, arrow).

In contrast to repeated measurements of maps for a given neuron, there was high variability for maps recorded from different cells, even in nearly identical locations within the barrel column (Figures 4A and 4B). Despite this variability, the maps shared certain features. The overall organization was generally columnar, although subsets of inputs arose from neighboring barrel columns (Figures 4Ba and 4Bd). Some neurons received strong input from layer 2/3 (Figure 4Ba), while others were dominated by ascending input (Figure 4Bc). Using barrels for alignment, we averaged input maps across cells (n = 10). The resulting average input map revealed clear columnar organization (Figure 4C).

Layer 2/3 Neurons above Septa Are Weakly Coupled to Layer 4
Does the segmentation of layer 4 to layer 2/3 also extend to septa? To address this issue, we recorded input maps of layer 2/3 cells situated directly above septa (Figures...
Figure 4. Input Maps Measured in Layer 2/3 Neurons under Control Conditions

Pixels contaminated by direct responses have been blacked out. Single-cell maps are averages of two to six map repetitions. Here and in Figure 5 the white triangles mark the soma positions of individual cells, and the red triangles mark the average positions. (A and D) Schematics indicating positions of recorded neurons (triangles and arrows). (B) Maps for single neurons (a–d) directly above barrels. (C) Averaged map for neurons above barrels (n = 10). (E) Maps for single neurons (a–d) directly above septa. (F) Averaged map for neurons above septa (n = 10).

Figure 5. Input Maps Measured in Layer 2/3 Neurons under Deprived Conditions

(A) Maps for single neurons (a–d) directly above barrels. (B) Averaged map for neurons above barrels (n = 8). (C) Maps for single neurons (a–d) directly above septa. (D) Averaged map for neurons above septa (n = 8).
4D and 4E). Maps from individual cells suggest a distribution of inputs distinct from cells above barrels. The distributions of layer 4 inputs were much more variable, not respecting barrel boundaries and often arising in a sparse distribution from several regions. Often layer 4 was almost absent from input maps (Figures 4Ea, 4Ec, and 4Ed). Local supragranular inputs were stronger than for above-barrel neurons, as were infragranular inputs (see Figures 4Ec and 4Ed). These general features, (i) absence of strong layer 4 input and (ii) more pronounced layer 2/3 and layer 5 inputs, are apparent in the average map (n = 10) (Figure 4F). We conclude that layer 2/3 neurons vary with respect to their input maps depending on their horizontal position in the barrel map.

Deprivation Decouples Layer 2/3 Neurons from Layer 4 within Barrel Columns
Previous receptive field measurements using full-field unilateral deprivation revealed that the development of layer 2/3 receptive fields is experience dependent, while layer 4 receptive fields are stable (Stemp et al., 2001). These results suggest that experience-dependent plasticity involves synapses onto layer 2/3 neurons. Are patterns of inputs to layer 2/3 neurons experience dependent? Could changes in these patterns account for receptive field plasticity? To test this, we compared input maps measured in slices from deprived animals with control slices.

We measured the input maps of layer 2/3 neurons above barrels in slices from deprived animals, using the same deprivation paradigm. We found a markedly different cortical organization (Figure 5A). For these cells, input maps often had reduced input from layer 4 (Figures 5Aa–5Ac) and increased local input from layer 2/3 (Figures 5Aa and 5Ac), as compared to controls (Figure 4B). These differences were evident in the average map (n = 8) (cf. Figures 5B and 4C).

Deprivation Couples Layer 2/3 Neurons to Layer 4 within Septal Regions
Are the circuit properties of neurons above septa, which are normally poorly coupled to layer 4 (Figures 4E and 4F), affected by sensory deprivation? We measured the input maps of layer 2/3 neurons above septa in slices from deprived animals. Surprisingly, these maps showed experience-dependent changes that were complementary to those of above-barrel cells (Figure 5C). Compared to controls, the input maps from these cells often had increased input from layer 4 (Figures 5Ca, 5Cb, and 5Cd). This enhanced columnar coupling, centered on the septal region of layer 4, was evident in the average map (n = 8) (cf. Figures 5D and 4F).

Complementary Circuit Plasticity in Barrels and Septa
We analyzed these data at multiple levels (Figure 6). First, we computed difference maps to determine the loci of plasticity, sites where the effects of deprivation were greatest. A pronounced locus for above-barrel cells was in layer 4 directly beneath the recorded neurons (Figure 6A). Additional plasticity occurred in supragranular and, to a lesser extent, infragranular areas. For above-septal cells, the primary locus of plasticity also was in layer 4 directly beneath the recorded neurons, but the change was opposite in sign (Figure 6B). The difference maps thus indicate the complementary nature of layer 4 input plasticity for these two positionally defined cell types.

We then focused our analysis on layer 4 inputs by performing region-of-interest analyses. First, we examined the input strength as a function of horizontal position by calculating column means (green zone in Figure 6C). For above-barrel neurons, this demonstrated that the experience-dependent loss of layer 4 inputs was most acute directly below the recorded cells (region C in Figure 6C). Furthermore, this analysis revealed that layer 4 inputs to control cells were narrowly distributed: the half-maximal width of the response profile (blue lines, Figure 6D) is ~200 μm, less than a typical barrel diameter (~300 μm). The surrounding regions in layer 4 to the left and right of this central region (shown as regions L and R in Figure 6C) did not appear involved in plasticity for these above-barrel cells (analyzed further below). In contrast, for cells above septa (Figure 6E), the same analysis revealed both a complementary gain of layer 4 inputs directly below the recorded cells and some gain of inputs from the surrounding regions.

We also divided layer 4 into three regions: left, center, and right (regions L, C, and R; see Figure 6C). Plotting the average current evoked in all three regions for each cell (Figures 6F and 6G) reveals the overall group-wide effects of deprivation and position. Inputs from layer 4 were more narrowly columnar for cells above barrels than above septa; individual septal cells (control or deprived) in some cases received inputs distributed very asymmetrically below them in layer 4. The effect of deprivation in general was one of "retraction" of amplitudes toward the origin for above-barrel cells, and of "expansion" for above-septal cells. Thus, although deprivation caused highly complementary changes in the primary locus of plasticity (region C) for above-barrel and above-septal cells, some intrinsic differences in input maps for these two cell types were also conserved.

We further analyzed the experience dependence of responses in the primary locus of plasticity, directly below recorded neurons (region C). The average responses in region C (filled symbols in Figure 6H) again showed the complementary nature of input plasticity: for deprived cells above barrels, inputs from this region diminished substantially (53% of control levels), whereas for septa, inputs increased (83% greater than controls). Pooling pixel values (current amplitudes) from region C by group and plotting them as cumulative distributions (Figure 6I) revealed the aggregate effect of deprivation on current amplitudes. In particular, deprivation caused complementary redistributions of current amplitudes, such that above-barrel cells' response amplitudes became strikingly like those of above-septal cells, and vice versa. These treatment-dependent differences (i.e., control versus deprived) were highly significant, as were the position-dependent differences (i.e., above-barrel versus above-septal) within each treatment group (p < 0.001, rank sum test).

While excitation profiles provide information about the excitability and resolution of photostimulation, they cannot address the density of excitatory neurons, a separate and potentially important parameter in LPS con-
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Figure 6. Analysis of Circuit Plasticity

(A and B) Difference maps were made by subtracting the averaged control from deprived maps (Figures 4 and 5). Difference maps for above-barrel cells (A). Difference map for above-septum cells (B).

(C–I) Region-of-interest comparisons. (C) Schematic showing regions in layer 4 that were analyzed. (D) Mean amplitudes of layer 4 inputs (green rectangle in [C]) for control and deprived above-barrel cells. (E) Layer 4 mean amplitudes for above-septum cells. (F) Three-dimensional plot of each control (blue) and deprived (red) cell’s mean response amplitudes calculated for each region (middle, left, and right regions of layer 4), as depicted in [C]. (G) Same analysis, for cells above septa. (H) Mean amplitudes of responses in midlayer 4 (i.e., barrel or septal) regions (region “C” in [C]). Open circles represent individual cells’ mean values, and filled squares represent group means, for control (blue) and deprived (red) cells. (I) Cumulative distributions of response amplitudes, according to experimental group as indicated in the legend.

Excitatory neuronal density appeared relatively constant as follows. In slices from deprived and control animals, we recorded DIC images at a depth of 50 μm in the slice, in barrels and in septa. Labels were scrambled, and two blinded examiners counted the number of excitatory neurons (pyramidal and stellate morphology) per high power field (117 × 105 μm). The two data sets were statistically indistinguishable and therefore averaged. A total of 343 excitatory neurons were identified in 45 images (0.6 mm²). The average number per field did not vary significantly by group (control, barrels: 6.8 ± 0.7, n = 13 images; control, septa: 8.0 ± 0.4, n = 10; deprived, barrels: 8.5 ± 0.8, n = 12; deprived, septa: 7.3 ± 0.8, n = 10). Thus, for the experiments in this study, excitatory neuronal density appeared relatively constant across the cytoarchitectonic boundaries of layer 4 in barrel cortex and was not changed by deprivation.

Inhibitory Inputs

Is the horizontal segmentation of layer 4 further reflected in inhibitory circuits in layer 2/3? Do changes in inhibition contribute to plasticity? The circuit analysis performed for excitatory transmission can similarly be performed for inhibition. We mapped inhibitory currents by setting
Figure 7. Average Input Maps of Inhibition

(A) Sample inhibitory inputs, recorded at the reversal potential for glutamatergic currents.

(B, E, and H) Schematics indicating position of the recorded neurons. Neurons recorded in layers 2/3 were pyramidal cells (B–G) and in layer 4 stellate cells (H–J). Note that for the stellate cells only, a different alignment procedure was used, because cells were recorded in a variety of positions within barrels and not at a specific location with respect to barrels as in all other maps in this study. Therefore, the stellate maps only (I and J) are aligned to a common soma position (at the origin). The eccentrically placed barrel outline (dashed line in the schematic in [H]) reflects the slight overrepresentation of right-barrel cells in our samples.

(C) Input map above barrels, control (average of n = 3).

(D) Input map above barrels, deprived (n = 3).

(F) Input map above septa, control (n = 6).

(G) Input map above septa, deprived (n = 6).

(I) Input map in barrel, control (n = 3).

(J) Input map in barrel, deprived (n = 3).

the holding potential to the reversal potential for glutamatergic currents (Figure 7). Inhibitory synaptic events appeared as large outward currents (Figure 7A). Inhibitory inputs to layer 2/3 pyramidal neurons were spatially much more local than excitatory inputs (Figures 7B–7J), consistent with interneuron axonal architecture (Porter et al., 2001; Wang et al., 2002). We found no obvious dependence either on treatment (control or deprived, cf. Figures 7C and 7F versus 7D and 7G) or position (barrel or septal, cf. Figures 7C and 7D versus 7F and 7G). Changes in inhibition in layer 4 could in principle contribute to the experience-dependent differences in excitatory maps measured in layer 2/3 neurons, for example via shunting. We therefore examined inhibition in layer 4 by recording from stellate cells under similar conditions as described above. Inhibitory input maps in barrels did not show clear differences with deprivation (Figures 7I and 7J).

Discussion

The goal of this study was to identify circuitry changes underlying experience-dependent layer 2/3 receptive field plasticity in the developing rat barrel cortex. Laser scanning photostimulation (LSPS) allowed us to rapidly map intracortical synaptic connectivity in brain slices. We found that the input maps of layer 2/3 neurons vary systematically: neurons directly above barrels received,
on average, significantly more layer 4 input than those above the septa separating barrels. This pattern was reversed with deprivation: neurons above septa were strongly coupled to septal regions of layer 4, while connectivity between barrel regions and layer 2/3 was reduced.

Laser Scanning Photostimulation for Circuit Analysis
Consistent with previous studies (Callaway and Katz, 1993; Katz and D'Avella, 1994; Dantzker and Callaway, 2000; Roerig and Chen, 2002; Schubert et al., 2001), we find that LSPS is a powerful tool for analyzing the structure of cortical circuits. This method has important advantages over other methods that have been used to analyze cortical circuits. In contrast to paired recordings (Markram et al., 1997; Feldmeyer et al., 2002), LSPS is efficient, allowing an exhaustive survey of the spatial distributions of synaptic inputs converging on an individual recorded neuron. In contrast to extracellular stimulation, LSPS excites somata as opposed to axons of passage, simplifying the interpretation of input maps. The optical beam can also be scanned rapidly and at high resolution of a large presynaptic region of interest. Imaging methods have also been used for circuit analysis. These typically analyze outflow (Kozloski et al., 2001; Petersen and Sakmann, 2001; Laaris and Keller, 2002) and are thus less well suited to understand circuit changes involving a particular postsynaptic target (i.e., layer 2/3 pyramidal neurons).

Our results extend the prior characterizations (Callaway and Katz, 1993; Dantzker and Callaway, 2000; Kötter et al., 1998; Roerig and Chen, 2002; Schubert et al., 2001) of LSPS as a tool for mapping synaptic circuits in brain slices. First, we further explored the resolution of photostimulation, previously estimated as at least sufficient for laminar and columnar analysis (Dantzker and Callaway, 2000; Schubert et al., 2001), showing that in this system it is better than 100 μm (Figure 2). Second, we found that input maps from individual cells have complex high-resolution features that vary little over repeat measurements (Figure 3). Third, maps from different cells exhibited great heterogeneity, but group averages revealed connectivities that clearly depended on the cells' horizontal positions in cortex and on their sensory history (Figure 4). Thus, this in vitro technique provides an effective way to detect changes in the local (<1 mm) circuitry of a given cell type following experimental manipulations such as sensory deprivation.

Distinct Barrel and Septal Circuits in Layer 2/3
Barrel cortex is segmented horizontally in layer 4 into barrels, separated by septal regions (Woolsey and Van der Loos, 1970). Barrels and septa are cortical stations for two separate thalamocortical circuits. Thalamic inputs to barrels arise mainly from VPM, whereas thalamic inputs to septa arise primarily from POM (Koralek et al., 1988; Chmielowska et al., 1989; Lu and Lin, 1992; Brumberg et al., 1999). The VPM-barrel system (lemniscal pathway) subserves high-acuity, short-latency, and precisely somatotopic representation of vibrissal signals. In contrast, the more widely connecting POM-septal system (paralemniscal pathway) mediates slower, multiwhisker, complex processing (Chiaia et al., 1991; Diamond et al., 1992b; Ahissar et al., 2000) in a cortex-dependent manner (Diamond et al., 1992a). In layer 4, receptive fields in septa are much broader than in barrels (Brecht and Sakmann, 2002), and septa connect widely to adjacent barrels, other septa, and numerous other cortical areas both ipsi- and contralaterally (Chapin et al., 1987; Olavarria et al., 1984; Kim and Ebner, 1999). The distinct circuitry of the VPM-barrel and POM-septal systems begs the question of how the horizontal segmentation of layer 4 into barrels and septa is transferred to layer 2/3.

Strong coupling between layer 4 barrels and layer 2/3 cells in the same column is predicted based on previous studies (Petersen and Sakmann, 2000, 2001; Feldmeyer et al., 2002; Laaris and Keller, 2002). Layer 4 barrel cells direct most of their axonal arbor vertically to layer 2/3 (Lübke et al., 2000; Brecht and Sakmann, 2002; Petersen and Sakmann, 2000). Our data show that the converse is also true: cells directly above barrels do receive substantial inputs from layer 4 barrels (Figures 3 and 4A–4C). Despite substantial heterogeneity, our findings for control cells above barrels are consistent with the notion of a strong vertical organization within a barrel-related column (Simons, 1978; Bernardo et al., 1990).

For the septa, anatomical studies have suggested the existence of “septal-related columns” extending into layer 2/3 (Kim and Ebner, 1999). Surprisingly, our data indicates that cells above septa received less layer 4 input than cells above barrels (Figure 4); some patterns of input from layer 4 to above-septal layer 2/3 cells, such as inputs from either or both of the barrels next to the septum, were observed in isolated examples but were less evident in average maps. In contrast, local layer 2/3 connections were relatively strong for the above-septum cells, meaning that these cells were often functionally connected to other neurons in at least two barrel-related columns. Thus, the connectivity of cells above septa is biased toward local supragranular connections rather than sampling from layer 4.

It would be of great interest to know whether there also exist barrel/septal differences both in the outputs of supragranular cells and in their inputs from the infragranular layers (largely outside the area sampled here), particularly in light of observations that output cells from infragranular layers in granular cortex appear vertically in register with the septa (Crandall et al., 1986; Bourassa et al., 1995). It will also be important to find out if systematic differences in synaptic input fields in layer 2/3 exist in other cortices. Indeed, LSPS experiments in primary visual cortex indicate that layer 3B neurons as a group have highly polymorphic input maps (Sawatari and Callaway, 2000), much like the group of layer 2/3 neurons studied here, but that the average laminar distribution of inputs differed for projecting versus nonprojecting pyramidal neurons, and for pyramidal neurons situated in blobs versus those in interblobs. Finally, the extent to which barrel/septal circuits differ along barrel arcs (studied here) versus barrel rows merits evaluation, since septa along rows are smaller (Welker and Woolsey, 1974) and may have different response properties.
Experience-Dependent Plasticity in Supragranular Circuits

Sensory deprivation by trimming all whiskers on the contralateral face reduces spine motility (Lendvai et al., 2000) and disrupts receptive field structure (Stern et al., 2001) of layer 2/3 neurons in barrel cortex. On the other hand, receptive fields of layer 4 neurons are stable (Stern et al., 2001). These in vivo observations implicate synaptic circuits converging onto layer 2/3 neurons as the locus of experience-dependent plasticity (Stern et al., 2001; Feldman, 2001). Results using different deprivation paradigms during development (Fox, 1992; Finnerty et al., 1999; Trachtenberg et al., 2000) and in the adult (Diamond et al., 1994), such as selective trimming of subsets of whiskers, also implicate layer 2/3 as a primary site of plasticity.

LSPS mapping showed that sensory deprivation caused complementary changes in the input maps of layer 2/3 neurons above barrels and above septa. Coupling to layer 4 was reduced for cells above barrels and augmented for cells above septa. In addition, all layer 2/3 neurons had wider ranging input from other layer 2/3 cells in deprived animals. These circuit level changes could explain the broadening of layer 2/3 receptive fields observed in vivo following the same paradigm of whisker trimming (Stern et al., 2001). After deprivation, layer 2/3 lost input from the high-acuity VPM-barrel system and gained input from the low-acuity/multiwhisker POm-septal system, resulting in broader layer 2/3 receptive fields. However, further experiments involving layer 2/3 neurons at different horizontal and vertical positions will be required to settle this issue.

An intriguing aspect of the circuitry plasticity observed here is that it appears to reflect competition between two thalamocortical circuits, the VPM-barrel and POm-septal systems (Jeannin et al., 1981; Rice, 1995; Kaas and Ebner, 1998). These two systems are well segregated in layer 4 (Kim and Ebner, 1999). Because septa and septal columns are the targets not only of POm projections but also numerous others pathways (see above), the augmentation in the septal circuitry observed here may be driven by inputs from POm, VPM, ipsilateral motor cortex, contralateral areas (such as the other barrel field), or other sources. In the deprived brain, the septal neurons may thus experience less of a reduction of patterned input than barrel neurons, and would therefore more effectively compete for postsynaptic targets in layer 2/3. Interactions between barrel and septal circuits are not without precedent. At the level of layer 4 cytoarchitecture, broadening of septa has been described following whisker lesioning of mice at P4–P5, interpreted as reflecting a “competitive advantage” of septal components over the VPM-barrel projection (Jeannin et al., 1981; Rice, 1995).

What cellular substrates underlie experience-dependent changes in synaptic circuits and receptive fields? Our measurements have not detected systematic experience-dependent changes in inhibition. Changes at excitatory connections could be of several types. First, structural plasticity in dendrites or axons could be involved. Preliminary data indicate that dendritic branching patterns are perturbed by deprivation (K.S. and M. Maravall, unpublished data). A second involves local synaptic changes, either by growth of new synapses or plasticity of existing synapses (Finnerty et al., 1999). Consistent with this possibility, measurements of glutamate receptor content have revealed that deprivation impairs delivery of AMPA type glutamate receptors (Takahashi et al., 2003). Combinations of these and other expression mechanisms are likely involved.

Experimental Procedures

Slice Preparation

Animal care and experimentation followed institutional guidelines. For sensory deprivation experiments, all left-sided mystacial vibrissae of Wistar rats were trimmed daily, from P9 until sacrifice at P14–P16. Across-whisker-barrel row slices (Finnerty et al., 1999) (Figure 1A), 300 μm in thickness, were cut by vibrating-microtome (VT1000S, Leica Microsystems) from the right hemisphere of deprived or control animals. Chilled cutting solution consisted of 110 mM choline chloride, 25 mM NaHCO3, 25 mM D-glucose, 11.6 mM sodium ascorbate, 7 mM MgSO4, 31.1 mM sodium pyruvate, 2.5 mM KCl, 1.25 mM NaH2PO4, and 0.5 mM CaCl2. Slices were then transferred to artificial cerebrospinal fluid (ACSF) consisting of 127 mM NaCl, 25 mM NaHCO3, 25 mM D-glucose, 2.5 mM KCl, 4 mM MgCl2, 4 mM CaCl2, and 1.25 mM NaH2PO4, aerated with 95% O2/5% CO2. Slices in ACSF were first incubated at 34°C for ~0.5 hr, then maintained at room temperature prior to use. In the recording chamber, barrels, septa, and laminae were readily identified (Figure 1B). For the above-barrel configuration, only slices with at least three clear, full-sized barrels (i.e., a center and two side barrels) were used, while for the above-septum configuration, at least two were required; typically one to three slices per animal fulfilled these criteria. To minimize any possible overlap of barrels and septa along the z axis of the slice, we used thin slices and also routinely examined adjacent slices to ensure that the selected slices contained the barrels “cores.” Recorded neurons were situated in barrel rows in arcs 2–4, and vertically in the center of layers 2/3, at an average distance of 203 ± 44 μm (±SD) from layer 4 (no significant intergroup differences).

Electrophysiology

Neurons were visualized with infrared differential interference contrast optics, and patched using borosilicate electrodes (resistances, 4–7 MΩ). Neurons were 40–80 μm deep in the slice, with no significant differences between groups (control/above-barrel, 45 ± 3 μm; control/above-septum, 52 ± 5 μm; deprived/above-barrel, 48 ± 5 μm; deprived/above-septum, 49 ± 4 μm; mean ± SEM, pair-wise t tests). Access resistances were in the range 10–40 MΩ. For current recordings, intracellular solution consisted of 120 mM CsMeSO4, 20 mM CsCl, 4 mM NaCl, 10 mM HEPES, 1 mM EGTA, 4 mM MgATP, and 0.3 mM Na3GTP, 14 mM sodium phosphocreatine, 3 mM ascorbate, and 0.1 Alexa-594 (Molecular Probes); pH was adjusted to 7.25 with CsOH. For voltage recordings, the solution was identical except for substitution of potassium for cesium. Excitatory currents were measured at a holding potential of ~65mV, close to reversal for fast inhibition. Further isolation of excitatory currents by blocking GABA receptors was precluded by paroxysmal activity, even when activity was dampened by high divalents and adenosine (in agreement with prior studies, e.g., Laaris et al., [2000], but see Petersen and Sakmann, [2001]). Inhibitory currents were measured at the empirically determined reversal potential for glutamatergic currents (5mV–10mV at the soma, determined for each cell). Recordings were at room temperature. Responses were amplified (Axopatch 200B, Axon Instruments), filtered at 5 kHz, and digitized at 10 kHz. Custom software written in Matlab (MathWorks, Inc.) was used for electrophysiological acquisition, laser scanning, and data analysis.

Glutamate Uncaging

Nitroindolyl (Ni)-caged glutamate (Sigma-RBI; [Canepari et al., 2001]) was added to recirculating ACSF to a concentration of 0.37 mM. Once whole-cell recording was established, focal photolysis of caged glutamate was accomplished by Q switching a DPSS laser (355 nm wavelength, frequency-tripled Nd:YVO4, 100 kHz pulse repetition rate; DPSS Lasers, Inc.), to give a 1 ms light stimulus.
consisting of 100 pulses. Laser power was controlled using neutral density filters and measured with each stimulus presentation by a photodiode. In preliminary experiments, we tested a range of laser powers and empirically determined that 5 mW (at the specimen) provided reliable excitation of neurons while staying well below the threshold for photo- and excitotoxicity-related damage. In separate experiments (data not shown), we further characterized the effects of intensity on LSPS mapping, finding that mapping resolution remained constant over a wide range of intensities and that the number of action potentials fired scaled with intensity for different cell types. Thus, above a threshold level of intensity, connectivity imaging appears to yield comparable connectivity patterns over a fairly wide range of stimulus intensities.

The beam’s position was controlled with mirror galvanometers (Cambridge Scanning, Inc.). The beam entered the microscope via a dichroic mirror, and was focused using a low-numerical aperture (NA), low-magnification objective lens (10×/0.3 NA/water UMPlanFI, or 4×/0.13 NA/AIR UMPlanFI; Olympus). The optics were designed to generate a nearly cylindrical beam in the slice so as to keep the mapping two dimensional (i.e., avoiding three-dimensional effects due to rays turning). We typically performed control experiments performed by uncaging glutamate on small dendritic branches revealed a beam diameter of ~15 μm and a depth of field of ~500 μm.

**Stimulus Patterns**

The standard stimulus pattern for LSPS mapping consisted of 256 positions on a 16 × 16 grid. Spacing was set to 50 μm between adjacent rows and columns, giving a 750 × 750 μm mapping region. For loose-seal spike recordings, stimulus patterns with 64 positions (8 × 8 grid) were used. To avoid receptor desensitization, glutamate toxicity, and local cage depletion, we designed a basic stimulus sequence pattern that avoided the neighborhoods of recently visited sites (Figure 1E), and alternated among a set of flipped and rotated variants of this sequence during consecutive mapping. UV stimuli were presented at ~1 Hz. Traces consisted of 100 ms of baseline prior to the stimulus, a 500 ms response interval, and a test pulse for measuring electrophysiological parameters.

**Analysis**

Responses were analyzed within 75 ms after the UV stimulus (Figure 1D). Control experiments (data not shown) confirmed the previously reported temporal separation of direct and synaptic responses at 9–10 ms (Schubert et al., 2001). Thus, responses occurring within the first 10 ms of the analysis window were categorized as direct (i.e., purely postsynaptic), and later responses were categorized as synaptic. Individual input maps were constructed by computing the mean current amplitude (10–75 ms poststimulus) for each location of photostimulation; maps constructed from measurements of the maximal currents gave similar results (data not shown). Typically two to five maps were obtained per cell and averaged (Figures 3, 4B, 4E, 5A, and 5C). These averaged single-cell maps were used to obtain group-averaged maps according to treatment condition (control versus deprived) and cell position (above-barrel versus above-septum) (Figures 4C, 4F, 5B, and 5D). Direct responses were excluded from calculations of averages (blacked-out pixels). For display only, minimal smoothing was performed on group average images using a 3 × 3 Gaussian kernel with standard deviation of ~25 μm (Figures 4B, 4D, 5B, 5D, 6A, and 6B). Statistical comparisons were performed on raw data and, except where noted, were made using Student’s t test. Significance level was p = 0.05. Error bars in plots signify standard errors of the mean.

Spontaneous synaptic activity, which could potentially degrade the signal-to-noise ratio and thus the quality of synaptic input maps, was addressed as follows. We included high concentrations of divalent cations in the bath solution (4 mM Ca²⁺ and 4 mM Mg²⁺), reducing neuronal excitability while maintaining a high probability of release at synapses. Under these conditions, estimates of the frequency of spontaneous events, based on analysis of the prestimulus baseline interval in a subset of cells (n = 10), indicated that mean spontaneous event rates are low (0.7/s for events with peak amplitudes ~10 pA). Most importantly, we obtained multiple maps per cell and averaged them (see above), thereby greatly reducing the impact of spontaneous events and providing a pixel-by-pixel measure of the trial-to-trial variability (Figure 3). Normalization methods (Dantzker and Callaway, 2000) were not necessary (see “SD” and mean maps, Figure 3) and thus not used.

The possibility that some disinaptically activating sites occur locally, very close to the soma, was assessed in the following way. Excitation profiles were recorded from cells first with synaptic transmission blocked by reducing the concentration of calcium ions in the ACSF mapping fluid to zero (0 mM Ca²⁺, 8 mM Mg²⁺), then with synaptic transmission enabled (4 mM Ca²⁺, 4 mM Mg²⁺). In 4/4 cells (layer 2/3) the addition of Ca²⁺ did not significantly change either the total number of APs elicited (96% ± 8% compared to 0 Ca²⁺) or the mean distance of APs from the soma (89% ± 13% compared to 0 Ca²⁺).

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