Experience-dependent Changes in Basal Dendritic Branching of Layer 2/3 Pyramidal Neurons During a Critical Period for Developmental Plasticity in Rat Barrel Cortex

In rat barrel cortex, development of layer 2/3 receptive fields can be disrupted by sensory deprivation, with a critical period ending around postnatal day (PND) 14. To determine if experience-dependent plasticity of dendritic morphology could contribute to the reorganization of synaptic inputs, we analyzed dendritic structure in acute brain slices using two-photon laser scanning microscopy (2PLSM) and automated segmentation and analysis software. Layer 2/3 pyramidal cells from control and deprived rats were imaged from PND 9 to PND 20, spanning the critical period. Detailed analyses were performed on basal arbors, which receive the majority of synaptic input from layer 4. Some parameters (number of primary dendrites, volume subtended, aspect ratios) were stable, suggesting that development of several important properties of basal arbors has ceased by age PND 9. However, the spatial organization of secondary basal branching changed with age and experience. In older neurons there was a larger fraction of branch points farther from the soma. Deprivation from age PND 9 delayed these changes in secondary branching. This effect of deprivation was rapid (detectable at PND 10) and present at all ages observed. Deprivation initiated at PND 15 had no effect on basal branching measured at PND 20. Thus the spatial organization of secondary dendritic branching is experience-dependent and shares a critical period with receptive field plasticity.

Keywords: dendrite, deprivation, morphology, neocortex, two-photon

Introduction

The development of neocortical neuronal circuits is dependent on sensory experience (Katz and Shatz, 1996) and probably involves a multitude of mechanisms (Crair and Malenka, 1995; Fox, 1995; Kirkwood et al., 1995; Hensch et al., 1998; Huang et al., 1999; Quinlan et al., 1999; Lu et al., 2001; Philpot et al., 2001). The barrel cortex (BC) of rodents is an ideal system for studying experience-dependent cortical development (Simons and Land, 1987; Fox, 1992). A topographical sensory map from the whiskers to primary somatosensory cortex (S1) is established early during postnatal development. Barrels are discrete aggregates of neurons in S1 layer 4 (Woolsey and Loos, 1970; Welker and Woolsey, 1974) that correspond to representations of individual whiskers (principal whisker, PW) (Welker, 1971; Simons, 1978; Armstrong-James and Fox, 1987): in animals with normal sensory experience, the electrophysiological map mirrors the anatomical map. Barrels form soon after birth (Schlaggar and O’Leary, 1994) and are resistant to sensory manipulation after an early critical period (postnatal day [PND] 5) (Rice and Van der Loos, 1977; Henderson et al., 1992; Fox et al., 1996). However, sensory deprivation by whisker clipping can still perturb the normal whisker selectivity of BC responses (Hand, 1982; Simons and Land, 1987; Fox, 1992) in a layer-specific manner: while layer 4 responses become less sensitive to whisker clipping at around PND 7 (Fox, 1992), layer 2/3 and layer 5 receptive fields remain plastic (Armstrong-James et al., 1994; Diamond et al., 1994; Glazewski and Fox, 1996; Lendvai et al., 2000).

Layer-specific plasticity differences probably reflect different time courses for maturation of thalamocortical and intracortical neurons and synapses (Crair and Malenka, 1995; Kirkwood et al., 1995). Intracortical synaptic circuitry undergoes massive development after thalamocortical innervation and barrel formation: cortical synaptic density increases severalfold between PND 9 and PND 15 (Micheva and Beaulieu, 1996; De Felipe et al., 1997).

We recently analyzed the experience-dependent development of layer 2/3 receptive fields using in vivo intracellular recordings from PND 12 to PND 20 (Stern et al., 2001). Trimming all contralateral whiskers at PND 9 caused remarkable whisker map disorganization: responses to PW stimulation decreased while surround responses increased, resulting in a significant loss in acuity. Deprivation initiated at PND 15 failed to change receptive fields measured at PND 20. A recent study using laser scanning photostimulation (LSPS) revealed that in the normal brain layer 4 barrels project more strongly to layer 2/3 than septal regions. In the deprived brain this relationship is reversed: septal neurons dominate the projection to layer 2/3. In addition, in the deprived brain there is stronger connectivity within layer 2/3. These studies provide a circuit-level explanation of the expression of plasticity (Shepherd et al., 2005). In contrast, cellular correlates of plasticity are poorly understood. For example, layer 4 to layer 2/3 axonal arbors appear to develop in an experience-independent manner (Bender et al., 2003).

We have analyzed layer 2/3 dendritic development over ages spanning the layer 2/3 critical period. We focused on basal arbors, since they receive the majority of layer 4 input (Feldmeyer et al., 2002). We found that basal dendritic branching develops in a stereotyped sequence that is delayed by sensory deprivation during but not after the critical period.

Materials and Methods

Animal Deprivation Protocol

All procedures were in accordance with animal care and use guidelines of the Cold Spring Harbor Laboratory. Rat pups were deprived by clipping all whiskers on one side of the snout to <1 mm length. Clipping began at ages PND 9, 12 or 15 and was repeated at intervals of <48 h. Clipping was performed with no anesthesia and brief handling; control animals were also handled during clipping sessions. Deprived animals were kept in the same cages as their mothers and control
littermates until the experiment. Recordings were made at PND 9, 10, 12, 14, 17 and 20.

Preparation
Cortical slices were prepared from the hemisphere contralateral to the clipped whiskers (see Fig. 1G for a schematic). The brain was removed and blocked at an angle intermediate between coronal and sagittal (40°–45° from the midline, anteromedial to posterolateral) and inclined ~10° from the vertical plane. This angle was approximately parallel to barrel arcs (Finnerty et al., 1999; Feldman, 2000; Shepherd et al., 2003). The most posterior slices that showed barrels under bright-field illumination contained the large barrels of the posterior medial barrel subfield (PMBSF), corresponding to the longer vibrissae at the posterior edge of the whisker pad. Slices (300 µm thick) were cut on a VT-100 microtome (Leica, Wetzlar, Germany) with the brain submerged in a chilled (2–5°C) cutting solution bubbled with carbogen (95% O2/5% CO2). The solution contained (in mM): 110 choline chloride, 25 NaHCO3, 25 tris-glucose, 11.6 sodium ascorbate, 7 MgSO4, 3.1 sodium pyruvate, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2. After cutting, slices were transferred to a submerged holding chamber containing artificial cerebrospinal fluid (ACSF) and incubated at 35°C for 25–50 min and then held at room temperature until used. The composition of the ACSF was (in mM) 127 NaCl, 25 NaHCO3, 25 tris-glucose, 2.5 KCl, 2 CaCl2, 1 MgSO4, 1.25 NaH2PO4. All chemicals were from Sigma (St Louis, MO) unless otherwise noted.

Recording Conditions
Neurons were labeled using whole cell patch recordings. Either calcine (200 µM, Sigma) or Alexa 594 (50–200 µM, Molecular Probes) were added to an internal solution containing (in mM): 135 potassium methylsulphonate, 10 HEPES, 10 sodium phosphocreatine, 4 MgCl2, 4 Na2ATP, 0.4 Na-GTP. Electrodes were pulled to a resistance of 3–6 MΩ.

Recordings were performed only within slice regions that contained barrels of the PMBSF identified under transillumination. To limit the effects of morphological variability across cells situated at different depths, all layer 2/3 pyramids were 200–500 µm from the pia (Peters and Jones, 1984). Neurons with transverse apical arbors were not included (van Bredereode et al., 2000).

For imaging, we used a custom-built two-photon laser scanning microscope (2PLSM) (Mainen et al., 1999). Images had 512 × 512 pixels (0.4–0.57 µm/pixel) with a field of view of 292 µm (e.g. Fig. 1A–F). Image stacks of 70–150 frames were collected in 1 µm steps (Fig. 1G) separately for the apical and basal dendritic trees. Laser power was adjusted as imaging depth varied.

Analysis of Truncation Artifacts
The single most important source of systematic error in slice studies of dendritic morphology is abor truncation. We took the following steps to check for the effects of truncation on our results. For all experiments, the depth from the top of the stack to the cell body was measured (mean ± SD = 44.7 ± 9.08 µm; this depth was always smaller than the actual depth from the slice surface). Comparisons of depths were made across every pair of groups for which morphological data were also compared (e.g. control and deprived groups for each age, or pairs of control groups at all ages; see Results), using t-tests. None of these pairwise comparisons of imaging depth were significant. Therefore, statistically significant differences across our data groups could not be traced back to differences in truncation. In addition, all ANOVA tests involving more than two groups of data (see Results) were replicated on depths, with no significant differences found. Therefore, non-significant differences should have been the default result for all comparisons of data across groups: truncation artifacts would mask statistical differences (give false negative results) rather than produce false positives. Further, comparison of full stacks (including the slice surface) with more truncated stacks used for image analysis showed that no branch points were affected by truncation. This implies that the majority of basal branch points are relatively close to the soma, as found previously (Larkman, 1991).
Figure 1. Analysis of basal dendritic morphology. Neurons were filled with a fluorescent dye, imaged with 2PLSM, and analyzed with automated 3-D segmentation software. (A–F) Maximal projections of image stacks showing the cell body, basal arbor and part of the apical arbor of layer 2/3 pyramidal neurons. Ages and conditions are indicated in each panel (d9, deprived from PND 9; d15, deprived from PND 15). Scale is the same for all panels. (D) includes the pipette used to fill the neuron. (G) Schematic of preparation. Slices were cut from the hemisphere contralateral to the clipped whiskers and oriented along cortical barrel columns. Image stack is projected onto three planes for the neuron shown in (E). (H) Projection of the segmented arbor from the neuron shown in (G). (I) Projection of the trimmed arbor, showing the dendritic tree used for morphological analysis. The ring marks the cell body region. (J) Same as (I) for the neuron shown in (B).
Results

Two-photon Imaging of Basal Dendritic Morphology
We measured the development and plasticity of basal dendritic morphology during a period of rapid synaptogenesis and enhanced whisker map plasticity in rat barrel cortex. Layer 2/3 pyramidal neurons from control and deprived animals were imaged at PND 9, 10, 12, 14, 17 and 20 (Fig. 1) and analyzed using segmentation software.

We concentrated our analyses on basal arbors because they are the main locus for ascending synaptic input onto layer 2/3 pyramidal neurons (Feldmeyer et al., 2002), and hence constitute the main pathway for afferent input from whiskers. Furthermore, the structure and shape of apical arbors are extremely variable (Lubke et al., 2003). Image stacks were recorded from \( n = 102 \) neurons, of which \( n = 65 \) were included in the analysis, with four to six neurons analyzed per group. The main criteria influencing whether a neuron was included in the analysis were the extent of truncation assessed by visual inspection, and whether dye spillage and image quality allowed proper segmentation of the dendritic arbor (Fig. 1H–J; see Materials and Methods).

To characterize dendritic arbors we first considered large-scale measures of structure (number of primary dendrites, subtended volume, ratios of moments of inertia). We then considered local arbor structure (spatial branch point distributions, Sholl diagrams). We first present the results of our analyses of the large-scale measures and then describe the effects of age and of deprivation on the detailed branching properties of the basal arbor.
Large-scale Features of Arbor Structure are Independent of Development and Sensory Experience

After age PND 9–10, dendritic structure did not obviously change as a function of development and experience (e.g. Fig. 1A–F). We first counted the number of primary basal dendrites, defined as those emerging from the soma. This number did not change with age or experience (Fig. 2). These results are consistent with other studies (see Discussion) and suggest that the full complement of primary dendrites is assembled at the start of the period of maximum intracortical synaptogenesis.

The total spatial extent of the basal arbor was measured using the minimum volume subtended by a convex polygon containing the arbor. This measure varied widely from neuron to neuron, as the presence of a single ‘outlier’ branch often dominated the size of the convex polygon. No differences due to age or experience were detected (data not shown). Similarly, analysis of the moments of inertia of arbors showed large variability, and no developmental or experience-dependent effects were found. Thus the arrangement of primary dendrites and the overall shape of the basal arbor do not substantially change from PND 9 to PND 20, remaining relatively stable throughout the period of maximal synaptogenesis.

Secondary Basal Branching Changes with Development

Spatial Branch Point Distributions

We next analyzed measures of local changes in dendritic structure. The spatial branch point distribution is more robust than large-scale measures against the presence of rare, long outlier branches and against variability due to arbor truncation. Histograms of three-dimensional radial distances of branch points to the soma were constructed and integrated, to provide cumulative distributions of branch point distances (Fig. 3A). Branch point distributions at the youngest age (PND 9) had a larger fraction of their weight concentrated at a shorter distance (20–25 µm from the soma) than later in development (Fig. 3A). At later ages (e.g. PND 20) distributions had a substantial part of their weight further away (50–60 µm from the soma). This development of branching distributions occurred gradually. Differences between branch point distributions at PND 9 and all other ages were significant, as were differences between distributions at PND 20 and most other ages (Table 1).

Correlations with Number of Branch Tips: Are Branches Pruned or Only Added?

What are the processes underlying developmental changes in the distributions of branch points? One possibility is that new branch growth would add branch points selectively farther from the soma. If this were enough to explain the change in branch point distributions, one would expect systematic developmental increases in the total number of branch points and of dendritic tips (which are linearly related). However, we found that the number of branch tips did not increase with age (Fig. 3B). This suggests that other processes such as selective branch retraction close to the soma, and perhaps overall arbor growth, contribute to developmental rearrangements of dendritic structure.

Support for the possibility of branch retraction came from developmental changes in the spatial organization of branching. For each neuron, we measured the median distance from soma to branch points. For each age group, we then computed the correlation over all neurons of this median branch point distance with the number of branch tips. The result was that, in each of the older age groups (PND 14–20), total branch tip number was highly correlated with median branch point distance (Fig. 3D). Thus, at older ages, a larger number of branches was directly related to a larger median distance, i.e. neurons with many branches had relatively more branches farther from the soma. Therefore, within these groups, just one of these variables (total number or median distance) could account for the full variability in branching patterns; in this sense branching was highly organized. However, for the younger age groups (PND 9–10, Fig. 3C) this organization was not present: there was little correlation between median distance and number of branches. In these age groups, neurons could have many branches but arranged comparatively close to the soma, or have few branches sprouting at relatively large distances (compare representations of younger and older age groups in Fig. 3G).

This developmental change in the correlations between median branch point distance and number of branches (Fig. 3E) suggests that at earlier ages dendritic branching is less organized spatially. Developmental rearrangements would include retraction of branches close to the soma, as well as addition of new branches farther from the soma. Figure 3G shows a representation of the effects of these rearrangements on spatial branch point distributions.

The remaining possibility is that branch points are pushed outward as a result of arbor growth (including primary and secondary branch growth). However, this possibility would cause a collective shift of branch points away from the soma: for instance, primary dendrite growth would imply an increased distance to all branch points, including the closest ones. Our data suggest otherwise, as there was no noticeable growth either in the distance from the soma to the closest branch points (Fig. 3A), or in the minimum median branch point distance found in each age group (except from PND 9; Fig. 3C,D). In addition, a significant effect of arbor growth would be unlikely because of the dense structure of the neuropil, which consists of highly entangled neuronal processes.

Table 1

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Cells show P-values for comparisons between the corresponding ages.

Sholl Diagram Analyses

A complementary approach to branch point distance analysis is Sholl diagram analysis (Sholl, 1953). This involves counting the number of dendrites intersecting a series of concentric spherical shells of increasing radius, centered on the cell body, and plotting these counts against the distance to the cell body. These analyses gave results consistent with the changes
observed in spatial branch point distributions (Fig. 3F): development did not cause a systematic increase in the maximum number of dendritic intersections with a shell. However, age had a clear effect in that it tended to increase the number of shells with a large number of intersections, that is, the peak became wider. In addition, the distance from the soma to the Sholl diagram peak increased with age (Fig. 3F).

**Correlations with Total Dendritic Length**

We next analyzed the total dendritic length of basal arbors, quantified by summing dendritic segment lengths over all the segments constituting each tree. Branch point distributions with greater relative weight at increased distances might correlate with increased total arbor length: if changes in the branch point distribution were purely due to addition of new branch points at relatively distant positions, we would expect to observe systematic increases in total length from the new branches; retraction of other branches, if present, would tend to counteract these increases.

Consistent with this possibility, we did observe apparently age-dependent changes in basal length. In control animals, basal arbors were significantly shorter at PND 9 and 10 than at the oldest ages (PND 17–20) (data not shown; one-way ANOVA test, \( P < 0.03 \)), consistent with the increased width of Sholl diagrams at older ages (Fig. 3F). Intermediate ages (PND 12 and 14) were not however significantly different from either the younger age groups or the older ones, suggesting that growth in dendritic length was gradual.

**Secondary Basal Branching Changes with Sensory Experience**

**Spatial Branch Point Distributions**

Differences in experience had a marked effect on the distributions of branch points (Fig. 4A). In neurons from animals deprived starting at age PND 9, dendritic branching tended to occur at shorter distances than in neurons from control animals at the same ages. This effect could be observed already at PND 10, one day after deprivation (Fig. 4A), and was present at all ages analyzed, up to PND 17 (Fig. 4A). At every age there was a significant difference between control and deprived cumulative branch point distance distributions, after deprivation at PND 9 (Table 2). Depressed distributions at later ages (e.g. PND 10) tended to resemble control distributions at earlier ages (e.g. PND 9).

**Figure 4.** Dependence of secondary basal branching on sensory experience. (A) Spatial branch point distributions at different ages. Experimental age is shown over each panel. In blue, control distributions; in red, distributions after deprivation from PND 9; in green, distributions after deprivation from PND 12. Asterisks denote significant differences caused by deprivation at corresponding ages. Distributions after deprivation at PND 9 and PND 12 were weighted towards significantly lower distances than control distributions. (B) Correlation coefficients between the number of tips and the median branch point distance, computed across all neurons in the control groups and deprived distributions. (C) Correlation did not increase systematically with age after deprivation at PND 9, and was delayed compared to correlation in normal development. (D) Spatial branch point distributions measured at PND 20. In blue, control distribution; in violet, distribution after deprivation from PND 15. There was no significant difference between the two groups: sensory deprivation had no effect on branching if started after PND 14. (E) Average Sholl diagrams for neurons in control and deprived groups at different ages. Experimental age is shown over each panel. In blue, control distributions; in red, distributions after deprivation from PND 9; in green, distributions after deprivation from PND 12; in violet, distributions after deprivation from PND 15. After deprivation from PND 9, Sholl diagrams peaked closer to the cell body than in control neurons.

The effects of experience on branch point distributions were further tested. For each neuron we measured the median branch point distance and performed a non-parametric ANOVA two-way test on the resulting distribution of medians, over all ages at which arbors were imaged both under control conditions and after deprivation at PND 9 [ages PND 10–17; median distances for each group (mean and SEM): PND 10 control, 29.1 ± 2.6 µm; PND 10 deprived, 21.9 ± 1.5 µm; PND 12 control, 24.4 ± 2.5 µm; PND 12 deprived, 19.0 ± 3.3 µm; PND 14 control, 25.2 ± 3.1 µm; PND 14 deprived, 16.5 ± 1.4 µm; PND 17 control, 25.3 ± 3.0 µm; PND 17 deprived, 22.1 ± 1.2 µm]. The effects of deprivation were highly significant (\( P < 0.001 \)).
Correlations with Number of Branch Tips: Does Deprivation Delay Development?

Deprivation did not change the total number of branch tips (data not shown). However, deprivation did affect the spatial organization of dendritic branching, as it affected the correlations between median branch point distances and numbers of branches (Fig. 4B). The developmental increase in correlations was delayed compared to control conditions: the correlation coefficients of deprived groups resembled those of control groups at earlier ages, consistent with the changes observed in spatial branch point distributions. Our data are therefore compatible with the idea that the secondary structure of basal dendritic arbors matures more slowly in the deprived brain than under conditions of normal experience.

Critical Period for Dendritic Structure Plasticity

To determine if plasticity of dendritic structure reflects a critical period we analyzed the structure of neurons from animals deprived at various ages. At PND 17, neurons were imaged from control animals and from animals deprived at PND 9 and PND 12 (Fig. 4A). Deprivation at PND 12 also disrupted dendritic arbor development, with branching distributions more similar to those from neurons deprived at PND 9 than to those from the control group (Table 2). However, deprivation initiated at PND 15 failed to disrupt branching distributions observed at PND 20 (Fig. 4C; Table 2). Thus the experience-dependent plasticity of dendritic arbor remodeling shares a critical period with receptive field plasticity.

Sholl Diagram Analyses

Over all ages from PND 10 to PND 17, Sholl diagrams for neurons from animals deprived at PND 9 peaked at slightly shorter distances from the cell body than Sholl diagrams for control neurons (Fig. 4D). In one group (PND 12) the maximum number of intersections recorded was also appreciably smaller. (At this age, and consistent with this result, the number of tips was slightly larger for the control group than for the deprived group; this was not systematically true over all ages, as mentioned above.) In all other groups (PND 10, 14, 17) the smaller distance to the peak in Sholl diagrams for deprived groups as compared to controls was partly due to a slightly larger number of intersections at very short distances, accompanied by a smaller spread of the peak towards longer distances (~65–80 μm from the soma). Therefore, a deprived group for a given age tended to have a Sholl diagram more like that of a younger-age control group. These changes were always localized within comparatively proximal distances (~80 μm) and were consistent with the quantitative statistical tests performed on branch point distributions and with the hypothesis that deprivation delayed maturation of secondary dendritic structure.

Discussion

Layer 2/3 BC sensory maps (Lendvai et al., 2000; Stern et al., 2001) and circuits (Shepherd et al., 2003) display rapid developmental and experience-dependent plasticity during a critical period around PND 10–14. Here we show that refinement of secondary basal dendritic branching matures over this developmental period and that sensory deprivation interferes with this maturation. These findings suggest a link between dendritic structural plasticity and the experience-dependent development of cortical circuits.

Experience-dependent Development of Secondary Dendritic Structure

Our results rely primarily on analyses of the spatial distribution of basal dendritic branch points (Figs 3A, 4A,C). Arrangements in basal branch point distributions occur progressively from PND 9 to PND 17 and are confined to secondary branching. The number of primary basal dendrites is established earlier (by PND 9; Fig. 2), consistent with the idea that dendritic development and plasticity occurs in stages (see below). The number of branch tips does not vary significantly with age, indicating that rearrangements include branch retractions and additions. Secondary branches are selectively pruned close to the soma, while branches are selectively added farther from the soma.

The effects of deprivation started at PND 9 on the developmental redistribution of secondary branches can be detected rapidly: at PND 10 deprived neurons are already different from control neurons (Fig. 4A), indicating that dendritic rearrangements occur over times as short as one day. This is consistent with the pronounced dendritic motility observed at these developmental ages in vivo (Lendvai et al., 2000). Deprived neurons tend to have a reduced relative fraction of secondary branching at longer distances from the cell body, suggesting that deprivation reduces branch addition and subtraction. This is consistent with a previous study showing that deprivation ‘freezes’ dendritic structure at these ages (Lendvai et al., 2000). Plasticity of dendritic arbors obeyed a similar critical period to plasticity of receptive fields (Fig. 4C; Table 2).

Furthermore, the locations of the corresponding dendritic branches were consistent with a role as scaffolding for vertical synapses from layer 4. The basal arbor of layer 2/3 pyramidal dendrites is the chief locus for ascending intracortical synaptic input from layer 4 (Feldmeyer et al., 2002), and is therefore the main pathway for ascending excitation after whisker stimulation. Our results are also consistent with recent glutamate uncaging data indicating that the proximal (<100 mm) basal tufts of PND 14–16 layer 2/3 pyramidal neurons deprived at PND 9 are less sensitive to glutamate stimulation than those of control cells (Shepherd et al., 2003).

Table 2

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Cells show P-values for comparisons at the ages shown in the corresponding row; comparisons are across control groups and groups deprived at the age denoted in the row after deprivation at the age denoted in the column.
Comparison to Other Studies of Dendritic Morphology

Dendritic development proceeds along a series of well-defined stages. Primary dendrites are established before PND 9. The growth of dendrites toward their adult length occurs slowly; length changes are hardly detectable over the period PND 9–17. Rearrangement of secondary branches, including growth and retraction, occurs over a period of several days (PND 9–15). Consistent with the earlier establishment of primary dendrites and slow dendritic growth, we find that changes in organization of secondary branches are the only process sensitive to experience at these ages.

Earlier data on the dendritic morphology of supragranular neurons were obtained mainly using Golgi stains and HRP-DAB reactions of biocytin-filled cells. Different methods lead to quantitative differences. However, many of our conclusions are similar to previous studies. First, there is large variability in the number of dendritic tips per basal arbor: in our data the range is 20–138. In the visual cortex of the adult cat the range was found to be 13–49 (Larkman, 1991). Although in our data the number of tips per arbor has a wide range, our distribution is heavily biased towards lower values (80% of our neurons have fewer than 60 tips; 87% have fewer than 70 tips) and has a long tail. Recent studies in adult rat S1 layer 2/3 cells also gave wide ranges (Gottlieb and Keller, 1997; Schroder and Luhmann, 1997). Second, the peak number of intersections in Sholl diagrams is in the range 20–50 (Fig. 3F, 4D) (Larkman, 1991; Gottlieb and Keller, 1997; Schroder and Luhmann, 1997). According to some studies (Petit et al., 1988) there may still be small developmental increases in peak number of intersections from PND 17–20 to adulthood.

Our mechanistic conclusions on dendritic development are also similar across studies (McAllister, 2000). Primary dendritic branching is complete before secondary branching and refinement occur (rat: Petit et al., 1988; kitten: Zec and Tieman, 1994). Elaboration of secondary branching precedes and overlaps with sap synaptogenesis and sensitivity to effects of early sensory experience (Juraska and Fikova, 1979). In the rat, after 2–3 weeks of age there are no changes in dendritic branch number (Petit et al., 1988): all further arbor development is in the form of terminal segment growth (Juraska, 1982; Petit et al., 1988).

Our results are consistent with published studies showing that axon and network activity plays a fundamental role in postnatal dendritic arbor development, particularly patterned activity provided by sensory input (McAllister, 2000; Cline, 2001; Wong and Ghosh, 2002). In the Xenopus retina, sensory input, sensory stimulation affects dendritic rate of growth (Sin et al., 2002), through glutamate receptor-dependent extension of existing branches and regulation of branch addition and retraction (Rajan and Cline, 1998). The dendritic arbor expands rapidly early on; its rate of growth slows down as its complexity increases and synapses mature, implying changes in the roles of activity in arbor development and stability (Wu et al., 1999). In our results, during the second postnatal week, changes in sensory input to barrel cortex layer 2/3 pyramidal neurons affect elaboration of secondary branching rather than growth of existing dendrites. A recent paper has shown that activity blockade can impair basal dendritic development in CA1 pyramidal neurons by interfering with secondary branching rather than by preventing the elongation of existing dendrites (Groc et al., 2002). It may be possible that drastic and long-lasting environmental manipulations still induce changes in secondary basal dendritic branching in the adult cortex (Volkmar and Greenough, 1972; Greenough and Volkmar, 1973; Uylings et al., 1978; Greenough et al., 1979).

Plasticity of Dendritic Structure as a Locus for Plasticity of Sensory Maps

Experience-dependent plasticity of layer 2/3 receptive fields involves layer 4–layer 2/3 synapses (Lendvai et al., 2000; Stern et al., 2001; Shepherd et al., 2003). At the cellular level a multitude of mechanisms are likely involved, including experience-dependent maturation of excitatory synapses (Cairn and Malenka, 1995; Takahashi et al., 2003) and synapse formation and elimination (Lendvai et al., 2000; Trachtenberg et al., 2002). The fact that experience-dependent plasticity includes rearrangements in dendritic structure at the level of branches implies a high rate of synapse formation and elimination, consistent with the rapid experience-dependent dendritic spine turnover observed in vivo (Lendvai et al., 2000). The rearrangement of dendritic trees may reflect competition of layer 4 septal and barrel neurons for cortical territory in layer 2/3 (Shepherd et al., 2003).

The critical period in dendritic plasticity may reveal fundamental differences between developmental and adult plasticity. Although in the adult cortex synapse formation and elimination occurs and is regulated by experience (Trachtenberg et al., 2002), these synaptic changes are local, without growth or elimination of axons or dendritic branches. In other words, a fixed complement of potential synapses is involved (Stepanyants et al., 2002). Thus plasticity by synapse formation and elimination induced in the adult cortex would be limited and reversible. In contrast, plasticity involving large-scale rearrangements of dendritic and axonal branches during the critical period may be irreversible.

Notes

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