Neonatal Whisker Clipping Alters Intracortical, But Not Thalamocortical Projections, in Rat Barrel Cortex

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ABSTRACT

Retrograde axonal transport of cholera toxin B subunit (CTB) was used to compare the development of intracortical and thalamocortical connections in normal rats with those in rats in which all of the whiskers were trimmed continuously from birth. In normal animals, injections of CTB into a single barrel column resulted in an asymmetrical labeling of cells that were distributed preferentially within columns related to the same row in which the injection was placed. This anisotropy in the patterns of intracortical connections was not observed in whisker-clipped animals. In these animals, there was a significant reduction in the mean number of labeled cells in the infragranular layers, and labeled cells were distributed symmetrically around the injection site. The same injections of CTB also labeled thalamocortical neurons in the ventrobasal thalamus. Analysis of the distribution of these cells revealed that, in both control and experimental animals, the vast majority of labeled cells were restricted to a homologous (i.e., corresponding to the injected cortical barrel) thalamic barreloid. These findings indicate that manipulations of sensory experience alter patterns of intracortical, but not thalamocortical, connections. J. Comp. Neurol. 412:83–94, 1999.

The sensory system related to the mystacial vibrissae (whiskers) of mammals is unique, in that it samples information from an array of discrete peripheral receptor units. In certain rodent species, the whiskers map in a one-to-one, topographic manner onto corresponding arrays of discrete cellular aggregates at three consecutive relays: the trigeminal nucleus, the ventrobasal thalamic nucleus, and layer IV of the primary somatosensory cortex (for review see Jones and Diamond, 1995). In the primary somatosensory cortex, these aggregates are known as “barrels” and comprise the postremedial barrel subfield (PMBSF; Woolsey and Van der Loos, 1970). In the ventrobasal nucleus of the thalamus, these aggregates are called “barreloids” (Van der Loos, 1976; Woolsey et al., 1979; Land and Simons, 1985). Both the barrels and the barreloids are arranged as arrays of rows and arcs that correspond in number and organization to the rows and arcs of whiskers on the animal’s snout. Neurons in a barrel and barreloid that correspond to a particular whisker respond preferentially to stimulation of that whisker (for review see Jones and Diamond, 1995). Thus, the receptive field properties of neurons in individual barrels and barreloids can be predicted simply by determining the location of a cell within this anatomical map.

The discrete nature of the whisker/barrel system—having discrete sensory receptors (whisker follicles) and central representations—is particularly advantageous for investigating the effects of sensory experience on the development of central representation of peripheral receptors. A number of studies have demonstrated that sensory denervation induces anatomical and functional changes in these central representations (for reviews, see Woolsey, 1990; Kossut, 1992b). Dramatic developmental changes also can be induced after the relatively innocuous procedure of sensory deprivation by whisker clipping. These include severe impairments in tactile discrimination abilities (Symons and Tees, 1990; Carvell and Simons, 1996), alterations in γ-aminobutyric-containing (GABAergic) inhibition within the barrel cortex (Akhtar and Land, 1991; Micheva and Beaulieu, 1995a,b), and changes in the number and size of dendritic spines (Vees et al., 1998). In
addition, the removal from postnatal day 1 (P1; day of birth) of all but a single whisker causes neurons in the deprived barrels to respond more vigorously to the “inappropriate,” spared whisker (Simons and Land, 1987; Fox, 1992, 1994). This plasticity can be observed even when deprivation starts during the second postnatal week (Fox, 1992): Fox provided electrophysiological evidence that the locus of this plasticity is intracortical and that the plasticity is not related to changes in structures afferent to the barrel cortex.

The aim of this study was to investigate the anatomical correlates associated with functional plasticity induced by sensory deprivation. Specifically, we tested the hypothesis that this sensory manipulation affects the development of intracortical and thalamocortical connections in the barrel cortex. We used neuroanatomical tract-tracing techniques in control and whisker-clipped animals to compare the number and distribution of neurons contributing to intracortical and thalamocortical connections in the barrel cortex.

**MATERIALS AND METHODS**

**Animals**

Experimental protocols were approved by the institutional animal care and use committee and conformed to pertinent institutional and National Institutes of Health guidelines. Timed-pregnant Wistar rats gave birth in our facilities to all rats used in this study. Litter mates of both sexes were assigned randomly to either a control group or an experimental group. Animals belonging to the experimental group had their mystacial vibrissae (whiskers) trimmed continuously bilaterally to a length of <1 mm from the day of birth (P0) until they were subjected to surgical procedures (at P42–P47). Naive litter mates were from the day of birth until they were subjected to surgical procedures (at P42–P47). Naive litter mates were from the day of birth until they were subjected to surgical procedures (at P42–P47). Naive litter mates were from the day of birth until they were subjected to surgical procedures (at P42–P47). Naive litter mates were from the day of birth until they were subjected to surgical procedures (at P42–P47). Naive litter mates were from the day of birth until they were subjected to surgical procedures (at P42–P47).

**Surgery**

Experimental and control animals were anesthetized with ketamine HCl (100 mg/kg, i.m.) and xylazine HCl (0.5–1.0 mg, i.m.). Dexamethasone (0.4 mg, i.m.) was administered to prevent cortical edema, and bupiracaine HCl was applied locally to surgical wounds. Animals were placed on a regulated heating blanket and mounted in a stereotaxic apparatus. The somatosensory cortex over the left hemisphere was exposed through a craniotomy, the dura was cut and reflected, and the exposed cortex was covered with warm mineral oil. The cisterna magnum was opened to prevent cortical pulsation and edema. A glass pipette (10 µm tip diameter) filled with 0.5% cholera toxin B subunit (CTB; List Biological Laboratories, Campbell, CA) was advanced into the barrel cortex. Iontophoretic injections (5 µA positive current, 3 minutes alternating on/off at 50% duty cycle) were made at depths 0.4 mm and 0.8 mm from the pial surface. After the injections, the pipette was withdrawn, the skin was sutured, and the animals recovered.

Seventy-two hours after the injections, the animals were anesthetized with 35 mg/kg chloral hydrate and perfused transcardially with phosphate-buffered saline, pH 7.4 (PBS; 0.9% NaCl) followed by a 4% paraformaldehyde solution, pH 7.4. The brains were removed, the left somatosensory cortex was dissected and flattened between glass slides, and horizontal sections (50-um-thick) through the entire barrel cortex were cut with a Vibratome. Oblique sagittal (50-um thick) sections through the left thalamus were cut with a Vibratome, as described previously (Land et al., 1995): we chose this unconventional plane, because it produces sections through a complete arc of barreloids (Land et al., 1995).

Between each of the following steps, five washes were performed with PBS for 5 minutes each. Unless indicated otherwise, all incubations were performed at room temperature. Sections through the thalamus and cortex were incubated overnight at 4°C in goat anti-chick IgG (1:1,000 in PBS containing 0.2% Triton X-100 and 1% bovine serum albumin [BSA]). They were then incubated for 1 hour in donkey anti-goat immunoglobulin G (IgG; Jackson ImmunoResearch Laboratories, West Grove, PA; 1:1,000 in PBS containing 0.2% Triton X-100 and 1% BSA). An avidin-biotin-peroxidase complex (ABC Elite kit; Vector Laboratories, Burlingame, CA) solution was then applied for one hour. The sections were then incubated in the dark for 10 minutes in a urea-H2O2 and 3,3′-diaminobenzidine tetrahydrochloride (0.7 mg/ml) solution dissolved in 0.06 M Tris buffer, pH 7.5 (Sigma, St. Louis, MO), containing 1% nickel-ammonium sulfate. Every other section through the thalamus and cortex was processed for cytochrome oxidase histochemistry (Wong-Riley and Welt, 1980). All sections were mounted in order on gelatin-subbed glass slides and postfixed in 1% osmium tetroxide for further signal enhancement. They were then dehydrated in graded alcohol solutions, defatted in xylene, and coverslipped by using DPX mounting medium (Electron Microscopy Sciences, Fort Washington, PA). The barrel cortex from four additional animals (two control and two experimental) that did not receive CTB injections was sectioned by using a cryostat into 2-um-thick sections in the coronal plane, and the sections were stained by using cresyl violet.

**Analysis**

**Intracortical connections.** Horizontal sections through the PMBSF cortex from 15 animals (seven control and eight experimental) were used for analyses of intracortical connections. This is the cortical region that contains the somatotopic representation of the mystacial vibrissae (White and DeAmicis, 1977). Layer IV of the PMBSF cortex contains large barrels arranged in five rows, labeled A–E, corresponding to the five rows of whiskers on the animal’s snout, and in several arcs labeled with Arabic numerals (Woolsey and Van der Loos, 1970). In addition, the four large caudal whiskers (labeled α through δ) are represented by four corresponding barrels straddling the first arc (Figs. 1, 3; and see Woolsey and Van der Loos, 1970).

The boundaries of the PMBSF barrels, as they appear in cytochrome oxidase-labeled sections through layer IV, were plotted with the use of a computer-aided morphometry system (Neurolucida; MicroBrightField, Colchester, VT). The locations of CTB-labeled somata throughout all layers of the PMBSF cortex were plotted by using the Neurolucida system. Consecutive sections were aligned relative one another with the use of fiduciary landmarks, such as cross sections through small blood vessels. The
The numerical density ($N_v$) of labeled neurons was calculated according to the following formula:

$$N_v = \frac{\sum Q}{\sum V_{DIS}}$$

where $\sum Q$ is the total number of nuclei counted in the dissectors from each animal, and $\sum V_{DIS}$ is the total volume of all the dissectors in each block. Dissector volume was calculated by multiplying the area of the reference plane by twice the section thickness. Finally, surface density plots of labeled cells were generated by calculating the number of labeled cells in 50-µm$^3$ bins and plotting interpolated images (Transform; Fortner Research, Sterling VA).

**Thalamocortical connections.** Oblique sagittal sections through the PMBSF cortex from 11 animals (six control and five experimental) were used for analyses of thalamocortical connections. The boundaries of individual barreloids, as they appear in cytochrome oxidase-labeled sections, were reconstructed from serial sections with the use of the Neurolucida system. The locations of CTB-labeled somata throughout the ventrobasal thalamus were then plotted as described above, excluding cells that appeared in consecutive sections. The total number of labeled cells and their location within particular barreloids was computed by using the Neurolucida package. In addition, surface density plots of labeled cells were generated by calculating the number of labeled cells in 100-µm$^3$ bins and plotting interpolated images.

**Numerical density of barrel neurons.** Semithin, Nissl-stained coronal sections were used to compare the numerical density ($N_v$) of cells in control ($n=2$) and experimental ($n=2$) animals with the use of Sterio’s (1984) unbiased counting method. From each animal, four pairs of serial sections through the PMBSF cortex were chosen. Pairs of adjacent sections were designated as a single dissector. Three identical counting frames, 150 µm high and 100 µm wide, were selected for each dissector. Counting frames were placed over layers II, IV, and Vb in such a way that the layer IV counting frame was aligned so that its center was placed over the C2/C3 barrel border, and the other two counting frames were immediately above and below the layer IV frame. Nissl-stained nuclei within a counting frame were plotted with the Neurolucida package; nuclei that intersected one of the frame’s edges were excluded. Computation of the $N_v$ of these nuclei was performed as described above.

Quantitative data are presented as mean ± standard error of the means (S.E.M.). The significance of differences between data parameters was assessed with a one-way analysis of variance (ANOVA; Fisher’s PLDS analysis, with a significance level of 5% (StatView; Abacus Concepts, Berkeley, CA). High-resolution digital photomicrographs were obtained with a PowerPhase (PhaseOne, Copenhagen, Denmark) charge-coupled device (CCD) and stored on a Power Macintosh (Apple Computers, Cupertino, CA). All digitized images were analyzed on a G3-Macintosh computer using Photoshop software (Adobe Systems, Mountain View, CA). Image manipulations were restricted to linear level adjustments and image cropping.

**RESULTS**

$N_v$ of cortical neurons

Unilateral whisker clipping has been reported to result in a reduction in the numerical density ($N_v$) of GABA-containing neurons in layer IV contralateral to the trimmed whiskers and an increase in the $N_v$ of these cells in barrels ipsilateral to the deprivation (Micheva and Beaulieu, 1995b). Because changes in the overall $N_v$ of neurons may affect the number and distribution of neurons labeled after cortical injections, we first compared the $N_v$ of Nissl-stained nuclei in the barrel cortex of normal and experimental animals (see Materials and Methods). The $N_v$ of all cells in experimental animals (76,770 ± 15,390 per 1 mm$^3$; mean ± S.E.M.) was not significantly different ($P=0.36$) than that in control animals (79,130 ± 26,440 per 1 mm$^3$). There were also no significant differences in the $N_v$ of cells in individual layers of the barrel cortex between experimental and control animals: supragranular layer, 73,530 ± 14,400 vs. 69,290 ± 11,660 per 1 mm$^3$, respectively; granular layer, 108,640 ± 38,330 vs. 111,830 ± 27,750, per 1 mm$^3$; infragranular layer, 48,140 ± 9,250 vs. 55,930 ± 8,730 per 1 mm$^3$. Therefore, we concluded that any differences in the number or distribution of labeled cells will not be due to an overall reduction in neuronal density in experimental animals.

**CTB injections**

We used retrograde transport of CTB from barrel injections to compare the patterns of intracortical and thalamocortical connections in control and whisker-clipped (“experimental”) animals. For these comparisons to be valid, it was necessary to make uniform injections of the tracer in all subjects. We therefore included in the analysis only those cases in which the injection was restricted to a single barrel column: a columnar area extending vertically throughout the cortical layers in register with a single layer IV barrel (see Keller, 1995). Injections sites were defined as the area containing dense reaction product in which no labeled cells could be discerned. Included in this study were cases that satisfied the following criteria: 1) CTB injection sites were perpendicular to the pial surface and extended throughout cortical layers II–V, without invading the subcortical white matter; and 2) within layer IV, the injection sites were restricted to a single cyto-
chrome oxidase-labeled barrel hollow. Surrounding each layer IV injection site were labeled cells located in the barrel septa (Fig. 1A). We included in the analysis cases in which injections were centered on the following barrels: C2 (three control animals and three experimental animals), C3 (two control animals and three experimental animals), and B2 (two control animals and two experimental animals). A total of seven control animals and eight experimental animals that satisfied these criteria were included in this study.

Figure 1A depicts an example of a CTB injection site as it appears in a horizontal section through layer IV. Because the injection sites involved most cortical layers, we restrict our analysis to the distribution of labeled cells located horizontal to the injection sites and make no attempt to identify vertical, interlaminar pathways labeled by our paradigm. Because tracer injections are likely to result in labeling of axons of passage, we also restrict our analysis to that of retrogradely labeled cells, which can be identified unequivocally as projecting to the core of the injection site.
sites, and we do not describe the distribution of anterogradely labeled axons.

Intracortical connections

In control animals, retrogradely labeled somata were distributed at distances up to 3 mm from the injection sites. Most labeled cells were in the PMBSF region of the primary somatosensory cortex (SmI). Retrogradely labeled cells also were found in more anterolateral regions of SmI, the secondary somatosensory cortex (SII), the motor cortex, and the perirhinal cortex, all areas known to project to the PMBSF (Nussbaumer and Van der Loos, 1985; Welker et al., 1988; Keller, 1995).

To compare patterns of intracortical connections in control and experimental animals, we plotted the location of retrogradely-labeled cells in all layers of the PMBSF. We first compared the numerical density \(N_v\) of labeled cells in experimental and control animals by using an unbiased counting procedure (see Materials and Methods). Because laminar boundaries are not easily distinguishable in horizontal sections, we grouped data from the supragranular (layers I–III), granular (the layer IV barrels), and infragranular (V–VI) layers. The mean \(N_v\) of labeled cells in experimental animals was 13.7% lower \(P = 0.012\) than in control animals (Fig. 2). To determine whether this reduction occurred in specific layers, we compared the \(N_v\) of labeled cells in individual layers of the barrel cortex (Fig. 2). A significant reduction in the mean \(N_v\) of labeled cells occurred in the infragranular layers (24.4% reduction; \(P = 0.019\)). Reductions in the mean \(N_v\) of labeled cells also occurred in the supragranular (9.4%) and granular (5.9%) layers, but these were not statistically significant \(P = 0.12\) and 0.24, respectively.

To compare the distribution of intracortical connections in experimental and control animals, we generated spatial density maps from three-dimensional reconstructions of the locations of all labeled cells in the PMBSF cortex (see Materials and Methods). These maps provide a two-dimensional display of spatial density data obtained from three-dimensional reconstructions of the distribution of cells. Figure 3 depicts a comparison of the distribution of labeled cells in representative experimental and control animals. In both control and experimental animals, the highest density of labeled cells occurred in close proximity to the injection sites. In control animals, the distribution of labeled cells was highly asymmetrical, in that most cells were within barrel columns associated with the same row in which the injection was placed. This anisotropy occurred in all layers and was most pronounced in the infragranular layers (Fig. 3). In comparison, labeled cells in whisker-clipped animals were distributed more symmetrically relative to the injection site (Fig. 3).

To quantify the difference in the distribution of labeled cells in experimental and control animals, we calculated the proportion of labeled cells within columns associated with the row of barrels containing the injection site (parent row). Cells were assigned to a particular barrel row if their soma was within the cytochrome oxidase-labeled row in layer IV, or in register with that row in the infragranular or supragranular layers. In all layers of the barrel cortex from control animals, the percentage of labeled neurons in the parent barrel row was significantly higher that that in neighboring barrel rows (Fig. 4). Thus, although barrel columns associated with a single row of barrels represent less than 20% of the total volume of the barrel cortex, most labeled cells occurred within the rows associated with the injection site. In comparison, in whisker-clipped animals, labeled cells in the infragranular and supragranular layers were distributed evenly in parent rows and in other barrel rows; The preferential distribution of labeled cells in parent rows was maintained only in layer IV (Fig. 4). Furthermore, in all layers, the percentage of labeled cells in parent rows was significantly lower in whisker-clipped animals (Fig. 4). These findings indicate that the anisotropy characterizing intracortical connec-

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![Fig. 2. The numerical density of retrogradely labeled cells in different layers of the barrel cortex labeled after injection of CTB into a single barrel column. Data are from whisker-clipped and control animals. In this and subsequent figures, data are presented as mean ± S.E.M., and P values are derived from a Fisher's PLDS analysis, with a significance level of 5%. Note that significant differences in numerical density occur only in the infragranular layers. All, cumulative data from all layers.](image-url)
Fig. 3. Spatial density maps comparing the distribution of retrogradely labeled cells in different layers of a control rat and a whisker-clipped rat. The maps were generated by computing the number of cells in 50 µm³ bins, assigning a color value to each bin, and interpolating the resulting image. In control animals, labeled cells are distributed asymmetrically, particularly in the infragranular layers, such that they are distributed preferentially along the row of barrels containing the injection site (I.S.). This asymmetry does not develop in whisker-clipped animals. Barrels are designated by letters and numbers, as described in the text. Scale bar = 500 µm.
tions in the normal barrel cortex does not develop in whisker-clipped animals.

**Thalamocortical connections**

Neurons within a thalamic barreloid project almost exclusively to a single, topologically corresponding (homologous) cortical barrel (Land and Simons, 1985; Land et al., 1995). To determine whether whisker clipping affects the development of these precise topological connections, we examined the distribution of thalamocortical barreloid neurons labeled retrogradely after CTB injections into a single cortical barrel. We used histologic material from the same control and experimental animals described above.

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**Fig. 4.** Comparison of the distribution of labeled cells in different cortical layers from control and whisker-clipped animals. In control animals, labeled neurons are distributed preferentially in the parent barrel row (containing the injection site) and in the infragranular and supragranular regions in register with that row. In experimental animals, this anisotropy is lost in all layers except layer IV. Furthermore, in all layers, the proportion of labeled cells associated with the parent row is significantly lower in experimental animals.
Individual barreloids were identified in oblique sagittal sections through the thalamus that had been stained for cytochrome oxidase (see Materials and Methods). In this plane, individual barreloids corresponding to the same arc appear in a single section. Individual barreloids appear as curved, tapering cylinders, previously termed "rods" (Land et al., 1995). Figure 1B depicts a schematic of the organization of these rods as they appear in an oblique sagittal section. In all cases, it was necessary to reconstruct the cytochrome oxidase-labeled barreloids from multiple sections to identify reliably the borders of these rods. In four cases, the borders of individual barreloids could not be identified readily. We excluded these cases from the analysis. The analysis described below was derived from six control animals in which CTB was injected into barrel C2 (n = 3) or barrel C3 (n = 1), or barrel B2 (n = 2).

In both control and experimental animals, CTB-labeled somata and axons were distributed as a dense cluster within the ventrobasal thalamus (Fig. 1B, C). We limit our analysis to the distribution of retrogradely labeled thalamic cells, i.e., thalamocortical neurons. To study the relation between these clusters and individual thalamic barreloids, we combined three-dimensional reconstructions of the distribution of labeled cells with similar reconstructions of cytochrome oxidase-labeled barreloids obtained from adjacent sections (see Materials and Methods).

In all cases, clusters of retrogradely labeled thalamocortical cells coincided with individual cytochrome oxidase-dense rods. Labeled thalamocortical neurons were not distributed homogeneously within a barreloid. Rather, the highest density of labeled cells was in the dorsomedial portion of a barreloid, and significant portions of the homologous barreloids were devoid of labeled neurons. (Figs. 1A, 5).

Figure 5 compares spatial density maps of the distribution of labeled thalamocortical neurons in control and experimental animals after CTB injections restricted to the C2 cortical barrel. In both cases, the majority of labeled thalamocortical neurons were distributed within the confines of the homologous C2 barreloid.

To quantify the topologic relation between thalamocortical neurons and their cortical targets (the layer IV barrel), we calculated the number and percentage of labeled thalamocortical neurons in homologous (corresponding to the injected barrel) and adjacent barreloids (Fig. 6). Homologous barreloids in control animals (n = 6), on average, had 143.8 \( \pm \) 12.1 labeled thalamocortical cells compared with 127.4 \( \pm \) 11.4 labeled cells in homologous barreloids of experimental animals (n = 5). Small numbers of labeled cells were located in one or two barreloids immediately adjacent to the homologous one (13.7 \( \pm \) 6.7 cells in control animals; 25.2 \( \pm \) 4.3 cells in experimental animals). Thus, the vast majority of labeled cells in both control (91.3% \( \pm \) 5.7%; mean \( \pm \) S.E.M.) and experimental animals (83.5% \( \pm \) 16.5) were restricted to a homologous barreloid (Fig. 6). Although the dispersion of labeled thalamocortical cells in experimental animals was slightly higher than in control animals, this difference was not statistically significant (P = 0.43). We conclude that whisker clipping produced no overt changes in the specific patterns of thalamocortical inputs to the barrel cortex.

**DISCUSSION**

Effects of whisker clipping on the development of intracortical connections

In control animals, injections of CTB into a single barrel column results in retrograde labeling of neurons over large
areas of the PMBSF cortex. These represent horizontal, intracortical connections that link neurons in different barrel columns. These connections are asymmetrical, in that they occur preferentially between neurons associated with the same row of barrel columns. A similar anisotropy in the patterns of horizontal, intrinsic connections in the barrel cortex was described previously (Bernardo et al., 1990; Fabri and Burton, 1991; Hoeflinger et al., 1995). In both rats (present study) and mice (McCasland et al., 1992), this anisotropy is most pronounced in the infragranular layers, whereas horizontal connections in the supragranular layers are more widespread and symmetrical. We have reported that most of the neurons contributing to these horizontal connections are excitatory and that inhibitory neurons are responsible primarily for short-range connections between immediately adjacent barrel columns (Aroniadou-Anderjaska and Keller, 1996).

In animals in which whiskers were clipped from birth, the patterns of intracortical connections are significantly different from control animals. Retrogradely labeled neurons are distributed symmetrically around the injections site, and the anisotropy characterizing these patterns in normal animals is not apparent. This effect is most prominent in the infragranular and supragranular layers (Figs. 3, 4) and is associated with a reduction in the numerical density of labeled cells in these layers (Fig. 2).

The observed differences in labeling between whisker-clipped and control animals may be due to differences in uptake or transport of CTB, resulting from reduced neuronal activity in whisker-clipped animals. We consider this possibility unlikely for at least two reasons: 1) uptake and transport of other retrograde tracers, such as horseradish peroxidase, are unaffected by the degree of neuronal activity (Sato et al., 1989); and 2) if uptake and transport of CTB is affected by whisker-clipping, then labeling of both intracortical and thalamocortical pathways is likely to be affected to a similar extent. Our finding that thalamocortical connections are not affected by whisker clipping argues against this possibility.

Two previous studies examined the effects of early transection of the infraorbital nerve on the development of intracortical connections in the barrel cortex. In both studies, deafferentation was produced during the second postnatal week, because earlier nerve lesions are known to affect the development of thalamocortical afferents and of the barrels themselves (Woolsey and Wann, 1976; Jensen and Killackey, 1987b; Catalano et al., 1995). McCasland et al. (1992) reported that, after this procedure in neonatal mice, the spatial extent of horizontal intracortical connections is significantly reduced, and the anisotropy in these connections does not develop. In contrast, Rhoades et al. (1996) found that a similar deafferentation in neonatal mice...
Effects of specifically and selectively altering the intracortical connections. This procedure also differs markedly from the barrel cortex (by application of tetrodotoxin over the pial surface) has no effect on the development of intracortical connections. This procedure also differs markedly from our approach of clipping the whiskers. We chose whisker clipping because it allowed us to examine the effects of specifically and selectively altering the patterns of sensory activity that occur during normal whisking behavior. Furthermore, neonatal whisker clipping or plucking is known to modify dramatically the response properties of barrel cortex neurons (Simons and Land, 1987; Fox, 1992, 1994), and we were interested in identifying anatomical correlates associated with this plasticity.

Our finding that whisker clipping is associated with abnormal development of intracortical connections is in agreement with the results of several related studies. Kossut (1992a) showed that plucking a single whisker from birth results in reduced metabolic activity in the area surrounding the homologous cortical barrel and suggested that this decrease is due to a reduction in synaptic interactions mediated by intracortical axons. Fox (1994) demonstrated that plasticity in the response properties of barrel neurons induced by whisker removal is dependent on interactions among neighboring barrel columns.

Our finding that sensory deprivation affects the development of intracortical connections also is consistent with findings in the cat visual cortex. Changes in normal visual experience, such as those associated with dark rearing (Lübke and Albus, 1992), binocular deprivation (Callaway and Katz, 1991), or artificially induced strabismus (Lowed and Singer, 1992), dramatically alter the development of normal patterns of intrinsic axon collaterals in the primary visual cortex.

The anisotropy in the patterns of intracortical connections in normal animals is thought to be involved in the generation of activity gradients within the barrel cortex (see Simons, 1995). These are manifested as gradients in metabolic activity across the array of barrel columns (Durham and Woolsey, 1985; McCasland and Woolsey, 1988) as well as in gradients in the degree of cross-whisker inhibition among neurons located in different rows and arcs (Simons and Carvell, 1989). The lack of anisotropy in the patterns of intracortical connections after whisker-clipping suggests that patterned sensory inputs are necessary for the generation of these activity gradients. One possibility is that, during development, patterned whisking activity generates gradients of neurotrophic factors that regulate the development of this anisotropy. This possibility will be examined in subsequent studies.

The changes in the number and distribution of labeled cells reported here were not associated with changes in the Nv of the total number of barrel cortex neurons. The lack of observed differences in the Nv of cells in our study is in contrast to the finding by Micheva and Beaulieu (1995b) of changes in the Nv of GABAergic cells after whisker clipping. This difference may be due to the fact that our paradigm involved bilateral whisker clipping, whereas Micheva and Beaulieu employed unilateral whisker clipping. Furthermore, whereas Micheva and Beaulieu focused on the number of GABA-containing neurons, we computed the number of all cells (including nonneuronal cells). Because GABAergic neurons account for <10% of the cells in the barrel cortex (Micheva and Beaulieu, 1995b), our analysis may have failed to detect small changes in the Nv, of this relatively small neuronal subpopulation. This potential omission is unlikely to affect our results, because GABAergic neurons make an insignificant contribution to horizontal intracortical connections in the barrel cortex (Aroniadou-Anderjaska and Keller, 1996), which are the focus of this study.

Effects of whisker clipping on thalamocortical connections

We also investigated the possibility that sensory deprivation affects the development of thalamocortical afferents to the barrel cortex. In normal animals, individual thalamocortical axons from the ventrobasal thalamus project almost exclusively to a single barrel column, terminating in the hollow of a single layer IV barrel and in the infragranular layers immediately below that barrel (Jensen and Killackey, 1987a; Senft and Woolsey, 1991). Furthermore, essentially all thalamic inputs to a particular cortical barrel originate from a single, homologous cortical barreloid (Land and Simons, 1985; Land et al., 1995). We found a similar degree of specificity in the topographic arrangement of thalamic afferents: >90% of thalamic neurons that were labeled retrogradely after CTB injections into a single barrel were found in a single, homologous barreloid (Figs. 1, 5, 6).

Both in the present work and in previous studies (Land and Simons, 1985; Land et al., 1995), only a subpopulation of neurons in a given thalamic barreloid was labeled by retrograde transport from the cortex. In the present study, we identified in control animals an average of 144 labeled thalamic neurons per barreloid. Land et al. estimated that each barreloid may contain as many as 300 neurons, indicating that <50% of the neurons were labeled in our study. This is surprising, because an earlier report suggested that all neurons in the ventrobasal thalamus project to the barrel cortex (Saporta and Kruger, 1977). Land et al. provided evidence suggesting that some thalamic neurons may project exclusively to the deep cortical layers and, thus, may not be labeled by tracer injection into the more superficial layers. Therefore, it is possible that a larger proportion of thalamic neurons would have been labeled if our injections had included the deeper layers of the barrel cortex. We chose not to do so, because we wanted to limit our injections to the cortical gray matter and to avoid the possibility of labeling the subcortical white matter directly.

Land et al. (1995) reported that tracer injections restricted to layer IV of the barrel cortex labels neurons in the more ventrolateral portion of a thalamic barreloid, whereas injections into the deep cortical layers labels neurons concentrated more dorsomedially in a barreloid. In the present study, injections that targeted layers II–V resulted in a more dense retrograde label in the dorsomedial portion of the barreloids (Figs. 1, 5). It is possible that
our finding of a higher density of labeled cells in the dorsomedial portion of barreloids is due to the inclusion of layer V in our injections sites. However, we cannot exclude the possibility that this difference may be related to differences in experimental approaches, such as the use of different neuroanatomical tracers.

In contrast to the effects of whisker clipping on the development of intracortical connections, we observed no changes in the number or distribution of thalamic neurons projecting to the barrel cortex. This indicates that the patterns of thalamocortical afferents to the barrel cortex are not affected by sensory deprivation. Pertinent to this finding are reports that pharmacological suppression of cortical activity does not affect the development of thalamic afferents to the barrel cortex (Slaugger et al., 1993; Chiaia et al., 1994a,b). This is not surprising, because, already at birth, thalamic afferents exhibit a high degree of topographic organization (Dawson and Killackey, 1985; Agmon et al., 1993, 1995).

In contrast, several studies have demonstrated that sensory denervation, induced by transection of the infraorbital nerve or by coagulating the whisker follicles, dramatically affects the development of these thalamocortical arborizations. These changes include the appearance of projections to the barrel cortex from thalamic regions that normally do not project to the barrel cortex (Verney et al., 1982) and abnormal projections of individual thalamic axons to multiple cortical barrels (Jensen and Killackey, 1987b; Catalano et al., 1995).

Our finding that neonatal whisker clipping does not affect the topography of thalamocortical connections is in contrast to findings in the visual system, demonstrating that monocular deprivation can alter the morphology of thalamic afferents to the cat visual cortex (Antonini and Stryker, 1993, 1996; Antonini et al., 1998). The difference between the effects of sensory deprivation on thalamic afferents in the visual system versus the somatosensory system may be related to the fact that thalamocortical projections to the barrel cortex are topographically-specific already at birth (see above), whereas thalamic afferents to the visual cortex develop their topographic specificity postnatally (see, e.g., Friedlander and Martin, 1989). We should also note that monocular deprivation and whisker clipping are not analogous manipulations. The former may affect competition for "cortical space" by thalamic afferents arising from the two eyes (see Antonini et al., 1998), whereas our manipulation affected sensory inputs from the entire set of whiskers. It is possible, therefore, that selective whisker clipping that spares a subset of the vibrissae may result in alteration in thalamocortical interactions. This possibility will be investigated in subsequent studies.

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LITERATURE CITED


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