Spatial Organization of Thalamocortical and Corticothalamic Projection Systems in the Rat SmI Barrel Cortex

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ABSTRACT
Axonal tracing techniques were used to examine the distribution of corticothalamic projection neurons in relation to the organization of the thalamocortical recipient zones in the whisker representation of the rat first somatic sensory cortex. Following injection of horseradish peroxidase into the physiologically defined vibrissa area in the ventrobasal complex of the thalamus, labeling in the cortex had a columnar appearance. Dense patches of anterograde labeling were located within the centers of the layer IV barrels and extended superficially through lamina III; the septa between barrels contained considerably less reaction product. Retrogradely labeled neurons were observed in lower layer V and layer VI where they were concentrated preferentially deep to the barrel centers. Regions deep to the septa displayed less overall labeling and a lower relative number of thalamic projecting neurons. Zones having the larger numbers of retrogradely labeled cells also contained terminallike labeling of either corticothalamic or thalamocortical origin. Following an injection that included the posterior group medial to the ventrobasal complex, anterograde labeling in layer IV was located largely in the septa. In conjunction with previous findings concerning the origin and termination of other projection systems in the barrel cortex, these results suggest that a vibrissal column contains a central core zone intimately linked with the ventrobasal thalamus that is bounded by narrower regions of more diverse inputs and outputs that form an interface between adjacent cortical columns.

Key words: cortical columns, vibrissae, trigeminal, HRP

The first somatic sensory cortex of many rodent species is characterized by a cytoarchitectonically distinct modular organization. Within layer IV of the face area, there is a clear one-to-one relationship between the physiological representation of individual vibrissae on the contralateral mystacial pad and consistently identifiable aggregates of neurons called barrels (Woolsey and Van der Loos, '70; Welker, '71, '76). Electrophysiological (Simons and Woolsey, '79; Armstrong-James and Fox, '87) and 2-deoxyglucose (Durham and Woolsey, '77; Chmielowska et al., '86) studies have provided evidence that each barrel is the morphological correlate of a single functional column that extends throughout the thickness of the cortex (Woolsey and Van der Loos, '70). One function of a cortical column is the integration of information arising from the array of whiskers on the face (see Simons et al., '88). For example, though all driveable neurons within a column are activated by a particular whisker, cells at different depths respond differentially to movements of adjacent vibrissae, deflected alone or in combination with the columnar whisker (Simons, '78, '85). These properties are thought to reflect in part connections both among neurons located within an individual column and among neurons in neighboring columns.

Recently we have demonstrated a substantial transformation within the layer IV barrel of afferent information from the ventrobasal thalamus (Simons and Carvell, '89). Response properties of barrel neurons are characterized by single-whisker receptive fields having strong inhibitory sur-
The remainder of the experiment. Inserted into the external jugular vein (Harms and Ojeda, '74) for supplemental administration of Nembutal during the rat's head was fixed to the exposed skull by using dental trophysiologically by using tungsten microelectrodes for the vibrissa representation in VB was then identified electrophysiologically by using a stepping microdrive. HRP injection was terminated at the depth at which the dorsal border of VB had been identified by using the recording barrel during the initial advance of the electrode. Constant negative current was applied during further withdrawal from the brain. In order to label corticothalamic cells corresponding to several rows of whiskers, up to ten such injections were made at 75 μm rostral-caudal intervals.

Following HRP deposition the scalp wound margins were sutured closed. Neosporin ointment was applied to the incision sites, and the animal was allowed to recover from the barbiturate under observation. After a 36–48-hour survival, the rats were anesthetized with a lethal dose of Nembutal, the descending aorta was clamped, and the animal was perfused transcardially with 150 ml of warm, heparinized saline (1 ml heparin/50 ml saline) followed by 300 ml of cold fixative. The fixative consisted of 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were extracted from the skull, postfixed for 3–5 hours, and then sunk in 30% sucrose phosphate buffer at 4°C.

**Histochemistry**

Brains were sectioned on a freezing microtome. Prior to cutting, the right cerebral hemisphere was separated from the diencephalon. Thalami were sectioned at 80 μm in the coronal plane. Cortices were cut in one of three planes at 60 μm. In six cases the cortex was flattened on a plane tangential to the pial surface overlying the postero medial barrel subfield (PMBSF). Cortices from the other specimens were sectioned in oblique coronal planes perpendicular to the pial surface (Fig. 1). In seven cases the brain was oriented so that the microtome stage during freezing and cut in a plane tangential to the pial surface of the coronal plane (Fig. 1). In seven cases the brain was oriented so that the microtome stage during freezing and cut in a plane tangential to the pial surface. Cortices from the other specimens were sectioned in oblique coronal planes perpendicular to the pial surface (Fig. 1). In seven cases the brain was oriented so that the microtome stage during freezing and cut in a plane tangential to the pial surface. Cortices from the other specimens were sectioned in oblique coronal planes perpendicular to the pial surface (Fig. 1). In seven cases the brain was oriented so that the microtome stage during freezing and cut in a plane tangential to the pial surface.
In 77 sections from nine specimens, the locations of all retrogradely labeled cells were plotted on acetate transparencies with the aid of a camera lucida. As described above, the boundaries of the layer IV barrels were projected deep through the infragranular layers where the retrogradely labeled cell bodies were observed. In five specimens, areal measures were made of regions in register with the layer IV barrels and of regions in register with the septa between them, and the numbers of retrogradely labeled cells per 10,000 um² were determined. The upper and lower borders of the measured infragranular zones were defined by the most superficial location of retrogradely labeled cell bodies and the white matter. Selected sections were photographed with a Leitz-Dialux photomicrography system.

RESULTS

Injection sites in the thalamus

Figure 2A is a Nissl-stained section through the rat diencephalon showing the ventrobasal complex and its relationship to some other thalamic nuclei. Panels B–D illustrate the size and location of three representative iontophoretic injection sites. All injections were centered within the dorsal and medial aspects of VB, corresponding to the representation of the large mystacial vibrissae on the contralateral face. In these and other specimens there appeared to be relatively little effective spread of HRP into the nearby posterior group (PO) which also contains a representation of the whiskers (see Carvell and Simons, '87). Even with injections that appeared to fully encompass the VB whisker representation, the transport of HRP to the cortex was typically circumscribed in that anterograde labeling of barrels was observed only in that part of the PMBSF that corresponded to the vibrissae represented physiologically at the thalamic injection sites. Indeed it was necessary to make multiple injection penetrations spanning the representation of several whisker rows in order to obtain substantial labeling of barrels in adjacent rows in the cortex. In addition a distinctly different pattern of cortical labeling was observed when a large injection clearly included PO just medial to VB. In no case was labeling observed in visual cortex even though in some thalami the reaction product halo around the injection site extended into the dorsal lateral geniculate nucleus. We therefore assume that the zone of effective uptake of the injected HRP was confined to VB in all cases but one, which will be discussed below.

Appearance of cortical labeling in tangential sections

HRP reaction product in the first somatic sensory cortex had a patchy, discontinuous distribution that reflected the spatial organization of barrels in layer IV. In tangential sections the densest anterograde labeling was observed in the centers of individual layer IV barrels. The term "center" here refers to the cytochrome-oxidase-rich component of the barrel which in mice is relatively cell sparse and called the barrel "hollow" (see Land and Simons, '85a). Anterograde labeling extended superficially through layer III. In sections through lower layer V and layer VI barrellike patches of labeling were also apparent. Areas of densest labeling were located in register with the overlying layer IV barrels and consisted of labeled somata, dendrites, and axons. Retrogradely labeled cell bodies were distributed over a broader expanse of cortex than the anterograde labeling that could be used to help define barrel centers.
Fig. 2. Photomicrographs of coronal sections of the rat diencephalon through the ventrobasal complex (VB). **Panel A:** A Nissl-stained section showing VB, the posterior group medial to VB (PO), and the thalamic reticular nucleus (R). The whisker representation is located in the medial two-thirds of VB, with arc 1 vibrissae represented dorsally and row A vibrissae caudally. **Panels B–D:** The size and location of HRP injections in three specimens. Tissue in panel B was processed by using TMB as the chromagen; specimens in C and D were processed with DAB/cobalt. Scale in A = 500 µm; also applies B–D.

Fig. 3. Appearance of cortical labeling in tangential sections following the thalamic injection shown in Figure 2B. **Panel A:** Photomicrograph of a section through layer IV of the PMBSF. Note the high density of labeling in the barrel centers, particularly those of row C and D, and the relatively sparse labeling in the septa, especially between rows C and D. Anterogradely labeled barrels in row C are C1–C3 (upper left to lower right). **Panel B:** Photomicrograph of a section from the same hemisphere through layer VI. Arrows denote the zone corresponding to the septum between rows C and D that is shown at higher magnification in **panel C.** All sections were reacted with DAB/cobalt. Scale in A = 200 µm, applies also to panel B. Scale in C = 100 µm. Orientation, medial up; anterior right.
Figure 3 shows cortical labeling following the thalamic injection illustrated in Figure 2B. In this experiment HRP was iontophoretically injected during seven closely spaced penetrations (see Materials and Methods). Responses from whiskers in row D, arcs 2–4, and/or in row C, arcs 1–3, were recorded in all of the penetrations, and in one penetration some responses from row B whiskers were observed. As shown in Figure 3A the densest anterograde labeling was found in the corresponding row C and D barrels, with considerably less dense labeling in row B. Labeling was concentrated within the barrel centers. There, reaction product was not uniformly distributed; rather, the barrel center was crisscrossed by narrow bands of very dense labeling that appeared to segment the barrel neuropil into a latticelike arrangement of different compartments. In contrast to the barrel center, the septum contained far less reaction product. This was most apparent for septa between barrel rows. As shown in Figure 3A the densest anterograde labeling was shown at the same magnification in Figure 3B. The pattern, including the “bridge” between the rostral C and D barrels, is similar to that seen in panel A. Segmentation of the overall labeling is less pronounced, particularly within the zones corresponding to the whisker rows. In addition reaction product here is located within somata and dendrites. As shown at higher magnification in Figure 3C the retrogradely labeled cell bodies are observed more frequently deep to the barrel centers than deep to the between-row septum. Numerous horizontally running processes can be distinguished in the sparsely labeled zone. These consist of dendrites of retrogradely labeled corticothermal cells and axons of either these neurons and/or of thalamocortical cells. Even in zones containing the highest concentration of labeled cells, somata with and without reaction product were intermingled. With respect to the distribution of labeled cell bodies visual inspection of the specimens can be misleading since attention is drawn to regions containing the most reaction product. Consequently, the locations of retrogradely labeled somata only were plotted with the aid of a camera lucida. Figure 4 is a line drawing from a single section of a tangentially cut hemisphere different from that shown in Figure 3 illustrating the distribution of labeled cell bodies with respect to the overlying barrels. Because of the difficulty in precisely aligning many sections and because the cortex is organized in radial, not parallel, lines, projecting the boundaries of the layer IV barrels deep into the cortex in this section plane is at best an approximation. Nevertheless, labeled somata appear to be concentrated more densely beneath the barrel centers than beneath the septa. Cell counts showed that there were 20% more labeled cells bodies per unit area deep to the barrel centers than deep to the—that is, the barrel:septum ratio was 1:2.0. The pattern of anterograde labeling observed in tangential sections was distinctly different in the one experiment in which a relatively large injection clearly included PO just medial to VB. A photomicrograph of a section through layer IV is shown in Figure 5. Anterograde labeling in the anterolateral aspect of the barrel field is located within both the barrel centers and the septa. More posteriorly, only the septa contain substantial amounts of reaction product. 

Appearance of labeling in oblique coronal sections

In sections cut normal to the pial surface labeling in the cortex displayed a distinctly vertical organization. This is nicely illustrated in Figure 6A, where the HRP reaction product is confined largely within a single vertical column. In this experiment HRP was injected during five penetrations centered in the representation of the D1 and D2 vibrissae. The plane of this section is across the barrel rows (see Fig. 1A). The zone of anterograde labeling in layer IV is highly circumscribed, and as in the tangential section of Figure 3A, labeling within the barrel center itself is heterogeneously distributed. Labeled axons extend superficially through lamina III. The densest and most superficially located reaction product is present above the centralmost aspect of the layer IV barrel. Immediately deep to the barrel, in upper and middle layer V, labeling consists primarily of vertically oriented processes. These processes include apical dendrites of retrogradely labeled corticothermal cells whose somata are in the deeper aspects of the cortex. Labeled cell bodies are located in lower layer V and in layer VI where they are observed more often in the upper half of the lamina. Labeled cells tend to be clustered deep to the layer IV barrel. Compared to the more superficial aspects of layer V, the zone of retrogradely labeled cell bodies contains considerably denser and more widely distributed reaction product. This parallels the tangential extent of the labeling in layer IV. Thus the overall pattern of labeling within this section has a vertically oriented hourglass appearance.

The preferential distribution of labeled corticothermal cells deep to the barrel centers is best appreciated in sections where two or more adjacent columns of labeling are visible. Figure 6B and C are photomicrographs of sections from the same specimen shown in Figure 6A. Sections in panels A–C are located in progressively more anterior and...
lateral aspects of the barrel field and show increasing density of labeling in the adjacent row C and E barrels. Clustering of the retrogradely labeled neurons is most apparent where the patches of label in layer IV are most widely separated—that is, between the row D and E barrels.

A similar columnar pattern was observed in oblique coronal planes oriented parallel to or along the whisker rows (see Fig. 1B). A section along row B is shown in Figure 6D. Thalamic injections were centered in the physiological representation of row B whiskers, arcs 1–3. Anterograde labeling is densest in the B2 and B3 barrels. The septa are more narrow than in panels A–C in part because within-row septa are normally quite narrow and in part because the plane of section was not exactly parallel to the whisker rows. Nevertheless, retrogradely labeled corticothalamic cells are more common deep to the barrel centers. Regions underlying the narrow septa between barrels in row B contain comparatively fewer numbers of labeled somata per unit area (see also below). Note that because of slight differences in the section plane with respect to the pial surface, the relative thicknesses of supragranular and infragranular laminae appear different in this specimen compared to that of Figure 6A–C. With the relative compression of the deepest aspects of the cortex, the retrogradely labeled cells appear to be closer to the white matter than in the other panels.

**Apical dendrites of labeled corticothalamic cells**

HRP-containing apical dendrites were observed often. These processes emanated from retrogradely labeled somata and ascended through layer V where there was little evidence for extensive terminal branching. Because of the dense anterograde labeling of thalamocortical axon terminals within layer IV, it was not possible to follow individual dendrites within the labeled barrels. In columns containing less anterograde labeling, apical dendrites of labeled corticothalamic cells could, however, be clearly seen to have terminal arbors within layer IV. Figure 7 is a photomicrograph illustrating the morphology of some apical dendrites. At the right side of the figure, a pyramidal cell can be seen to project its apical dendrite into an overlying, anterogradely labeled barrel. Because of the relative absence of reaction product in the barrel centers at the left, the terminal apical arbors of dendrites from two other neurons are visible in layer IV. As shown here and in other figures, apical dendrites of cells lying deep to the barrel centers ascended directly to the barrel in its parent column. Cells located deep to the septa also had vertically directed apical dendrites; it is not clear from our material whether these terminated only within the overlying septum and/or whether some of their arbors terminated within one or more of the barrel centers belonging to neighboring columns.

**Quantitative measures of corticothalamic cells**

Qualitatively the somata of labeled corticothalamic cells appeared to be most densely clustered deep to the barrel centers. To determine whether the relative numbers of labeled cell bodies, as distinct from dendrites and axonal processes, were in fact greater deep to the barrel center than deep to the septa, cell counts/unit area were made. Figure 8 illustrates the method used to delineate the measured areas (see Materials and Methods).

Data were obtained only from oblique coronal sections containing at least two well-labeled adjacent barrels in layer...
Fig. 6. Appearance of labeling in oblique coronal sections. Photomicrographs in panels A–C show sections from a single hemisphere cut in a plane across the barrel rows. Labeling corresponding to barrel rows C–E has a columnar appearance. Panel D: Photomicrograph of a section from a specimen oriented along row B; columns B2 and B3 are indicated.

Laminar boundaries are shown in panel A and apply also to B and C. The relative thicknesses of the laminae are somewhat different in panel D because of the orientation of the section with respect to the pial surface. Scale in A = 200 μm applies also to B–D.
IV. A total of 7,780 cells in 48 serial sections from four specimens were examined. Results are presented in Table 1, which shows the mean number (±1 standard deviation) of retrogradely labeled corticothalamic cells per 10,000 μm² for the indicated number of sections from the four specimens. “Barrel:septum” ratios were obtained by dividing the mean for the zone deep to the barrel centers by the mean for the zone deep to the septa. The observed ratios were 1.22–1.45, indicating that regions deep to the barrels contained approximately 20–40% more labeled corticothalamic cells per unit area than regions deep to the septa. Note that a similar ratio (1.20) was observed in the tangentially sectioned specimen of Figure 4. Paired t-tests for the cases of Table 1 showed that differences between the barrel and septum means were statistically significant in three specimens (P’s < .02, two tail). For the fourth case, the probability associated with the observed t value was .055.

Within individual specimens there was variability in the overall number of labeled cells and in the relative numbers of labeled cell cells deep to the barrel vs. those deep to the septa. This is illustrated by the line drawings of Figure 9. Each panel shows data from a single section. Panels A and C and panels B and D are from cases 4 and 2, respectively, in Table 1. For each hemisphere, the two sections were selected to illustrate the range of barrel:septum ratios; these sections were not adjacent to one another. For each specimen sections in panels A and B contain the highest relative numbers of labeled somata in the zones deep to the barrel; those of panels C and D had the lowest. Thus, in panel A the zone deep to the barrel centers contained 8.54 cells/10,000 μm² whereas the zone deep to the septum contained no labeled cells. In panel C there were 4.17 cells/10,000 μm² in the regions deep to the barrels and 5.50 cells/10,000 μm² deep to the septum. In panel B, the barrel:septum ratio was 1.86 (4.18/2.24); that of panel D was 0.81 (7.47/9.25). Through the series of adjacent sections there were no clear periodic fluctuations in barrel:septum ratios suggestive of a greater concentration of labeled cells deep to the geometric center of a barrel or to its outer margins. Similarly, in tangential specimens, such as that of Figure 4, there was no apparent preferential distribution of labeled cells with respect to specific aspects of the barrel centers.

Table 1 reveals substantial differences among the four specimens in terms of the overall numbers of labeled cells. These are likely due to differential tissue shrinkage, to vagaries of the HRP technique, and/or to plane of section relative to the pial surface. With respect to the latter, for example, the comparatively large number of labeled cells in case 1 may reflect the fact that the lower layers, e.g., layer VI, appeared compressed relative to the more superficial laminae (see Fig. 6). Importantly, however, differences among the specimens in overall cell numbers were not reflected in differences in the barrel:septum ratios. Specifically, a one-way analysis of variance comparing these ratios.
Fig. 8. Photomicrographs illustrating the method used to delineate zones for determining numbers of corticothalamic cells per unit area. Panel A: A section oriented across the barrel rows that contains three well-labeled barrel columns. The same section is shown in panel B with solid lines in layer IV denoting barrel boundaries which were projected radially into infragranular laminae to delineate zones deep to the barrel centers (boxes) from zones deep to the septa; see text. Scale in A = 200 μm, applies also to B–D.

Fig. 9. Line drawings illustrating the spatial distribution of labeled corticothalamic cells from each of four sections. Shading denotes the zone deep to the overlying septa, determined as illustrated in Figure 8. Panels A and C and panels B and D illustrate locations of labeled somata in nonadjacent sections from two specimens, respectively. Panels A and B are from sections containing the greatest barrel:septum ratios; those of C and D contain the lowest; see text. Bar in A = 50 μm, applies also to B–D.

**TABLE 1. Numbers of Corticothalamic Cells/10,000 μm² Deep to the Barrels and Septa**

<table>
<thead>
<tr>
<th>Case</th>
<th>Plane</th>
<th>N</th>
<th>&quot;Barrel&quot;</th>
<th>&quot;Septum&quot;</th>
<th>Ratio</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Along</td>
<td>11</td>
<td>10.98 ± 2.56</td>
<td>8.97 ± 3.38</td>
<td>1.22</td>
<td>2.75</td>
<td>.02</td>
</tr>
<tr>
<td>2</td>
<td>Across</td>
<td>14</td>
<td>5.20 ± 1.19</td>
<td>4.19 ± 1.68</td>
<td>1.24</td>
<td>3.84</td>
<td>.002</td>
</tr>
<tr>
<td>3</td>
<td>Across</td>
<td>12</td>
<td>3.35 ± 0.78</td>
<td>2.35 ± 0.59</td>
<td>1.43</td>
<td>3.56</td>
<td>.004</td>
</tr>
<tr>
<td>4</td>
<td>Across</td>
<td>11</td>
<td>6.73 ± 2.04</td>
<td>4.64 ± 2.51</td>
<td>1.45</td>
<td>2.77</td>
<td>.005</td>
</tr>
</tbody>
</table>

1Number of sections.
2Paired t-test.
3Two tail probability.

for the 48 sections and four cases that compose the data of Table 1 failed to reveal a significant difference among the four cases (F = 1.86, P = .15). Thus the relative numbers of corticothalamic cells deep to the barrels vs. the septa are similar in these specimens regardless of whether the overlying septa are between barrels in the same row or between barrels in different rows.

Soma diameters of labeled corticothalamic cells were measured in oblique coronal sections. Measurements were made across the greatest width of the soma in a plane perpendicular to the apex of the neuron. Data were obtained from all labeled cells in single sections from two different specimens. Mean somal diameters were 10.30 μm ± 1.94 s.d. (N = 106) and 10.17 μm ± 1.86 (N = 329). Though the distributions were unimodal, the larger cells tended to be located most often in layer V.

**DISCUSSION**

The present findings demonstrate a distinctive pattern of anterograde and retrograde labeling in the first somatic sensory cortex of rats following HRP injections into the physiologically defined vibrissae representation in the ipsilateral VB thalamus. The termination of thalamic afferents and the origin of the reciprocal efferent projections are distributed in a laminar-dependent fashion, and at different cortical depths the tangential organization of both systems largely parallels the known physiological and cytoarchitectonic representation of the whiskers on the contralateral face. The close correspondence between these afferent and efferent systems suggests that an individual column in the barrel...
cortex contains functionally related neuronal networks that are intimately associated with the major source of discriminative tactile information to this cortical area.

**Thalamocortical projections**

Numerous studies using a variety of axonal tracing techniques have shown that projections from VB to the barrel field in layer IV are organized into discrete patches that correspond to the cytoarchitectural organization of the barrels (Killackey, '73; Killackey and Leshin, '75; Wise and Jones, '77; Woolsey and Dierker, '78). Axons ramify largely though not exclusively in the barrel centers, which are also distinctive for their high levels of metabolic activity (Wong-Riley and Welt, '80; Land and Simons, '85a; Chmielowska et al., '86). Recently, Jensen and Killackey ('87) reported that arbors of individual thalamocortical axons in the rat barrel field extend superficially into layer III and in some cases to lamina II (see also Lorente de Nó, '49). In addition, for some axons less extensive arborizations were observed in lower layer V and upper layer VI. Similar observations have been made in mice (Bernardo and Woolsey, '87). Our results are entirely consistent with these observations. An interesting finding to emerge from our material is an apparent nonuniformity in the density of reaction product in the barrel centers. Similarly, using computer-assisted analyses of silver grain densities following thalamocortical transport of triitated amino acids in mice, Woolsey and Dierker ('78) observed clustering of presumed axon terminals within the barrel. Moreover, within the barrel centers of rats cytochrome oxidase reactivity is distributed in a nonuniform fashion (Land and Simons, '85a), and there is some physiological evidence suggestive of functional heterogeneity within individual cortical barrels (Simons and Land, '86; Chmielowska et al., '86; McCasland and Woolsey, '89).

**Characteristics of corticothalamic neurons**

Corticothalamic neurons were located in lower layer V and in layer VI where the largest concentration was in the upper half of the lamina. These findings accord with others in the first somatic sensory cortex of mice (Hersch and White, '81; White and Hersch, '82) and rats (Wise, '75; Wise and Jones, '77; for a review see Jones, '84). Wise and Jones observed additionally some labeled cells in upper layer V, following large pressure injections of HRP into the diencephalon. White and Hersch ('82), who also employed pressure injections, suggested that the presence of retrogradely labeled layer V neurons might be due to the spread of HRP into PO. In the present study, labeling in lower layer V was observed even with small iontophoretic injections that appeared to be restricted to VB. Although we cannot rule out the possibility of HRP uptake by fibers of passage from PO, the different patterns of anterograde labeling observed with VB vs. VB plus PO injections (see also Koralek et al., '88) suggest that relatively few, if any, of the retrogradely labeled corticothalamic cells studied here represent cells projecting only to PO that were labeled because their axons pass through the VB injection site.

Retrogradely labeled neurons had apical dendrites and were of the pyramidal or the modified pyramidal variety. Mean somal diameters, which were approximately 10 μm, are similar to those in mice (White and Hersch, '82), but smaller than those reported in rats by Wise and Jones, even in layer VI. This discrepancy may reflect differences in tissue shrinkage and/or in the method of measurement. For example, our measures were based on cell diameter perpendicular to the apex of the soma whereas data in the study by Wise and Jones appear to have been derived secondarily from somal area measures obtained with a computer microscope. Labeled apical dendrites ascended to layer IV and, when visible, were observed to terminate there. Similar findings have been reported for corticothalamic cells in mice (White and Hersch, '82) and are consistent with the more general observation that apical dendrites of layer VI pyramidal cells terminate largely within the thalamic recipient zones in the middle cortical depths (Hendry and Jones, '83; Escobal et al., '86). In mice corticothalamic apical dendrites in layer IV receive synapses from thalamocortical axons (White and Hersch, '82).

**Columnar organization**

Afferent input from VB to the face region of the first somatic sensory cortex is organized into discrete zones corresponding to barrel centers within layer IV. Input to an individual barrel arises largely from its corresponding “barreloid” in the VB thalamus (Land et al., '86; Land and Simons, '85b). Anterograde labeling of presumed thalamocortical origin is observed also in the deeper layers as patches that are in register with the overlying barrels (see also Bernardo and Woolsey, '87). Individual thalamocortical axons in rodents (Bernardo and Woolsey, '87; Jensen and Killackey, '87) and cats (Landry and Deschênes, '81) traverse the cortical gray matter by a roughly radial trajectory and distribute terminal arbors to granular and deep infragranular zones that are vertically aligned.

The present findings show that corticothalamic neurons projecting back to VB are distributed in the deeper layers in a similarly patchy fashion. Cell counts per unit area showed a larger relative number of these cells deep to the barrel centers than deep to the septa. This does not seem to be correlated with an overall cytoarchitecture in the deeper layers suggestive of barreloid aggregations of neurons. Rather, it appears that corticothalamic cells are most likely to be located within regions traversed by the incoming thalamocortical fibers. This is supported by the observation that the clustering of retrogradely labeled somata is most readily apparent where the separation of barrels in layer IV is greatest, i.e., between barrels in different rows. This is supported also by the data of Table 1; excluding case 4, t values for the two “across”-row cases are larger than for the “along”-row case. Nevertheless, data obtained in sections cut along the rows must be interpreted with caution because, unless the section plane is optimal, the narrow within-row septa are difficult to visualize. A patchy pattern of corticothalamic cells might be even less readily apparent in mice, which lack wide septa between barrels in the same or in different rows.

The present findings might imply a reciprocal one-to-one correspondence between a single cortical column and an individual thalamic barreloid. A recent study of corticothalamic projection patterns, however, indicates that this is not entirely the case because corticothalamic projections from a single cortical column diverge to terminate within many barreloids (Hoogland et al., '87). Divergence in corticothalamic feedback may provide a capacity for sensory systems to selectively enhance activity within a topographically organized projection system on the basis of predictions about the trajectory of a stimulus across the receptor array (see, for example, Anderson and Van Essen, '87; Koch, '87).

The pattern of anterograde and retrograde labeling observed following HRP injections into VB is highly reminisc-
cent of the pattern of cytochrome oxidase (CO) reactivity in this cortex (Land and Simons, '85a). Specifically, CO reactivity is greatest in the barrel centers, extending superficially into supragranular laminae, and is heightened also in lower layer V and layer VI deep to the barrel centers. The CO-rich infragranular zones thus appear to be coextensive with regions containing the largest population of corticothalamic projection neurons. Our observation of substantial, particularly into supragranular laminae, and is heightened also in extracellular microscopic evidence of thalamocortical synapses here, and, in fact, some corticothalamic neurons receive such inputs in the deeper layers (White and Hersch, '82). Extra-cellular unit recordings have demonstrated that some cells in the deeper aspect of the rat barrel cortex are particularly well-driven by whisker vibrations (Simons, '78), and latencies of excitatory postsynaptic potentials evoked by whisker deflections are among the shortest observed in this cortex (Carvell and Simons, '88).

Barrels in layer IV and the CO-rich, corticothalamic infragranular zones are linked by axon collaterals of corticothalamic neurons which synapse preferentially on smooth, presumably GABAergic and inhibitory, barrel cells (White and Keller, '87). Also, though both the granular and infragranular zones contain synapses of thalamocortical and of corticothalamic origin, the relative proportions of such synapses are somewhat reciprocal. The barrel neuropil contains a greater incidence of thalamocortical synapses and the infragranular zones have a greater incidence of corticothalamic ones. Thus, a functional column in this cortex may be characterized by a central core zone containing at least two linked neural networks each of which is intimately associated with the VB thalamus.

There is increasing evidence that some afferent and efferent projection systems other than those associated with VB are organized in a fashion complementary to the VB thalamocortical/corticocortical system. For example, diencephalic inputs from PO tend to avoid the barrel centers, being concentrated most heavily in the layer IV septa and in layer Va deep to the barrel (Lu and Lin, '86; Lin et al., '87; Koralek et al., '88; see also Herkenham,'80). Axonal terminations of some corticocortical systems within the somatosensory cortex itself largely avoid barrel centers (Chapin et al., '87). Callosal inputs are likewise largely excluded from the barrels and are distributed more to the septa and the regions immediately superficial and deep to them; these regions also contain somata of callosally projecting neurons (Olavarria et al., '84). In mice efferent neurons of layer V that project to ipsilateral motor cortex or to the brainstem tend to be located more often deep to the outer margins of the barrel centers and deep to the septa (Crandall et al., '86). The targets of neurons in layer VI that were not labeled by our VB injections are not yet known.

An unresolved issue concerns the targets of projection neurons situated superficial to the barrel centers in layer III and deep to them in middle and upper layer V. For example, though corticotrigeminal projecting pyramidal cells in layer V are located deep to the barrel fields, it is not clear how they are distributed relative to the overlying centers of individual barrels (see Wise et al., '79). In addition, patterns of local corticocortical connections within and among barrel columns have not been extensively studied. In spite of the absence of these important data, available anatomical evidence is consistent with the idea that an individual cortical column in the rodent barrel cortex contains a central "core" zone closely associated with a specific extrinsic source, i.e., VB, that is surrounded by a narrower region of more diverse inputs and outputs that forms the interface between adjacent columns. There is some evidence that the receptive field properties of neurons in these different compartments are functionally distinctive. Response properties of cells in the barrel cortex differ in a laminar-dependent fashion (Simons, '78; Chapin, '86; Armstrong-James and Fox, '87), and where examined, in tangential fashion as well. For example, in layer IV barrel neurons are known to have smaller receptive fields than those in the septa (Armstrong-James and Fox, '87). Thus, information processing within a vibrissa column may be understandable in terms of functionally and anatomically identifiable subsets of neurons that are differentially engaged by peripheral stimuli and the behavioral context in which they occur.

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LITERATURE CITED


