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Septal Columns in Rodent Barrel Cortex: Functional Circuits for Modulating Whisking Behavior

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ABSTRACT

In rodents, each mystacial whisker is represented in the granular layer of primary somatosensory (SI) cortex by a compact cluster of cells known as a barrel, and barrels are separated from each other by domains that are called septa. Vertical columns of neurons aligned with each barrel act as a functional assembly to process information from a "principal" whisker, but a functional role has not been identified for vertical columns of neurons that are aligned with the septa. To determine whether these septal columns provide the main source of projections to primary motor (MI) cortex, we placed retrograde tracers in MI cortex and analyzed the location of the retrogradely labeled neurons with respect to the septal and barrel compartments of SI barrel cortex. In cases in which SI barrel cortex was sectioned tangentially, retrogradely labeled neurons in the extragranular layers of SI were plotted and superimposed onto reconstructions of the layer IV barrel field. In each of these cases, most labeled neurons were located above or below the septal regions of layer IV. When SI barrel cortex was sectioned coronally, we observed multiple columns of labeled SI neurons that were vertically aligned with the septal zones of layer IV. These results indicate that columns of neurons that are vertically aligned with the septa, or septal columns, are functionally linked by virtue of their projections to MI cortex. We hypothesize that these septal columns represent an interconnected and functionally distinct circuit that transmits information to MI and other brain regions involved in motor control. J. Comp. Neurol. 480:299–309, 2004.

Indexing terms: columnar organization; motor cortex; retrograde tracing; sensorimotor integration, somatosensory cortex

In rodents, the primary somatosensory (SI) and motor (MI) cortices are interconnected to facilitate somesthesis-guided behaviors. Consistent with the importance of whisking behavior for acquiring sensory information to modulate exploratory movements and other behaviors (Welker, 1964; Jenkinson and Glickstein, 2000), single tracer studies indicate that the SI and MI vibrissal representations are connected by topographically organized projections (Welker et al., 1988; Miyashita et al., 1994; Izraeli and Porter, 1995). By using a dual anterograde tracer paradigm, we recently characterized the detailed topography of projections from SI barrel cortex to MI and concluded that their divergent patterns facilitated the integration of inputs from whiskers in the same row (Hoffer et al., 2003).

Despite these studies on the interconnections between SI barrel cortex and the MI whisker representation, many important details remain unclear. For example, few data indicate whether SI projections to MI originate in regions associated with either the barrel or septal compartments in layer IV. Tracer injections in MI reportedly produced retrogradely labeled neurons in corresponding barrel columns of SI (Izraeli and Porter, 1995), but photomicrographs depicting the location of labeled neurons with respect to the barrel boundaries were not provided. In another study, we found that SI projections to the MI

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whisker representation appeared to originate from the septal regions of barrel cortex (Hoffer and Alloway, 2001). That study, however, was focused on characterizing corticostriatal projections, and we did not systematically analyze SI projections to MI with respect to the barrel field compartments.

Determining the location of SI projection neurons with respect to its histochemically defined compartments has an important bearing on the functional principles of cortical organization. A major tenet of cortical organization maintains that sensory cortex is organized into vertical modules or columns of neurons that operate as functional processing units (Mountcastle, 1997). Rodent barrel cortex has been used to advance this conceptual view, in part because the barrel field has an isomorphic relationship with the peripheral whiskers (Woolsey and Van der Loos, 1970), and because neurons vertically aligned with the SI whisker barrels respond primarily to inputs from a single whisker (Welker, 1971, 1976; Simons, 1978; Ito, 1985; Chapin, 1986; Chmielowska et al., 1986).

Consistent with the principles of columnar organization, accumulating data suggest that the barrel and septal regions in layer IV represent the cortical input stages for two functional circuits that are largely segregated throughout the other layers of SI barrel cortex. Whereas layer IV barrels receive thalamic inputs from the ventral posteromedial (VPM) nucleus, the septal regions receive inputs mainly from the posterior medial (POm) nucleus (Koralek et al., 1988; Lu and Lin, 1993). Furthermore, the layer IV barrels project primarily to supragranular sites in the parent and neighboring barrel columns, whereas the layer IV septal regions project longer distances and terminate mainly in other groups of septa-related neurons (Chapin et al., 1987; Kim and Ebner, 1999). Similar patterns of segregation have also been observed for local projections originating from the extragranular layers associated with the barrel or septal regions (Kim and Ebner, 1999).

Although these facts reinforce the view that barrel cortex has a columnar organization, a functional role for columns of septal neurons has not been established. Connections between septa-related neurons become stronger after whisker deprivation (Shepard et al., 2003), but no study has identified a specific functional attribute that is shared by populations of neurons that form a septal column. One possibility is that septal columns are functionally linked by virtue of their connections with surrounding cortical areas or other brain regions that have a specific function. As one test of this hypothesis, we placed retrograde tracers in the MI whisker representation and examined the laminar topography of labeled SI neurons with respect to the whisker barrels and intervening septa in layer IV. Our results indicate that SI projections to MI originate predominantly from vertical columns of neurons that are aligned with the layer IV septal regions.

MATERIALS AND METHODS

Experiments were performed on 14 male Sprague-Dawley rats ranging between 300 and 600 g. All procedures complied with NIH guidelines for the use and care of laboratory animals and were approved by our institutional animal welfare committee.

Animal surgery

Each rat received an intramuscular (IM) injection of ketamine (20 mg/kg) and xylazine (6 mg/kg) to induce anesthesia. Additional anesthesia was administered approximately every 45–60 minutes or when withdrawal reflexes were evoked. Atropine methyl nitrate (0.05 mg/kg, IM) was administered to reduce bronchial secretions, and chloramphenicol was also administered (50 mg/kg, IM) to prevent bacterial infection. The rats were intubated through the oral cavity, placed in a stereotaxic frame, and artificially ventilated with a 2:1 mixture of nitrous oxide and oxygen. Body temperature, expired CO_2, and heart rate were monitored continuously throughout the surgery. After exposing the skull, the wound margins were infiltrated with 2% lidocaine. A craniotomy was made over MI at coordinates consistent with previous maps of the MI whisker representations (Hall and Lindholm, 1974; Neafsey et al., 1986; Hoffer et al., 2003).

Tracer injections

Retrograde tracers were injected in 19 hemispheres at stereotaxic coordinates (1.7–2.0 mm rostral and 1.5–1.8 mm lateral to bregma) that were consistent with the MI whisker representation (Hoffer and Alloway, 2001; Hoffer et al., 2003). In 14 hemispheres, intracortical microstimulation (ICMS) was used before tracer injection to evoke whisker twitches, thereby confirming that the injected site was within the MI whisker representation. For these cases, glass micropipettes filled with a 3 M sodium chloride solution and having impedances of 0.5 MΩ were used to microstimulate the MI whisker representation. The stimulating micropipette was lowered approximately 1.7 mm below the pial surface to activate layer V pyramidal cells. Cathodal pulse trains lasting 70 msec (0.7-msec pulses and 3.3-msec interpulse intervals) were administered to evoke small contralateral whisker movements. Initially, current pulses started at 100–150 μA but were reduced to threshold levels (25–100 μA) that evoked small movements of a few mystacial whiskers. After locating an appropriate whisker representation in MI cortex, either Diamidino Yellow (DY), True Blue, or cholera toxin subunit B (CTb) was deposited in MI cortex.

For CTb injections, a 1% solution of the tracer (List Biological Lab; Campbell, CA) in 0.1 M phosphate buffer (PB) at a pH of 6.0 was loaded into a glass pipette with a tip diameter of 10–20 μm. The pipette was lowered into MI cortex to a depth of 1.0–1.4 mm. The CTb was iontophoretically injected using positive current (4–5 μA) pulses with a duty cycle of 7 seconds. Iontophoresis of CTb continued for 15–20 minutes at one or two depths within the same vertical column of MI cortex. These parameters produced tracer deposits that labeled a region of MI cortex that was 1 mm in diameter.

For DY or True Blue injections, each fluorescent tracer was pressure injected into MI cortex using a Hamilton syringe having a 75- to 100-μm glass tip cemented to the needle. The syringe was placed in a mechanical pressure-injection syringe holder (Kopf Instruments, model 5000; Tujunga, CA) and, after filling the glass injection pipette with tracer, the pipette was lowered to a depth of 1.0–1.4 mm in MI cortex. Approximately 120–140 nl of a fluorescent tracer was slowly injected throughout the vertical extent of MI cortex. This volume typically labeled a region of MI cortex that was 1 mm wide. After completing the

injection, the wound margins were sutured and the animal was returned to its home cage for 4–8 days.

Histology

Each rat was deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame so that fiduciary marks could be placed at specific stereotaxic coordinates. Subsequently, the animal was transcardially perfused with 4% paraformaldehyde (pH 7.4) in 0.1 M PB, and then with 500 ml of 4% paraformaldehyde containing 10% sucrose. The brain was removed and placed overnight in cold fixative with 30% sucrose. In six cases, however, the cortex was dissected from the hemisphere, and the resulting cortical slab was gently flattened between two glass slides and placed in cold fixative with 30% sucrose. On the following day, the brain tissue was sectioned with a sliding microtome at a thickness of 50 or 60 μm. In the case of whole hemispheres, the brain was sectioned coronally. In the case of cortical slabs, the slab was placed pia-side down on a plane of frozen substrate so that sectioning commenced in the infragranular layers of the cortical slab. This procedure increased the likelihood of obtaining tangential cortical sections that were precisely parallel to the pia, and it facilitated our ability to acquire at least one section that contained the entire layer IV barrel field. Depending on section thickness, barrels were located in sections 6 through 9 (where section 1 includes the pia), and these sections were specifically processed for cytochrome oxidase (CO). All remaining sections were processed in serial order for retrograde tracing.

Serially ordered sections were processed for CTb labeling as described by others (Luppi et al., 1990; Dederen et al., 1994). Briefly, the sections were preincubated in phosphate buffered saline (PBS) containing 0.3% Triton X-100 (PBS-T) with 5% normal rabbit serum and 0.5% bovine serum albumin for 1 hour. After rinsing in 0.1 M PBS, the sections were incubated with polyclonal anti-goat CTb (1:10,000–20,000, List Biological Lab, Campbell, CA) in PBS-T with 0.5% bovine serum albumin for 1 day at room temperature or 2–3 days at 4°C. Thereafter, sections were incubated in biotinylated rabbit anti-goat IgG (1:2,000, Jackson Immunoresearch Lab, West Grove, PA) and then in avidin–biotin complexes (diluted in PBS [1:100], Vector Lab, Burlingame, CA) for 1.5 to 2.0 hours with intervening rinses. The sections were then reacted 5–10 minutes with diaminobenzidine (DAB) intensified with nickel chloride to produce a black reaction product. The processed sections were mounted on gel-dipped slides, dried overnight, and cover-slipped with Cytoseal. Standard light microscopy was used to visualize the CTb injection sites and labeled neurons.

Sections from the hemispheres injected with DY or True Blue were washed in 0.1 M PB and then mounted on gel-coated slides. The sections were briefly dehydrated in alcohol, defatted in xylene, and then cover-slipped with Cytoseal. The injection site and labeled soma were visualized by viewing them with light transmitted through a filter cube (11000v2, Chroma Technology; Rockingham, VT) with excitation filters permitting transmission from 320 to 490 nm and emission filters permitting transmission above 420 nm.

Tangential sections through cortical layer IV were processed for cytochrome oxidase (CO) according to previous reports (Wong-Riley, 1979; Land and Simons, 1985). Sections were washed twice in a PB solution and then incubated at 35°C for 5–6 hours in a PB solution that contained 4% sucrose, 0.05% DAB, and 0.05% cytochrome C (Sigma Laboratories, C-2506; St. Louis, MO). Sections were then washed in PB before being mounted on gel-coated slides and dried. Mounted sections were post-fixed in neutral formalin, dehydrated in a series of alcohol, defatted in xylene, and cover-slipped with Cytoseal.

Anatomic reconstructions

Labeled soma in SI and surrounding cortical regions were plotted with a microscopic reconstruction system (AccuStage; St. Paul, MN) connected to an Olympus light microscope (BH-2) with 1×, 2×, 10×, 20×, and 40× objectives. Digital reconstructions of labeled soma and surrounding anatomical landmarks, including prominent blood vessels, were saved to computer files. Likewise, tangential sections through the SI barrel field, as determined by high concentrations of CO labeling, were digitally reconstructed and saved to a computer file. Subsequently, the digital reconstruction of the CO-labeled barrel field was superimposed on each digital reconstruction of the labeled neurons in the extragranular layers so that blood vessels common to both sections were aligned. Finally, the number of labeled neurons in the barrel and septal regions were separately counted for statistical analysis. Software modules in the Accustage reconstruction system were used to measure compartmental areas in layer IV and to calculate the density of labeled neurons in the extragranular layers.

A Cool Snap HQ CCD digital camera (Roper Scientific, Tucson, AZ) was used to capture images of neuronal labeling. Image acquisition was controlled by a software program (IP-Lab, version 3.52, Scanalytics, Inc., Fairfax, VA) that was also used to store the images as TIF files. Subsequently, the raw TIF files were imported into another software program (Deneba Systems, Inc., Canvas 8.0; Miami, FL) that was used to adjust image brightness and contrast so that labeling appeared as it did during microscopic visualization.

RESULTS

A total of 14 animals received tracer injections in MI cortex. In nine of these animals, a single tracer was injected into one hemisphere, whereas the remaining five animals received a different tracer in each hemisphere. Thus, a total of 19 hemispheres were injected with either CTb (n = 11), DY (n = 7), or True Blue (n = 1) as the retrograde tracer. Data from three hemispheres were excluded from our analysis, because histologic inspection of the cortex and thalamus indicated that the tracer was deposited outside the MI whisker representation or that too little tracer was deposited to produce labeling in SI.

Regardless of the tracer that was used, tracer injections that labeled a 1-mm patch of MI cortex produced a large number of retrogradely labeled neurons throughout SI cortex and the surrounding cortical regions. In addition to observing distributed populations of labeled soma in SI barrel cortex, we frequently observed dense populations of labeled neurons in a broad band of cortex (> 1 mm wide) lying between the SI whisker and forelimb representations, which were both identified on the basis of CO labeling. Based on this location, this broad band of labeling is likely to represent the dysgranular zone of SI (Donoghue

and Parham, 1983; Chapin and Lin, 1984; Chapin et al., 1987). We also observed populations of labeled neurons in the region lying immediately lateral to the SI barrel field. The exact position of this labeled area varied across animals, but either adjoined the barrel field or was slightly separated from it, and suggests that this labeling represents the whisker representation of the secondary somatosensory (SII) cortex (Fabri and Burton, 1991; Izraeli and Porter, 1995; Alloway et al., 2000). Further lateral to SII cortex, but always separated by a sparsely labeled region, we observed groups of labeled neurons that represent the parietal ventral (PV) and perirhinal (PR) somatosensory cortical regions (Krubitzer et al., 1986; Fabri and Burton, 1991; Miyashita et al., 1994). Finally, a dense population of labeled neurons was usually observed caudal to the SI barrel field and, presumably, represents the posteromedial parietal cortical (PM) area (Koralek et al., 1990; Fabri and Burton, 1991; Kim and Ebner, 1999).

Topography of labeling in SI barrel cortex

To compare the topography of neuronal labeling in SI barrel cortex and the surrounding areas, we plotted and reconstructed the distribution of labeled neurons in hemispheres in which the cortex had been flattened and sectioned tangentially (n = 6). The reconstructed labeling patterns in these cases revealed some important topographic distinctions between SI and the surrounding cortical regions. Although neuronal labeling in SII, PV, PR, or PM displayed some variations in labeling density both across and within cases, the labeling patterns in these areas were devoid of obvious patchiness or other well-defined topographic features. By contrast, labeled neurons in SI barrel cortex were concentrated in specific regions and displayed spatial patterns suggesting the presence of a network of functional modules that project to MI cortex. The remainder of this analysis is focused on characterizing these labeling patterns in SI barrel cortex.

The spatial distributions of SI barrel neurons that project to MI are illustrated by case SM0 in Figure 1. As this figure indicates, tangential reconstructions of labeled neurons in SI barrel cortex revealed clusters of densely packed neurons that formed parallel strips of cortical labeling (see Fig. 1, row A). Photomicrographs of these regions (see Fig. 1, rows B and C) indicate that the labeling produced by CTb was dominated by dense clusters of large neuronal soma, many of which appeared to be pyramidal in shape. Some CTb labeling was also due to the presence of the tracer in the neuropil surrounding the labeled soma, but it was not possible to distinguish whether this labeling consisted mainly of axonal terminals or dendritic processes.

The retrogradely labeled neurons in SI barrel cortex were distributed primarily among sites located directly above or below the septal regions of the barrel field. As indicated by Figure 2, a comparison of the CO-labeled barrel field with the distribution of labeled neurons in case SM0 indicates that the majority of CTb-labeled neurons were concentrated in three parallel strips that corresponded to the septal regions lying between the C, D, and E barrel rows and between row E and the dysgranular zone. Some labeled neurons appeared to be within the boundaries of a barrel column, but virtually all of these were distributed along the edges of those columns. Very few labeled neurons appeared in the center of a barrel column, and the lack of labeled neurons is demonstrated by comparing the CO-labeled barrel field in layer IV with photomicrographs depicting the distribution of CTb labeling in the extragranular layers. Thus, sections that were processed for CTb labeling (see Fig. 1, row B) contain circular pale regions that are devoid of labeled neurons; these regions correspond to the position of the CO-labeled barrels in rows D and E of layer IV (see Fig. 2, left panel).

When fluorescent retrograde tracers were placed in MI cortex, similar row-like patterns of labeled neurons were observed in SI barrel cortex. In those cases, the strips of cortical labeling in SI produced by DY or True Blue were not quite as prominent as those produced by CTb, because these fluorescent tracers did not label the axonal or dendritic processes that frequently surround the retrogradely labeled soma. Nonetheless, as indicated by case SM1 in Figure 3, densely packed clusters of fluorescent soma were observed predominantly in areas that were aligned with the septa zones of layer IV.

The strong association between the septal regions and the labeled neurons that project to MI is underscored by examining those parts of SI barrel cortex in which the septal regions are relatively large. Variations in the spatial extent of the septal regions are common across animals, and some septal regions are wider than others within the same animal. Depending on the whisker representations that were injected in MI cortex, retrograde labeling in SI occasionally corresponded with regions that contained the widest septal zones. Figure 4, for example, depicts a layer IV barrel field that was characterized by a relatively wide septal region just lateral to the C barrel row. Moreover, a large gap in the C barrel row was observed just rostral to barrel C3. This animal (case SM2) had received a large deposit of CTb in the MI whisker representation, and examination of the regions below the enlarged septal regions revealed several dense clusters of CTb-labeled neurons. Consistent with other cases, adjacent circular regions that were devoid of labeled neurons were aligned with the CO-labeled barrels of layer IV (see Fig. 4D, D′, and D″).

Columns of septal labeling

Our observations strongly suggest that SI projections to the MI whisker representation originate from columns of neurons that are vertically aligned with the layer IV septal regions. Thus, populations of SI neurons labeled by tracer deposits in MI were observed in the same septa-related regions at several depths throughout barrel cortex. Whereas the density of labeling varied across different cortical depths, direct comparison of tangential sections obtained from different layers, often separated by a millimeter or more, did not reveal any major difference in the topography of the labeling (see Figs. 1, 4). Indeed, the topographic distribution of labeled neurons in the supragranular layers was virtually identical to that observed in the deepest infragranular layers (in Fig. 2, compare panels for sections 3, 5, 7, and sections 16, 18, 20).

To confirm that SI projections to the MI whisker representation originate from columns of neurons that are vertically aligned with the septal regions of layer IV, we also examined the distribution of CTb-labeled neurons in coronal sections of SI cortex. As shown in Figures 5 and 6, injections of CTb that infiltrated a 1-mm wide patch of MI cortex produced several vertical columns of retrogradely labeled neurons in SI cortex. The labeled columns, which varied from 100 to 300 μm in width, contained hundreds of

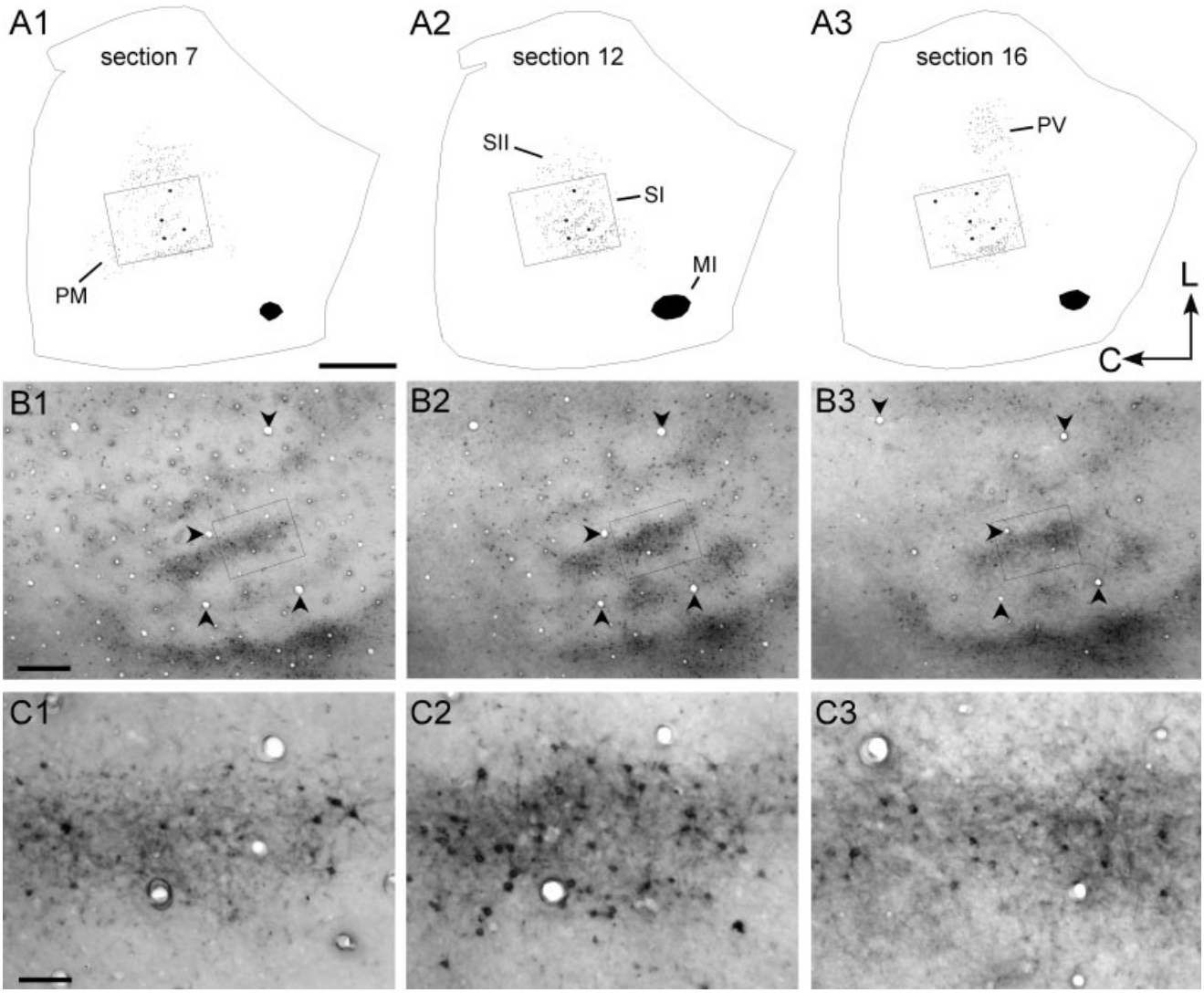

Fig. 1. Distribution of labeled projection neurons in the septal regions of primary somatosensory (SI) barrel cortex (case SM0). **Row A:** Reconstructed tangential sections showing the row-like distributions of labeled neurons (black dots) in SI cortex after depositing cholera toxin subunit B (CTb) in the primary motor (MI) cortex whisker representation. Successively deeper layers of SI cortex are represented by reconstructions of sections 7, 12, and 16. In each reconstruction, boxes indicate areas shown by photomicrographs in row B, black ovals represent the CTb deposit in MI, and filled circles represent blood vessels. **Row B:** Photomicrographs of the CTb-labeled neurons in the septal regions of SI barrel cortex. Boxes indicate areas shown by photomicrographs in row C, and arrowheads indicate the blood vessels depicted in row A. **Row C:** Magnified views of CTb-labeled processes in one septal region. PV, parietal ventral somatosensory cortical regions; PM, posteromedial parietal cortical area. Scale bars = 3 mm in row A; 500 μm in row B; 100 μm in row C.

labeled pyramidal neurons as well as their dendrites and other labeled fibers. While the density of labeling varied across layers, labeled neurons were densest in layer V. Many neurons were also observed in layer VI and in the supragranular layers, but very few neurons were observed in either compartment of the layer IV barrel field. As shown in Figure 6, when the CTb-labeled columns were compared with the location of CO-labeled barrels in adjacent sections, it was apparent that the vertical columns of labeled neurons were closely aligned with layer IV septal regions that were devoid of CO labeling. A few labeled neurons were occasionally observed in regions aligned with the CO-dense barrels of layer IV. As Figure 6 indicates, however, most of the labeled neurons appearing within a barrel column were distributed within layer V, and their density was substantially lower than that seen in the septal columns. Furthermore, we frequently observed many barrel columns in which the central portion of the column was completely devoid of labeled neurons in all of the cortical layers.

Statistical analysis

To characterize the extent to which SI projections to MI originate from septal columns, we quantified the distribution of labeled neurons according to their association with the barrel or septal compartments of layer IV. This analysis was performed only on hemispheres in which the cortex was sectioned tangentially (n = 6). In each case,

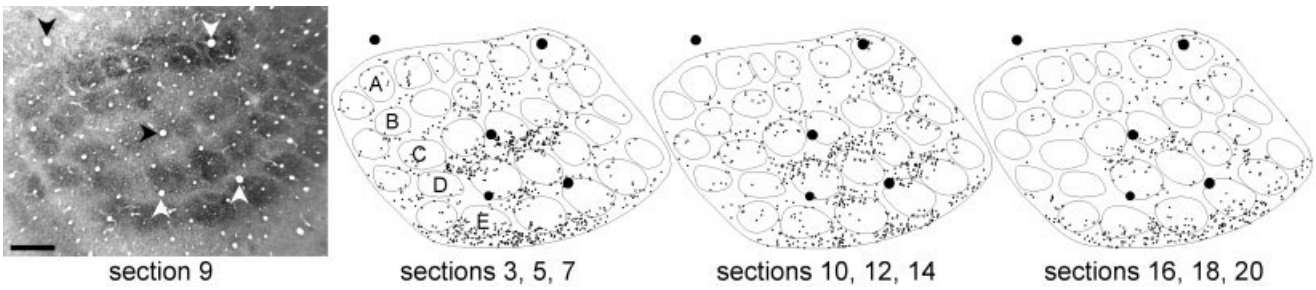

Fig. 2. Relative topography of cholera toxin subunit B (CTb) -labeled neurons and the layer IV barrel field in case SM0, which was also presented in Figure 1. Photomicrograph of tangential section 9 shows the location of high concentrations of cytochrome oxidase (CO). Arrowheads indicate the same set of blood vessels shown in Figure 1. Right panels indicate the location of CTb-labeled neurons with respect to the outlines of the CO-labeled barrels in layer IV. Each panel represents three superimposed reconstructions of sections through the supragranular (sections 3, 5, and 7) or deeper (sections 10, 12, 14, 16, 18, and 20) layers of cortex. Filled circles represent the same blood vessels indicated by arrowheads in the photomicrograph. Specific barrel rows are indicated by letters (i.e., A, B, C, D, and E). Scale bar = 500 μm.

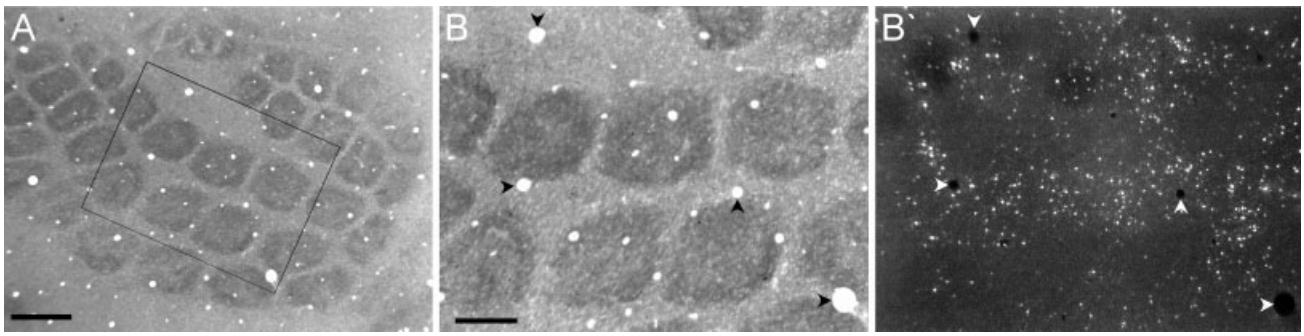

Fig. 3. Relative topography of fluorescent-labeled soma and the layer IV barrel field in case SM1. **A:** Photomicrograph depicting the cytochrome oxidase–labeled barrels in layer IV. The boxed area indicates the regions appearing in B and B′. **B and B′:** Comparison of selected parts of the layer IV barrel field with the location of fluorescent neurons in layer V that were labeled by depositing Diamidino Yellow in the primary motor cortex whisker representation. Arrowheads in both panels depict the same blood vessels. Scale bars = 500 μm in A; 250 μm in B (applies to B,B′).

labeled neurons in the extragranular layers and the outlines of the CO-labeled barrels in layer IV were plotted with respect to nearby blood vessels. A boundary enclosing the entire barrel field was drawn around the barrels and intervening septa, and this area included the septal regions lying medial to row E or lateral to row A. These latter septal zones were included because they represent perigranular zones (Chapin et al., 1987). In addition, the neurons in these perigranular regions are whisker-sensitive and can be distinguished from the unresponsive neurons recorded in the dysgranular zone (Chapin and Lin, 1984).

With the aid of the plotted blood vessels, each reconstructed extragranular section was aligned and superimposed upon the reconstruction of the layer IV barrel field. Labeled neurons appearing in the septal and barrel regions were separately counted, and then expressed as a proportion of the total sum of labeled neurons that appeared within the boundaries of the SI barrel field. This procedure normalized the variations in the number of labeled neurons in SI cortex that were produced because of differences in tracer injection volumes or other factors.

In each of the six cases, the majority of labeled neurons in SI barrel cortex resided in areas that were vertically aligned with the layer IV septa. Figure 7 indicates that, on average, 64.3% of the labeled neurons were located in these septal columns, whereas only 35.7% of the neurons appeared within the barrel columns. A matched-sample analysis indicated that this difference was statistically significant (paired $t = 5.34$, $P < 0.01$). The association of the projection neurons with the septal regions of layer IV is even more compelling, because the barrels occupy more space than the septa. In the six hemispheres that we analyzed, measurements of the barrel field area in layer IV revealed that, on average, 54.1% of the total area was contained in the CO-labeled barrels (see Fig. 7). If the neurons projecting to MI were uniformly distributed throughout SI barrel cortex, then the relative number of labeled neurons within each compartmental column should be proportional to the relative size of the barrel and septal regions in layer IV. To test this hypothesis, a chi-square goodness-of-fit test was conducted on each of the six reconstructed hemispheres (Hays, 1973). In each case, the χ^2 statistic exceeded 87 (1 degree of freedom, $P < 0.001$), indicating that the observed number of labeled neurons in each compartment diverged significantly from what would be expected if the projection neurons were uniformly distributed throughout SI barrel cortex.

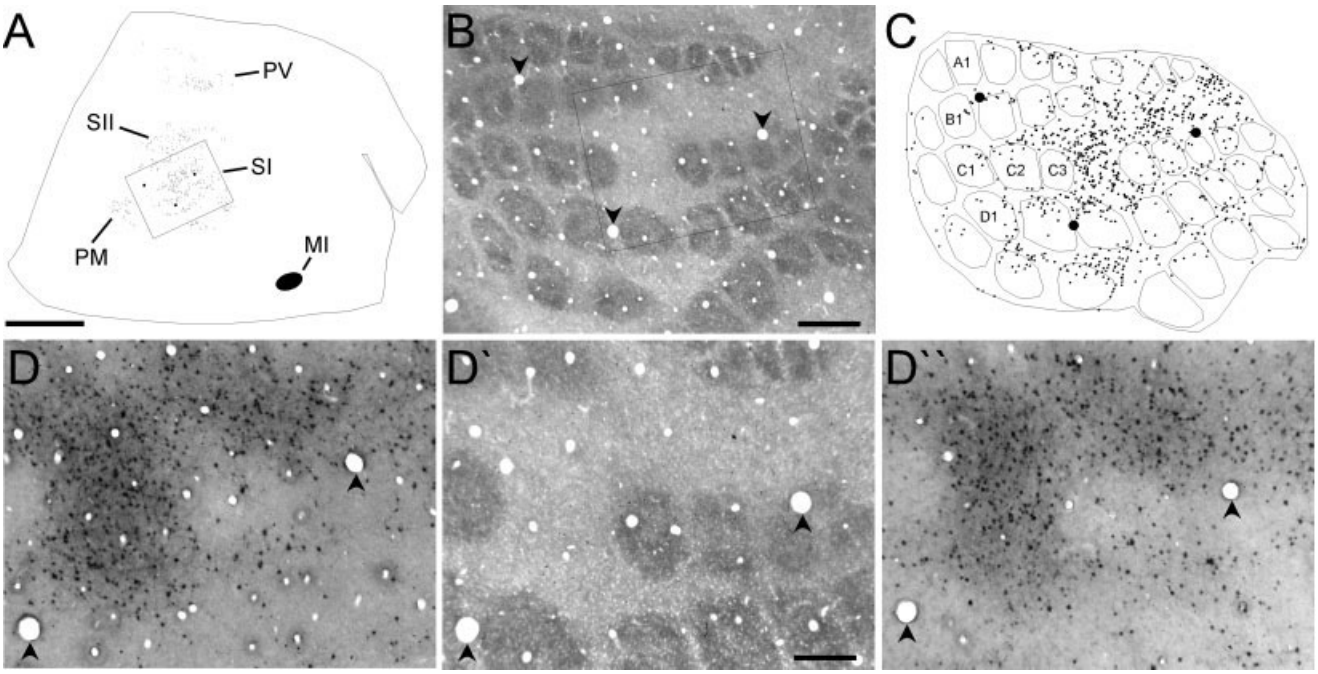

Fig. 4. Association of cholera toxin subunit B (CTb) -labeled projection neurons with the primary somatosensory cortex (SI) septal regions in case SM2. **A:** Reconstruction of the topography of CTb-labeled neurons in SI barrel cortex and surrounding cortical regions in the infragranular layers (tangential section 12). The boxed area indicates the location of the layer IV region that appears in B. **B:** Photomicrograph of cytochrome oxidase (CO) -labeled barrels in layer IV (tangential section 8). Arrowheads indicate blood vessels represented in subsequent panels; the boxed area indicates the region represented in D, D′, and D″. **C:** Plotted location of CTb-labeled neurons shown with respect to outlines of the CO-labeled barrels. For this panel, reconstructions of supragranular section 5 and infragranular section 12 were aligned and superimposed. Specific barrels are indicated by letters and numbers (i.e., A1, B1, C1, C2, C3, and D1). **D,D′,D″:** Photomicrographs from tangential sections 5, 8, and 12, respectively, that depict dense clusters of CTb-labeled neurons that are associated with the layer IV septal region. Arrowheads indicate blood vessels common to all panels. Scale bars = 3 mm in A; 500 μm in B (applies to B,C); 250 μm in D′ (applies to D,D′,D″).

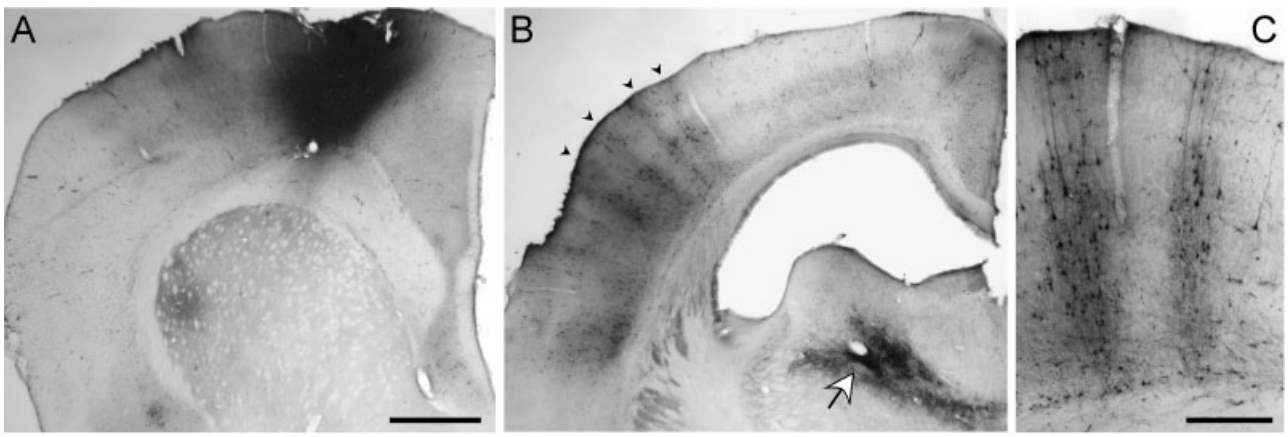

Fig. 5. Multiple columns of cholera toxin subunit B (CTb) labeling in primary somatosensory cortex (SI) barrel cortex (case SM3L). **A:** Injection site in primary motor cortex (MI) located 1.9 mm rostral to bregma. **B:** Coronal section through SI shows multiple columns of CTb labeling in the barrel field (arrowheads). Labeling in the nucleus posterior medial nucleus of the thalamus is indicated by an arrow. **C:** Magnified view of a pair of CTb-labeled columns showing the laminar position of individual CTb-labeled neurons. This section was obtained 360 μm caudal to that in B. Scale bars = 1 mm in A (applies to A,B); 250 μm in C.

Consistent with these findings, the mean density of labeled neurons in the septal columns (37.5 neurons/mm^2) was more than twice the value measured for the barrel columns (17.1 neurons/mm^2). As shown by Figure 8, a matched-sample analysis indicated that the density of labeled neurons was significantly greater in the regions aligned with the septa than in regions aligned with the barrels (paired $t = 4.96$, $P < 0.01$). Mean density measure-

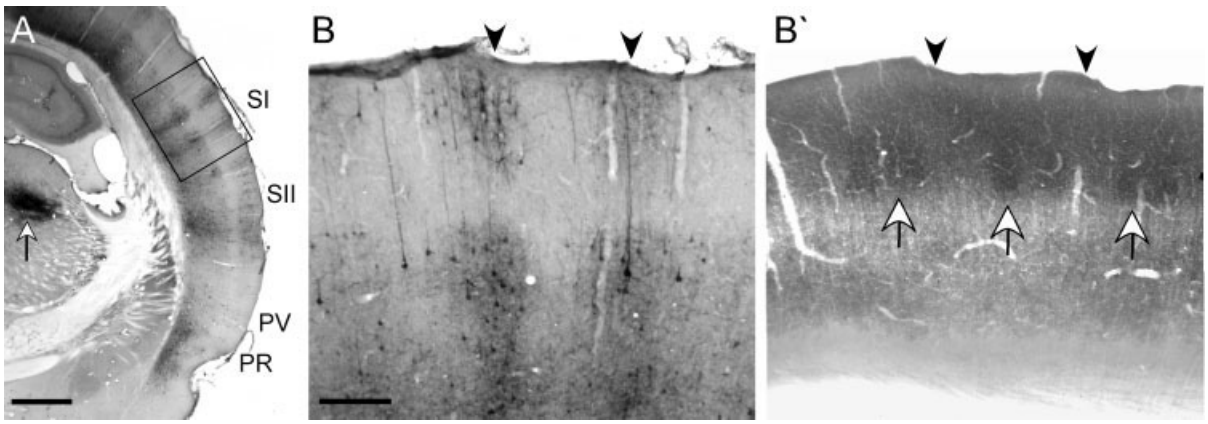

Fig. 6. Alternation of cholera toxin subunit B (CTb) labeling and cytochrome oxidase (CO) -labeled barrels in case SM13. **A:** Coronal section showing vertical columns of CTb-labeled neurons in primary somatosensory cortex (SI) and additional populations of CTb-labeled neurons in cortical areas SII, parietal ventral (PV) and perirhinal (PR) somatosensory cortical regions, and in the posterior medial nucleus (arrow). The boxed area indicates the region represented in B and B′. **B,B′:** Magnified view of vertical columns of CTb-labeled neurons and CO-labeled barrels in SI barrel cortex. Arrowheads indicate the same surface variations aligned with the columns of CTb-labeled neurons; arrows indicate CO-labeled barrels. Scale bars = 1 mm in A; 250μm in B (applies to B,B′).

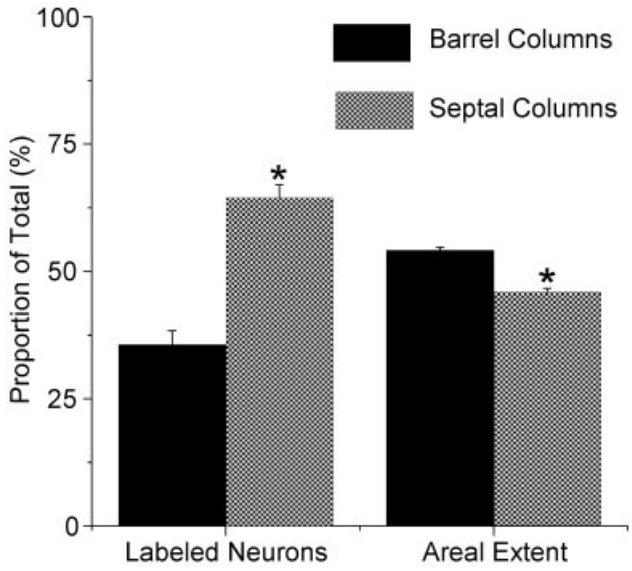

Fig. 7. Statistical comparisons of the retrograde labeling patterns and areal extent of the barrel and septal regions. The left bar indicates the mean proportion of the total number of labeled neurons in the extragranular layers of primary somatosensory barrel cortex that were aligned with either the barrel or septal regions of layer IV. The right bar indicates the mean proportion of the total area of the barrel field that was devoted to the barrel or septal compartments. Asterisks indicate significant differences between barrel and septal regions as determined by a paired t test, $P < 0.01$; error bars indicate SEM.

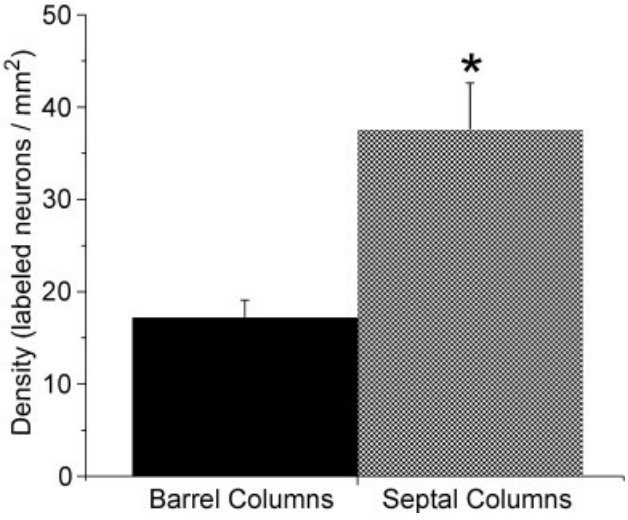

Fig. 8. Comparison of labeling density in the barrel and septal columns. Each bar indicates the mean density of labeled neurons across the summed area of the barrel and septal columns of each plotted section. The asterisk indicates significant differences between barrel and septal columns as determined by a paired t test, $P < 0.01$; error bars indicate SEM.

ments can be misleading, however, because this value also includes sparsely labeled septal regions that did not project to the MI injection site. When we focused our analysis on the densely labeled septal regions depicted in Figure 4D and D″ (i.e., the gap in the C barrel row), we found that the maximum density of labeled neurons in tangential sections through this region was 300–350 neurons/mm^2.

DISCUSSION

Placing relatively large deposits of a retrograde tracer into the MI whisker representation produced extensive labeling among SI neurons that are aligned with the septal regions of SI barrel cortex. In tangential sections through SI cortex, we observed dense strips of labeled neurons that matched the orientation, width, and other spatial variations of the septal regions in layer IV. In coronal sections, we observed dense, vertical columns of labeled neurons that alternated with the CO-labeled barrels of layer IV. Our quantitative analysis showed that nearly two thirds of the SI neurons that project to MI were

located in these septal columns. Among the labeled neurons observed within the boundaries of a barrel column, the vast majority of these were located near the imaginary border separating the septal and barrel columns. Collectively, these results indicate that columns of septal neurons are functionally linked by virtue of their shared projections to MI cortex.

Interpretative considerations

Our results demonstrate that SI projections to the MI whisker representation originate mainly from columns of neurons that are aligned with the layer IV septal regions, but this characterization of multiple septal columns might not be equivalent to the characteristics that define multiple barrel columns. Although a barrel column is defined by a circumscribed region that processes inputs from a single "principal" whisker, the parameters that define a septal column remain unclear. One difficulty with defining a functional septal column concerns its spatial extent and topographic relationship with other cortical areas. Most evidence for columnar organization suggests that functional columns are approximately 300–600 microns in diameter (Mountcastle, 1997), yet our results consist of multiple row-like strips of retrogradely labeled neurons that extend for 2 or 3 mm. This extensive labeling was probably due to the large tracer injections that we made in MI. Smaller, more discrete injections that involve a single whisker representation in MI might label fewer SI neurons that extend across a small part of the barrel field. Alternatively, it is conceivable that multiple septal columns converge onto focal sites in MI cortex, and row-like strips of retrogradely labeled neurons would still be observed in SI after placing a tracer into a single column of MI cortex. Even though a one-to-one topographic relationship might not exist between functional columns in SI and MI, substantial evidence indicates that primary sensory areas in the neocortex consist of functional modules that have a columnar organization (Mountcastle, 1997). Although additional empirical studies may cause this conceptual view to be modified, the available evidence suggests that it is parsimonious to consider our row-like strips of retrogradely labeled neurons as representing multiple septal columns in which each column is a functional entity for processing a limited amount of sensory information.

Functional significance of septal columns

Several studies indicate that the whisker representations of SI and MI are interconnected, but the specificity of these connections with columns of septal neurons was not reported previously (White and DeAmicis, 1977; Akers and Killackey, 1978; Porter and White, 1983). Tracer injections in rat MI cortex produce dense labeling in all layers of the dysgranular zone (Donoghue and Parham, 1983), but this region is usually viewed as a distinct and separate subfield of rodent SI cortex (Chapin et al., 1987). Within SI barrel cortex, retrograde-tracing studies indicated the presence of clusters of labeled neurons in the infragranular layers (Donoghue and Parham, 1983) or in both the supragranular and infragranular layers (Porter and White, 1983; Miyashita et al., 1994; Izraeli and Porter, 1995). Whereas some investigators noted that these clusters resembled cortical columns (Miyashita et al., 1994), their spatial positions were not characterized with respect to the histochemically defined compartments of the layer IV barrel field.

The association between septal columns and projections to the MI whisker representation is similar to that reported for other sets of projections from SI barrel cortex. In an early study of projections from rat SI cortex, lesions of the SI dysgranular (or "agranular") zone produced degeneration of callosal fibers that terminated in the dysgranular zone of the contralateral hemisphere (Akers and Killackey, 1978). Subsequent experiments with retrograde tracers revealed that callosal projections originate both from the dysgranular zones and from multiple layers of neurons that are aligned with the layer IV septal regions (Olavarria et al., 1984). Similarly, placing retrograde tracers in the pons, superior colliculus, pyramidal tract, MI, or contralateral SI caused dense labeling in layer V sites located below the walls and intervening septa of the SI barrel field (Crandall et al., 1986). In this latter study, labeling patterns were not described for the remaining cortical layers, possibly because many of these projection systems originate exclusively from layer V.

That corticopontine, corticotectal, and callosal neurons are aligned with the septal regions indicates that septal columns contain heterogeneous populations of neurons that project to multiple cortical areas. Despite this functional heterogeneity, it is apparent that these different neuronal populations have functional similarities of a broader nature. Accordingly, septal columns consist of neuronal populations that transmit information to brain regions that are not involved in the discriminative aspects of somatosensory processing. Thus, septal neurons respond to multiple whiskers, show much smaller responses to the principal whisker than barrel column neurons, and do not exhibit the selective directional responses that characterize barrel neurons (Armstrong-James and Fox, 1987; Bruno et al., 2003). Instead, layer IV septal regions are aligned with functional sets of neurons that project to brain regions that use certain aspects of somesthesis for the purpose of guiding or modulating motor behavior. This view is consistent with recent suggestions that the septal regions should be reclassified as a high-order cortical area rather than being considered as primary sensory cortex (Killackey and Sherman, 2003).

Hypothetical mechanisms of septal columns

Converging pieces of anatomical evidence indicate that septal columns represent functional circuits that are partially segregated from the barrel columns. Thalamic inputs to the septa originate mainly from the nucleus POm and, to a lesser extent, from a small ventral region in the nucleus VPM (Koralek et al., 1988; Lu and Lin, 1993; Pierret et al., 2000). By comparison, the layer IV barrels receive inputs exclusively from the nucleus VPM. Analysis of intracortical projections within SI barrel cortex indicates that septal neurons and the extragranular neurons located above or below the septal regions are densely interconnected with each other but not with the neurons of the barrel columns (Chapin et al., 1987; Hoeflinger et al., 1995; Kim and Ebner, 1999). Moreover, in contrast to the short-ranged projections between neighboring barrel columns, septal columns have intracortical projections that extend over longer distances before terminating in other septal columns (Kim and Ebner, 1999). Finally, our results reinforce the concept of a partially segregated septal circuit by demonstrating that SI projections to the MI

whisker representation are largely limited to vertical columns aligned with the layer IV septal regions.

Recent findings from our laboratory indicate that projections from SI barrel cortex to MI have an anisotropic organization. Using a dual anterograde tracing paradigm, we showed that neighboring regions in the same whisker barrel row send more overlapping projections to MI cortex than neighboring parts of barrel cortex that represent different whisker barrel rows (Hoffer et al., 2003). The present results suggest that the MI labeling observed in our previous study must represent projections from columns of septal neurons. Thus, the overlapping labeled terminals that we previously observed in MI were probably due to injecting tracers into neighboring septal columns in the same row-like strip of cortex. This finding is significant because tracer injections in the septa produce dense walls of transported labeling in the septa that extend much further along the barrel rows than along the barrel arcs (Chapin et al., 1987; Hoeflinger et al., 1995; Kim and Ebner, 1999). These long row-like septal projections appear to provide an anatomical substrate for linking multiple columns of septal neurons. We hypothesize that this and other intrinsic features of the septal circuitry increase the probability that multiple columns of septal neurons are synchronously active during whisking behavior. In conjunction with the anisotropic pattern of overlapping projections to MI (Hoffer et al., 2003), synchronous activation of multiple columns of septal neurons in the same row-like strip should facilitate the activation of their common targets in brain regions such as MI cortex.

Although this hypothesis remains to be tested, it is consistent with physiologic data. Neurons in the nucleus POm, which provides thalamic inputs to the septal regions, display small amplitude responses to vibrissal stimulation (Diamond et al., 1992; Sosnik et al., 2001). The latency of these responses vary according to the frequency of whisker stimulation, prompting the view that POm provides feedback to the cortical whisker representations about the rate of whisker movements (Ahissar et al., 2001; Sosnik et al., 2001). Similarly, MI neurons exhibit small amplitude sinusoidal responses that reflect the fundamental frequency of vibrissal stimulation (Kleinfeld et al., 2002). These facts suggest that sensory modulation of MI neural activity during whisking behavior is mediated largely by projections from the SI septal column circuitry.

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