

Cortical, Callosal, and Thalamic Connections From Primary Somatosensory Cortex in the Naked Mole-Rat (*Heterocephalus glaber*), With Special Emphasis on the Connectivity of the Incisor Representation

ERIN C. HENRY¹ AND KENNETH C. CATANIA^{2*}

¹Neuroscience Graduate Program, Vanderbilt University, Nashville, Tennessee

²Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee

ABSTRACT

We investigated the distribution of cortical, callosal, and thalamic connections from the primary somatosensory area (S1) in naked mole-rats, concentrating on lower incisor and forelimb representations. A neuronal tracer (WGA-HRP) was injected into the center of each respective representation under guidance from microelectrode recordings of neuronal activity. The locations of cells and terminals were determined by aligning plots of labeled cells with flattened cortical sections reacted for cytochrome oxidase. The S1 lower incisor area was found to have locally confined intrahemispheric connections and longer connections to a small cluster of cells in the presumptive secondary somatosensory (S2) and parietal ventral (PV) incisor fields. The S1 incisor area also had sparse connections with anterior cortex, in presumptive primary motor cortex. Homotopic callosal projections were identified between the S1 lower incisor areas in each hemisphere. Thalamocortical connections related to the incisor were confined to ventromedial portions of the ventral posterior medial subnucleus (VPM) and posterior medial nucleus (Po). Injections into the S1 forelimb area revealed reciprocal intrahemispheric connections to S2 and PV, to two areas in frontal cortex, and to two areas posterior to S1 that appear homologous to posterior lateral area and posterior medial area in rats. The S1 forelimb representation also had callosal projections to the contralateral S1 limb area and to contralateral S2 and PV. Thalamic distribution of label from forelimb injections included ventral portions of the ventral posterior lateral subnucleus (VPL), dorsolateral Po, the ventral lateral nucleus, and the ventral medial nucleus and neighboring intralaminar nuclei. Anat Rec Part A, 288A:626–645, 2006. © 2006 Wiley-Liss, Inc.

Key words: neocortex; teeth; S1; S2; VPM

Abbreviations *Cortical Areas*: AGI, agranular lateral field; AGm, agranular medial field; M1, primary motor area; PFA, prefrontal area; PL, posterior lateral area; PM, posterior medial area; PV, parietal ventral area; S1, primary somatosensory area; S2, secondary somatosensory area *Body Representations*: BP, buccal pad; Ch, chin; FL, forelimb; HL, hindlimb; LIM1, lower incisor module one; LIM2, lower incisor module two; TM, tongue module; UIM, upper incisor module; Vib, vibrissae. *Thalamic subdivisions*: CL, central lateral thalamic nucleus; cp, cerebral peduncle; fr, fasciculus retroflexus; ic, internal capsule; ns, nigrostriatal bundle; ME, median eminence; MGP, medial globus pallidus; ml, medial lemniscus; MM, medial mammillary nucleus; mt, mamillothalamic tract; PF, parafascicular thalamic nucleus; Po, posterior medial nucleus; Rt, reticular thalamic nucleus; SNR, substantia nigra, reticular; STh, subthalamic nu-

cleus; VL, ventral lateral nucleus; VM, ventral medial nucleus; VP, ventral posterior complex; VPL, ventral posterior lateral subnucleus; VPM, ventral posterior medial subnucleus.

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*Correspondence to: Kenneth C. Catania, Department of Biological Sciences, Vanderbilt University, VU Station B, 351634, Nashville, TN 37235. Fax: 615-343-6707. E-mail: ken.catania@vanderbilt.edu

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Naked mole-rats are subterranean rodents from eastern Africa that rely on their sense of touch to navigate underground tunnels. Although they have reduced eyes and ears, their bodies are covered with sensitive body vibrissae and their unusually large incisors provide important tactile cues as they dig tunnels and manipulate objects (Lacey et al., 1991; Catania and Remple, 2002; Crish et al., 2003). This reliance on touch is reflected in the organization of their neocortex. Mole-rats have a disproportionately large somatosensory area that extends caudally into cortical regions that normally subserve vision (Catania and Remple, 2002). But the most surprising and seemingly unique feature of their somatosensory cortex is the greatly magnified representation of the incisors. Over 30% of primary somatosensory cortex (S1) is devoted to the representation of the two front teeth (Catania and Remple, 2002). This can be contrasted with the much smaller proportion of somatosensory cortex (7%) devoted to the incisors in closely related laboratory rats (data from Remple et al., 2003).

More recent investigations of mole-rat somatosensory cortex have revealed a number of cytochrome oxidase (CO)-dense cell aggregates in layer IV that correspond to the representations of body parts including the vibrissae, chin, forelimb, tongue, and teeth (Henry et al., 2006). In addition, microelectrode recordings from lateral parietal cortex have provided evidence for the secondary somatosensory (S2) and parietal ventral (PV) areas, as described in other species (Henry et al., 2006). In these respects, mole-rats appear to share common features of brain organization with laboratory rats. However, the unusually large and medially located dental representations in mole-rats remain unique among mammals and provide a favorable system for investigating how the teeth are represented in the CNS.

In this investigation, we explore the connectivity of the primary somatosensory cortex, concentrating on the cortical representations of the dentition and forelimb. Our primary interest was to determine how the dental areas and corresponding cortical modules are organized and how these areas compare to the representations of the body. The central representation of the dentition has been largely overlooked in mammals. This is probably due to the difficulty of accessing these lateral areas in most species, along with the small size of the representations. However, there are a number of questions that may be addressed by examining the connections of these regions. For example, it has been hypothesized that callosal projections between the cortical representations of midline body parts may help to unify sensory perceptions across the hemispheres (Manzoni et al., 1980; Gould and Kaas, 1981; Innocenti, 1986; Hayama and Ogawa, 1997). Because mole-rat incisors are behaviorally important midline structures, their representation may require connections between the two hemispheres to coordinate sensory inputs, and this in turn may be true of dental representations in mammals generally.

In addition to interhemispheric connectivity, we were also interested in the connections between S1 and the presumptive S2 and PV areas in lateral cortex. Cortical areas are best defined by a number of criteria (Kaas, 1983) and by comparing electrophysiological maps of lateral cortex to the connections of this region we may more confidently describe how this region is organized. By placing injections into the S1 lower forelimb representation,

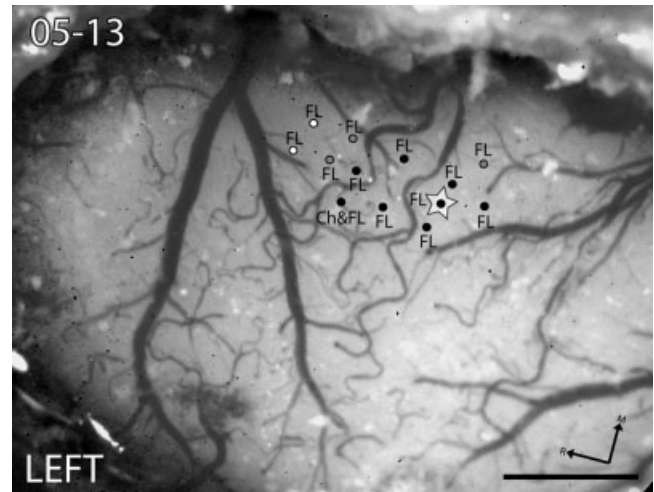


Fig. 1. Surface view of parietal cortex where microelectrode recordings were made for case 05-13 prior to injection. In this case, the injection was placed into the forelimb representation. The circles mark the microelectrode penetration sites. The shade of the circles indicates the magnitude of the elicited neuronal response (white, weak response; gray, clear response; black, robust response). The white star marks the injection site. FL, forelimb; Ch, chin. Scale bar = 1 mm.

which is separated from the secondary areas by the face representations in S1 and S2, we were able to examine the connections between S1 and more lateral somatosensory representations.

Finally, we investigated the relationship between cortex and thalamus in order to determine how this component of the dental representation is organized and how this organization compares with similar areas in other rodent species.

MATERIALS AND METHODS

Seven adult mole-rats were used to determine the connectivity of the S1 lower incisor and forelimb representations. All animal procedures followed National Institutes of Health guidelines and were performed according to the standards set by the Animal Welfare Act and the Vanderbilt University Institutional Animal Care and Use Committee. The animals were anesthetized with ketamine hydrochloride and xylazine diluted in distilled water at a dosage of 15–20 mg/kg and 0.6–1.0 mg/kg, respectively. Body temperature was maintained with a heating pad and hot water bottles. A head post was attached to the right side of the skull using dental cement and a craniotomy was performed to expose the left cortex. The cortex was covered with saline and a digital photograph of the cortical surface was taken to mark penetration sites during the recording session.

Multiunit recordings were carried out to locate the approximate boundary of the S1 lower incisor or forelimb areas (Fig. 1). Recording electrodes were low-impedance tungsten (1.0 M Ω at 1,000 Hz), which were lowered into the cortex to depths ranging from approximately 400 to 500 μ m. Soft probes were used to identify the receptive fields for each penetration. The boundary of the receptive field was defined as the skin surface or enamel surface (in the case of the incisors) where light contact produced a

detectable increase in neural activity as visualized with an oscilloscope combined with auditory monitoring through a speaker. Once a map of the area was determined, a Hamilton syringe mounted to a stereotaxic unit was used to inject 0.05–0.1 μ l of 1–2% wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) into the center of the targeted representation. The syringe was lowered into the cortex to a depth of approximately 400–600 μ m and was left in the brain for 10–15 min to allow time for uptake. The brain was then washed three times with saline and covered with a cap made from dental adhesive. The cap was reinforced with fluid dental adhesive to secure it to the skull. The skin was sutured using 5-0 Ethilon nylon suture thread (Somerville, NJ) and the animal was allowed to recover from anesthesia. After 48 hrs, the animal was given an overdose of sodium pentobarbital (100 mg/kg) and perfused with 0.9% saline followed by 2–4% paraformaldehyde. The brain was removed and the cortex was separated from thalamus and flattened between glass slides. The cortices and thalamic tissue were immersed in 30% sucrose in phosphate buffer for 12 hrs. Sections were cut parallel to the flattened cortical surface on a freezing microtome at 50 μ m. The thalamic sections were cut coronally on the freezing microtome at 40–50 μ m. Cortical and thalamic sections were alternatively processed for the metabolic enzyme cytochrome oxidase (Wong-Riley, 1979) or processed for HRP label with tetramethylbenzidine (TMB) using the protocol developed by Gibson et al. (1984). Selected thalamic sections were also processed for acetylcholinesterase (AChE) (Stepniewska and Kaas, 1997), allowing for a more detailed analysis of architectonic boundaries.

Data Analysis

Analysis was conducted by digitally plotting HRP-labeled cells and terminals using Igor Pro (WaveMetrics, Lake Oswego, Canada). The resulting EPS files were imported into Adobe Illustrator 10 (Adobe, San Jose, CA) from Igor Pro and aligned with images from the adjacent sections processed for cytochrome oxidase. The images were captured with a Spot 2 camera (Diagnostic Instruments, Sterling Heights, MI) mounted on a Nikon E800 microscope (Nikon, Melville, NY) using the ACT-1 image acquisition program (Nikon). Images were adjusted for brightness and contrast in Adobe Photoshop CS. Alignment of the sections processed for TMB with sections processed for cytochrome oxidase was achieved using injection sites, blood vessel patterns, and section contours as landmarks (analysis methods are a modification of procedures from Lyon and Kaas, 2001).

RESULTS

The first series of injections was placed into the cortical lower incisor representation to reveal the connectivity of S1 dentition area (Figs. 2–6). We selected the lower incisor representation as our target because it was located in a rostral and medial position that separated it from the presumptive S2 and PV areas, thus facilitating differentiation of the S1 and S2/PV incisor territories. The second group of injections was placed into the cortical forelimb representation, providing a reference for the connective pattern of the body representation and allowing for comparison to the tooth area (Figs. 7–10).

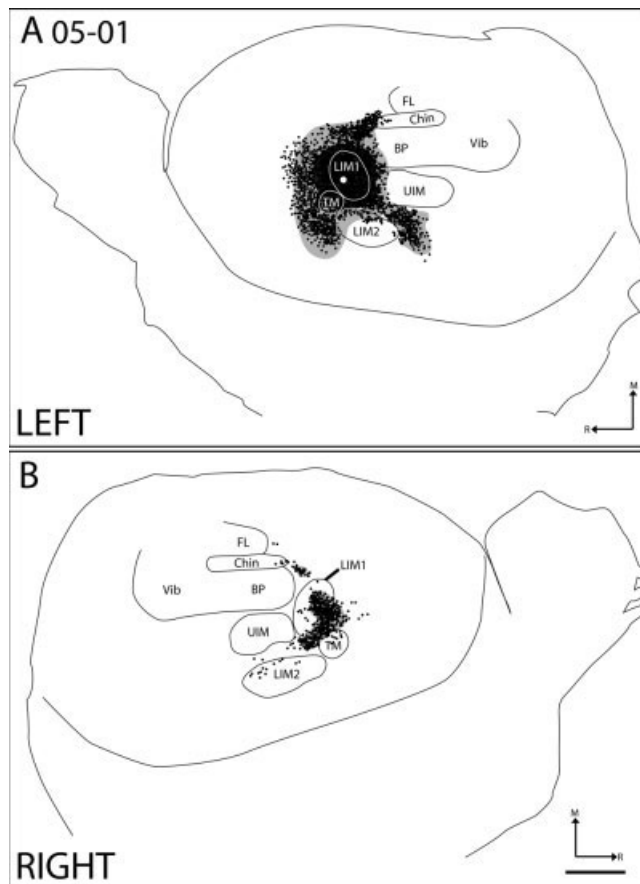


Fig. 2. Connections of the S1 lower incisor representation. **A:** Intra-hemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. The white circle marks the injection site. **B:** Callosal connections in the contralateral hemisphere at the level of layer III/IV. LIM1, lower incisor module one; LIM2, lower incisor module two; TM, tongue module; UIM, upper incisor module; BP, buccal pad; Vib, vibrissae. Scale bar = 1 mm.

Intrahemispheric Cortical Connections

All of the injections placed in S1 were guided by electrophysiological recordings of neuronal activity. To determine the boundary of our selected area, we recorded neuronal responses to gentle peripheral stimulation from approximately 10–20 penetration sites in either the S1 lower incisor or forelimb representations (Fig. 1). Injections were made into the center of physiologically defined areas such that injection sites were surrounded by neurons responding to stimulation from the peripheral body structure of interest (either the lower tooth or forepaw). The connectivity of these regions was revealed by flattening cortex and cutting sections parallel to the surface so that the distribution of label could be overlaid with adjacent sections of tissue reacted for cytochrome oxidase revealing the S1 body and facial regions.

Intrahemispheric Connections of S1 Lower Incisor Representation

Injections made in the lower incisor representation produced round dense injection cores of intense and uniform

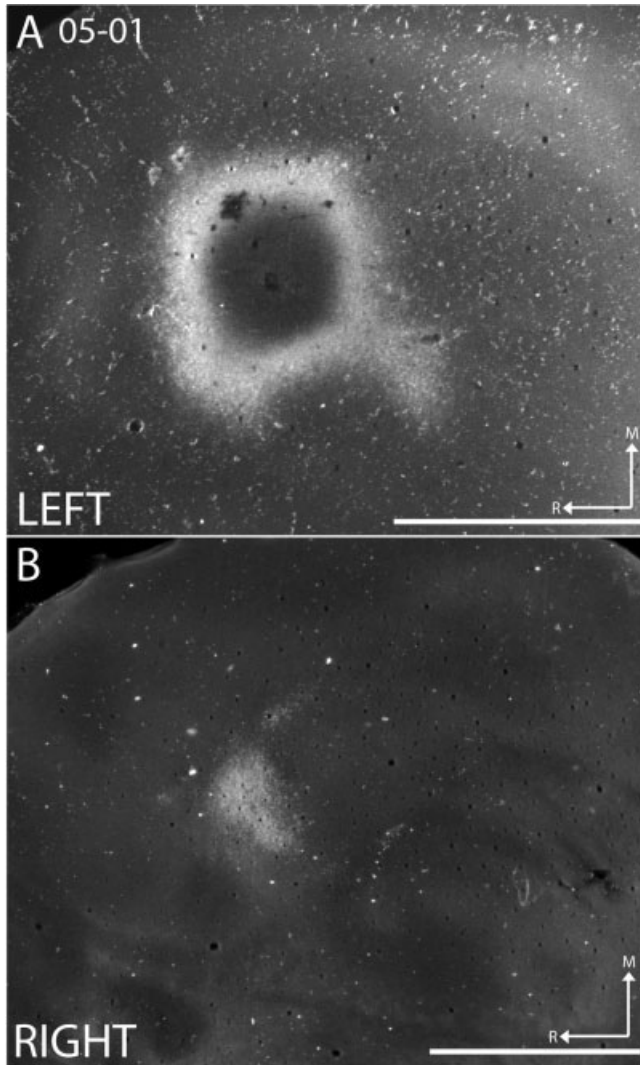


Fig. 3. Dark-field photomicrographs of cortical sections processed for TMB. **A:** Labeled cells and terminals after an injection into the S1 lower incisor representation. **B:** Callosal connections are distributed in an arced pattern within the incisor representation. Scale bar = 2 mm.

label surrounding the site of entry (with approximate diameters ranging from 0.5 to 1.25 mm). This injection core was considered the region of effective tracer uptake (Mesulam, 1982). The label in the injection core was spread throughout the cortical layers, although the deep cortical layers (approximately layers V/VI) had fewer labeled cells confined to areas closely associated with the injection site. The reconstructions in the adjoining figures were derived from the section of cortical tissue reacted for TMB immediately adjacent to the section with the most visible CO-dense cell aggregates in S1 (approximately layer IV).

Surrounding the injection site, local neuronal labeling was reciprocal, containing both cells and terminals. Case 05-01 shows the extent of labeling for an injection made into the center of the S1 lower incisor representation, which aligned with the center of the lower incisor module one (LIM1), the more medial of two CO-dense modules within the S1 lower incisor area (Figs. 2 and 3). Labeled

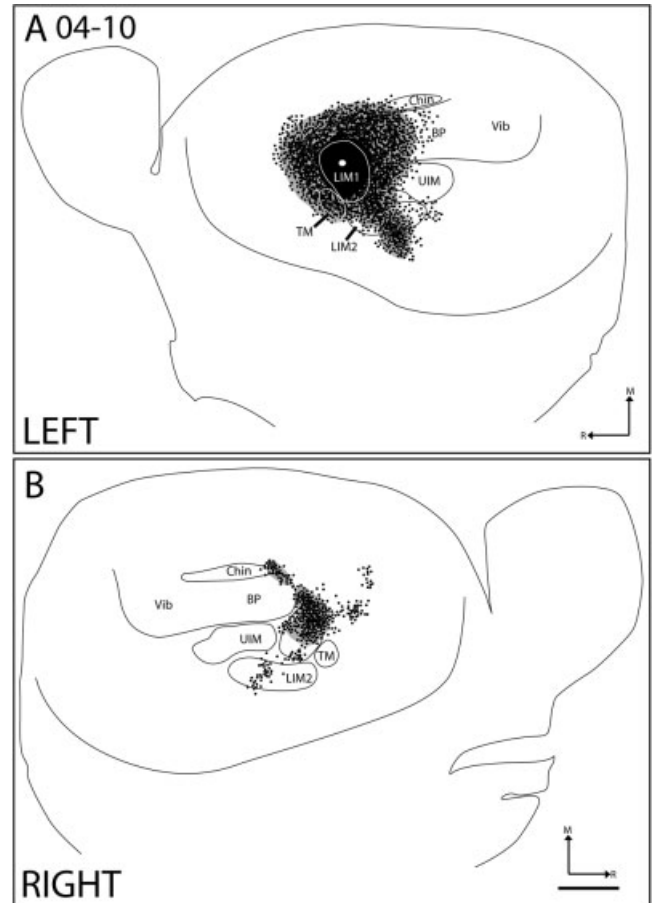


Fig. 4. Connections of the S1 lower incisor representation. **A:** Intra-hemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. The white circle marks the injection site. **B:** Callosal connections in the contralateral hemisphere at the level of layer III/IV. Scale bar = 1 mm.

cells were distributed throughout the medial portion of the lower incisor representation tapering to a narrow cluster of cells that flanked the rostral-most aspects of the buccal pad and chin representations. Reciprocal projections between the lower incisor representation and the rostral portions of the buccal pad and chin were also found, but the degree of label within the areas was variable.

Injections into the S1 lower incisor representation consistently labeled the entire ipsilateral S1 tongue representation. The label was reciprocal and dense. The location of the tongue area can be identified by histological means as a roughly circular CO-dense module (TM) in the rostralateral-most portion of S1, adjacent to the rostral border of the lower incisor representation (Henry et. al, 2006). In all the incisor injection cases, WGA-HRP label filled this module and extended beyond its rostral border into neighboring frontal cortex (for example, see Fig. 5).

Labeling in the lower incisor module two (LIM2), the more lateral of the two incisor modules, was inconsistent across the cases and often depended on the spread of the injection. In case 05-01, the majority of the LIM2 module was devoid of label, with only a sparse distribution of

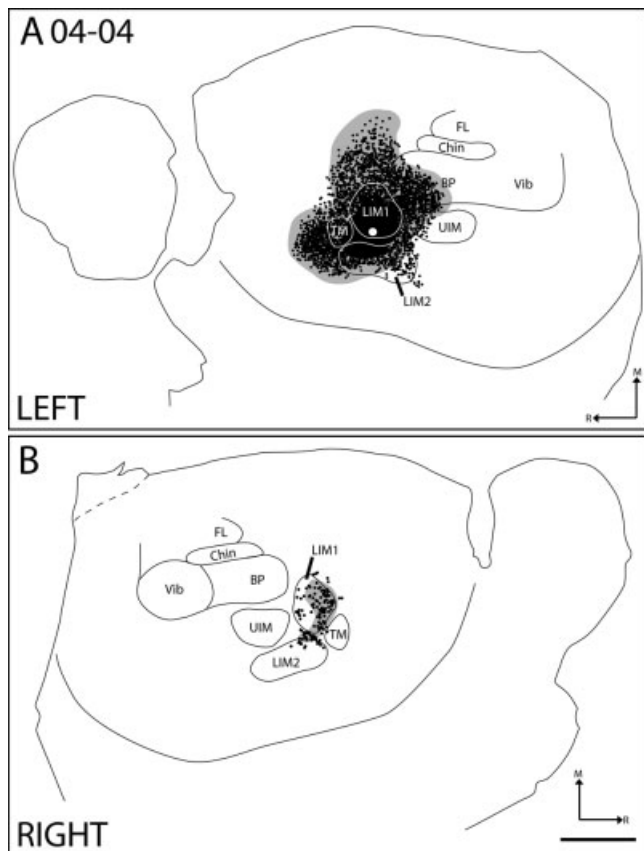


Fig. 5. Connections of the S1 lower incisor representation. **A:** Intra-hemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. The white circle marks the injection site. **B:** Callosal connections in the contralateral hemisphere at the level of layer III/IV. Scale bar = 1 mm.

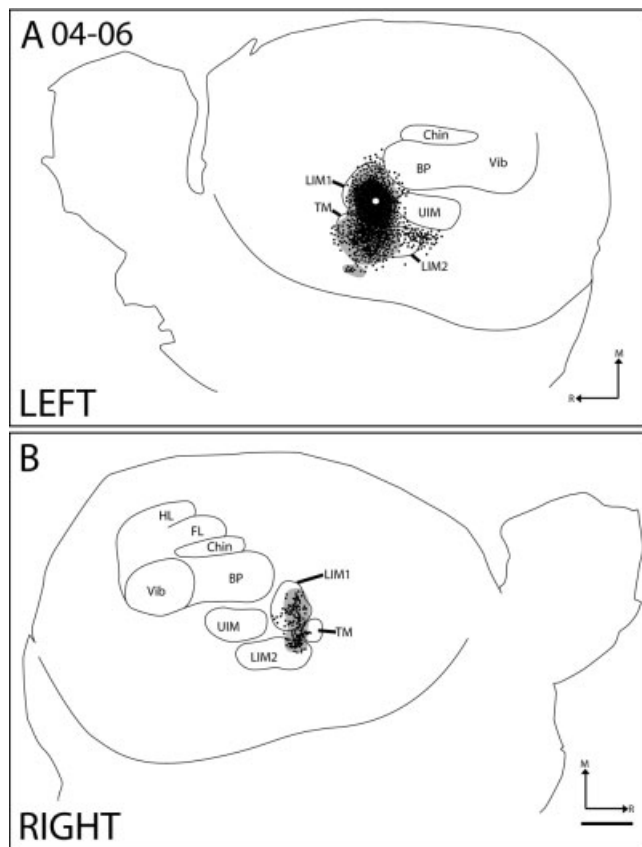


Fig. 6. Connections of the S1 lower incisor representation. **A:** Intra-hemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. The white circle marks the injection site. **B:** Callosal connections in the contralateral hemisphere at the level of layer III/IV. Scale bar = 1 mm.

labeled cells and terminals in the medial-most part of the area (Fig. 2). In case 04-10, the center of the LIM2 module received reciprocal label from the injection in LIM1. However, the rostral and caudal portions of the module contained only a few labeled cells (Fig. 4). In cases 04-04 and 04-06, injections were placed further lateral, close to the border between the LIM1 and LIM2 modules (Figs. 5 and 6). In these cases, the LIM2 module did receive some label. However, the pattern of label was inconsistent and patches of sparse or absent label within the module were consistently observed. For example, in case 04-04, the label was confined to the medial portion of the module with the lateral-most portion largely spared, except for a sparse collection of terminals. In case 04-06, the label is mostly confined to the rostral portion of the module, much of which was likely due to the proximity to the injection site.

The upper incisor module (UIM), located directly caudal to the LIM1 module, received only sparse projections in the rostral-most portion of the area; the remainder of the module was consistently devoid of label (Figs. 2–6). The degree of label in the upper incisor area depended largely on the size and spread of the injection. The reconstructed section from case 04-06 had less than 10 labeled cells in

UIM (Fig. 6), while larger injections placed in cases 04-10 and 04-04 had a greater spread of label into the upper incisor territory (Figs. 4 and 5). This difference in labeling could be accounted for by diffusion of the tracer beyond the lower incisor area in the cases with larger injections.

In addition to the label in S1, all of the cases showed some dispersion of label in caudolateral cortex, behind and between the LIM2 and UIM modules. For example, case 05-01 shows label in the perigranular septal area between the two modules with label continuing caudal in an arc around the caudal boundary of the LIM2 module (Fig. 2). Case 04-06 shows a similar pattern of label (Fig. 6). In addition to label in the septal regions, cases 04-10 and 04-04 also had label through the center of the LIM2 module and in caudolateral cortex (Figs. 4 and 5). This label appears to be in area of cortex identified functionally as the S2/PV mixed incisor area (Henry et al., 2006).

Label from the S1 lower incisor area injections was also found rostral to the S1 orofacial territory. The pattern of label in frontal cortex formed an arc along the rostral border of the lower incisor, tongue, buccal pad, and chin representations. Three of the cases (05-01, 04-10, and 04-04) had dense label in this area, most of which was reciprocal (Figs. 2–5). The greatest density of label rostral

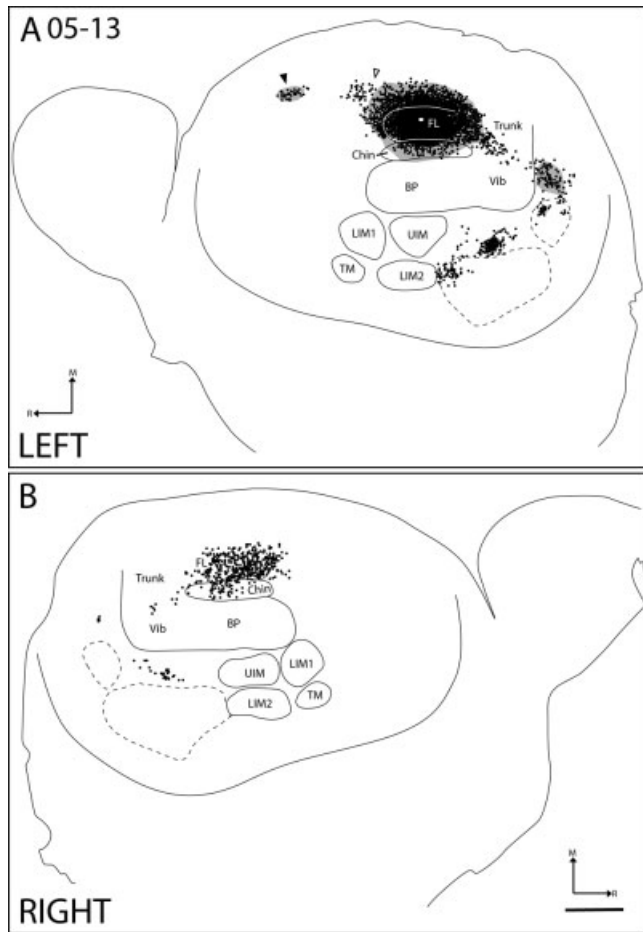


Fig. 7. Connections of the S1 forelimb representation. **A:** Intrahemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. Note the label rostrally adjacent to S1 (open arrow) and the further anterior patch of label in frontal cortex (black arrow) as well as the multiple foci of label in caudolateral parietal cortex. The white circle marks the injection site. **B:** Callosal connections in contralateral cortex at the level of layer III/IV. Dotted lines outline CO-dense territories with unknown physiological properties. Scale bar = 1 mm.

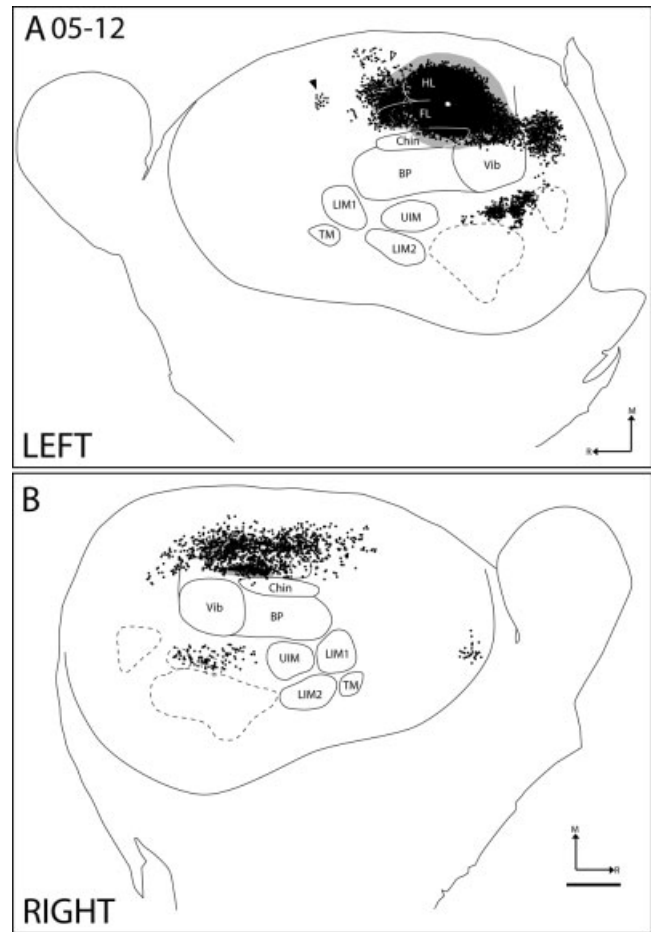


Fig. 8. Connections of the S1 forelimb representation. **A:** Intrahemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. Note the label rostrally adjacent to S1 (open arrow) and the further anterior patch of label in frontal cortex (black arrow) as well as the multiple foci of label in caudolateral parietal cortex. The white circle marks the injection site. **B:** Callosal connections in contralateral cortex at the level of layer III/IV. Dotted lines outline CO-dense territories with unknown physiological properties. Scale bar = 1 mm.

to the lower incisor representation was found in case 04-10, where it occupied approximately 0.75 mm of cortical territory anterior to the rostral border of S1 (Fig. 4). In case 04-04, two clusters of label in rostralateral cortex were located rostral to the S1 buccal pad, chin, and forelimb representations, running roughly parallel to the areas but separated by 200 μ m from the chin area and 400 μ m from the forelimb area (Fig. 5). The second anterior cluster of label was coextensive with the S1 tongue and lower incisor representations.

Intrahemispheric Connections of S1 Forelimb Representation

Injections of WGA-HRP were placed in the forelimb representation in three mole-rats to reveal connections within S1 and to other cortical areas. The injections into the S1 forelimb representation produced a densely labeled

injection core, with diameters 1 to 2 mm wide. Local label near the injection site was distributed in a radial pattern, filling the entire S1 forelimb representation, and in larger injections, also filling portions of the granular region related to the hindlimb and trunk representations.

In case 05-13, a small injection was made in the center of the S1 forelimb representation, at a cortical site where neuronal activation was elicited by peripheral stimulation of the proximal paw and wrist skin surfaces (Figs. 1 and 7). The CO-dense granular portion of the forepaw representation was completely filled with labeled cell bodies and terminals. These local reciprocal connections flanked the injection site and included projections to the medial portion of the chin representation and the lateral portion of the hindlimb area. In cases 05-12 and 05-08, larger injections resulted in label that expanded into the hindlimb and rostral trunk representations (Figs. 8 and 10). In

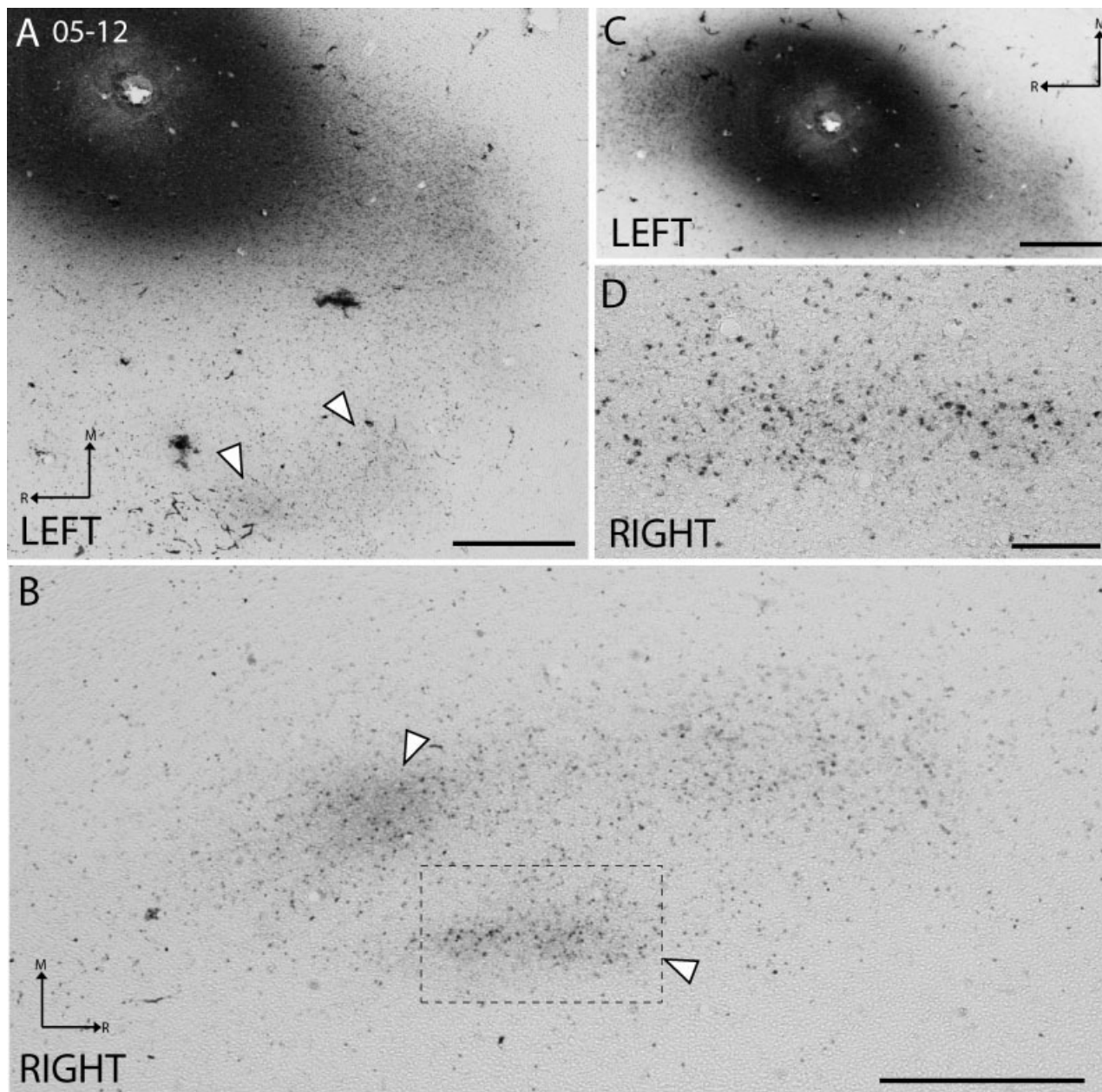


Fig. 9. Bright-field photomicrographs of label distribution from the S1 forelimb area in case 05-12. **A:** Injection site and neighboring patches of label in caudal parietal cortex. Arrows highlight label in S2/PV corresponding to the limbs. **B:** Callosal connections in the contralateral S1 forelimb representation. Patches of terminals (see arrows) are restricted

to border zones flanking the granular region of the forelimb area. **C:** Injection site, with projections to anterior and posterior cortical areas. **D:** Enlarged image of framed region in B showing the reciprocal connections within the border region separating the forelimb and chin representations. Scale bars = 0.5 mm (A–C); = 100 μ m (D).

case 05-08, the diffuse spread of the tracer filled both the forelimb and hindlimb CO-dense granular regions entirely (Fig. 10). The injection in case 05-12 was slightly smaller and alignment with CO-stained tissue showed the injection site was close to the forelimb/hindlimb border, which may explain the medial spread into the hindlimb representation (however, neurons at the injection site had responded exclusively to light peripheral stimulation of the forelimb). In all three cases, intrinsic connections existed

between the forelimb and the neighboring trunk area, with label largely located in the lateral portion of the trunk representation adjacent to the vibrissae area. This region of the trunk representation is typically responsive to stimulation from the shoulder, neck, and posterior head (Henry et al., 2006). In case 05-13, sparse label was found in this trunk region. However, relatively dense label was found in this region for cases 05-12 and 05-08 (Figs. 7, 8, and 10). These larger injections (cases 05-12 and 05-08)

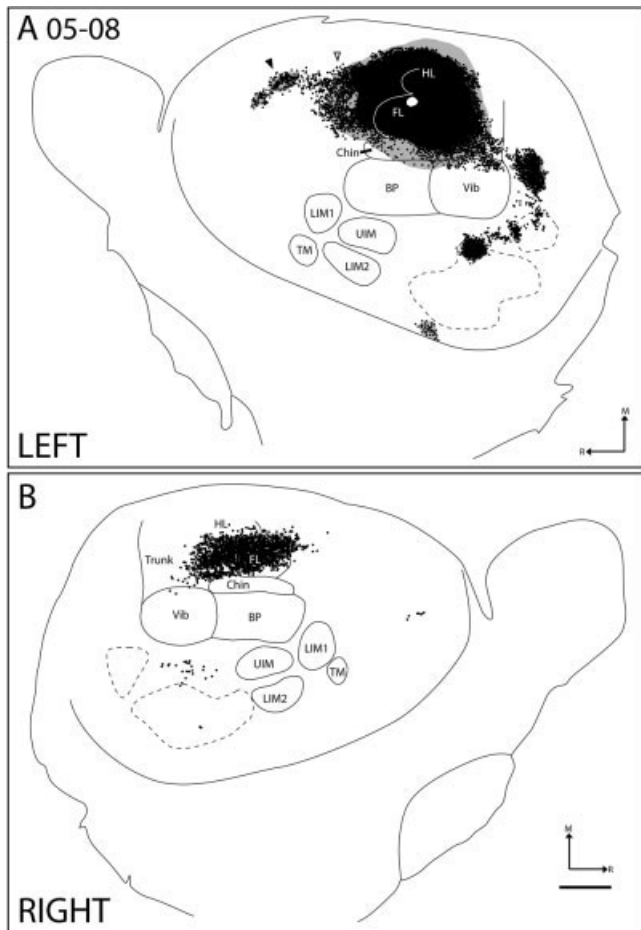


Fig. 10. Connections of the S1 forelimb representation. **A:** Intrahemispheric distribution of labeled cells (black dots) and terminals (gray shading) reconstructed from a single cortical section adjacent to the CO-dense granular aggregates of layer IV. Note the label rostrally adjacent to S1 (open arrow) and the further anterior patch of label in frontal cortex (black arrow) as well as the multiple foci of label in caudolateral parietal cortex. The white circle marks the injection site. **B:** Callosal connections in contralateral cortex at the level of layer III/IV. Dotted lines outline CO-dense territories with unknown physiological properties. Scale bar = 1 mm.

also had connections to more rostromedial areas of trunk, neighboring the hindlimb area. This area of the trunk representation is typically responsive to stimulation of the ventrum and hind flank (Henry et al., 2006).

Multiple patches of label were found outside the borders of S1. In parietal cortex caudal to S1, a dense circular concentration of label was identified behind the lateral S1 trunk and vibrissae areas. This distribution of label containing fibers and cell bodies was found in all cases in which the forelimb was injected. A particularly clear example of this was found in case 05-08, where the area of label was obviously separated from the granular border of the S1 trunk and vibrissae representations (Fig. 10). This region has not been previously identified in naked mole-rats. However, it appears to be homologous to the posterior medial (PM) area identified in rats (Koralek et al., 1990; Li et al., 1990; Fabri and Burton, 1991a) and squirrels (Krubitzer et al., 1986).

Below the presumptive PM area, a number of additional patches of label were found outside the boundary of S1. Directly lateral to PM, small labeled cell clusters coincided with a CO-dense region of cortex. The patches were mostly confined to the rostromedial portion of the CO-dense region. However, there was variation across the cases. For example, in case 05-13, three small clusters of cells were located in the medial portions of the CO-dense region (Fig. 7). In case 05-08, the cell clusters were found along the medial and rostral portions of the CO-dense patch (Fig. 10). This area has not been defined by electrophysiological means in the mole-rat, but it shares similarities in location to an area identified in rats as posterior lateral cortex (PL) (Fabri and Burton, 1991a).

Additional clusters of label were located lateral to the S1 face area in the region of the S2/PV limb and trunk representations. For instance, in case 05-12, the injection filled both S1 limb representations and parts of the trunk representation and resulted in multiple clusters of label in lateral cortex (Fig. 8). These projections in S2/PV are consistent with multiunit microelectrode maps of the area that indicated the forelimb and hindlimb are represented in this region of S2 and PV (Henry et al., 2006; for rats, see Rempel et al., 2003). In case 05-13, where the label was largely confined to the S1 forelimb area, the distribution of label was restricted to a single cluster, located in the same region as the rostral-most cluster in case 05-12 (Figs. 7 and 8).

Tracer label was also found in frontal cortex anterior to S1. However, there was variability between the three cases. All three S1 forelimb injection cases showed connections with an adjacent rostral cortical area (Figs. 7, 8, and 10). Label in this area was spread in an arced pattern around the rostral extent of the S1 limb area and extended to varying degrees in the rostromedial direction. Case 05-13 provides a good example of this labeling pattern (Fig. 7). This labeled area probably corresponds to primary motor cortex (M1), also known as the agranular lateral field (AGl), as the pattern of label is similar to that seen in rat frontal cortex after S1 limb area injections (Fabri and Burton, 1991a). This area in the mole-rat cortex is CO-sparse as compared to neighboring S1, and this is consistent with the appearance of M1 in rats. However, conclusions must remain tentative in the absence of motor stimulation studies and detailed cytoarchitectural analysis.

In all three cases, a second focus of label was identified anterior to the presumptive M1 in frontal cortex. In cases 05-13 and 05-08, this anterior area of label contained labeled cells and terminals, indicating reciprocal connections with S1 (Figs. 7 and 10). However, it should be noted that in case 05-12, only sparse cellular label was found in this area (Fig. 8). In cases 05-13 and 05-12, the pattern of label was restricted to a single site, whereas in case 05-08 (the largest injection of the three), two adjacent clusters were identifiable. This anterior label suggests the presence of a second area in frontal cortex that may be homologous to the agranular medial field (AGm) or the prefrontal area (PFA) observed in rats (Neafsey and Sievert, 1982; Fabri and Burton, 1991a).

Callosal Connections

For each of the injection cases, the contralateral hemisphere was investigated to determine the pattern of connectivity between S1 representations. In all of the cases,

transported label was found in the corresponding S1 body part representation in the opposite hemisphere, but distribution patterns within the labeled area were varied. For the injections placed into the S1 lower incisor area, the majority of transported label was found in the rostral portion of the LIM1 module. The transported label was identified in both cells and terminals. Case 05-01 provides a good example of this transport with label curved around the rostral half of LIM1 and sparing the caudal portion of the module (Fig. 2). Reciprocal connections were also found rostral to the buccal pad. Further medial, a sparse number of cells were found near the rostral border of the chin and forelimb representations. The lower incisor-responsive area (as identified by microelectrode recordings) includes these medial locations near the chin and buccal pad (Catania and Remple, 2002; Henry et al., 2006), suggesting connections seen in this area are within the incisor representation. In lateral cortex, the tongue and LIM2 modules were largely devoid of label, with only a small number of labeled cells observed in the medial-most portion of LIM2 (Fig. 2). Labeled cells were also found in the CO-sparse area separating the upper (UIM) and lower incisor (LIM2) modules.

In case 04-10, the pattern of label was similar to that in case 05-01. However, there were also labeled cells in frontal cortex, rostral to the lower incisor module, LIM1 (Fig. 3). This was the only case with this pattern so conclusions regarding this label are difficult to make. In comparison to the other two cases, cases 04-04 and 04-06 had very sparse callosal label in the contralateral hemisphere, which was restricted to the rostral half of LIM1 and the CO-sparse septal area lateral to LIM1 (Figs. 5 and 6). In case 04-06, this lateral area of label extended into the rostromedial portion of LIM2 (Fig. 6).

In cases with injections placed in the S1 forelimb representation, separate patches of label were found in the corresponding limb representations in the opposite hemisphere. Case 05-12 provides the best example of this because label was densest at the medial and lateral borders of the forelimb representation, with sparser label in the center of the representation (Figs. 8 and 9). In addition, only the border regions that flanked the CO-dense forelimb area contained labeled terminals, indicating that anterograde projections from the opposite hemisphere avoided the center of the forelimb representation (Fig. 9B and D). Label distribution in the other two cases was not as well defined, making it hard to determine differences between the amounts of label in the border and center portions of the forelimb area. In case 05-08, the majority of label appeared in the border area between the S1 forelimb and hindlimb representations. However, the injection site included portions of the border zone so this could reflect reciprocal callosal connections for this region of S1.

Callosally transported label was also found in lateral cortex of the contralateral hemisphere in the location of the S2/PV limb representations (Catania and Remple, 2002; Henry et al., 2006). Label in this area was sparse in all cases and no terminals were found (Figs. 7, 8, and 10). The greatest population of cells in lateral cortex was found in case 05-12. This region was clearly separated from S1 by a strip of CO-sparse cortex where the S2 face representation is located (Fig. 8) (Henry et al., 2006).

Sparse label was also found rostral to the S1 limb area in the opposite hemisphere with particularly extensive anterior label in case 05-12 (Fig. 8). No terminals were

identified rostral to S1, implying the connectivity is exclusively unidirectional from the contralateral presumptive M1 area to ipsilateral S1. Weak projections were also found to the contralateral S1 trunk representation in approximately the same distribution observed in the ipsilateral hemisphere.

Thalamic Anatomy in Naked Mole-Rat

In order to facilitate interpretation of the connectational results and highlight similarities and differences between the thalamic complexes of mole-rats and other rodent species, the basic anatomy of the mole-rat thalamic somatosensory nuclei is reviewed here.

In each of the cortical S1 injection cases, the thalamus was isolated from the cortical tissue after perfusion (48 hrs postinjection) and sectioned on a freezing microtome in the coronal plane. Sections were alternatively reacted for TMB, CO, and occasionally AChE to allow for identification of the major ascending somatosensory nuclei in the naked mole-rat. Cytoarchitectural subdivisions of the thalamic nuclei were identified by comparison to mouse and rat brain thalamic subdivisions characterized previously (Paxinos and Watson, 1986; Franklin and Paxinos, 1997).

The thalamic somatosensory nuclei in the naked mole-rat are structurally similar to corresponding thalamic nuclei in the mouse and rat (Fig. 11). As in the thalamus of the mouse and rat, the mole-rat thalamus contains a prominent ventroposterior nucleus (VP) composed of two nuclear subdivisions: the ventral posterior medial subnucleus (VPM) and the ventral posterior lateral subnucleus (VPL). VPM comprises the dorsomedial portion of VP with VPL nestled around it in the ventrolateral portion of VP. Both VPM and VPL are CO-dense as compared to neighboring structures and have a patchy appearance (Fig. 11). The dorsomedial portion of VPL can be differentiated from VPM by its denser appearance and smaller patchy regions. In mole-rats, the lateral extent of VP resides close to the lateral border of thalamus because the lateral geniculate nuclei are significantly reduced as compared to corresponding areas in the rat and mouse (Fig. 11). This reduction in thalamic visual nuclei has also been reported in the blind mole-rat (*Spalax ehrenbergi*) and is consistent with the overall reduction of most CNS visual areas observed in various mole-rat species (Bronchti et al., 1991; Cooper et al., 1993a, 1993b; Rehkamper et al., 1994; Mann et al., 1996; Negroni et al., 2003; Crish et al., 2005).

The reticular thalamic nucleus (Rt) was ventrolateral to VPL, separated from VPL by a light septal region, which is continuous with VPL in anterior thalamus and with the medial lemniscus (ml) in posterior thalamus. The posterior medial nucleus (Po) neighbors VPM's dorsal border and is distinguishable from VPM by its lower CO density and homogeneous cellular appearance (Fig. 11). A collection of intralaminar nuclei were identified dorsomedial to Po, including the central lateral nucleus (CL) and the parafascicular nucleus (PF). These nuclei are CO-dense and can be visually differentiated from the medial border of Po. In the rat, these intralaminar nuclei have projections to areas in motor and somatosensory cortex, as well as to the striatum, creating a circuit for the coordination of sensory-motor and limbic responses (Berendse and Groenewegen, 1990; LeDoux et al., 1990; Fabri and Burton, 1991b; Price, 1995).

The two major motor nuclei in the mole-rat thalamus, the ventrolateral nucleus (VL) and the ventromedial nu-

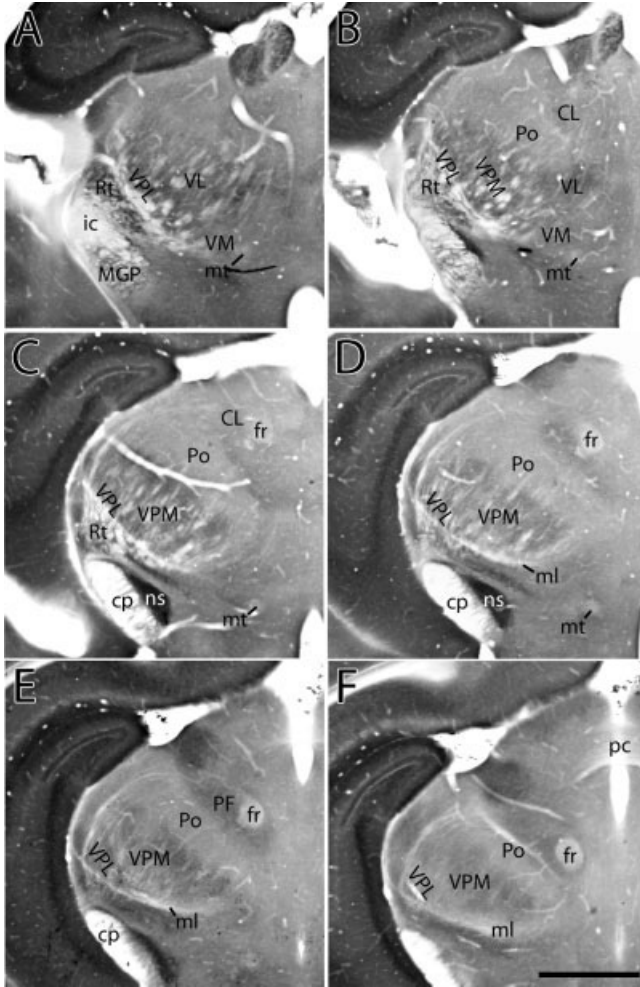


Fig. 11. Coronal rostrocaudal cross-sections through somatosensory thalamus reacted for cytochrome-oxidase. **A–F:** Photomicrographs of sections illustrate the ability to delineate thalamic subdivisions related to somatosensory processing by comparing differences in CO density and cellular appearance. For example, the VP nuclear complex is significantly more CO-dense than the neighboring Po nucleus. The cellular appearance of Po is also more homogeneous than that seen in VP. In all images, the dorsal aspect is toward the top, and lateral is to the left. Scale bar = 1 mm.

cleus (VM), flank the somatosensory nuclei. VL is found anterior to the VP complex, with the caudal-most portions of the nucleus residing in medial thalamus, neighboring Po and VPM (Fig. 11A and B). In rats, VL sends the majority of its projections to motor cortex but also projects to S1, particularly to the S1 limb representations (Donoghue et al., 1979; Cicirata et al., 1986; Aldes, 1988). The VM nucleus is found in ventromedial thalamus neighboring VPL. VM sends projections to many neocortical destinations, with particularly dense connections to rostral neocortex (Herkenham, 1979; Price, 1995).

Thalamic Connections From S1

The distribution of label related to the S1 incisor and forelimb representations provided general organizational details for the arrangement and topography of body part

representations in the ventrobasal complex and Po. The arrangement of label from the S1 lower incisor representation was of particular interest because it occupies approximately 15–18% of S1 (Catania and Remple, 2002). We hypothesized that this cortical magnification would be reflected in a large subcortical incisor representation in thalamus. Although the tooth representation of VPM has seldom been investigated in rodents, Tabata et al. (2002) were able to record neuron responses in this area related to the periodontal mechanoreceptors in rats. They found neurons responsive to the periodontal mechanoreceptors resided in the medial rostroventral portion of VPM, which corresponds well with our tracer distribution from the S1 lower incisor representation in the mole-rat.

All of our cortical S1 incisor injections produced concentrated label in ventromedial VPM (Figs. 12–15). These clusters of label included both terminals and cells, indicating that VPM and S1 were reciprocally interconnected. Po had a more irregular distribution of label from case to case, but generally label was confined to the ventromedial portion of the nucleus. Most of the label in Po was cellular, with only sparse terminal projections found along the ventral border of Po, neighboring areas of terminal label in VPM. Case 04-06 shows this distribution of label in VPM and Po (Fig. 12).

Sparse label was also identified in neighboring thalamic nuclei including VM, VL, CL, and PF. Label in all these areas was found to be exclusively retrograde, suggesting feed-forward connectivity with S1 cortex. In cases 04-06, 04-10, and 05-01, labeled cells were found only in the caudal portions of VL, sparing the rostral portions of the nucleus (Figs. 12–14).

In cases with injections from the S1 forelimb representation, there were consistent patterns of WGA-HRP distribution in thalamus (Figs. 16–19). Because the injections in cases 05-08 and 05-12 were larger and filled portions of both S1 limb areas, labeled projections in thalamus were more robust than in case 05-13. In all of the cases, label was identified in both the VPL portion of VP and Po. In case 05-13, label composed of both cells and terminals was identified as an elongated cluster in the center of ventral VPL, nestled against the ventral border of VPM (Fig. 18). Label was completely absent in the dorsal portion of the nucleus. In the cases with larger injections (cases 05-08 and 05-12), this cluster of label in VPL was greatly expanded, with cell bodies occupying all of ventral VPL and terminals filling the entire nucleus (Figs. 16 and 19).

In Po, reciprocal label corresponding to the S1 forelimb representation was clustered in the dorsolateral portion of the nuclei (Figs. 16, 18, and 19). Label contributions from the S1 hindlimb area in Po neighbored the forelimb cluster of cells in dorsolateral thalamus. For example, in case 05-13, a single discrete cluster of cells was found in dorsolateral Po, but in cases 05-12 and 05-08, there were two dorsal clusters in Po consisting of label cells and terminals.

Dense label was also found in VL in all of the S1 forelimb injection cases. This label was concentrated in the rostral and central portions of the nucleus, filling these areas heavily with both cells and terminals. This apparent direct connection between VL and the S1 forelimb representation is consistent with previous investigations in the rat (Donoghue et al., 1979; Cicirata et al., 1986; Aldes, 1988).

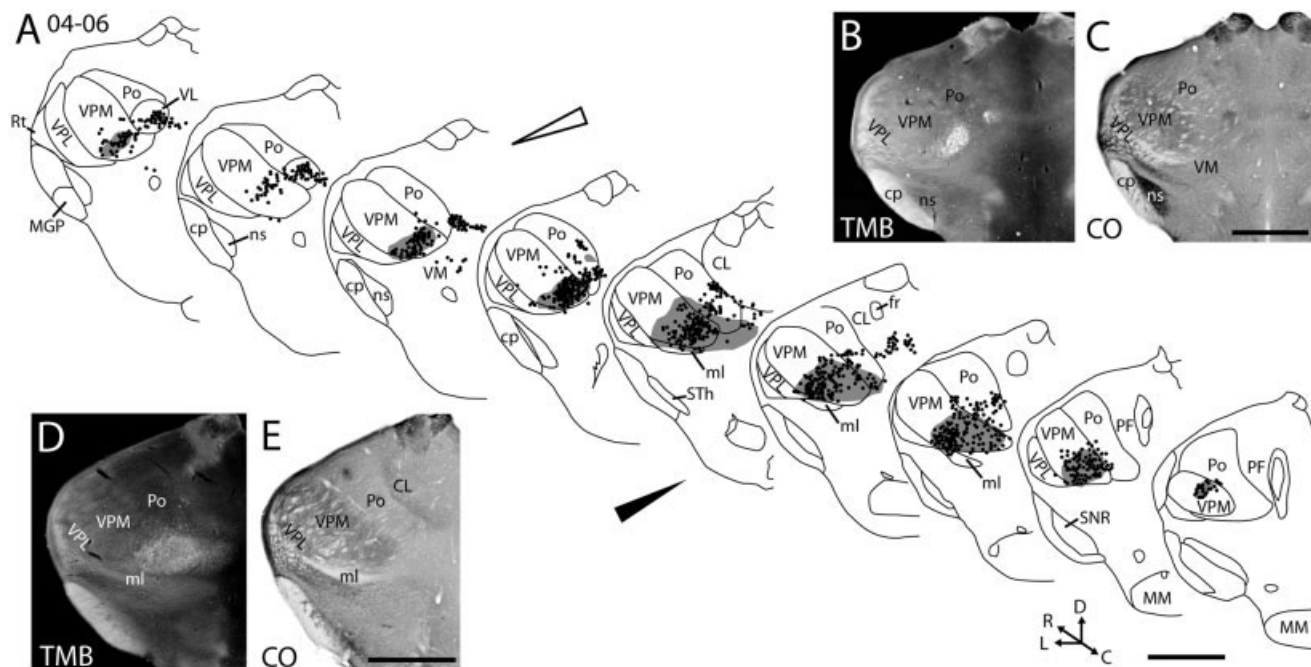


Fig. 12. Thalamocortical reconstruction of connections corresponding to the lower incisor representation. **A**: Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower incisor area.

B and **C**: Dark-field image of TMB-reacted label and adjacent CO-stained section corresponding to reconstructed section with the white arrow in **A**. **D** and **E**: Images corresponding to the thalamic section with the black arrow in **A**. Scale bars = 1 mm.

The ventral medial nucleus (VM) was consistently shown to have sparse retrograde connections to the S1 forelimb area. In case 05-13, this label was found medial to the ventromedial portion of VPL (Fig. 18). In case 05-08, there also appeared to be anterograde transport to VM, but this was not seen in other cases (Fig. 17). The boundaries for VM were difficult to determine with the stains used in this study as the subdivisions medial to the VP complex had a uniformly homogeneous cellular appearance.

Sparse label was also found in the lateral intralaminar nuclei, CL and PF. In addition, labeled fibers were identified in the internal capsule and cerebral peduncle. Terminals were also lightly distributed in the reticular nucleus (Rt) in cases 05-12 and 05-08 (Figs. 16 and 19).

DISCUSSION

The results of this study are illustrated in Figure 20 and can be summarized as follows.

One, the S1 lower incisor representation had reciprocal intrahemispheric connections to neighboring S1 orofacial areas (including tongue, buccal pad, and chin), motor cortex adjacent to S1, and parts of lateral parietal cortex related to the S2/PV tooth area.

Two, injections into the S1 forelimb representation revealed reciprocal intrahemispheric connections to neighboring S1 body areas (including hindlimb, trunk, and chin), two discrete motor areas in frontal cortex, two areas in caudal cortex likely homologous to PM and PL in rats, and the S2/PV limb representation in lateral parietal cortex.

Three, callosal connections from the S1 lower incisor representation project to contralateral S1 within the ros-

tral portion of LIM1, the septal regions between the CO-dense modules related to the tongue and teeth, and a small patch of cortex rostral to the S1 chin and buccal pad representations.

Four, callosal projections from the S1 forelimb representation retrogradely labeled cell bodies throughout the CO-dense S1 forelimb area in the contralateral hemisphere, but anterogradely filled terminals were restricted to the medial and lateral boundary regions of the limb area. Callosally derived label was also observed in the S2/PV limb area and in motor cortex immediately rostral to the S1 limb representations.

Five, the somatosensory thalamic nuclei, VP and Po, are reciprocally connected to S1 and each nucleus is organized topographically, with discrete representations for the body parts we investigated. Thalamocortical projections to S1 were also found in VL, VM, CL, and PF.

Technical Considerations

Although all of the injections were guided by microelectrode neuronal recordings to locate the center of the region of interest, the boundaries of the effective uptake zone of WGA-HRP were not always confined to a single S1 body part representation. This variation in injection size and spread resulted in different amounts of transported label across the cases. Larger injections (with injection zones greater than 1 mm in diameter) maximized visualization of weak projection systems, such as the callosal projections related to the S2/PV area in the opposite hemisphere. Smaller injections confined to a single cortical S1 body region provided more discrete transport of label and helped with interpreting the relevance of labeled areas revealed by the larger injections. For instance, it is likely

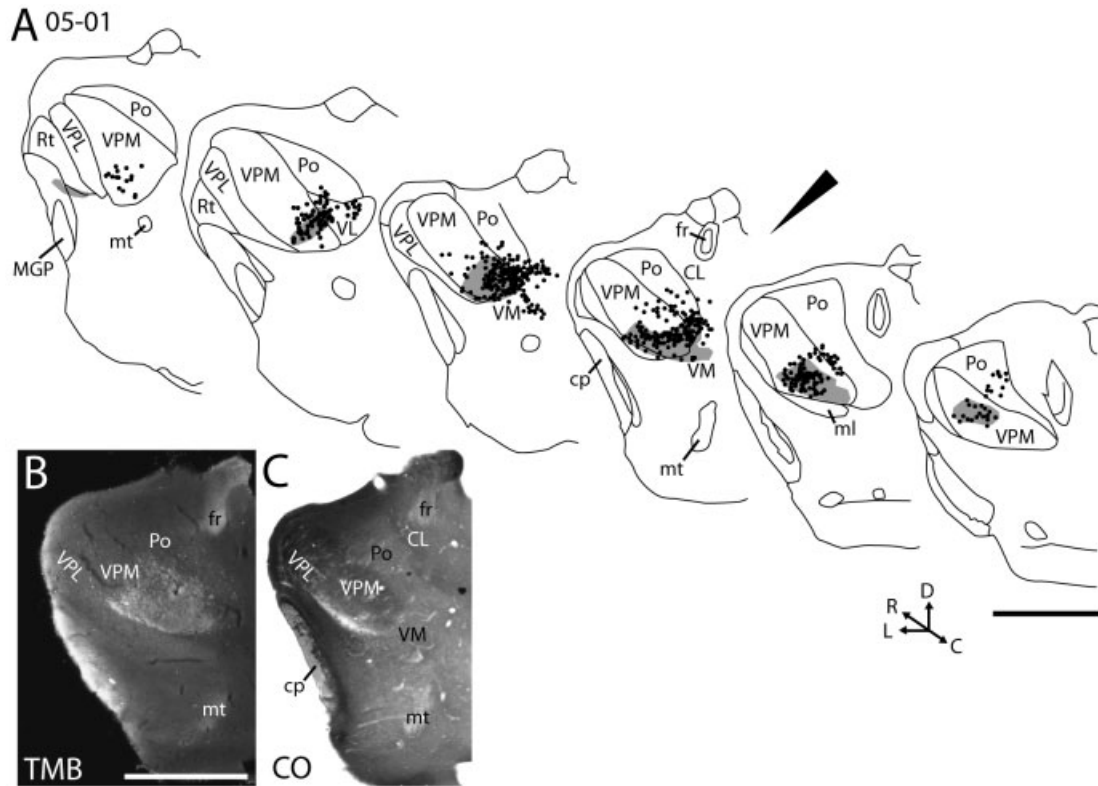


Fig. 13. Thalamic reconstruction of connections corresponding to the lower incisor representation. **A**: Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower incisor area.

B and **C**: Dark-field image of TMB-reacted label and adjacent CO-stained section corresponding to reconstructed section with the black arrow in **A**. Scale bars = 1 mm.

that the injections in cases 05-08 and 05-12 resulted in tracer uptake in the hindlimb and trunk areas (in addition to forelimb) as thalamic patterns of label in these cases showed two foci of label within Po and an expanded population of label in dorsal portions of VPL as compared to smaller injections confined to the S1 forelimb area (see case 05-13 for comparison; Figs. 7, 8, and 10). Patterns of label in VP and Po are good indicators of the extent of cortical uptake zones, as projections to these nuclei are highly specific (Saporta and Kruger, 1977; Chapin et al., 1987; Fabri and Burton, 1991b).

Connections of S1 Lower Incisor Representation

The distribution of ipsilateral and contralateral cortical label in S1 and S2/PV tooth areas was consistent with recent investigations of mole-rat cortex based on electrophysiological recordings from cortex (Catania and Remple, 2002; Henry et al., 2006). The results are discussed in separate sections in the following order: ipsilateral label, contralateral (callosal) label, and thalamic label.

Connections in Ipsilateral Cortex

The connections from the LIM1 module in the S1 lower tooth area were found in neighboring representations of the face including the tongue, buccal pad, and chin. Cortical connections between neighboring or functionally as-

sociated body areas have been reported in a variety of rodent species (rat: Chapin et al., 1987; Fabri and Burton, 1991a; squirrel: Krubitzer et al., 1986; and mice: Bernardo et al., 1989). This connectivity suggests a coordination of cortical inputs between areas commonly costimulated during a behavioral task (Fabri and Burton, 1991a). In the case of the mole-rat, the incisors are used for digging, carrying objects, and chewing food items (Jarvis and Bennett, 1991). When a mole-rat performs these tooth-related tasks, surrounding facial structures such as the whiskers on the chin and buccal pad are simultaneously stimulated, providing input complimentary to the periodontal mechanoreceptors. Coordination of inputs from the tongue and teeth would also be important for successful mastication and a variety of other jaw movements.

Although most of the S1 orofacial areas neighboring the injection site received projections, there were areas that did not have robust connections to the LIM1 region of the lower incisor representation. One of these areas was LIM2, the more lateral CO-dense module related to the S1 lower incisor area (Henry et al., 2006). Label was only sporadically found in LIM2 and was sparse in those cases. This segregation of label suggests that the S1 incisor area might have functionally distinct subdivisions within the cortical representation. In monkeys, cortical neurons receiving input from different classes of peripheral mechanoreceptors are segregated in bands within the hand

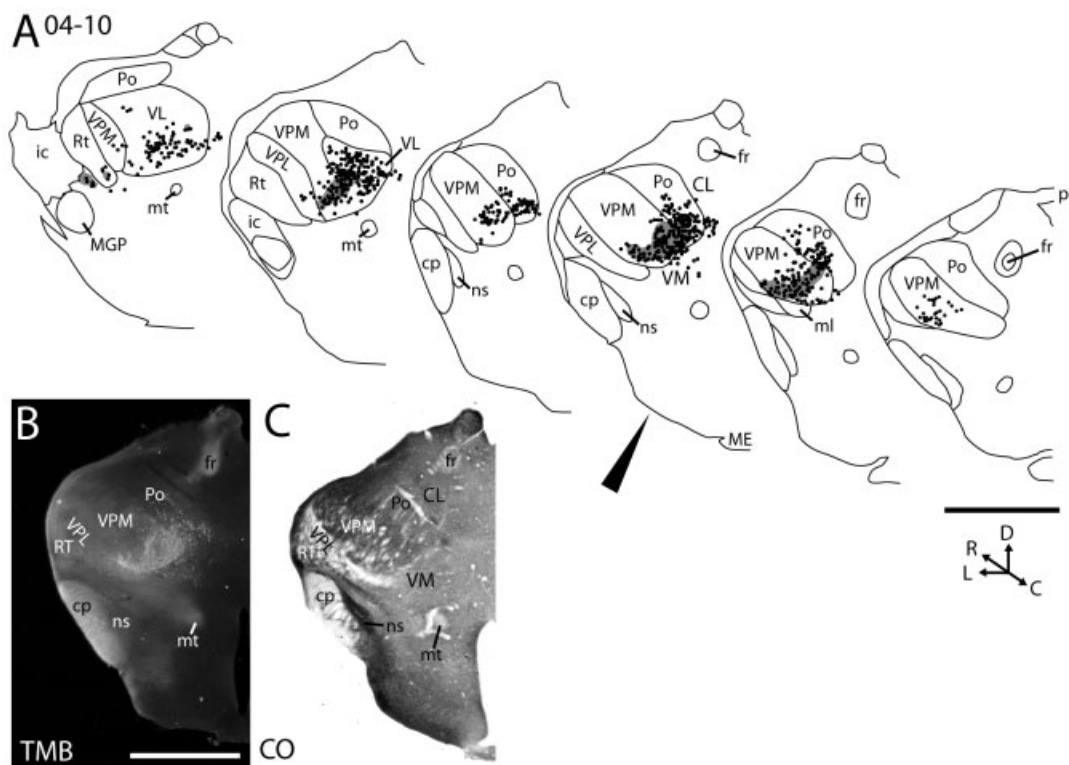


Fig. 14. Thalamus reconstruction of connections corresponding to the lower incisor representation. **A:** Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower incisor area.

B and C: Dark-field image of TMB-reacted label and adjacent CO-stained section corresponding to reconstructed section with the black arrow in A. Scale bars = 1 mm.

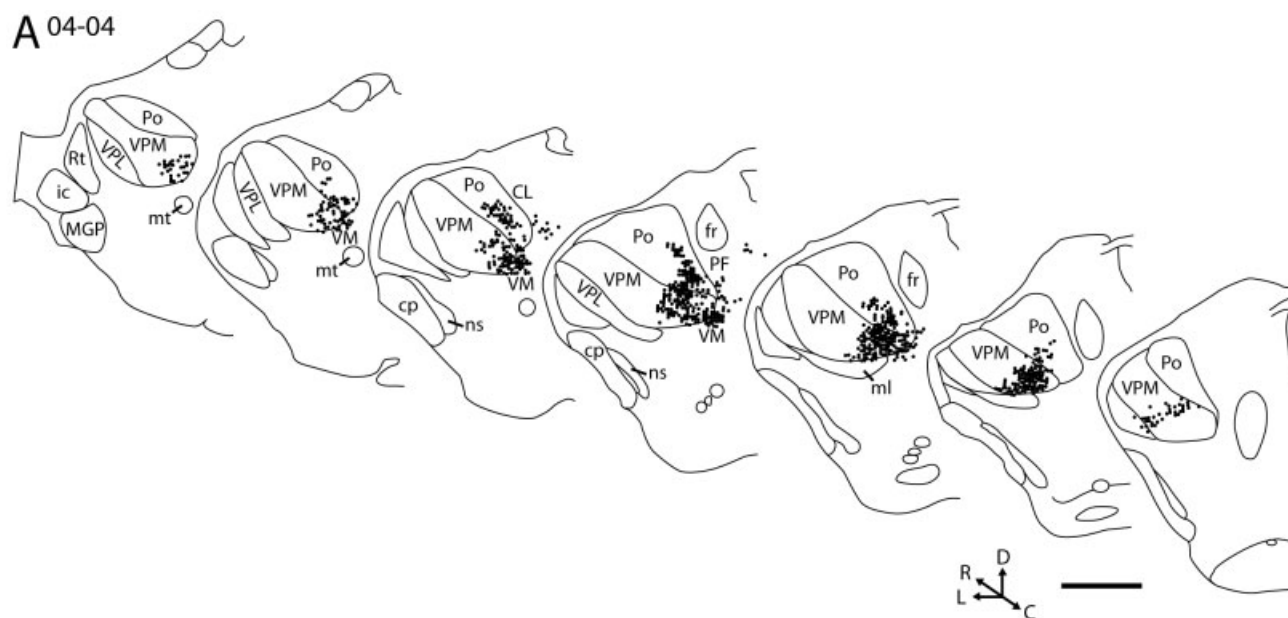


Fig. 15. Thalamus reconstruction of connections corresponding to the lower incisor representation. Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) after an injection into the S1 lower incisor area. This reconstruction case did not include terminal labeling. Scale bar = 1 mm.

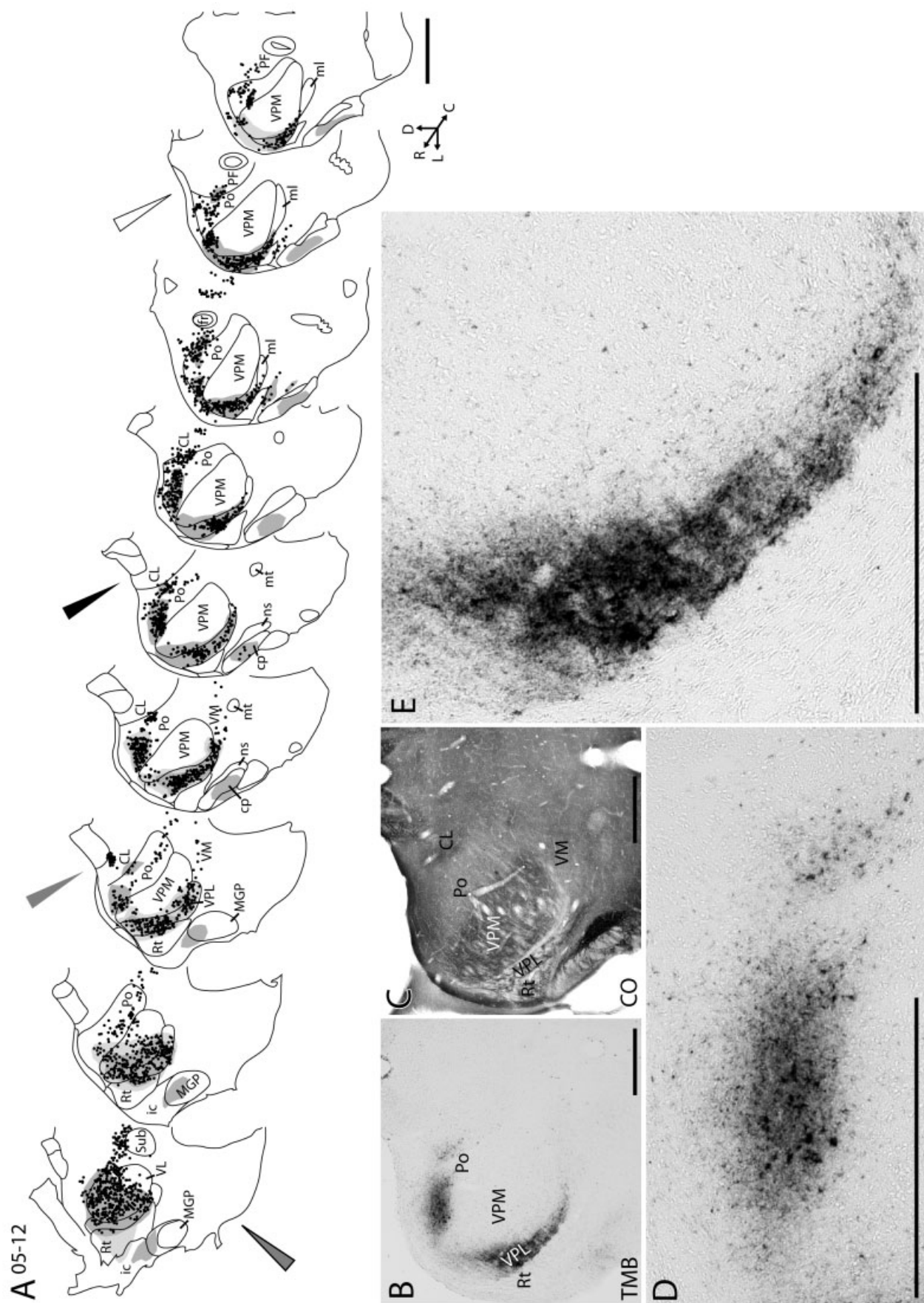


Fig. 16. Thalamic reconstruction of connections corresponding to the S1 limb representation. **A:** Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower forelimb area.

B: Bright-field photomicrograph of label distribution corresponding to the section with the black arrow. **C:** CO-reacted tissue section adjacent to section in B. **D** and **E:** Enlarged images of thalamic label from B within Po and VPL, respectively. Scale bars = 1 mm (A); 0.5 mm (B-E).

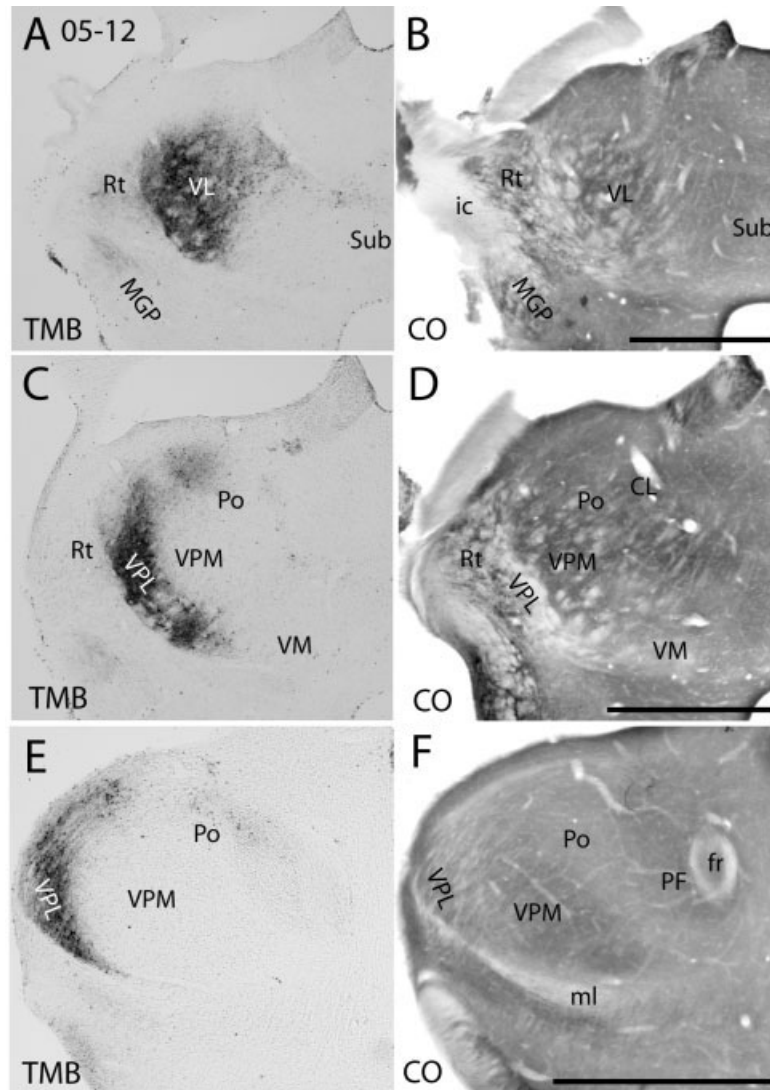


Fig. 17. Photomicrographs of TMB- or CO-reacted sections corresponding to reconstructed sections in Figure 16 A. **A** and **B**: Images corresponding to the rostral-most section (marked by a gray arrow outlined in black) in Figure 16A. **C** and **D**: Relates to section with gray arrow in upper left quadrant. **E** and **F**: Images corresponding to section with white arrow. Scale bars = 1 mm.

representation of area 3b (Sur et al., 1981). This functional segregation in cortex appears to be a feature of behaviorally important sensory structures where fine discriminations are needed. Perhaps the segregation of connections in the mole-rat lower incisor representation is similarly related to functional segregation of different inputs. Although the receptor population of the mole-rat periodontal ligament is uncharacterized, in rats the predominant mechanoreceptor in the periodontal ligament of the incisors is the unencapsulated ruffini-like ending (Chambers et al., 1972; Sato et al., 1988; Byers and Dong, 1989; Takahashi-Iwanaga et al., 1997). This receptor has been demonstrated to have directional sensitivity (Chambers et al., 1972; Tabata et al., 2002). Evidence for neurons with differing stimulation preferences in mole-rat S1 was observed during the physiological mapping of the incisor areas. Some neurons in the S1 incisor area responded to

light taps on the enamel surface, whereas other neurons were maximally activated by light brush strokes of the probe over the length of the tooth (Henry et al., 2006). These different types of stimulation could stretch the periodontal ligament in different directions and thus activate mechanoreceptors. Finer physiological mapping of the lower incisor area is needed to determine whether stimulation preferences may be segregated to specific areas of cortex and if such preferences could relate to the CO-dense regions within the area.

The S1 upper incisor representation also lacked intrinsic connections to the lower incisor area. This was intriguing because the incisors are usually activated in unison and must be coordinated when performing behavioral tasks such as chewing or digging. However, connections were present between the S1 lower incisor representation and a neighboring caudolateral area of cortex that con-

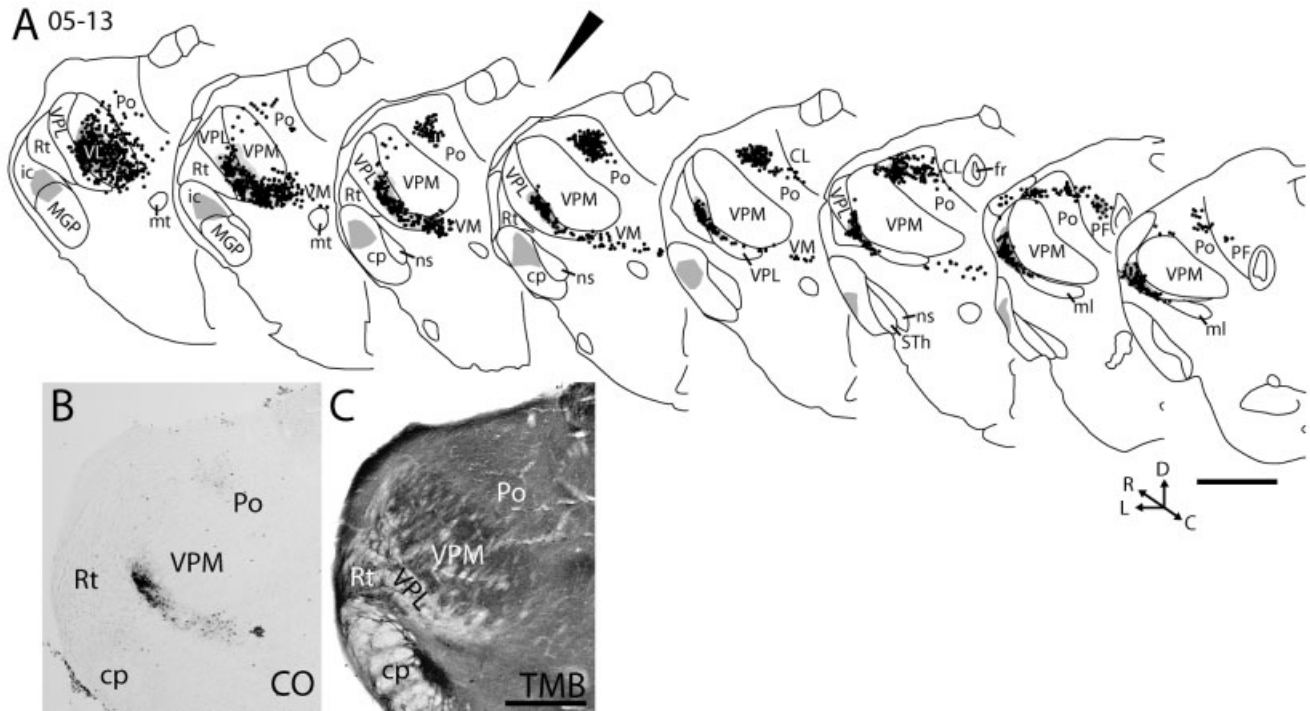


Fig. 18. Thalamus reconstruction of connections corresponding to the S1 forelimb representation. **A:** Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower forelimb area.

B: Bright-field photomicrograph of TMB label distribution corresponding to the section with the black arrow. **C:** CO-reacted tissue section adjacent to section in B. Scale bars = 1 mm (A); 0.5 mm (B and C).

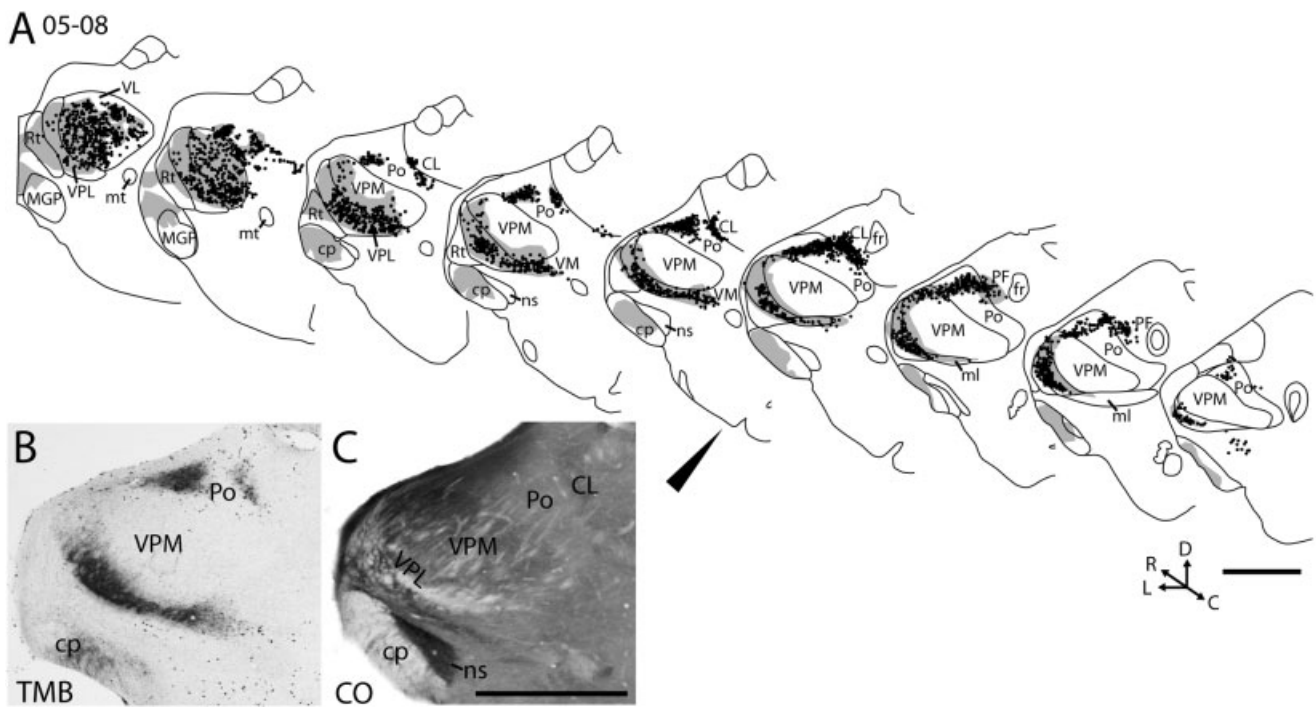


Fig. 19. Thalamus reconstruction of connections corresponding to the S1 forelimb/hindlimb representation. **A:** Rostrocaudal cross-sections through thalamus demonstrating the location of cell bodies (black dots) and terminals (gray shading) after an injection into the S1 lower forelimb

area. **B:** Bright-field photomicrograph of label distribution corresponding to the section with the black arrow. **C:** CO-reacted tissue section adjacent to section in panel B. Scale bars = 1 mm.

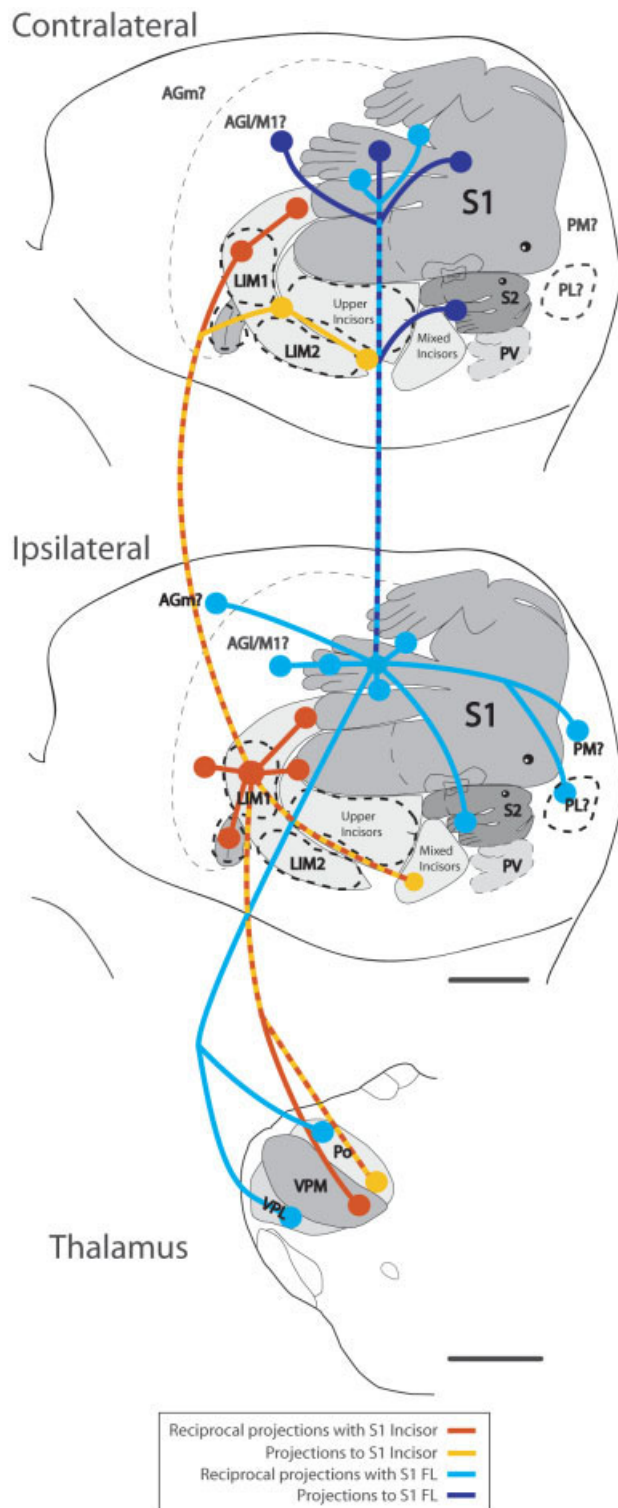


Fig. 20. Summary diagram of cortical and thalamic connectivity corresponding to the S1 lower incisor and forelimb representations. Reciprocal (red and light blue) and exclusively retrograde (yellow and dark blue) connections are aligned with the physiologically derived map of somatosensory space. Projection lines with mixed colors reflect the sparse nature of anterograde connections. Thick black dotted lines indicate areas of CO-dense modules. Thin black dotted lines designate proposed borders for presumptive cortical territories in frontal or lateral parietal cortex. Round colored circles indicate cortical territories that had connections to other areas as indicated by colored lines. Scale bars = 1 mm.

tains the presumptive S2/PV tooth area functionally identified by multiunit electrophysiology methods (Henry et al., 2006). This secondary tooth representation has also been identified in rats (Remple et al., 2003). Label distributed in this area often included cells in the caudal septal regions between the lower and upper tooth CO-dense modules.

Connections in Contralateral Cortex

The cortical representations of midline body parts typically have homotopic connections with the corresponding S1 representations in the opposite hemisphere (Hayama and Ogawa, 1997, 2001). Distal body areas represented cortically tend to have more segregated connections from thalamic and callosal inputs, with the callosal connections largely restricted to dysgranular zones and thalamic projections terminating in granular areas (Wise and Jones, 1976; Akers and Killackey, 1978; Olavarria et al., 1984; Hayama and Ogawa, 1997).

The S1 incisor areas were found to be reciprocally interconnected within the CO-dense granular regions of LIM1. However, portions of the module were consistently devoid of label. This incomplete filling of the LIM1 module in contralateral S1 suggests some limited segregation of callosal and thalamic inputs for this midline body area. Septal regions between LIM1 and LIM2 often contained terminals and cell bodies and there was also label in septal areas medial to LIM1 anterior to the buccal and chin representations. It is unclear whether this medial label is related to the face or to the functionally defined CO-sparse portion of the lower incisor area medial to LIM1 (Catania and Remple, 2002; Henry et al., 2006).

Sporadic interhemispheric connections were also found in LIM2 and neighboring caudal cortical territory near the S2/PV tooth area, suggesting weak projections exist to the S1 LIM1 area in the contralateral hemisphere.

Connections in Thalamus

The distribution of label related to the S1 incisor area in mole-rat thalamus is consistent with thalamic organization of S1 facial projections characterized in the rat and mouse (Waite, 1973; Erzurumlu and Killackey, 1980; Peschanski, 1984; Kosar et al., 1986; Fabri and Burton, 1991b). Functional studies have shown periodontal mechanoreceptor-sensitive neurons reside in ventromedial VPM in rats (Tabata et al., 2002). The majority of the VPM neurons in this area responded to mechanical stimulation of the rat's contralateral lower incisor. In the mouse, the pattern of connections from the mandibular incisor tooth pulp to the ventromedial portion of VPM closely resembles the pattern described in our present results in mole-rats (Barnett et al., 1995).

Trigeminal-related inputs to Po are more diffuse than those to VPM (Peschanski, 1984; Chiaia et al., 1991a, 1991b; Fabri and Burton, 1991b) and this lack of segregation was reflected in the more variable location of Po incisor-related neurons observed in the mole-rats. In mice, tooth pulp-related neurons in Po were found further dorsolateral (Barnett et al., 1995) than in mole-rats. But as the neurons labeled in the mole-rat Po nucleus reflect projections that include both tooth pulp and periodontal mechanoreceptor inputs, investigation of both populations of inputs in mice would be needed to make a more accurate comparison between the two species.

Thalamocortical connectivity has also been explored in the blind mole-rat (*Spalax ehrenbergi*), another subterranean mole-rat species with an expanded somatosensory system (Necker et al., 1992). Although the S1 tooth representation was not the focus of their investigation, they did place injections in the lateral S1 face territory, producing a pattern of label in thalamus consistent with what we observed in VPM and Po of naked mole-rats (Rehkaemper et al., 1994). Rehkaemper et al. (1994) placed their injections after recording cortical responses to air puff stimulation on the face. This method is unlikely to elicit tooth-related neuronal activity. We speculate that the effective tracer uptake zone in those studies included portions of the incisor area because the thalamic label was concentrated in the ventromedial-most portions of VPM and Po.

Connectivity of S1 Forelimb Representation

The connectivity of the S1 forelimb area was investigated in this study to provide a comparison with the unique connections of the incisor representation. This injection site also revealed connections to presumptive motor areas in frontal cortex, to areas in lateral parietal cortex, and to caudal somatosensory areas previously unidentified in the naked mole-rat. The results are discussed in separate sections in the following order: ipsilateral label, contralateral (callosal) label, and thalamic label.

Connections in Ipsilateral Cortex

The widespread pattern of intrinsic ipsilateral connections from the CO-dense S1 forelimb representation to motor and secondary somatosensory areas is similar to that described for the rat (Akers and Killackey, 1978; Koralek et al., 1990; Fabri and Burton, 1991a). The label in the more rostral areas of cortex indicates the likely location of motor areas related to the body representation in the naked mole-rat. CO staining in these rostral regions is uniformly sparse and homogeneous in cellular appearance (Henry et al., 2006). Although microstimulation techniques are needed to determine how these anterior areas are organized, the labeling in mole-rat anterior cortex is remarkably similar to patterns of motor connections in other rodents. For example, the S1 forelimb injections produced two distinct areas of label anterior to S1. The more caudal patch of label neighboring S1 is likely homologous to M1 or AGI described in rats (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Fabri and Burton, 1991a). The more anterior patch of label is likely related to AGm or the supplementary motor area in rats (Reep et al., 1990). Fabri and Burton (1991a) described AGm as a small motor area with minimal topographic organization, which may explain why we did not see connections to this region in the S1 incisor injection cases.

Our results from the S1 forelimb injection cases also revealed the presence of label in caudal parietal areas. A large distinct cluster of cells was found caudal to the trunk and vibrissae areas, in an area of cortex that has been identified as PM in rats and squirrels (rat: Koralek et al., 1990; Li et al., 1990; Fabri and Burton, 1991a; squirrel: Krubitzer et al., 1986). A second sparse and variable distribution of label was found below PM, usually within the borders of a CO-dense area near the posterior boundary of the S2/PV region. This label could relate to an area described in rats as PL (Fabri and Burton, 1991a). It has

been suggested that these areas are sensory association areas that integrate inputs from multiple modalities and may be homologous to primate area 5 (Chapin and Woodward, 1982; Fabri and Burton, 1991a).

The connections between S1 and the S2/PV limb area are consistent with the physiologically derived functional map of lateral somatosensory cortex (Henry et al., 2006). The results from these connectional studies support the hypothesis that an upright representation of the body (i.e., upper body represented medially and lower body laterally) characterizes mole-rat S2, as has also been reported for rat S2 (Fabri and Burton, 1991a; Remple et al., 2003). We were unable to find two distinct foci of label for the forelimb representation, making it difficult to comment on the presence of a third area in lateral cortex suggested by our physiological results (Henry et al., 2006). However, because physiological results place the forelimb and hindlimb at the border region between S2 and PV, connections to these body areas would be expected to produce a single patch of label following injections into S1 limb representations (e.g., Fabri and Burton, 1991a).

Connections Between S1 Forelimb and Contralateral Cortex

Callosal connections projecting to the contralateral cortical hemisphere produced label within the forelimb region of areas S1 and S2/PV. Anterograde label in contralateral S1 tended to be confined to the border areas between the forelimb representation and adjacent body areas. This segregation of terminal zones related to callosal and thalamic inputs is similar to connectional patterns described for rats (Akers and Killackey, 1978; Olavarria et al., 1984; Koralek et al., 1990; Hayama and Ogawa, 1997). We did, however, find many labeled cells within the contralateral granular forelimb representation. Olavarria et al. (1984) also reported scattered neuronal labeling within the granular zones of S1, suggesting that the generalization of a complete segregation of thalamic and callosal inputs may be an oversimplification. For example, Killackey et al. (1983) described callosal projections within the cortical somatosensory hand representation in monkeys, despite this area being heavily innervated by thalamic projections. These results suggest callosal and thalamic projections within the S1 area are somewhat coextensive, providing a substrate for integration of the two inputs.

Connections in Thalamus

Fabri and Burton (1991b) extensively mapped the projections from the S1 body area to VP and Po in the rat and found a topographical map of the contralateral body surface in each nucleus. The VP nucleus contained a body representation with an upright orientation (with the head and distal limbs pointed medially) (Saporta and Kruger, 1977; Nothias et al., 1988; Fabri and Burton, 1991b). The smaller body representation in Po was inverted, with a congruent border along the VPM vibrissae and face areas. Our results in the naked mole-rat are consistent with this pattern of thalamic connectivity and suggest similar orientations of the body areas in these nuclei. Rehkaemper et al. (1994) also found a similar arrangement of corticothalamic projections in the blind mole-rat. Such mirror image representations, sharing a common border, are often observed at the cortical level in mammals but have also

been reported for nuclei in nonmammalian sensory systems (e.g., for electric fish; see Heiligenberg and Bastian, 1984). This common observation for diverse brain areas in different species suggests that mirror image representations are a particularly efficient design.

There is a considerable difference in the size and location of the thalamic nuclei of mole-rats and rats. Because the visual thalamic nuclei are dramatically reduced in naked mole-rats, the thalamic somatosensory nuclei have shifted to a more lateral location, possibly providing room for expansion of these areas. This lateral expansion of the thalamic somatosensory nuclei at the expense of visual nuclei was also described in the blind mole-rat (Mann et al., 1996). Mann et al. (1996) reported that the thalamic somatosensory nuclei are larger relative to total thalamic territory in the blind mole-rat as compared to the rat. VP and Po also appear enlarged in naked mole-rats (Fig. 11C and D), suggesting the thalamic somatosensory nuclei are enlarged in mole-rats generally. However, this thalamic expansion is far less dramatic than what has been observed cortically for both mole-rat species (Necker et al., 1992; Rehkamper et al., 1994; Catania and Remple, 2002). This suggests that the dramatic cortical expansions of somatosensory areas in mole-rats are independent of the subcortical magnification factors and do not reflect equivalent expansion patterns throughout the hierarchical levels of somatosensory processing in the CNS.

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