BILATERAL TOPOGRAPHY OF CORTICAL PROJECTIONS TO MI CORTEX

A Thesis in
Neuroscience
by
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ABSTRACT

To determine the cortical regions that project to primary motor (MI) cortex, we injected retrograde tracers into physiologically-identified sites in rat MI. Microscopic examination of tangential sections revealed dense populations of labeled neuronal soma in multiple areas of the ipsilateral cortex and in the contralateral MI. Neurons labeled by different tracers were usually segregated from each other when the two tracers were injected separately into whisker and forelimb regions. The contralateral MI region contained more labeled neurons than any other cortical region when the tracer was injected into the MI whisker region. By contrast, when the tracer was injected into the MI forepaw representation, similar percentages of labeled neurons were present in the contralateral MI and ipsilateral SI cortical areas. The higher proportion of neurons in the contralateral MI whisker region suggests that callosal connections between the MI whisker regions may contribute to bilateral coordination of whisking behavior.
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“Differentiation of function implies differentiation of structure and differentiation of structure means differentiation of function. So far as the comparative anatomist detects homological cerebral structures, so far, it is to be hoped, will the physiologist be able to develop the homological functions, and so far as the anatomist discerns different structures, so far will the physiologist be able to detect different functions.”

- J. Hughlings Jackson
Chapter 1. Introduction

Many behaviors depend on somatosensory feedback to modulate motor activity (Jenkinson and Glickstein, 2000; Ahissar and Kleinfeld, 2003). Consistent with this fact, interconnections between the somatosensory and motor cortices have been demonstrated in a wide range of mammalian species (Burton and Kopf, 1984; Krubitzer et al., 1986; Huerta and Pons, 1990; Krubitzer and Kaas, 1990). In rodents, for example, both the primary (SI) and secondary (SII) somatosensory cortical areas project to primary motor (MI) cortex (Donoghue and Parham, 1983; Cicirata et al., 1986; Reep et al., 1990; Arimatsu et al., 1999; Gu et al., 1999; Hoffer et al., 2003; Alloway et al., 2004; Chakrabarti and Alloway, 2006).

Additional corticocortical projections to MI cortex indicate that motor behavior is also modulated by other sensory and related systems. In rats, both the primary and secondary visual cortical areas (Miller and Vogt, 1984), as well as parts of the auditory cortex, project to motor cortex (Ermolaeva and Borgest, 1980; Ermolaeva et al., 1982; Kimura et al., 2004). Rat motor cortex also receives inputs from the posterior parietal cortex (PPC), which processes spatial information and other salient stimulus features that direct and organize motor behavior (Reep et al., 1994; Tees, 1999). Furthermore, information from the hippocampus can reach MI via the entorhinal and perirhinal regions (Swanson and Kohler, 1986; McIntyre et al., 1996; Insausti et al., 1997; Kyuhou and Gemba, 2002).

Despite substantial knowledge about the cortical areas that project to rat MI cortex, there is little information regarding the relative contribution of these areas to the functional subdivisions in MI cortex. The MI cortex is subdivided into medial (Agm) and lateral (Agl) agranular zones (Donoghue and Parham, 1983), which correspond to frontal cortical regions 1 (Fr1) and 2 (Fr2), respectively (Paxinos and Watson, 1986; Weiss and Keller, 1994). Although, conventionally, Agm has been considered a premotor cortical area (Donoghue and Wise, 1982; Neafsey et al., 1986; Rouiller et al., 1993; Hoover and Vertes, 2007), detailed mapping of Agm and Agl with intracranial microstimulation (ICMS) indicates that Agm contains the whisker representation whereas Agl contains the limb and trunk representations (Brecht, Krauss et al., 2004; Brecht, Schneider et al., 2004). Some reports have described cortical projections to Agm or Agl (Reep et al., 1990), but no study has quantitatively compared the relative contributions of the callosal and ipsilateral cortical projections to both of these motor regions. This issue is significant because whisking behavior and limb movements differ kinematically in several respects, including their relative degree of...
bilateral coordination. Consistent with such differences, we recently reported that the whisker region in Agm has more interhemispheric projections than the forelimb region in Agl (Alloway et al, 2009).

Another unresolved issue concerns the relative topography of the afferent cortical projections to rat MI cortex. Virtually all previous tract-tracing studies of MI cortex used single tracer injections to characterize the distribution of cortical areas that contribute inputs to Agm or Agl. Injecting two different tracers into the MI whisker and forelimb regions of each rat would reveal the differential patterns of projections to each of these functional subdivisions.

Therefore, to characterize the relative contribution and topographic organization of the cortical projections to the different subdivisions of rat MI cortex, we placed retrograde tracers into the MI whisker and forepaw regions. In some rats we placed a single tracer into one of these physiologically-defined MI regions; in others we separately placed different tracers into each MI cortical region. In all rats, we plotted the distribution of retrogradely-labeled neurons with respect to a map of cytochrome oxidase (CO) labeling in both the ipsilateral and contralateral cortical hemispheres. Our results indicate significant differences in the relative contribution and topographic distribution of the cortical regions that project to the whisker and forepaw regions of MI cortex.
Chapter 2. Materials and Methods

Experiments were performed on adult male Sprague-Dawley rats (Charles River Co., Wilmington, MA). All procedures conformed to NIH guidelines for the care and use of laboratory animals and were approved by the Penn State Institutional Animal Care and Use Committee.

Animal Surgery

Each rat was anesthetized with an intramuscular (IM) injection of ketamine (20 mg/kg) and xylazine (6 mg/kg); supplemental injections were administered, as needed, to suppress withdrawal reflexes for the duration of the procedure. Following injections of atropine sulfate (0.05 mg/kg, IM), chloramphenicol (50 mg/kg, IM), and dexamethasone sodium phosphate (5 mg/kg, IM), each rat was mounted in a stereotaxic device (Kopf Instruments, Tujunga, CA). Heart rate and body temperature were monitored continuously throughout the surgical procedure, and body temperature was maintained between 35 and 38°C by a homeothermic heating blanket over the rat's trunk. The skin over the cranium was resected and the wound margins were infiltrated with 2% lidocaine. After exposing the skull, a craniotomy was made at stereotaxic coordinates consistent with the location of the MI whisker and forepaw representations (Hall and Lindholm, 1974; Neafsey et al., 1986; Hoffer et al., 2003; Brecht, Krauss, et al., 2004).

Tracer Injections

Intracranial microstimulation (ICMS) was used to locate the whisker and forelimb representations in MI cortex. Cathodal pulse trains of 80 ms (0.7-ms pulses and 3.3-ms interpulse intervals; Master 8, A.M.P.I., Jerusalem, Israel) were delivered through glass micropipettes that contained 3 M sodium chloride solution and had impedances of 0.4 to 1.5 MΩ. The micropipette was advanced to a depth of 1.7 mm and pulses of 100-150 μA were initially delivered to evoke twitches of the contralateral whiskers. The current was gradually reduced to threshold levels (15-100 μA) that produced discrete twitches of one or just a few whiskers. To locate the forepaw MI region, ICMS was sequentially tested at more lateral penetrations, until dorsiflexion of the contralateral forepaw was noted (Gu et al., 1999). Among all cases that were analyzed, MI whisker injections were made 0.89-2.42 mm lateral and 1.22-2.97 mm rostral to bregma, whereas MI forepaw injections were made 2.21-3.41 mm lateral and 0.97-2.58 mm rostral to bregma. Within each case, the whisker MI injection was always medial to the forepaw MI injection.

Red (RB) latex beads (LumaFluor, Inc., Naples, FL, catalog #R-145) and True Blue (TB) (Molecular Probes, Invitrogen Corp., Carlsbad, CA, catalog #T-1323) were pressure-injected from a 2-μl Hamilton microsyringe (model 7002KH, Hamilton Co., Reno, NV) in which a glass pipette (tip diameter, 60-100 μm) was cemented to the syringe needle. Latex beads were used in many experiments because they do not
diffuse far from the injection pipette (Katz et al., 1984). Tracer volume (80-140 nl) was manually controlled by a calibrated injector holder (model 5000, Kopf Instruments, Tujunga, CA) that was attached to the stereotaxic frame by a micromanipulator. The diameter of all pressure-injected tracer deposits ranged from 100 to 1000 micrometers (μm).

Fluoro-Gold (FG) (Fluoro-Chrome, LLC, Denver, CO, catalog #H-22845) was deposited in MI cortex by iontophoretic administration. A glass pipette with a tip diameter of 20-60 μm was filled with the tracer, and positive current (0.8-1.5 μA) pulses were applied at alternating on-off intervals of 7 seconds for 25 to 40 min. This produced deposits that ranged between 0.5 and 1 mm in diameter.

Tracer deposits were made 1.6 mm below the cortical surface, which approximately corresponds to the deep layers of cortex. Following tracer injections and suturing of the wound margins, each animal was returned to the animal colony and housed individually for 1-2 weeks.

**Histology**

Each rat was deeply anesthetized with sodium pentobarbital (100 mg/kg, IP) and transcardially perfused with physiological saline (500 ml) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (500 ml) and then 4% paraformaldehyde with 10% sucrose (350-400 ml). The brain was removed and refrigerated 4-6 hours in 4% paraformaldehyde with 30% sucrose. Cortical slabs from both the injected and the contralateral hemispheres were dissected and stored in cold fixative overnight before being sectioned horizontally at a thickness of 60 μm. To obtain a complete section through the layer IV barrel field, the pial surface of the cortical slab was placed on a plane of frozen substrate and sectioning began in the infragranular layers. Tangential sections located 6-9 sections below the pia contained the layer IV barrel field, and only these sections were processed for cytochrome oxidase (CO) (Alloway et al., 2004; Land and Simons, 1985; Wong-Riley, 1979). The ipsilateral subcortical tissue was sectioned coronally at 60 μm to reveal the location of retrogradely-labeled neurons in the thalamus.

Tissue sections used for plotting retrogradely labeled neurons were washed in 0.1 M phosphate buffer, mounted on gel-dipped slides, and then dried overnight. Subsequently, the mounted sections were dehydrated and defatted for visualization of the tracer injection sites and retrogradely-labeled neurons. For tissue containing RB, the mounted tissue was cover-slipped using Krystalon (Fisher Scientific, Pittsburgh, PA); otherwise, Cytoseal (VWR, Inc., West Chester, PA) was used. Alternate subcortical sections were stained with thionin to reveal Nissl material and the thalamic nuclear boundaries.
Anatomical Reconstructions

Sections with retrogradely-labeled neurons were digitally reconstructed using the AccuStage (Minnesota Datametrics, St. Paul, MN) plotting system mounted onto the stage of an Olympus light microscope (BH-2). Each labeled neuron was plotted with respect to the major blood vessels and the outlines of the tissue section. Photomicrographs were obtained by using a microscope-mounted digital camera (Cool Snap HQ CCD, Roper Scientific, Tucson, AZ) controlled by IP-Lab software (version 3.7, Scanalytics, Inc., Fairfax, VA).

Digital reconstructions and photomicrographs of the CO labeling were superimposed by importing the digital files into a software program (Deneba Systems, Inc., Canvas X; Miami, FL) that enabled alignment of the major blood vessels in adjacent sections. The boundaries of several cortical regions, including the primary (SI) and secondary somatosensory (SII) cortices, parietal ventral (PV), posterior parietal (PPC), and temporal (TC) regions, were delineated with respect to CO labeling, as described in previous reports (Fabri and Burton, 1991; Koralek et al., 1990; Remple et al., 2003). Likewise, digital photomicrographs of Nissl-stained sections through the thalamus were imported into the same software program, and the boundaries of the major thalamic nuclei were identified and traced. Subsequently, these reconstructions of the thalamic boundaries were superimposed on the digital reconstructions of retrogradely-labeled neurons.

Quantitative Analysis

The number of labeled neurons in each cortical area was counted in both cortical hemispheres. After counting all labeled neurons across both hemispheres, the proportion of labeled neurons in each cortical area was calculated. Abbreviations are defined in Table 1. To calculate the mean density of labeled neurons in each cortical area, each plotted reconstruction was subdivided into an array of square bins. The mean labeling density was then calculated by dividing the number of labeled neurons in each cortical area by the volume of tissue that contained the labeled neurons:

\[
\text{Density} = \frac{\text{# of labeled neurons}}{\text{# of labeled bins} \times \text{bin size} \times \text{tissue thickness}}
\]

Tissue thickness of each section was always 60 μm, and each section was subdivided into a grid of square bins with 150 μm on each side.

In cases that received different tracer injections in both the MI whisker and forepaw regions, a custom software program calculated the proportion of the cortical area that contained both types of retrogradely-labeled neurons. Briefly, the plotted reconstructions were subdivided into bins that were 150, 200, or 250 μm² in size. Bins that contained only one type of retrogradely-labeled neurons were color coded to reflect
that tracer; bins that contained double-labeled neurons or both types of retrogradely-labeled neurons were colored white to indicate tracer overlap. The white bins were counted and then expressed as a percentage of all tracer-filled bins to allow statistical comparisons of the amount of overlap in each cortical region.
Table 1. Abbreviations.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Agl</td>
<td>Lateral Agranular Cortex</td>
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<tr>
<td>Agm</td>
<td>Medial Agranular Cortex</td>
</tr>
<tr>
<td>ALBSF</td>
<td>Anterolateral Barrel Subfield</td>
</tr>
<tr>
<td>CG</td>
<td>Cingulate Cortex</td>
</tr>
<tr>
<td>CS</td>
<td>Claustrum</td>
</tr>
<tr>
<td>EC</td>
<td>Entorhinal Cortex</td>
</tr>
<tr>
<td>Fp</td>
<td>Forepaw</td>
</tr>
<tr>
<td>IGr</td>
<td>Infragranular</td>
</tr>
<tr>
<td>MI</td>
<td>Primary Motor Cortex</td>
</tr>
<tr>
<td>PMBSF</td>
<td>Posteromedial Barrel Subfield</td>
</tr>
<tr>
<td>PPC</td>
<td>Posterior Parietal Cortex</td>
</tr>
<tr>
<td>PR</td>
<td>Perirhinal Cortex</td>
</tr>
<tr>
<td>PV</td>
<td>Parietal Ventral Cortex</td>
</tr>
<tr>
<td>RS</td>
<td>Retrosplenial Cortex</td>
</tr>
<tr>
<td>SGr</td>
<td>Supragranular</td>
</tr>
<tr>
<td>SI</td>
<td>Primary Somatosensory Cortex</td>
</tr>
<tr>
<td>SII</td>
<td>Secondary Somatosensory Cortex</td>
</tr>
<tr>
<td>TC</td>
<td>Temporal Cortex</td>
</tr>
<tr>
<td>VC</td>
<td>Visual Cortex</td>
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Chapter 3. Results

Microstimulation mapping and tracer injections were performed in 30 animals. Of these 30 cases, 9 were excluded from analysis because of failure to eject tracer from the pipette \((n = 4)\), premature death \((n = 2)\), spread of the MI forepaw (Fp) injection into SI Fp \((n = 2)\), or poor histology \((n = 1)\). In 4 animals, good retrograde labeling was observed, and the tissue was reserved for fluorescent photomicrography. The topographic distributions of cortical neurons that project to MI were quantitatively analyzed in the remaining 17 rats. In 10 of these cases, a single tracer was injected into the whisker \((n = 5)\) or forepaw \((n = 5)\) regions of MI. In the remaining 7 cases, two tracers were injected, one into the MI whisker region and another into the MI forepaw region. Each case and the tracer injection sites are listed in Table 2. Figure 1 shows the responses of microstimulation at each penetration in three representative examples; the appearance of the injection sites and retrograde labeling for each of these 3 cases will be further described in subsequent figures. In addition, we also injected FG into the MI whisker region of one rat whose brain was sectioned coronally so that we could examine the patterns of retrogradely-labeled neurons around the rhinal fissure (Rf).

Subcortical Labeling

In each dual-tracer-injection case, we verified that both tracers were injected into MI by inspecting the distribution of labeling in the ipsilateral thalamus. The ventrolateral (VL) nucleus contained both whisker- and forepaw-related neurons, in agreement with previous reports that VL has widespread projections to motor cortex (Shinoda and Kakei, 1989; Aumann et al., 1998). The medial part of the posterior thalamic nucleus (POm) also contained neurons labeled with each tracer (Cicirata et al., 1986). In contrast, many more neurons that projected to whisker than to forepaw MI appeared in the midline and intralaminar nuclei; this concurs with the finding that the axons of neurons in the mediodorsal (Krettek and Price, 1977) and paracentral nuclei (Berendese and Groenewegen, 1991) terminate mainly in Agm and medial Agl.

Identification of Multiple Cortical Areas

Tracer injections in MI cortex produced extensive retrograde labeling across several cortical areas in the ipsilateral hemisphere. In contrast, neuronal labeling in the contralateral hemisphere was restricted to a few cortical regions. In both hemispheres, labeling density varied noticeably from one cortical region to the next. In conjunction with variations in CO labeling, regional differences in the location and density of neuronal labeling was used to establish the location of several cortical regions as shown in Figures 2 and 3.
**SI cortex**

The CO-labeled barrel field and other CO-labeled regions in layer IV provide a complete view of the entire extent of SI cortex. All body part representations, including the whisker and forepaw representations, appeared in regions that corresponded with previous maps of SI (Chapin and Lin, 1984; Fabri and Burton, 1991). Therefore, the full spatial extent of SI was delineated by outlining these collective representations, as defined by the CO-labeled barrels (Woolsey and van der Loos, 1970), and other regions of dense CO labeling that represent specific body parts. All labeled neurons that appeared within this CO-defined boundary, including those in CO-poor areas, were counted as SI neurons.

Tracer injections into MI produced labeling in multiple parts of the ipsilateral SI cortex (see Fig. 2). Following injections in the MI whisker region, some of the labeled neurons in SI appeared rostrally in regions that are associated with the anterolateral barrel subfield (ALBSF). Many more labeled neurons, however, appeared in caudal SI and were concentrated in regions aligned with the CO-poor septa of the posteromedial barrel subfield (PMBSF) or with the CO-poor dysgranular zone that is located between the whisker and forelimb representations (Woolsey and van der Loos, 1970; Chapin and Lin, 1984).

Tracer injections in the MI forepaw region also produced labeling in SI that was scattered across multiple body part representations (see Fig. 3). In addition to labeled neurons appearing in the SI forepaw representation and related regions, such as the lower and upper arm representations, most of the SI projections to the MI forepaw injection sites were concentrated in regions that are aligned with the dysgranular zone. In addition, some SI projections to the MI forepaw region also originate from sites aligned with the septal zones in the PMBSF.

**Higher-order somatosensory regions**

Additional somesthesia-related areas were identified by their proximity and spatial configuration with respect to SI. The area located immediately caudal to SI cortex, for example, represents the posterior parietal cortex (PPC). Following tracer injections in the MI whisker region, we observed substantial labeling in PPC, which is consistent with other tracing studies that report connections between PPC and sensorimotor cortex (Chapin and Lin, 1984; Krubitzer et al., 1986; Brett-Green et
In contrast, MI forepaw injections resulted in sparse PPC labeling (Donoghue and Parham, 1983).

We also observed substantial labeling in three other somesthesia-related areas that are located lateral to SI. The secondary somatosensory (SII) cortex is located immediately lateral to SI (Chapin and Lin, 1984; Krubitzer et al., 1986; Alloway et al., 2000; Brett-Green et al., 2004), and it is bordered more laterally by the parietal ventral (PV) region (Chapin and Lin, 1984; Krubitzer et al., 1986; Brett-Green et al., 2004). The perirhinal (PR) region is located lateral to the PV region and, as its name indicates, is located along the rhinal fissure (Burwell and Amaral, 1998). The boundaries between the SII, PV, and PR regions were inferred by locating strips of cortex where the density of neuronal labeling was lowest. Consistent with this, these presumed boundaries followed the elongated rostrocaudal patterns that characterize neuronal labeling in the SII and PR regions, but not in PV. Hence, distinctions in the density and spatial configuration of neuronal labeling, as well as the proximity of labeling to SI or the Rf, provide several criteria for counting labeled neurons in SII, PV, or PR.

**Auditory and visual cortical areas**

The auditory and visual cortical areas were also identified on the basis of their location and proximity to elevations in CO labeling. Although some visual and auditory neurons were labeled after injecting the MI forepaw region, labeling in these sensory regions was relatively sparse and was more likely after tracers were placed in the MI whisker region. Caudal to SII and PV, an increase in CO signal marked the presence of primary auditory cortex or temporal area 1 (TE1), with TE2 and TE3 in the CO lighter regions located more caudally and laterally, respectively (Wallace, 1987; Gonzalez-Lima and Cada, 1994; Hevner et al., 1995). Labeled neurons in any of these TE areas were counted as temporal cortex (TC) neurons, and most TC labeling appeared as a tight cluster or band of neurons in the “posterodorsal” area that projects to frontal area 2, which corresponds to Agm (Kimura et al., 2004).

Primary visual cortex was characterized by a noticeable increase in CO labeling that appeared caudal to PPC (Wallace, 1987). As shown in Figure 2, a few labeled neurons were scattered in the central parts of visual cortex (VC) after injecting the MI whisker region, and this is consistent with a
report that this region has reciprocal connections with the posterior parts of motor area 8 (Miller and Vogt, 1984), which corresponds to AGm (Donoghue and Wise, 1982).

**Claustrum and entorhinal cortex**

In the rat, the claustrum (CS) is an elongated structure that is embedded in the infragranular layers near the Rf (Krieg, 1946; Caviness, 1975; Zilles et al., 1980). The most rostral part of the CS adjoins the orbital cortex (OC), and a distinct boundary between these regions could not be discerned in our flattened horizontal sections through the cortex. Therefore, because both OC and CS contain labeled neurons after tracers are placed in MI cortex (Reep et al., 1990; Miyashita et al., 1994), all labeled neurons plotted along the rostral part of the Rf were classified as being in the OC/CS region. Consistent with previous reports (Li et al., 1986; Slowniewski et al., 1986; Sadowski et al., 1997), neuronal labeling in the OC/CS was always denser in the ipsilateral than in the contralateral hemisphere, but the rostrocaudal extent of the labeling pattern was usually similar in both hemispheres. In most cases, a continuous strip of dense neuronal labeling was observed in the OC/CS that extended caudally for several millimeters until it reached the rostrocaudal level of the posteromedial barrel subfield (PMBSF) in SI. The rostral part of this strip of OC/CS labeling was located slightly medial to the Rf, but it gradually shifted more laterally as the labeling extended into more caudal levels. This description of the CS in horizontal sections is consistent with atlases in which coronal sections indicate that the CS is located slightly dorsal to the Rf in rostral sections but is located more ventrally with respect to the Rf in caudal sections (Krieg, 1946; Paxinos and Watson, 1986). To confirm that the strip of labeled neurons near Rf should be classified as being in the CS, we examined coronal brain sections of a rat in which we placed FG in the MI whisker region. Consistent with the photomicrographs shown in Figure 4, we observed dense neuronal labeling throughout the rostral two-thirds of the CS. Furthermore, the CS labeling shifted more ventrally with respect to the Rf in more caudal sections.

In rare instances, an unlabeled region appeared in the midst of the strip of OC/CS labeling (see Fig. 2). Because tangential sections through cortex were not always perfectly parallel to the cortical layers, parts of the OC/CS were occasionally included in horizontal sections that were processed for CO. In these rare cases, inspection of the CO-labeled sections revealed some faintly-labeled
neurons in locations that correspond to the OC/CS. We did not include these faintly-labeled neurons in our reconstructions. However, inspection of a brain cut coronally indicated that this gap in labeling was artifactual.

The entorhinal cortex (EC) appears in flattened tangential sections as a strip of cortex that forms the perimeter around the lateral and caudal edges of the PR and TC cortical areas. In many CO-labeled sections, the EC was distinguished from CO-rich regions in VC and TC by a band of light CO labeling known as the lamina dissecans (Insausti et al., 1997). We generally observed only a small amount of neuronal labeling in the EC, and this is consistent with data showing that only the dorsolateral and central parts of the EC project to MI (Insausti et al., 1997).

Motor cortex

The MI cortical region in the contralateral hemisphere was distinguished by low levels of CO labeling that are in marked contrast with the dense CO labeling that defines SI cortex. In contralateral MI, the densest neuronal labeling appeared in the homotopic region that corresponded to the tracer injection site in the injected hemisphere. Labeled cells were rarely seen in contralateral SI, and the boundary between SI and MI was marked by a steep decline in the density of labeled neurons. The border between the contralateral MI and the more caudal retrosplenial (RS) region was characterized by a transition from a wide, densely-labeled region to a narrower strip in which the density of labeled neurons was noticeably lower.

In the ipsilateral hemisphere, the MI region surrounding the injection site was densely packed with labeled neurons. Adjoining areas, such as the cingulate (CG) and RS regions, also contained many labeled neurons, and this is consistent with reports showing that these regions project to neighboring parts of MI (Vogt and Miller, 1983; Miyashita et al., 1994; Gu et al., 1999; Shibata et al., 2004). The CG and RS regions, however, do not have well-defined borders in CO-processed horizontal sections. Furthermore, because the dense band of labeling located medial to the SI boundary was not characterized by distinct changes in labeling density, reliable determination of the borders between the MI, RS, and CG regions in the ipsilateral hemisphere was not possible. Consequently, the number and density of labeled neurons in these regions was not analyzed.
Relative Contributions to the Whisker and Forepaw Regions in MI

Several findings indicate significant differences in the distributions of cortical neurons that project to the whisker and forepaw regions in MI. Figure 5, for example, shows the mean proportions of labeled neurons in 11 different cortical regions when retrograde tracers are injected into either the whisker or the forepaw representations of MI. As one might expect, the number of neurons that project to each MI region varies substantially across different cortical areas, and statistical analysis confirmed that the differences in cortical contributions are significant for both MI whisker \((F_{10.121} = 71.49; p < 0.01)\) and MI forepaw \((F_{10.121} = 92.47; p < 0.01)\) injections. In general, ipsilateral cortical projections to the MI forepaw region originate mainly from SI and, to a lesser extent, from SII and PV, both of which represent higher-order somatosensory cortical areas (Krubitzer and Kaas, 1990). By comparison, ipsilateral cortical projections to the MI whisker region originate mainly from SI and PPC, and then, to a lesser extent, from CS, SII, and PV.

Relative contributions of SI and contralateral MI

Statistical analysis of the data in Figure 5 indicates that some cortical areas differ significantly in the relative proportion of projections that they contribute to the whisker and forepaw regions in MI. The SI cortical area, for example, contains more than half (51.5%) of the projections to the MI forepaw region, but contributes only 17.6% of the projections to the MI whisker region \((t_{22} = 8.87; p = 5.09 \times 10^{-5})\). By comparison, more than half (51.6%) of the projections to the MI whisker region originate from contralateral MI, but the callosal projections from this area represent only 27.7% of the neurons that project to the MI forepaw region \((t_{22} = 4.88; p = 3.50 \times 10^{-5})\).

Consistent with these differential projection patterns, Figure 6 indicates that the mean density of labeled neurons in SI is much higher if the tracer is placed in the MI forepaw region than if it is placed in the MI whisker region \((t_{22} = 1.97; p < 0.05)\). Conversely, the mean density of neuronal labeling in contralateral MI is higher for MI whisker injections than for tracer injections in the MI forepaw region \((t_{22} = 3.22; p < 0.01)\).

Projections from PPC to the MI whisker region

As indicated by Figures 2, 3, and 5, the PPC contains more labeled neurons after injecting the MI whisker region than after injecting the MI forepaw region. Statistical analysis revealed significant differences in the relative proportion of the projections that the PPC contributes to the whisker or forepaw regions in MI \((t_{22} = 5.75; p = 4.36 \times 10^{-6})\). The PPC, on average, contains 7.7% of the neurons that project to the MI whisker region, but contains only 1.6% of the neurons that project to
the MI forepaw region. Furthermore, compared with tracer deposits in the forepaw region, Figure 6 indicates that the density of neuronal labeling in the PPC is significantly higher when tracers are placed in the MI whisker region ($t_{22} = 2.77; p < 0.01$).

**Projections from OC/CS to the MI whisker region**

The amount of neuronal labeling in the OC/CS depends on whether the MI tracers were deposited into the whisker or forepaw regions. Comparisons of the flattened reconstructions in Figures 2 and 3 suggest that OC/CS projects much more heavily to the MI whisker region than to the MI forepaw region. Because of the inherent difficulty associated with identifying the CS region in flattened tangential sections, we verified that the infragranular labeling near the Rf was contained in the CS (see Fig. 4).

Our statistical analysis confirmed differences in the projections from the OC/CS to the whisker or forepaw regions in MI. Compared to tracer injections in the MI forepaw region, MI whisker injections produced significantly greater proportions of labeling in the OC/CS of both hemispheres (ipsilateral: $t_{22} = 2.07; p < 0.05$; contralateral: $t_{22} = 2.75; p < 0.01$). The whisker MI injection cases also had denser labeling in the contralateral OC/CS ($t_{22} = 2.20; p = 0.02$), but we did not detect any density differences in the ipsilateral OC/CS.

**Topographic Organization of Projections to the Whisker and Forepaw Regions in MI**

In one group of rats ($n = 7$), different tracers were placed in the MI whisker and forepaw regions of each rat. The feasibility of injecting different tracers into separate parts of MI is illustrated in Figure 7. In this case (DT121), TB was injected into a site where ICMS evoked contralateral movements of whiskers B1-3, and FG was deposited into a site located 1.55 mm away where ICMS evoked dorsal flexion of the contralateral forepaw. The TB deposit was approximately 600 μm in diameter, which suggests that it may have diffused into multiple rows of whisker representations. Nonetheless, as shown by the photomicrographs in Figure 7, the tracers in this case had an edge-to-edge separation of nearly 1 mm. Among the 7 rats that received different tracers in the whisker and forepaw regions, the edge-to-edge separation between the injections was only $775 \pm 175$ μm (mean ± SEM).

Figure 8 illustrates the relative topography of cortical labeling in case DT121. The labeling patterns in this case indicated that most cortical areas were dominated by local regions in which
neurons were labeled exclusively by one tracer or the other. Although the topography of projections to each injection site was somewhat fragmented across non-contiguous regions, a crude somatotopic organization was seen in many cortical areas. In some sites, however, neurons that project to the MI whisker region were intermingled with neurons that project to the MI forepaw region. As shown by Figure 9, the areas that contained overlapping labeling comprised only a small fraction of the total cortical area that contained labeled neurons. The amount of labeled overlap was not constant, however, but varied significantly across the regions that we analyzed ($F_{11, 72} = 1.99966; p < .05$). Furthermore, labeled overlap appeared mainly where the whisker and forepaw representations adjoined each other. The following section describes the relative topography of the whisker and forepaw-related projections in each cortical area.

**SI cortex**

Neuronal labeling in SI cortex was characterized by a topographic organization that matched the somatotopic patterns produced by CO labeling. In both single and dual tracer cases, most of the SI neurons labeled by tracer injections in the MI whisker region were located in SI barrel cortex. As reported previously (Alloway et al., 2004; Chakrabarti and Alloway, 2006), most of the SI projections to MI originate from regions in barrel cortex that are aligned with the septal zones of the layer IV barrel field. Other SI projections to the MI whisker region originate from the CO-poor “unresponsive zone” that lies between the whisker and forepaw representations of SI (Chapin and Lin, 1984; Fabri and Burton, 1991). By comparison, most SI neurons labeled by tracer deposits in the MI forepaw region were associated with the CO-labeled regions that mark the forelimb, wrist, and forepaw representations of SI (Chapin and Lin, 1984). Other SI projections to the MI forepaw region, however, were aligned with the CO-poor unresponsive zone. Consequently, as seen in Figure 8, most of the labeled overlap in SI was in the unresponsive zone. In fact, double-labeled neurons were often observed in the unresponsive zone of SI along with other intermingled populations of neurons that were labeled by one tracer or the other (data not shown).

**Higher-order somatosensory regions**

In rats that received two tracer injections, most of the neuronal labeling in PPC was dominated by projections to the MI whisker region. In both single and dual tracer cases, tracer injections in the MI
whisker region produced widespread neuronal labeling throughout the PPC, especially in its lateral parts (see Figures 2 and 8). By comparison, PPC neurons that project to the MI forepaw region were usually grouped in a medial region that has been designated as the posteromedial (PM) area (Fabri and Burton, 1991). Based on these patterns and the relatively small number of PPC projections to the MI forepaw region, tracer overlap in the PPC was minimal and only appeared around the fringes of the forepaw representation in the PM area.

In SII and PV, the distributions of the labeled projections to the MI whisker and forepaw regions are consistent with the topographic maps for these cortical regions (Carvell and Simons, 1987; Fabri and Burton, 1991). Whereas tracer injections into the MI whisker region produced neuronal labeling just lateral to the PMBSF, the projections to the MI forepaw region were located more rostrally at sites lateral to the ALBSF. Furthermore, the forepaw-related neurons in SII adjoined the forepaw-related neurons in the PV region. The whisker-related neurons in PV were located lateral to the forepaw region but were medial to the Rf. Hence, the anatomical distribution of labeled neurons in the SII and PV regions of the dual tracer cases reflect the presence of multiple mirror-image body maps in these somatosensory cortical areas.

As illustrated by the selected sections for case DT121 (see Fig. 8), the density and distribution of neuronal labeling was more variable in PR than in SII or PV. Tracer injections in the MI whisker region produced considerably more labeling in PR than the tracer injections in the MI forepaw region. Consequently, PR did not appear to possess a clear topographic organization, and the amount of labeled overlap in the PR region was minimal. These findings are consistent with other results that suggest an absence of topographical organization in the PR area (Fabri and Burton, 1991).

**Auditory and visual cortical areas**

Although some labeled neurons appeared in the TC or VC after placing tracers in the MI whisker region, tracer injections in the MI forepaw produced little retrograde labeling in these areas. Consequently, labeled overlap in the TC and VC was virtually non-existent and the present study provided no evidence about the topography of the projections from these regions to MI cortex.
Clastrum and entorhinal cortex

Labeling in the OC/CS region did not follow a somatotopic pattern. Although a large number of neurons in the rostral half of the CS project to the MI whisker region, comparatively few CS neurons were labeled by tracer deposits in the MI forepaw region. The few neurons in the OC/CS that project to the MI forepaw region were scattered among those project to the MI whisker region and did not form any topographic pattern. This is consistent with previous reports showing a very general topography in which CS projections to sensorimotor cortex originate from the rostral half of this elongated structure, whereas those that project to the visual or auditory cortices originate from its caudal half (Li et al., 1986; Sadowski et al., 1997).

The EC contained a small number of labeled neurons even when both MI regions were injected in the same rat. Labeled overlap in the EC was about the same as in the OC/CS in percentage terms, but the EC area that contained intermingled populations of neurons labeled by different tracers was minimal.

Motor cortex

The relative topography of retrograde labeling in the contralateral MI cortex was consistent in both the single tracer and double tracer experiments. The bulk of the callosal neurons that project to the MI forepaw region are located caudally and laterally to the callosal neurons that project to the MI whisker region. These forepaw-related neurons are distributed along the medial edge of the CO-dense region that defines the SI forepaw representation. Furthermore, the MI forepaw region occupies a broad region in the mediolateral dimension that appears similar to the topography of Agl cortex. By comparison, the whisker-related neurons in the contralateral MI area occupy an elongated region along the midline of the hemisphere, and this distribution appears similar to the topography of Agm cortex.

In some sections through the contralateral MI region, a separate population of forepaw-related neurons appeared rostral to the “main forelimb” region. As shown in Figure 8C, this small neuronal population is separated from the “main forelimb” region by an area with very few labeled neurons. This pattern of two distinct regions that contained labeled projections to the MI forepaw region suggests that the rostral region may correspond to the “rostral forelimb area” that has been identified
by ICMS and anatomical tracing in some studies (Donoghue and Wise, 1982; Neafsey and Sievert, 1982; Sanderson et al., 1984; Neafsey et al., 1986; Rouiller et al., 1993; Tandon et al., 2008).

The amount of labeled overlap in the contralateral MI cortex was proportionately larger than in any other cortical area, and this difference was significant when contralateral MI overlap was compared with the labeled overlap in the EC, PR, VC, TC, or contralateral OC/CS. The location of overlap in the contralateral MI was not random, however, but was largely confined to the area where the rostral and caudal forelimb regions adjoin the region that contained labeled projections to the MI whisker area. Inspection of these overlapping areas revealed slender regions where neurons labeled by one tracer were intermingled with neurons labeled by the other tracer. In addition, as shown by Figure 10, these regions usually contained neurons that were double labeled because their axonal terminals innervated both injection sites (Weiss and Keller, 1994). Although some double-labeled neurons were occasionally seen in isolation among a small population of neurons labeled by only one tracer, most MI regions with labeled overlap contained several double-labeled neurons in close proximity to each other.
Table 2. Locations of retrograde tracer injections.

<table>
<thead>
<tr>
<th>Injected Tracer</th>
<th>ICMS Response</th>
<th>Tissue Volume (mm³)</th>
<th>Injected Tracer</th>
<th>ICMS Response</th>
<th>Tissue Volume (mm³)</th>
</tr>
</thead>
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<tr>
<td>DT 41</td>
<td>FG</td>
<td>Wh-B2</td>
<td>0.0912</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT 48</td>
<td>FG</td>
<td>Wh-C1,2</td>
<td>0.0594</td>
<td></td>
<td></td>
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<tr>
<td>DT 49</td>
<td>RB</td>
<td>Wh-B1</td>
<td>0.0279</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
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<td>RB</td>
<td>Wh-B1</td>
<td>0.0279</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
<td>DT 85</td>
<td>FG</td>
<td>Wh-D1,2</td>
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<td></td>
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</tr>
<tr>
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<td>Wh-C1</td>
<td>0.1618</td>
<td></td>
<td></td>
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<tr>
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<td>Wh-C row</td>
<td>0.0338</td>
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<td>Forepaw</td>
</tr>
<tr>
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<td>Wh-C2,3,4</td>
<td>0.2977</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
<td>DT 102</td>
<td>RB</td>
<td>Wh-C2,3,4</td>
<td>0.2977</td>
<td>RB</td>
<td>Forepaw</td>
</tr>
<tr>
<td>DT 104</td>
<td>RB</td>
<td>Wh-C2,3,4</td>
<td>0.2977</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
<td>DT 107</td>
<td>RB</td>
<td>Wh-C2,3,4</td>
<td>0.2977</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
<td>DT 109</td>
<td>RB</td>
<td>Wh-C2,3,4</td>
<td>0.2977</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
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<td>RB</td>
<td>Wh-B3,4</td>
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<td>FG</td>
<td>Forepaw</td>
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<tr>
<td>DT 116</td>
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<tr>
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<td>FG</td>
<td>Forepaw</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Wh-B1,2,3</td>
<td>0.1284</td>
<td>FG</td>
<td>Forepaw</td>
</tr>
</tbody>
</table>

mean (SEM)       | 0.1183 (0.027) | 0.1721 (0.060)
Fig. 1 Responses to ICMS and tracer injections in representative cases. 

a Responses to threshold-intensity (TI) ICMS in case DT83; red = RB injection (cf. Fig 2).

b Responses to TI ICMS in case DT109; green = FG injection (cf. Fig 3).

c Responses to TI ICMS in case DT121; blue = TB injection (cf. Fig 7).
Fig. 2 Distribution of callosal and corticocortical projections to the MI whisker region in case DT83.  

**a** Tangential sections through layer IV in the left and right hemispheres that were processed for cytochrome oxidase (CO). The boundaries of the cortical areas were outlined on the basis of CO labeling and previous studies, as explained in the text.  

**b** The appearance of the AGm injection site in an IGr tangential section after an RB injection into Layer V. The injection site and the surrounding tissue in the photomicrograph correspond to the insert in panel a.  

**c** Recontructions of the location of labeled neurons in the contralateral and ipsilateral hemispheres. Each hemispheric representation is based on superimposing the six reconstructed sections, two supragranular and four infragranular, located immediately adjacent to layer IV.  

**d** The appearance of dense retrograde labeling in the contralateral MI cortex. Scales, 1 mm (a and c); 250 μm (b); 25 μm (d).
Fig. 3 Distribution of callosal and corticocortical projections to the MI forepaw region in case DT109.

a Tangential sections through layer IV in the left and right hemispheres that were processed for CO. The boundaries of the cortical areas were outlined on the basis of CO labeling and previous studies, as explained in the text. b The appearance of the injection site in an IGr tangential section after FG was iontophoretically injected into Layer V. The injection site and the surrounding tissue in the photomicrograph correspond to the insert in panel a. Note that, in comparison to the whisker MI injection site shown in Fig 2b, this injection is more lateral, but of comparable size. c Reconstructions of the location of labeled neurons in the contralateral and ipsilateral hemispheres. Each hemispheric representation is based on superimposing the six reconstructed sections, two supragranular and four infragranular, located immediately adjacent to layer IV. d The appearance of dense retrograde labeling in the ipsilateral SI cortex, which is centered in the “forepaw” representation (Chapin and Lin, 1984). Scales, 1 mm (a and c); 250 μm (b); 25 μm (d).
Fig. 4 Rostrocaudal extent of retrograde labeling in the claustrum of a rat that received fluoro-gold (FG) in the MI whisker region. 
a Nissl-stained coronal section located 1.2 mm rostral to bregma; rectangle depicts the region in the next panel. 
b Cytoarchitecture of the claustrum and its proximity to the rhinal fissure Rf); rectangle depicts the region in the next panel. 

c, c’ Adjacent sections show the claustrum and its FG-labeled neurons; asterisks indicate identical blood vessels. 
d Coronal section through the same hemisphere, but located 1.3 mm caudal to bregma; rectangle depicts the region in the next panel.

e At caudal levels, the claustrum is more ventral with respect to the Rf. Rectangle depicts region in the next panel.

f, f’ Magnified views of the claustrum and FG-labeled neurons; arrows indicate the same blood vessels in both panels. Scale bar in a, b 1.0 mm, b’,c’,e 200 μm, c, c’ and f, f’ 50 μm.
Fig. 5 Mean proportion of labeled neurons observed in each cortical area following tracer injections in the whisker or forepaw regions in MI. For each tracer injection group (whiskers or forepaw), the sum of all proportions equals 100%. Bars represent mean proportion based on data reconstructed from 12 injections in each MI region; brackets represent SEM. Asterisks indicate cortical regions where group means were significantly different (Student’s t-test, *, p < .05; **, p < .01).
Fig. 6 Mean density of labeled neurons located in each cortical area following tracer injections in the whisker or forepaw regions in MI. Asterisks indicate cortical regions where group means were significantly different (Student’s t-test, *, p < .05; **, p < .01).
Fig. 7 Paired MI injections in a rat that received True Blue (TB) in B1-3, and FG in forepaw MI (DT121). 

a Photomicrograph of the two injection sites in an infragranular flattened horizontal section. Medial is left and caudal is “bottom.”

b A magnified view of the TB injection site in whisker MI. The area in this panel corresponds to the area enclosed by the rectangle on the left in a.

c A magnified view of the FG injection site in forepaw MI. This panel corresponds to the rectangle on the right in a. 

Scale bar in a, 500 µm, b and c, 250 µm.
Fig. 8 Bilateral distribution and topography of cortical neurons that project to whisker and forepaw MI in case DT121. a A pair of tangential sections located immediately superficial to the CO-processed sections. Blue neurons are TB-labeled and project to whisker MI; green neurons are FG-labeled and project to forepaw MI. As in Figures 2 and 3, multiple cortical areas have been delineated in sections from the ipsilateral hemisphere. b Overlap (150-μm² bins) in the sections shown in a. Blue bins contain at least one neuron labeled by TB, green bins contain at least one neuron labeled by FG, and white bins contain at least 1 neuron labeled by each tracer, or at least one double-labeled neuron. The white outlines represent SI cortex. c Anatomical reconstructions of two infragranular sections located immediately deep to the CO-processed sections. d Overlap (150-μm² bins) in the sections shown in b. Scale bar for all panels, 5 mm.
Fig. 9 Amount of overlap (bin width = 150 μm) between neurons that project to whisker MI and to forepaw MI. Overlap was low overall, but varied significantly across regions (p < .05). Neurons that project to MI whisker and to MI forepaw were significantly more intermingled in contralateral MI and ipsilateral SI than in TC and VC. Contralateral MI also had significantly more bins with overlap than EC and contralateral CS.
Fig. 10 Double-labeled neurons in contralateral MI following paired injections into whisker and forepaw MI (case DT121). a In the most medial part of the main Fp-related labeling in contralateral MI, some of the FG-labeled neurons were also labeled by TB. b This photomicrograph of double-labeled neurons was taken from the most caudal and lateral region of whisker-related labeling in contralateral MI. Scale, 25μm.
Chapter 4. Discussion

The present study used both single- and dual-tracer paradigms to characterize the distribution of cortical projections to the whisker and forepaw representations in MI cortex. Compared to the MI forepaw region, which receives most of its cortical inputs from ipsilateral SI, the MI whisker region receives significantly more cortical inputs from callosal projections that originate in the homotopic part of the contralateral MI cortex. Among the remaining cortical areas that were analyzed, the ipsilateral PPC and the OC/CS regions in both hemispheres contributed more projections to the MI whisker region than to the MI forepaw region. Finally, results from the dual-tracer experiments revealed minimal overlap among the populations of neurons projecting to the whisker or forepaw regions in MI. Most cortical regions contained a crude topographic organization in which labeled overlap was apparent where the whisker and forepaw regions adjoined each other.

**MI Callosal Connections May Modulate Bilateral Coordination**

Our data show that contralateral MI cortex contains the largest, densest population of neurons that project to whisker MI. Our finding that a higher proportion of contralateral MI neurons project to MI whisker than to MI forepaw concurs with reports that in cats (Pappas and Strick, 1981b) and primates (Pandya et al., 1969; Pandya et al., 1971; Kunzle, 1976; Jenny, 1979; Killackey et al., 1983; Rouiller et al., 1994; Fang et al., 2008) the distal forelimb areas of MI have sparse callosal connections but that more proximal representations have dense interhemispheric connections.

The significant population of contralateral MI neurons that project to whisker MI may mediate the synchrony of spontaneous rhythmic whisking (Sachdev et al., 2003). Alternatively, the callosal connections of whisker MI may support bilateral coordination of stimulus-related changes in whisking behavior (Mitchinson et al., 2007). When whiskers on one side of the face contact an object in the course of exploratory whisking, the amplitude of whisking on both sides of the face changes; specifically, whisking amplitude ipsilateral to the contact decreases, while whisking amplitude contralateral to the contact increases (Mitchinson et al., 2007). Rhythmic whisking depends not only on whisker MI activity (Lovick, 1972; Semba and Komisaruk, 1984; Gao et al., 2003), but also on the activity of a subcortical central pattern generator which seems to involve or be based in the facial nucleus (CPG) (Carvell et al., 1996; Hattox et al., 2002; Cramer and Keller, 2006). Primary motor
cortex can modify CPG output by controlling the number and firing rate of activated vibrissal motor neurons in the facial nucleus; thus MI can adjust whisking amplitude and frequency (Cramer and Keller, 2006). The dense callosal connections of whisker MI could facilitate quick, bilaterally-coordinated modulation of CPG output in response to unilateral stimulation, thus mediating a transition between high-amplitude, low-frequency exploratory whisking that is used to locate and make contact with an object, and low-amplitude, high-frequency discriminative whisking that is used to acquire information about an object’s texture, shape, and size (Harvey et al., 2001).

**PPC Participates in Sensory-Motor Cortical Circuits**

According to our data, more neurons in the ipsilateral PPC project to whisker than to forepaw MI. The PPC contains head-direction cells (Chen et al., 1994), and is critically involved in the brain’s representation of allocentric space and related orienting behaviors (Crowne et al., 1992). Most studies have focused on the functions of projections from visual or auditory cortical areas to PPC, but projections from SI to PPC implicate the region in somatosensory processing as well (Pons & Kaas, 1986; Kesner et al., 1989; Chen et al., 1994; Reep et al., 1994; Tees, 1999). It has been suggested that one of the PPC’s functions is to help to guide exploratory action based on tactile information (Dijkerman and de Haan, 2007).

The proposal that PPC helps to optimize exploratory movements based on somatosensory input is intriguing because of the relationship between whisker and head movement. Rats actively explore the tactile characteristics of their environment with their whiskers and alter the amplitude of whisking bilaterally when unilateral contact with an object is made (Kleinfeld et al., 2006; Mitchinson et al., 2007). Variations in bilateral whisking asymmetry precede horizontal head rotation by about 115 ms, or the duration of approximately one bout of whisking (Towal and Hartmann, 2006). Before the rat turns its head to explore a region of space, a circuit predicts what space the head will occupy if this movement is performed; the whiskers then verify that there is room to do so. To do this, the rat abbreviates whisks on the side of the face to which the head will turn, such that the whiskers achieve peak protraction sooner, and maintain a more caudal position, than the whiskers on the other side of the face (Towal and Hartmann, 2006). These modifications seem to fit with a system in which cortex is involved in the command to structure whisking about a relatively protracted or retracted “set point”
Thus the rat modifies amplitude and frequency to guide heading direction. It is possible that PPC’s projections to ipsilateral whisker MI reflect the involvement of this area in the septal circuits’ “regulation of whisker motion” (Alloway, 2008). In this model, bilateral coordination of whisking behavior with head turning could be accomplished by transmission of spatial- and tactile- related information from PPC to callosal projection neurons in whisker MI.

**Claustral Support for Bilateral Coordination**

The data presented here demonstrate that more neurons, both in the ipsilateral and the contralateral OC/CS, project to whisker MI than to forepaw MI. Previous work from our lab shows that whisker MI projects more heavily to the contralateral than to the ipsilateral CS, but forepaw MI projections preferentially target the ipsilateral CS (Alloway et al., 2009). Taken together, these findings are evidence of an indirect circuit by which whisker MI output may be bilaterally coordinated. Because of the convergence of motor and higher-order sensory information on the claustrum (Edelstein and Denaro, 2004), it is possible that the OC/CS conveys integrated sensorimotor information to whisker MI that modulates or reinforces the effects of whisker MI’s callosal connections.

**Technical Considerations**

**Histology**

Our conclusions are constrained by the fact that not all cortical regions can be reliably distinguished in horizontal sections. For example, both cingulate (Vogt and Miller, 1983) and retrosplenial (Shibata et al., 2004) cortices project to MI, but CO-stained horizontal sections do not allow differentiation between these areas and the adjacent MI. Thus, the labeling in these regions was excluded from our quantitative analysis. This could be problematic because we did observe substantial labeling in the area where Cg and Rs should be, particularly in cases where whisker MI was injected (see Fig. 2), so failure to collect and analyze data from these 2 areas might obscure important features of the distribution and topography of cortical neurons that project to MI cortex.
Electrophysiology

A major difference between the present study and many of the previous anatomical studies of cortical projections to MI is that we consider Agm to include vibrissal MI and made "whisker MI" injections at coordinates consistent with this area, whereas other authors (Donoghue and Wise, 1982; Gioanni and LAMarche, 1985; Neafsey et al., 1986; Rouiller et al., 1993; Hoover and Vertes, 2007) have designated this cytoarchitectonic region as a pre- or supplementary motor area and grouped it with the anterior cingulate, prelimbic, and infralimbic regions into the medial prefrontal cortex. However, all whisker MI injection sites were electrophysiologically defined by stimulus-evoked retraction of contralateral whiskers at low (≤ 50 μA) microstimulation currents. The ability to consistently evoke whisker twitches with low-amplitude stimulation confirms that our injections were made in whisker MI (Foerster, 1936; Penfield and Boldrey, 1937; Phillips, 1956; Woolsey et al., 1979; Pappas and Strick, 1981a; Strick and Preston, 1982; Gould et al., 1986; Preuss et al., 1996; Graziano et al., 2002; Brecht, Krauss, et al., 2004; Burish et al., 2008; Tandon et al., 2008).


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