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Corticocortical projections to area 1 in squirrel monkeys (*Saimiri sciureus*)

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Abstract

Cortical area 1 is a non-primary somatosensory area in the primate anterior parietal cortex that is critical to tactile discrimination. The corticocortical projections to area 1 in squirrel monkeys were determined by placing multiple injections of anatomical tracers into separate body part representations defined by multiunit microelectrode mapping in area 1. The pattern of labeled cells in the cortex indicated that area 1 has strong intrinsic connections within each body part representation, and has inputs from somatotopically matched regions of areas 3b, 3a, 2, and 5. Somatosensory areas in the lateral sulcus, including the second somatosensory area (S2), the parietal ventral area (PV), and the presumptive parietal rostral (PR) and ventral somatosensory (VS) areas also project to area 1. Topographically organized projections to area 1 also came from the primary motor cortex (M1), the dorsal and ventral premotor areas (PMd and PMv), and the supplementary motor area (SMA). Labeled cells were also found in cingulate motor and sensory areas on the medial wall of the hemisphere. Previous studies revealed a similar pattern of projections to area 1 in Old World macaque monkeys, suggesting a pattern of cortical inputs to area 1 that is common across anthropoid primates.

Graphical abstract

Somatotopic projections to area 1 arise from areas 3b, 3a, 2, and intrinsically within area 1 (red). Area 1 receives topographically organized inputs from sensory and motor areas in the posterior and lateral parietal cortex and the frontal lobe (blue). Additional cortical regions in the posterior

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Competing Interests

The authors have no conflicts of interest to declare.

Author Contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JHK, CMC Acquisition of data: CMC. Analysis and interpretation of data: CMC, JHK. Drafting of the manuscript: CMC, JHK. Obtained funding: JHK.

Data Accessibility

The corresponding author, Dr. Jon H. Kaas, will provide all supporting data necessary to reproduce the methodology described here upon request.

parietal cortex, upper bank of the lateral sulcus, medial wall and prefrontal cortex send additional, though less clearly organized, projections to area 1 (green).

Keywords

Area 1; Somatosensory Cortex; Parietal Cortex; Cortical Connections

Introduction

Area 1 forms a second systematic representation of cutaneous receptors of the contralateral half of the body that lies just caudal to the primary somatosensory area, 3b, of primates. While the physiological organization of the contralateral body map in area 1 across a number of primate species is well understood (Merzenich *et al.*, 1978; Nelson *et al.*, 1980; Sur *et al.*, 1982; Felleman *et al.*, 1983; Pons *et al.*, 1985b; Krubitzer & Kaas, 1990; Coq *et al.*, 2004; Padberg *et al.*, 2005), our understanding of cortical connections to area 1 is limited and focused largely on those of the hand representation (Pons & Kaas, 1986; Burton & Fabri, 1995; Coq *et al.*, 2004; Padberg *et al.*, 2005; Négyessy *et al.*, 2013). We identified a more complete complement of cortical regions projecting to area 1 by injecting anatomical tracers at sites from medial to lateral across contralateral body map in squirrel monkey area 1. Using multiple distinguishable tracers injected into physiologically defined body part representations also allowed us to determine the somatotopic organizations of these projections. Thus, the results more fully define the cortical inputs to area 1, while revealing the topographic organization of difficult to access sensory and motor areas buried in the lateral sulcus and along the medial wall.

Methods

Four adult squirrel monkeys (*Saimiri sciureus*) were used in this study. The experimental procedures were approved by the Vanderbilt University Animal Care and Use Committee

and adhered to National Institutes of Health guidelines. All surgical procedures were performed under aseptic conditions.

Like other primates, the map of the contralateral body in squirrel monkey area 1 is inverted, with the face and hand lying laterally and the leg and foot more medial (Fig. 1; Sur *et al.*, 1982; Jain *et al.*, 2001). Similar to macaques and cebus monkeys, the body map in area 1 mirrors that in area 3b of squirrel monkeys (Nelson *et al.*, 1980; Sur *et al.*, 1982; Felleman *et al.*, 1983). Multiunit microelectrode recording was used to guide the placement of injections of multiple, distinguishable tracers into distinct body part representations in area 1. Two multiunit microelectrode mapping sessions, one to determine targets for injections and a second to confirm the boundaries of the representations that were injected and determine the physiological borders between areas, were performed in each animal. During each session, the investigator delimiting the receptive field was blind to the location of the electrode in the brain due to the placement of sterile surgical drapes to maintain aseptic conditions around the craniotomy. Surgical draping did not interfere with the experimenter's ability to provide somatosensory stimulation during mapping.

Surgical procedures

The monkeys were anesthetized with an initial dose of ketamine hydrochloride (10-50 mg/kg, i.m.), secured in a stereotaxic frame, and maintained on 0.25-5% isoflurane during surgical procedures. Heart rate, blood oxygen levels, and temperature were monitored throughout the mapping procedure. Each monkey was placed on a heating pad or under a heating lamp to maintain the body temperature at 37° C. A local anesthetic, lidocaine hydrochloride, was applied to the ears and subcutaneous skin before the skin was incised to expose the skull. A craniotomy was performed to expose all of areas 1 and 3b from the midline to the lateral sulcus, and the dura was removed. Electrophysiological mapping was performed under intravenous ketamine anesthesia (20-40 mg/kg/h in sterile saline) delivered through either a leg or tail vein and supplemental injections of xylazine (0.2-0.4 mg/Kg i.m.). The exposed cortex was kept moist with regular application of sterile saline or covered with silicone oil. Photographs of the surface of the brain were used to mark the placement of microelectrodes, responses at different sites, and relative locations of tracer injections and electrolytic lesions using blood vessels and sulcal patterns as landmarks. Descriptions of receptive fields determined during physiological mapping were recorded and drawings of each receptive field were made on standard drawings of the squirrel monkey body.

Multiunit mapping and injections

The first mapping session was performed to target distinct body part representations in area 1 for tracer injections. Low-impedance tungsten microelectrodes (1.0 mΩ at 1,000 Hz) attached to a stereotaxic arm were inserted perpendicularly into the cortex at multiple sites in rows across areas 1 and 3b, while multiunit recording was used to map receptive fields based on activity driven by stimulation of the skin with glass or wooden probes. The mediolateral level of each injection site was chosen after distinct representations of different body parts were found, and the rostral and caudal boundaries of area 1 were identified in rows or columns of recording sites with receptive fields on the face, hand, forearm, trunk, leg, and foot. As areas 1 and 3b both responded to light touches on the skin, the area 1 rostral border

with 3b was defined by a reversal of the progression of the receptive fields across the body. In the hand, for example, the digit tips are represented caudally in area 1 and the proximal palm along the rostral border (Sur, 1980; Sur *et al.*, 1982; Chen *et al.*, 2005; 2009). This organization mirrors that of area 3b, where the palm is represented along the caudal border with area 1 and the digit tips rostral near the area 3b/area 3a boundary. Mapping of the face representation in area 1 has been limited (Sur *et al.*, 1982; Jain *et al.*, 2001; Iyengar *et al.*, 2007; Cervevich *et al.*, 2014). We defined representations of the face in area 1 by identifying clear receptive fields on the face in area 3b and moving the electrode laterally until a mirror reversal in receptive fields could be identified. Along the central sulcus where much of the arm, trunk, and leg are represented, it was impossible to insert the electrode perpendicular to layer 4. In these locations, the progression of receptive fields from those in area 1 to those in 3b was determined by moving the electrode in steps of 200–300 μm down the caudal bank of the central sulcus, parallel to layer IV. In anesthetized squirrel monkeys, area 2 is not highly responsive to touch (Sur *et al.*, 1982; present results). Thus, the caudal border of area 1 with area 2 was determined by a loss of response or a change in responses from being good and elicited by cutaneous stimulation to weak and elicited by tapping on the skin, pressing or squeezing of a muscle, and moving the joints to stimulate deep receptors.

Once discrete representations of different parts of the body in area 1 were determined, the electrode was removed from the stereotaxic arm and replaced with a Hamilton syringe with a glass pipette tip attached to ensure that the injections were placed along the same angle at which the receptive fields were mapped. Pressure injections of 0.04–0.6 μl of up to five of each of following tracers were made: Cholera Toxin Subunit-B (1% CTB in distilled water, Millipore Sigma, St. Louis, MO), CTB conjugated to green alexa fluor-488 (1% CTB-G in distilled water, Invitrogen, Carlsbad, CA), CTB conjugated to red alexa fluor-594 (1% CTB-R in distilled water, Invitrogen), Diamidino Yellow (2% DY in 0.1 M phosphate buffer, Millipore Sigma, St. Louis, MO, or Dr. Illing Plastic GmbH, Breuberg, Germany), and Fluororuby (10% FR in distilled water, Invitrogen). Following the injections, gel film was inserted to replace the opened dura, the craniotomy was closed with dental cement and skin sutured shut. The animal was then recovered from anesthesia, and treated with prophylactic antibiotic and analgesics.

A second mapping session following the same procedures as the first was performed to determine the full extent of each body part representation in areas 1 and 3b and physiological boundaries of each area after 10–14 days of tracer transport. During this session, electrolytic lesions were made by passing cathodal current (10 μA) at the approximate sites of mirror reversals between 3b and 1 body representations. These lesions were later used to relate the physiological maps to the cortical myeloarchitecture (Fig. 1). Once electrophysiological mapping was complete, the animals were given a lethal dose of sodium pentobarbital (80 mg/Kg), and perfused through the heart with 1 L of phosphate buffered saline followed by 500–800 mL of 2% paraformaldehyde in buffered saline and 500–800 ml of 2% paraformaldehyde with 10% sucrose.

The cortex was separated from the rest of the brain. The sulci were opened, and each hemisphere was flattened and blocked (Fig. 2). The cortex ipsilateral to the injections was cut into two blocks. The main block contained the frontal and parietal lobes, including as

much of the upper bank of the lateral sulcus and insula as possible. In some cases, parts of the upper bank of the lateral sulcus and insula stayed attached to a second block of cortex that included most of the temporal lobe and rostral parts of the occipital lobe. In case 10-19, the occipital lobe was retained and analyzed for projections to area 1. Since no labeled cells were found in the occipital lobe in case 10-19, the occipital lobe in other cases was removed and used in unrelated studies. Flattened blocks were held between two glass slides and stored for cryoprotection along with the thalamus from each case overnight in 30% sucrose at 4° C.

Tissue processing

The flattened cortical blocks were cut tangential to the surface at a thickness of 40 µm on a freezing microtome. Subsets of sections were mounted unstained for fluorescence microscopy. Alternate series were appropriately processed to reveal tracers. In all cases, one series was reacted for CTB immunohistochemistry (Bruce & Grofova, 1992; Angelucci *et al.*, 1996) to visualize CTB filled cells. Briefly, sections were rinsed in 0.05 M tris buffered saline plus 0.5% Triton X-100 (0.5 % TBS+), incubated in 2.5% normal rabbit serum in TBS+, and rinsed again. Sections were then incubated in 1:4000 dilution of CTB primary antibody (Product #703, List Biological Laboratories Inc., Campbell, CA) in 2.5% normal rabbit serum, 2% TBS+ for four days at 4° C. Following primary incubation, sections were rinsed in 0.5% TBS+, incubated in secondary antibody (1:200 biotinylated rabbit anti-goat IgG from Vector ABC kit pk-4005 in 1% TBS+) for two hours at room temperature, rinsed again, incubated in ABC (Vector ABC kit PK-4005, Vector Laboratories, Burlingame, CA) in 1% TBS for two hours, rinsed in 0.5% TBS+ and then 0.01 M phosphate buffered saline (PBS). Finally, sections are incubated in VIP solution (Vector VIP kit SK-4600, Vector) in PBS for 2–15 minutes to reveal the reaction CTB-immunoreactive (CTB-IR) cells. Sections were then rinsed in distilled water to end the reaction. CTB-IR cells included those labeled by injections of CTB and CTB conjugated to fluorescent alexas. This allowed us the advantage of essentially filling area 1 with a single tracer to reveal all projections to area 1, while also allowing us to see if these projections were topographically organized by comparing the locations of the distinct CTB-G and CTB-R filled cells seen with fluorescence microscopy to the locations of CTB-IR cells, anatomical boundaries, the results of our physiological mapping in the cortex, and published maps of the body in the cortex (Sur *et al.*, 1982). Additional series of sections were processed to reveal anatomical boundaries. A modified Gallyas (1979) method for silver staining of myelinated fibers was used to reveal boundaries of cortical areas and sulcal patterns. See Jain *et al.*, 1998a for a detailed description of silver staining.

Data analysis

Distributions of neurons filled by each tracer were plotted with a fluorescent/brightfield Leitz microscope coupled to a computer running Neurolucida™ plotting software (MBF Bioscience, Williston, VT). The injection cores and the diffusion zones around them were outlined. Landmarks, including blood vessels, electrode tracts, and micro-lesions, were also marked for use during reconstructions. Tracer labeled cells in each cortical area were counted to determine projection strength (Table 1).

Digital photomicrographs of sections stained to reveal architecture were taken using a Nikon DXM1200 camera mounted on a Nikon E800 microscope (Nikon, Melville, NY). These digital images were then adjusted for contrast, saturation, lightness, and curves with Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) but were not otherwise altered. When multiple photomicrographs were required to image the whole section, they were stitched together in Adobe Photoshop to create an image of a single whole section.

Reconstruction

Since a single section rarely revealed all areal boundaries, series of cortical sections stained for myelinated fibers were used to determine the borders of cortical areas and anatomically confirm the location of injection cores and labeled neurons. The outline of the myelin stained sections, areal borders, sulcal patterns, and landmarks, such as blood vessels, electrolytic lesions, and damage left after injections, were drawn. These landmarks were used to align architectonic boundaries throughout the depth of the cortex and create complete reconstructions of the cortical areas. As for other monkeys, a strip of dense myelin staining characterizes all of area 3b in squirrel monkeys (Fig. 1B; Krubitzer & Kaas, 1990; Jain *et al.*, 1998b; Jain *et al.*, 2001; Qi & Kaas, 2004). Caudal to area 3b myelin staining revealed a strip of cortex that stained moderately for myelinated fibers. This moderately stained region matched the locations of area 1 responses during our physiological mapping, and a similar myelin staining pattern has been described for area 1 in other New World monkey species (Krubitzer & Kaas, 1990; Coq *et al.*, 2004). The anatomical border between the representations of the hand and face is indicated by a myelin-poor septum known as the hand face border (HFB; Jain *et al.*, 1998a; Jain *et al.*, 2001; Qi & Kaas, 2004). Though often less distinct, myeloarchitecture of the cortex caudal to area 1 was used to identify area 2 (Seelke *et al.*, 2012) and the caudal border of this area was consistent with measurements from previously published studies in squirrel monkeys (Gharbawie *et al.*, 2011). The extents of other cortical areas were similarly based on variations in the distribution of myelin and borders recorded in previous studies. Physiological mapping data was added to the anatomical reconstructions by aligning penetrations along the trajectory of the electrode tracks that could be seen in the stained fiber sections and matching the locations of injection sites and lesions. Plotted cortex sections indicating the injection sites and distribution of backfilled cells were then aligned to the reconstructed borders and physiological maps using blood vessels, electrolytic lesions, and the injection cores as landmarks (Fig. 1). In cases 10-19 and 11-33, we also analyzed the density of neurons labeled by each tracer injection determine if tracer labeled cells were evenly distributed, or grouped in any way that might suggest the locations of cortical areas or specific body part representations within them. To do this, each cortical reconstruction was overlaid with a 250 μ m square grid. The number of labeled neurons within each grid square was counted using Adobe Illustrator's document info panel. All reconstructions and density maps were created with Adobe Illustrator CS5™ (Adobe Systems).

Results

Seventeen injections of distinguishable tracers were placed across area 1 in four squirrel monkeys to reveal organized projections from multiple cortical areas to area 1 (Fig. 1, Table

2). Not all of these injections were confined to area 1, and the results from the injections that spread into areas 3b and 2 are described as well.

Ipsilateral corticocortical projections

Case 10-19. Four injections were placed into representations of the contralateral body in area 1 in case 10-19 (Fig. 1C). The first was an injection of CTB centered on the distal digit 3 representation in caudal area 1. The zone of tracer diffusion around the core of this injection encompassed sites responsive to digits 2 and 4, parts of the palmar pads, and into the digit representation of presumptive area 2. The arm region in area 1 was the target for an injection of CTB-R that included representations of the forearm, upper arm, and armpit. A small injection of FR was placed rostrally in area 1 into representation of the dorsal and lateral middle trunk. The CTB-G injection was centered on representations of the leg, including the knee, upper leg, and hip. The core of this injection extended slightly into the adjacent representation of the nearby trunk. Immunohistochemical staining revealed a diffusion zone around the CTB-G injection that spread across area 1 foot, leg and trunk representations, and slightly overlapped the FR injection. The diffusion zone of the CTG-G injection extended somewhat into the 3b trunk representation.

The four tracers injected into area 1 in case 10-19 revealed a distinct pattern of projections from somatosensory areas within the anterior parietal cortex (APC), and parts of posterior parietal cortex (PPC). Immunohistochemical staining of the three CTB injections into area 1 showed that we essentially filled area 1, and revealed extensive intrinsic connections and projections from areas 3b, 3a, and 2 (Fig. 3). The distribution of fluorescent labeled cells in areas 1, 3b, 3a, and 2 was similar to, though more restricted than, that of the CTB-IR cells. Backfilled CTB-G neurons from an injection into the leg representation sat medial to those labeled by FR and CTB-R into the representations of the trunk and arm. No fluorescent tracer labeled cells were found lateral to the lateral tip of the central sulcus in any APC area. Labeled cells were also found in cortex caudal to area 2, a region presumed to include PPC area 5. The field of label continued into the PPC, though these projections were only clear in sections reacted to reveal CTB-IR neurons. Making individual markers that represent labeled cells large enough to be easily seen in our illustrations made it difficult to distinguish discrete groupings of filled neurons in each APC area, so an analysis of the density of the labeled cells was performed (Fig. 4). When depicted in this way, regions of high CTB-IR cell density could be seen at the approximate mediolateral level of each of the CTB injections in all four APC areas and area 5, suggesting the strongest projections come from the same mediolateral level as each injection. Since an individual body part tends to be represented at approximately the same mediolateral level in areas 3a, 3b, 1, 2, and 5, it is likely that dense projections labeled at three different mediolateral locations in each APC area indicate topographically organized projections from these somatosensory areas to area 1. Density analysis also suggested topographic organization of projections from within areas. The high density of CTB-IR cells near the digit 3 representation injections in lateral area 1 showed that the strongest intrinsic connections to the injected digit 3 representation arose in nearby area 1 where fingertips are represented. The density of projections is lower along border of areas 1 and 3b where the palm is represented, and increases again in rostral 3b where the distal digits are represented. Regions of dense labeling in area 3a along its

caudal border with area 3b and in rostral area 2 also correspond to the expected locations of digit representations in these areas. Similar dense patches that match the somatotopic location of the area 1 injections are seen in areas 3b, 3a, and 2 at the levels of the CTB-R and CTB-G injections. Density analysis of the projections revealed by the fluorescent injections indicated a similar pattern of dense label in mirrored locations in APC areas after the CTB-R and FR injections into the arm and trunk representations and the CTB-G injection centered on the area 1 leg representation.

Because CTB-IR neurons were unevenly distributed in each APC areas, and density analysis suggested inputs to area 1 come from matched representations in other areas, we aligned the body maps derived from our physiological mapping in areas 1 and 3b to confirm that these differences were result of topographically organized projections, and not uneven staining or weak signal under fluorescence microscopy (Fig. 5). Within area 1, the clusters of CTB-IR neurons appeared to be localized within the injected body part representation (Fig. 5C), e.g. there was a patch of CTB-IR cells centered on the digit representation that was separate from a patch that tended to be clustered in the arm representation. The CTB-G injection diffused across a wider region of area 1 and included parts of the leg, arm, and foot representations. A third, somewhat less distinct collection of CTB-IR cells was centered on the representation of the leg, but spread over a similar region of cortex. When plots of CTB-G, CTB-R and FR labeled neurons were matched to the electrophysiological maps in area 1, a similar pattern of constellations of backfilled neurons centered in individual body part representations was revealed (Fig. 5D). CTB-R and FR filled neurons were found across the area 1 arm and trunk representations, while none were found in the region responding to stimulation on the hand and few passed beyond the trunk representation in to the representations of the more distal leg and foot. In contrast, after an injection of CTB-G into a region representing parts of the leg and trunk, labeled cells were found in representations of the leg, foot, and trunk, but not the arm or hand. Responses to skin stimulation could be consistently evoked from area 3b, and revealed a mirrored body map in this area. Much like in area 1, patches of CTB-IR neurons found in area 3b tended to cluster in the representations of body that had been injected in area 1 (Fig. 5). Matching the 3b body map to the pattern of fluorescent filled cells showed that inputs from area 3b to the parts of the body represented at the center of each of these injections tended to arise in matched body representations. No CTB-R, FR, or CTB-G cells were found in the 3b hand region, suggesting that the CTB-IR cells found in this region resulted only from the CTB injection in the area 1 hand. Area 3a was less consistently responsive to stimulation, however the few responses found here indicated another parallel, mirrored map of the body in area 3a as has been described for other primate species (Krubitzer & Kaas, 1990; Huffman & Krubitzer, 2001; Wu & Kaas, 2003; Seelke *et al.*, 2012). Distinct patches of CTB-IR, FR, CTB-R, and CTB-G filled cells that roughly matched our incomplete map of the body in area 3a indicated somatotopically organized inputs from area 3a to area 1. Area 2 was largely unresponsive during mapping sessions, however a mirrored representation of the contralateral body has been described in area 2 in macaque monkeys (Pons *et al.*, 1985a; b), and the few responsive sites in area 2 in case 10-19 suggested a hand representation caudal to the hand representation in area 1 (Fig. 5A). Groups of CTB-IR neurons in area 2 fell at the same mediolateral level as each of our CTB injections in case 10-19. CTB-R, FR, and

CTB-G cells labeled as the result of injections in the arm, trunk, and leg regions were found in area 2, at the approximately matching mediolateral level of each injection (Fig. 5D). No fluorescent tracer filled neurons were found in the area 2 hand representation, while this region did contain CTB-IR cells that were likely labeled by the CTB injection into the area 1 digits. No mapping was performed in area 5 in any of our squirrel monkeys, but partial body maps have been demonstrated here in other primate species (Pearson & Powell, 1978; Pons *et al.*, 1985b; Seelke *et al.*, 2012). Separate foci of CTB-IR label and the few CTB-G and FR filled neurons here suggest that projections from area 5 to area 1 may also be topographically organized. Taken together, our results show that projections from other APC somatosensory areas tended to arise in somatotopically matched representations of the body in area 3b, 3a, and 2, and possibly area 5.

Somatosensory areas of the lateral parietal cortex that lie in the upper bank of the lateral sulcus contained both CTB-IR and fluorescent tracer filled cells. There was extensive CTB-IR labeling in the second somatosensory area (S2) and the parietal ventral area (PV). A region of slightly darker myelin staining did indicate the location of area S2 and PV; however, no border between them could be determined from the fiber stained sections. Comparing CTB-IR pattern to the distribution of CTB-R, FR, and CTB-G cells (Fig. 3) in this region indicated the location of each area, and that the projections from at least areas PV and S2 to area 1 are topographically arranged. A dense patch of CTB-IR label was found in what we presume to be the S2 and PV hand representations near the center of the myelin dense S2/PV region. Separate foci of CTB-G filled cells resulting from the injection into area 1 leg representation helped to distinguish projections coming from PV and S2. One CTB-G focus was more caudal and ventral to the cells we presume were labeled by the CTB injection into the digit representation, and was densest along the ventrocaudal border of S2. The second focus of CTB-G filled neurons overlapped the region of CTB-IR staining at the center of the S2/PV region, though it tended to cover only the ventral half of this density, a region that corresponds to the location of the leg and foot representations in the ventral half of PV (Coq *et al.*, 2004). When compared to the inputs from the leg, foci of cells filled by the CTB-R and FR injections matched the location of the trunk and arm in relation to the leg in published body maps of S2 and PV (Krubitzer *et al.*, 1995; Qi *et al.*, 2002; Coq *et al.*, 2004). Thus, we conclude that labeled neurons in the caudal half of the myelin dense region along the upper bank of the lateral sulcus were in S2, while those that were more rostral were in PV. Two more somatosensory areas, the ventral somatosensory (VS) and parietal rostral (PR) areas, have been identified in both New and Old World monkey lateral parietal cortex (Krubitzer *et al.*, 1995; Qi *et al.*, 2002; Coq *et al.*, 2004). Neither VS nor PR have been specifically described in squirrel monkeys, however cells filled by all injections were found in their presumptive locations. Cells in the presumptive VS were ventral to S2 and PV. Only CTB-IR neurons were found rostral to PV in the location of presumptive PR. Neither PR nor VS have been extensively explored physiologically and the fluorescent signal from our injections was weak in this region, so it remains unclear if projections from PR and VS to area 1 are somatotopically organized. A low number of tracer filled cells in the upper bank of the lateral sulcus were outside of the known somatosensory areas in regions that may have included parts of the insula (Table 1). In this case, the field of label did not extend

into the ventral block of the cortex containing the rest of the insula, the lower bank of the lateral sulcus, and temporal lobe.

Motor, premotor, and supplementary motor areas in the frontal lobe all contained neurons labeled by each of the four area 1 injections in case 10-19 (Fig. 3). Each of these areas has previously been demonstrated to have a mosaic somatotopic map of the body in squirrel monkeys and other primate species (Gould *et al.*, 1986; Stepniewska *et al.*, 1993; Ghosh & Gattera, 1995; Wu *et al.*, 2000; Graziano *et al.*, 2002; Stepniewska *et al.*, 2009; Gharbawie *et al.*, 2010), and the distribution of labeled cells suggests organized projections from each motor area to area 1. Primary motor cortex (M1) contained CTB-IR, CTB-R, FR, and CTB-G filled cells. CTB-IR density analysis showed dense foci of CTB-IR cells at mediolateral levels that roughly corresponded to the level of each CTB injection (Fig. 4). Neurons backfilled by the CTB-R and FR arm and trunk injections were restricted to the region of M1 that was at the same level as these representations in area 1 (Fig. 4). CTB-G labeled neurons were distributed in medial M1, at the same level as this injection into the leg and trunk representations (Fig. 3), and were often intermingled with the medial-most red labeled cells in M1. The regions containing cells labeled by our fluorescent tracers correspond to the mosaic somatotopic map of the body in M1, and indicate inputs from M1 to area 1 arise from at least roughly topographically matched regions. Dorsal and ventral premotor areas (PMd and PMv, respectively) both projected to area 1. The distribution of labeled cells in PMd suggests organized projections to area 1, with CTB-G, FR, and CTB-R labeled neurons overlapping CTB-IR cells in the medial half of the area, but not in the more lateral region devoted to movements of the hand. All injections labeled neurons in PMv with CTB-R, FR, and CTB-G backfilled neurons overlapping CTB-IR cells along only a small rostral portion of the CTB-IR grouping, suggesting a body representation in PMv. The supplementary motor area (SMA) was medial to M1 and PMd and extend onto the medial wall contained labeled neurons. There was CTB-IR labeling in across this area. More restricted projections from SMA to area 1 were also indicated by the presence of fluorescent labeled cells. In SMA, the CTB-G cells did not extend as far rostral as the overall CTB-IR labeled field, indicating projections to the lower half of the body likely come from caudal SMA. The few red fluorescent cells labeled by the CTB-R and FR injections into the arm and trunk representations were mixed with the more rostral CTB-G filled neurons in the likely location of the arm and trunk regions of SMA. In the most rostral SMA, only CTB-IR cells were present indicating a projection from the SMA hand representation to the hand region of area 1. Rostral to the defined motor areas of the frontal lobe, CTB-IR, CTB-R, FR, and DY were also found in the prefrontal cortex (PFC).

The presence of multiple foci of CTB-IR and fluorescent labeled cells along the medial wall suggest the location of motor and sensory areas that have not been explored in squirrel monkeys (Fig. 3–4). A number of CTB-IR and fluorescent labeled cells were found in presumptive pre-SMA. Deeper in the cingulate gyrus, labeled neurons from each of our injections were found in the caudal and rostral cingulate divisions (Cgc and Cgr, respectively) where cingulate motor areas have been described in other primates (Picard & Strick, 1996; Wu *et al.*, 2000). Along the medial wall, the densest distribution of cells for all tracers was found in a region of the cingulate gyrus medial to the regions of area 1 and 3b that cross onto the upper part of the medial wall (Fig. 4). This appears to correspond to the

location of the supplementary sensory area (SSA) that has been described in monkeys (Blomquist & Lorenzini, 1965; Murray & Coulter, 1982).

Case 11-33. Five injections were made in case 11-33 area 1 (Fig. 1D). In the hand representation, CTB was injected into representations of distal digits 2, 3, and 4 and extended into adjacent representations of pads 3 and 4. The tracer diffused to include other parts of the digit and palm representations, and slightly into the area 2 hand representation. A FR injection was contained entirely in the area 1 arm representation, and covered a zone that included responses to touch and stroking of hairs on the dorsal forearm and upper arm. An injection of CTB-G centered on representation of the occiput and dorsal trunk diffused to include the parts of the representations of the shoulder and neck. Mapping in this case was incomplete, so only responses at the center of the final two injections can be described. Representations of the knee and distal upper leg were at the center of a DY injection in case 11-33. Finally, CTB-R was injected into representations of the foot pads, insole of the foot, the fourth toe, and part of dorsal toe 1 along the inner edge of the foot.

Injections of CTB and CTB conjugates, FR, and DY in case 11-33 revealed a pattern of corticocortical projections similar to that in case 10-19. CTB-IR cells were unevenly distributed throughout area 1 and clustered near each injection site in the area 1 hand, occiput/shoulder, and foot cortex (Fig. 6). These CTB-IR foci corresponded to regions of area 1 that contained little to no FR or DY label from injections placed between the CTB injections. Neurons labeled by the FR injection into the arm representation were in the general location of the arm representation, and the filled cells extended into the adjacent representation of the dorsal hand. For the most part, the backfilled DY cells resulting from the injection into the region representing the leg were in the leg representation, but were also found in representations of the trunk, arm. There was a small focus of DY labeled cells in a region overlapping the FR cells in the occiput/shoulder representation. Microelectrode mapping in this case was incomplete, and the DY injection may have spread into the expected representations trunk and foot. The diffusion of the injection beyond the boundaries of the leg representation may explain the expansion of DY labeled cells into seemingly mismatched body representations in area 1. Uptake of CTB-R in this case was low, and both the immunohistochemical and fluorescent signals were weak; however, a very low number of CTB-R filled neurons were found with fluorescence microscopy around the region of the CTB-R injection that corresponded to weakly stained CTB-IR cells found in the area 1 foot representation in the CTB-processed sections. Given that non-adjacent representations were not shown to project to each other in case 10-19, we feel that these foci of dense CTB-IR cells and cells labeled by FR and DY injections indicate strong intrinsic projections within each of the injected representations and from nearby representations of adjacent body parts. Areas 3b, 3a, and 2 contained CTB-IR and DY filled cells that were consistently in matching representations. The pattern of dense patches of label in somatotopically matched representations indicated somatotopically arranged inputs from each of the other APC areas to area 1. CTB-IR and DY cells were found just caudal to the area 2 border in area 5 and in more caudal parts of PPC. The upper bank of the lateral sulcus was damaged during the flattening procedure making case 11-33 the only case with cells in the ventral block that contained parts of the upper bank of the lateral sulcus, insula, lower bank of the lateral sulcus, and temporal lobe in other cases. Despite the damage to the upper

bank of the lateral sulcus on the main block, labeled cells were found in the S2/PV region. S2 was the most intact area, and topographically organized projections were indicated when the locations of CTB-IR and DY filled cells were compared. Neurons labeled by the injection of DY into the leg representation were concentrated caudally in S2, in the location of a break in the CTB-IR field of cells in the likely location of the S2 leg representation. The parts of PV and VS that were intact contained both CTB-IR and DY filled cells. Given the damage to the ventral block and the low number of cells (93 CTB-IR, 51 DY, and no FR) found there, these cells were not included in any further analyses.

Labeled neurons were found in frontal motor areas and along the medial wall in case 11-33 (Fig. 6). M1 contained both CTB-IR and DY labeled cells. CTB-IR cells and DY collected in foci that paralleled the mediolateral level of injected area 1 representations. No DY filled cells were found in the expected location of the hand representation in motor cortex. Lateral M1 did, however, contain a high number of CTB-IR neurons, the likely result of the CTB injection into the area 1 hand representation. Thus, a rough somatotopic arrangement of projections from M1 to area 1 is again indicated. PMd contained CTB-IR cells with collections in the approximate locations of the hand and foot representations in area PMd. The few DY labeled cells in PMd were in the more medial part of PMd, and they were caudal and lateral to those CTB-IR neurons we presume were labeled by the CTB-R injection into the foot. Patches CTB-IR cells were found in PMv, as were neurons backfilled with DY. SMA and the PFC also contained CTB-IR and DY filled neurons. The CTB injections into the area 1 hand, occiput/shoulder, and foot representations and the DY injection into the area 1 leg also labeled cells along the medial wall. Backfilled neurons resulting from these injections were found in pre-SMA, Cgr, Cgc, and the presumptive SSA.

Case 12-40. In case 12-40, we placed injections of three tracers into area 1 (Fig. 1E). We attempted to make an injection into the face representation in the lateral extreme of area 1; however, the center of this DY injection into a representation of the contralateral upper lip was on the border of areas 1 and 3b. The diffusion zone included representations of the nose and muzzle. In this case, the center of a CTB injection was in a region of caudal area 1 representing distal digits 2, 3, and 4. The diffusion zone in area 1 continued into the rest of the digit 3 representation, but also spread into rostral area 2, the presumed location of digit tip representations in area 2. FR was injected largely into the 3b representation of the dorsal forearm, though a small part of the core and diffusion zone did extend into area 1's representation of the dorsal forearm and hand.

Injections into area 3b/1 face, area 1 hand, and area 3b arm backfilled cells throughout the cortex. The injection of CTB caudal in area 1 into the digit representation revealed a pattern of projections similar to the area 1 hand injections in our other cases (Fig. 7). Only one CTB injection was placed in case 12-40, and CTB-IR cells were found in presumptive hand representations in APC, upper bank of the lateral sulcus, and motor areas. Fewer CTB-IR neurons were labeled in SMA, pre-SMA, and cingulate areas than in other cases. Since the DY injection was made on the border of areas 1 and 3b, cells filled with DY in this case may project to either the area 1 or 3b. Descriptions of face representations in cortical somatosensory areas are limited due to the difficulty accessing this region for physiological mapping and anatomy (Cerkevich *et al.*, 2014). It is difficult to clearly define borders

between areas outside of area 3b's densely myelinated face ovals (Jain *et al.*, 2001; Iyengar *et al.*, 2007; Cervevich *et al.*, 2014). Thus, we chose to indicate the presumptive locations of face representations in unmapped portions of area 1 and areas 3a and 2 based on measurement and without drawing distinct borders in this region. In general, the pattern of projections to the face representations in areas 3b and 1 injected here was similar to that to other body parts. DY labeled neurons in areas 1 and 3b indicate intrinsic connections within both areas and connections between the two areas. Presumptive face cortex in both areas 3a and 2 also contained DY backfilled cells. Laterally, cells labeled with DY were in presumptive face regions of PV, S2, and VS, as well in PR. Lateral M1 and PMv projected to the 3b/1 face representations. Very few DY cells labeled by the injection into the 3b/1 face representation were found in PMd. A low number of neurons filled by the DY injection were scattered in PFC, SMA, and the cingulate gyrus. The FR injection into the representations of the forearm and dorsal hand in area 3b resulted in cells distributed in a pattern that was unlike our area 1 injections. FR labeled neurons were found in areas 3b, 1, 2, 5, and M1, with a few cells scattered in the upper bank of the lateral sulcus and frontal cortex. A concentration of backfilled FR neurons was found in lateral area 1, just medial to hand face border. Area 1 was not mapped this far lateral, but this region likely contains responses to D1 and the radial half of the dorsal hand.

Case 12-59. In case 12-59, we placed five injections of three tracers into area 1 (Fig. 1F). Two DY injections were made. The first was placed laterally into a representation of the anterior head above the brow. While the core of this injection remained in area 1, tracer did diffuse into part of area 3b representing the cheek. A second injection of DY was placed medially in the dorsal trunk and shoulder representation. Again, the bulk of this injection was in area 1, though it diffused slightly into the 3b middle trunk representation. Separate CTB injections were placed in the hand and leg representations. The hand injection was confined to area 1 and covered the representation of the second digit and adjacent representations of the first and third digits and palmar pads. The leg injection was centered on the representation of the anterior knee. The diffusion zone of this injection covered much of the rest of the leg representation in area 1 and spread slightly into the area 3b leg representation. Finally, a single small injection of FR was placed in the area 1 arm representation. This injection covered most of the forearm representation, though it also included the lateral upper arm, and parts of the ulnar wrist and ulnar margin of the hand.

The pattern of projections to injection sites in area 1 face, hand, arm, trunk and leg representations was similar to other cases (Fig. 8). Again, intrinsic connections revealed by each injection of CTB, DY, and FR were largely from adjacent representations in area 1. A distinct patch of DY filled cells was found in area 1 lateral to a DY injection into area 1 shoulder and upper trunk. This patch indicated strong intrinsic inputs from the area 1 representation of the back of the head. Projections from outside of area 1 were also like those in other cases. CTB injections in the area 1 hand representations and area 1/3b leg representations lead to backfilled cells in somatotopically matched regions of areas 3b, 3a, 2, 5, and the PPC. Again, CTB-IR cells were found in S2 and PV. This field of CTB-IR cells in the upper bank of the lateral sulcus may also extend into the presumptive location of PR. Generally, the cells projecting to digit and leg representations in area 1 sat more laterally in the bank of the sulcus, matching the expected location of hand and leg in S2 and PV. As with

other cases, projections from M1 suggested topographically matched projections from both the hand and leg regions to area 1. CTB-IR neurons were also found in PMv. Along the medial wall, patches of cells in the medial portion of the split leg representation (Sur *et al.*, 1982) were difficult to distinguish from those in the SSA, Cgc, and caudal SMA. Projections from Cgr and preSMA to area 1 hand and leg representations were also indicated. The FR injection into the area 1 forearm representation resulted in a limited number of filled cells. These few cells were found largely in the presumptive arm and hand representations of areas 3b, 3a, 2, 5, and M1. A patchy strip of DY filled cells extended across the presumptive trunk and occiput representations in the APC and area 5 and M1, matching the somatotopy of the more medial DY injection in case 12-59. The lateral DY injection largely in the area 1 upper head and face representation resulted in DY filled cells in adjacent 3b and area 2. In S2 and PV, DY filled cells were found closer to the crest of the lateral sulcus, indicating projections from the likely face representations in S2 and PV. A few DY neurons were scattered more laterally in S2/PV. These may represent neurons filled via projections to the more medial DY injection in the area 1 trunk. A few DY cells were found laterally in the presumptive M1 face representation, PMv, and lateral PMd. Only weak inputs from areas along the medial wall to area 1 trunk or face were indicated in this case.

Discussion

Overall, our injections of tracers revealed a widespread pattern of corticocortical projections to area 1. Labeled cells in the cortex indicated that area 1 contained strong intrinsic connections that are generally restricted to the injected representation and the nearby representations of adjacent body parts. Area 1 received somatotopic inputs from adjacent parts of area 3b, mostly from matched body part representations. Weaker, but still somatotopically organized, projections from area 3a were also present. The cortex caudal to area 1, including presumptive area 2, parts of area 5, and adjoining posterior parietal cortex, also provided topographically organized inputs to area 1. Somatosensory areas in the lateral sulcus, including the second somatosensory (S2), parietal ventral (PV), parietal rostral (PR), and ventral somatosensory (VS) areas sent topographically organized projections to area 1 as well. Primary motor (M1), dorsal and ventral premotor (PMd and PMv), supplementary motor (SMA), pre-SMA, and motor and sensory areas in cingulate cortex also projected area 1, often in an organized fashion. Ipsilateral corticocortical projections to area 1 are summarized in figure 9.

Projections from other cortical somatosensory areas to area 1 tend to arise in matched representations

Projections revealed by our injections into area 1 suggested organization within the anterior parietal cortex indicative of four somatotopically organized areas (Fig. 9). Intrinsic connections in area 1 tended to arise from within individual body part representations, with some input to a given representation coming from nearby representations of adjacent regions of the skin. Non-adjacent representations of non-adjacent body parts did not appear to have strong connections with each other within area 1. The primary somatosensory area, 3b, provided some of the densest projections to area 1, and these appeared to come largely from matched body part representations. Similarly, when small amounts of anterograde

anatomical tracers were injected into the digit representations in area 3b, terminal label was largely confined to the matching finger representation in area 1, consistent with previous results from squirrel monkeys (Négyessy *et al.*, 2013). Rostral to area 3b, proprioceptive area 3a also provided organized inputs to area 1. In the cortex immediately caudal to area 1, distinct foci of retrogradely labeled cells were found in the predicted locations of the injected body part, providing evidence for a parallel body map in area 2. However, given the limited responsiveness of the area 2 region in anesthetized New World monkeys, an alternate proposal is that New World monkeys do not have an area 2, and area 1 is adjoined by area 5 (Padberg *et al.*, 2005; Padberg *et al.*, 2007). A map of the body that mirrors that in area 1 has been described in area 2 in macaque monkeys (Pons *et al.*, 1985a; b), and our results suggest a similar arrangement in squirrel monkeys. The connections of foot and hand representations in squirrel monkey area 1 with foot and hand representations in area 2 were similar to those described in macaques (Pons & Kaas, 1986).

Somatosensory areas along the upper bank of the lateral sulcus are difficult to access for microelectrode mapping, and contain cells with large receptive fields that make interpreting the somatotopic organization in these areas difficult. Body maps in S2 and PV have been described in macaque (Krubitzer *et al.*, 1995), marmoset (Qi *et al.*, 2002), and titi monkeys (Coq *et al.*, 2004). The locations in PV and S2 of cells backfilled by our injections across area 1 suggested body maps in PV and S2 that are like those previously described in other monkey species. We found cells labeled by our area 1 injections in the presumptive locations of both PR and VS; however, these areas have not been extensively mapped. The projections from PR and VS to area 1 we revealed suggest topographic organization, but were too weak to determine if they were somatotopically organized.

Mosaic body maps in motor areas provide organized inputs to area 1

We found projections to area 1 from M1, PMd, PMv, and SMA (Fig. 9). Somatotopically organized, mosaic maps of the body have been described in each of these areas in several primate species (Gould *et al.*, 1986; Picard & Strick, 1996; Wu *et al.*, 2000; Gharbawie *et al.*, 2011). M1, PMd, PMv, and SMA all contained cells labeled by each of our injections across the body map in area 1. Cells projecting to different body part representations in area 1 were in the expected location of the matching representation in each of the motor, premotor, and supplementary motor areas. Our results also suggested a nearly full body map in PMv. A large representation of orofacial and forelimb movements has been described in PMv, with variable reports about the existence of other body part representations (see Wu *et al.*, 2000 for comparison among primates). Cells in PMv were generally labeled by each of our injections into area 1, including those in the trunk and leg representations, suggesting at least a limited representation of the lower half of the body in PMv. Organized inputs from multiple motor areas to area 1 may provide area 1 with information about the planning and execution of movements during tactile discrimination tasks.

The locations of motor and sensory areas along the medial wall are suggested by projections to area 1

Distinct foci of cells retrogradely labeled by our injections into area 1 were found in multiple locations along the medial wall in our squirrel monkeys (Fig. 9). The most rostral

focus of cells is in the location of the pre-SMA. No clear organization of inputs from pre-SMA was suggested by the pattern of projections to area 1. The pre-SMA, lacking responses to somatosensory stimuli and any clear somatotopic organization, has been implicated in motor preparation (Picard & Strick, 1996). Our injections of tracers into area 1 resulted in two collections of labeled neurons in the cingulate cortex. These locations may correspond to the rostral cingulate motor area and one or more of the subdivisions of the caudal cingulate motor area that have been described in macaques (Picard & Strick, 1996). This part of the cingulate has not been extensively explored in the squirrel monkey, thus we describe cells projecting to area 1 as simply in the rostral and caudal cingulate divisions (Cgr and Cgc) until further evidence in squirrel monkeys confirms homology with the cingulate motor areas of other species. Finally, we found cells labeled by our area 1 injections in a region medial to areas 3b and 1. Responses to somatosensory stimulation have described in this location in squirrel monkeys (Blomquist & Lorenzini, 1965), and it has been named the supplementary somatosensory area (SSA) (Blomquist & Lorenzini, 1965; Murray & Coulter, 1982). We have tentatively followed this naming scheme in our squirrel monkeys, and our connections to area 1 may also match the rostral and caudal locations of the upper and lower body representations that have been described in the SSA.

Conclusions

Our results, based on 17 injections of retrograde tracers placed at physiologically identified sites in area 1 of four squirrel monkey, allow several well supported conclusions. Intrinsic connections within area 1 arise largely from within a single body part representation, though connections between representations of adjacent body parts do exist. Our physiological mapping of area 3b in addition to area 1 confirmed that projections from 3b to area 1 are somatotopically organized. Neither areas 3a and 2 are particularly responsive to somatosensory stimulation in anesthetized squirrel monkeys. However, our limited mapping results and the somatotopic organizations of areas 3a and 2 described in other primates suggest that projections from areas 3a and 2 to area 1 are also somatotopically organized. Inputs to area 1 from somatosensory areas in the posterior and lateral parietal cortex, including area 5, the second somatosensory area (S2), parietal ventral area (PV), parietal rostral area (PR) and the ventral somatosensory area (VS) are topographically organized, though the somatotopy is less clear. Motor areas with known mosaic body maps (primary motor cortex (M1), the dorsal premotor area (PM), and ventral premotor area (PMv) also send topographic inputs to area 1. Finally, motor and sensory areas on the medial wall project to area 1. Little is known about these areas in squirrel monkeys, and further study is required to understand how they relate to cingulate motor and sensory areas described in other primate species.

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Abbreviations

APC	Anterior Parietal Cortex
Cgc	Caudal Cingulate
PMd	Dorsal Premotor Area
OFC	Orbitofrontal Cortex
PR	Parietal Rostral Area
PV	Parietal Ventral Area
Pre-SMA	Pre-Supplementary Motor Area
PFC	Prefrontal Cortex
PPC	Posterior Parietal Cortex
M1	Primary Motor Area
CS	Central Sulcus
CgS	Cingulate Sulcus
INS	Insula
LS	Lateral Sulcus
UBLS	Upper bank of the lateral sulcus
LBLS	Lower bank of the lateral sulcus
STS	Superior Temporal Sulcus
BDA	Biotinylated dextran amine
CTB	Cholera Toxin Subunit B
S2	Secondary Somatosensory Area
SMA	Supplementary Motor Area
SSA	Supplementary Sensory Area
PMv	Ventral Premotor Area
VS	Ventral Somatosensory Area
HFB	Hand Face Border
CTB-G	Cholera Toxin Subunit B conjugated to green alexa fluor-488
CTB-R	Cholera Toxin Subunit B conjugated to red alexa fluor-594

CTB-IR	Cholera Toxin Subunit B immunoreactive
DY	Diamidino Yellow
FR	Fluororuby

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Figure 1. Characterization of injection sites

A. The somatotopic map of area 1 (shaded in gray) and area 3b that was used for guidance during physiological mapping. Adapted from Sur et al., 1982 and Jain et al., 2001. Body part abbreviations: A- arm, D- digits, F- face, Ft- foot, H- hand, HFB- hand face border, L- leg, LL- lower lip, O- occiput, P- pads, T- toes, Th- teeth, Tn- tongue, Tr- trunk, UL- upper lip.

B. Photomicrograph of a section from case 10-19 stained to reveal myelinated fibers. Area 1 is a region with moderate staining for myelin caudal to the more densely stained area 3b. Regions of lighter myelin staining indicate the location of the central sulcus and the hand

face border. Darts mark the locations of electrolytic lesions and injection damage used for aligning physiological maps and plots of tracer filled cells to architectonic boundaries. The dashed box indicates the region of cortex seen in the reconstruction in C. C-F. Maps of responses to somatosensory stimulation on the contralateral body in areas 1, 3b, 3a, and 2 during multiunit microelectrode mapping. Physiological mapping data was added to anatomical reconstructions by aligning penetrations to landmarks including visible electrode tracks, tracer injection cores, and micro-lesions seen in sections processed to reveal myeloarchitecture and tracers. Black lines are areal boundaries indicated by myeloarchitecture. The central sulcus (CS) is shaded in gray. Circles are color coded to indicate the area of skin stimulated to elicit responses at that cortical location during mapping. Points linked by fine lines are on the same electrode track parallel to layer 4 along the banks of the CS. Injections sites are outlined and shaded in color. Colored stars are the locations of needle penetrations for injections made during the first mapping session. Gray stars are electrolytic lesions placed at the approximate location of the area 1/3b border during the second mapping session. Abbreviations as listed above. Rostral is left, and medial is upward. Scale bars are 2 mm.

Figure 2. Cortical flattening procedure

A. Lateral view of the left hemisphere of case 10-19. B-F. Opening of sulci, blocking of the hemisphere, and subsequent unfolding of the cortex. Black lines are the crests of sulci. White lines are the sulcal fundi. The dotted line in panel B indicates the approximate location where the occipital lobe was dissected away in cases 11-33, 12-40, and 12-59. The dotted line in C indicates when the frontal and parietal lobes were separated from the insula, temporal and occipital lobes in this case. The cortex was blocked in this manner so that sections cut tangential to the surface would fit on to glass slides for microscopy.

Figure 3. Distribution of cells labeled by CTB, CTB-R, FR, and CTB-G injections into area 1 digit, arm, trunk, and leg representations in case 10-19

A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of CTB-R and FR neurons. Each marker indicates a single labeled cell. Areal boundaries were determined using myeloarchitecture. Thin black lines indicate borders of areas. Thick black lines denote tears and the edges of the block. Gray shaded regions indicate the central sulcus, upper bank of the lateral sulcus (UBLS), OFC, and medial wall. The CTB, CTB-R, and CTB-G injection sites are outlined and shaded in blue, red, and green, respectively. Abbreviations as listed above. Rostral is left, and medial is up. Scale bar is 5 mm. Inputs to each injected representation were similar across area 1. There were strong intrinsic projections within area 1. Projections from APC arose from areas 3b, 3a, and area 2. The density of the labeled cells in APC areas suggests that projections arise in matched body part representations in area 3b, 3a, and 2. The field of label extended into PPC. There was extensive labeling in the somatosensory areas of the lateral sulcus including areas S2, PV, VS and PR. M1, PMd, PMv, SMA, pre-SMA and cingulate areas also contained back filled cells. Fluorescent labeled cells show that many of these inputs come from restricted parts of each cortical area that match the injected representation.

Figure 4. Density of projections labeled by CTB, CTB-R, FR, and CTB-G injections into area 1 digit, arm, trunk, and leg representations in case 10-19

A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The density of CTB-IR labeled cells. C. The density of CTB-G labeled cells. D. The density of CTB-R and FR labeled cells. Each colored square indicates the number of cells per 250 × 250 μm box. Darker colors indicate a higher number of cells. Abbreviations as listed above. Conventions as in figure 1. Density analysis reveals that the densest projections to a given injected region in area 1 arise in somatotopically matched

representations in other areas, and allowed us to distinguish the locations separate medial wall areas containing labeled cells.

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Figure 5. Topographic organization of projections in the anterior parietal cortex

A. The results of multiunit microelectrode mapping in anterior parietal areas in case 10-19. Conventions as in figure 1. B. Color coded physiological maps in areas 1, 3b, 3a, and 2. Sites responsive to the same body part were outlined in each area to create these color-coded body maps in each area. C. The distribution of CTB-IR cells related to the body maps in APC areas. D. The distribution of CTB-R, FR, and CTB-G filled cells related to body maps in APC areas. Each injected body representation in area 1 received inputs from somatotopically matching representations and nearby representations of adjacent body parts in areas 1, 3b, 3a, and 2.

Figure 6. Distribution of cells labeled by CTB, FR, CTB-G, DY, and CTB-R injections into area 1 digit, arm, trunk, leg and foot representations in case 11-33

A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of DY and FR filled neurons. Abbreviations as listed above. Conventions as in figure 1. Three injections of CTB across area 1 revealed strong intrinsic projections. Labeled neurons were also found in areas 3b, 3a, 2, 5, SV, PV, VP, M1, PMd, PMv, SMA, pre-SMA, PFC, and the cingulate gyrus. DY filled cells were found in the leg representations of the same areas labeled by CTB injections in this case. FR transport in this case was poor, however the intrinsic

projections in area 1 and the inputs from area 2 revealed by this injection suggest that projections to this region were from matching arm representations. A few cells labeled by the CTB-R injection into the foot representation are visible in medial area 1.

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Figure 7. Distribution of cells labeled by CTB, DY, and FR and DY injections into area 1 hand, area 1/3b face, and area 3b representation of the arm in case 12-40

A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of FR and DY filled neurons. Abbreviations as listed above. Conventions as in figure 1. Neurons labeled by injections into the area 1 hand and the 3b/1 face representations of were distributed similarly in somatosensory and motor areas. The FR injection into the 3b arm resulted in distribution of labeled cells typical after injections into area 3b.

Figure 8. Distribution of cells labeled by CTB, FR, and DY injections into area 1 hand, arm, trunk, leg, and face representations in case 12-59

A. The somatotopic maps of the parts of areas 3b (gray) and 1 with the locations of injections indicated. B. The distribution of CTB-IR neurons. C. The distribution of DY and FR filled neurons. Abbreviations as listed above. Conventions as in figure 1. Injections of CTB in the area 1 hand and leg representations revealed a similar pattern of projections as other cases with labeled cells found in all anterior parietal areas, the PPC, somatosensory areas in the lateral sulcus, and motor and premotor areas in the frontal lobe. FR transport after the injection in the area 1 arm was again limited. The few labeled cells were largely

distributed somatotopically in areas 1, 3b, 3a, 2, 5, and M1. Filled cells were found in somatotopically matched locations in the APC area 5 and M1 following DY injections in the area 1 trunk and face representations. The somatotopic pattern of projections to the face and trunk injection sites were less clear for projections from S2, PV, PMv, and PMd.

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Figure 9. Summary of corticocortical projections to area 1

Area 1 receives inputs from cortical somatosensory areas in the anterior parietal cortex and along the upper bank of the lateral sulcus, from multiple motor and premotor areas, and from the location of presumptive motor and sensory fields on the medial wall.

Table 1
Distribution of retrogradely labeled cells following tracer injections in area 1

Rows list case numbers, injected body region, types of labeled cells counted, and total number of cells. Cortical areas containing labeled cells are listed in the first column, and successive columns contain the percentage of cells labeled in each area. PPC includes all parietal areas caudal to area 5. LPC includes S2, PV, PR, and VS. UBLS/INS includes all labeled in the upper bank of the lateral sulcus and insula that were not in any part of the anterior or lateral parietal areas. Cingulate motor includes filled cells in the rostral and caudal cingulate motor regions. Other medial wall includes all labeled cells on the medial wall outside of the SMA, cingulate motor regions, and pre-SMA. Abbreviations as listed above.

	10-19			11-33			12-40			12-59		
	Hand, Arm, Leg	Leg	Arm & Trunk	Hand, Trunk, Foot	Leg	Arm & Foot	Hand	3b/1 Face	3b Arm	Hand & Leg	1/3b Face & Trunk	Arm
	CTB-IR	CTB-G	CTB-R & FR	CTB-IR	DY	FR & CTB-R	CTB-IR	DY	FR	CTB-IR	DY	FR
	n = 43,129	n = 4,482	n = 3,646	n = 38,206	n = 67,959	n = 897	n = 12,604	n = 48,314	n = 940	n = 31,391	n = 12,667	n = 490
Area 1	25.40	51.90	84.42	20.88	67.96	96.43	21.41	10.80	23.09	25.68	67.66	89.39
Area 3b	14.40	11.07	7.19	21.14	13.33	0.45	21.92	62.23	66.70	31.51	23.53	7.55
Area 3a	8.05	6.22	0.93	10.07	0.24	-	15.32	1.39	3.94	12.36	0.29	0.41
Area 2	12.22	2.92	2.28	16.05	12.50	2.79	21.66	13.89	3.51	14.99	1.68	0.82
Area 5	4.50	0.29	0.03	5.08	1.13	-	2.98	0.02	0.85	1.56	0.69	0.61
PPC	0.24	-	0.03	3.10	0.07	-	0.82	2.48	0.32	0.56	0.55	-
LPC	11.01	12.56	1.84	11.89	1.50	-	5.55	7.96	0.11	2.98	4.70	-
UBLS/INS	2.60	0.67	0.11	2.31	0.01	-	0.05	0.06	0.43	0.20	0.01	0.20
M1	12.39	6.38	2.08	6.51	1.01	-	9.39	0.62	0.74	5.33	0.82	1.02
PMd	1.89	1.25	0.16	0.76	0.00	-	0.06	<0.01	-	0.03	0.01	-
PMv	0.56	0.40	0.03	0.57	0.01	-	0.49	0.48	-	0.07	0.01	-
SMA	2.87	2.41	0.08	0.07	0.03	-	0.02	<0.01	-	0.39	0.01	-
Cingulate motor	1.25	0.42	0.14	0.17	0.14	0.11	0.17	<0.01	0.11	1.57	-	-
pre-SMA	0.75	0.20	0.05	0.02	0.03	-	0.05	-	-	0.45	0.01	-
Other medial wall	1.77	3.28	0.55	1.16	2.03	0.11	0.01	0.01	0.11	2.25	0.01	-
PFC	0.06	0.02	0.03	0.19	-	-	0.02	0.01	-	-	0.03	-
Other	0.04	-	0.05	0.03	-	0.11	0.10	0.04	0.11	0.09	0.01	-

Table 2**Injection sites**

The tracers and injected body representations are listed for each case. All injections are in area 1 unless otherwise indicated. D- digit, T- toe, other abbreviations as listed above.

<i>Body Region</i>	Case 10-19		Case 11-33		Case 12-40		Case 12-59	
	Tracer	Representation	Tracer	Representation	Tracer	Representation	Tracer	Representation
<i>Leg/Foot</i>	CTB-G	Knee, Upper Leg, Hip	CTB-R	Foot Pads, Insole, T4, T1, Inner Edge of Foot			CTB	Anterior Knee, Leg, Area 3b Leg
			DY	Distal Upper Leg				
<i>Trunk</i>	FR	Dorsal & Lateral Middle Trunk	CTB-G	Occiput, Dorsal Trunk, Shoulder, Neck			DY	Dorsal Trunk, Shoulder, 3b Middle Trunk
<i>Arm</i>	CTB-R	Forearm, Upper Arm, Armpit	FR	Dorsal Forearm, Upper Arm	FR	3b Dorsal Forearm, 1 Dorsal Forearm, Hand	FR	Forearm, Lateral Upper Arm, Ulnar Wrist, Ulnar Margin of Hand
<i>Hand</i>	CTB	D3, D2, D4, Palmar Pads, Area 2 Digit	CTB	Distal D2, 3, D4, Pads 3 & 4, Area 2 Hand	CTB	Distal D2, D3, D4, Area 2 Digits	CTB	D2, D1, D3, Palmar Pads
<i>Face</i>					DY	3b/1 Contralateral Upper Lip, Nose, Muzzle	DY	Forehead, Anterior Head, 3b Cheek